Written/Fax/E-mail Comments, Public Hearings on 11/7/07 Revised Proposed Rule for the Preble's Meadow Jumping Mouse

Updated 2/14/08

1	Index	
2	Dave Freudenthal Governor, State of Wyoming	Cheyenne, Wyoming
3	Thomas P. Nestler Colorado Division of Wildlife	Denver, Colorado
4	Vickie Brach USFS, South Park Ranger District	Fairplay, Colorado
5	Ellen Hodges USFS, Canyon Lakes Ranger District	Fort Collins, Colorado
6	Thomas A. Florich USFS, Laramie Ranger District	Laramie, Wyoming
7	Joe Reichardt Platte County Board of Commissioners	Wheatland, Wyoming
8	Cheryl Matthews Douglas County Open Space and Natural Resources	Castle Rock, Colorado
9	Dennis Hisey El Paso County Board of County Commissioners	Colorado Springs, Colorado
10	Bob Crifasi City of Boulder, OS&MP	Boulder, Colorado
11	John A. Kolanz City of Greeley	Greeley, Colorado
12	Rod Kuharich South Metro Water Supply Authority	Greenwood Village, Colorado
13	Don Britton Wheatland Irrigation District	Wheatland, Wyoming
14	Ken Hamilton Wyoming Farm Bureau Federation	Laramie, Wyoming

15	Tom Partin American Forest Research Council	Portland, Oregon
16	Ronald W. Opsahl Mountain States Legal Foundation	Lakewood, Colorado
17	Erin Robertson Center for Native Ecosystems	Denver, Colorado
18	Wm. Robert Irwin — Defenders of Wildlife Andrew E. Wetzer — Natural Resources Defense Council Michael J. Bean — Environmental Defense Jason Patlis — World Wildlife Fund	
19	Pauline P. Reetz Audubon Society of Greater Denver	Littleton, Colorado
20	Kent Holsinger Holsinger Law, LLC *Rescanned Documents/ New CD for Adjustments this only A-G; I-U; W-Y Holsinger	Denver, Colorado
21	Bob L. Ingels Housing and Building Association of Colorado Springs	Colorado Springs, Colorado
22	Jack Ekstrom Western Business Roundtable	Golden Colorado
23	David L. True True Ranches, LLC	Casper, Wyoming
24	Jack Saunders Saunders Company	Leadville, Colorado
25	William E. Gurski (1/08 FAX) KEM Homes	Fort Collins, Colorado
26	Rob Roy Ramey II	Nederland, Colorado
27	Kenneth R. Faux Jr.	Greenwood Village, Colorado
28	Jack Rick	
29	Howard Lind	Monument, Colorado

30	Coleen P. Abeyta	Colorado Springs, Colorado
31	Jane Rogers	Colorado Springs, Colorado
32	Ron and Mary Kunzelman	Colorado Springs, Colorado
33	Tom Nettles	
34	Michael and Claudette Stella	
35	Susan Majors	
36	Rata Clarke	
37	Mitchell Baldwin	
38	Marc Stein	Colorado Springs, Colorado
39	Martin Wolf	Colorado Springs, Colorado
40	Pete and May Weston	
41	Steve Tottosy	Colorado Springs, Colorado
42	Robert Hoff	Colorado Springs, Colorado
43	Doren Tenerowicz	Castle Rock, Colorado
44	Zachary Barr	Denver, Colorado
45	Gene Shannon	
46	Harvey Huston	Colorado Springs, Colorado
47	Pat Wells	Manitou Springs, Colorado

48	Dan Bjugstad	Colorado Springs, Colorado
49	Gerald and Marsh Federer	Cheyenne, Wyoming
50	Snookie Fatiuk	Colorado Springs, Colorado
51	Larry Banister1	Castle Rock, Colorado
52	Larry Banister2	Colorado Springs, Colorado
53	Patricia A. Smith	Colorado Springs, Colorado
54	Liz Klein	
55	12/10/07 Public Hearing	Lakewood, Colorado
56	12/12/07 Public Hearing	Wheatland, Wyoming

Holsinger Law, LLC

lands, wildlife and water law

Holsinger Law, LLC
104 Broadway, 3rd Floor
Denver, CO 80203
p: (303) 722-2828
f: (303) 496-1025
www.holsingerlaw.com

Kent Holsinger
kent@holsingerlaw.com

January 16, 2008

Ms. Susan Linner
Field Supervisor
Colorado Field Office
Ecological Services
U.S. Fish and Wildlife Service
P.O. Box 25486, MS-65412
Denver Federal Center
Denver, CO 80225

Re: Comments on Revised Proposed Rule To Amend the Listing for the Preble's Meadow Jumping Mouse ("Revised Rule")

Dear Ms. Linner:

On behalf of the Coloradans for Water Conservation and Development[1] ("CWCD"), we are writing in regards to the U.S. Fish and Wildlife Service ("FWS") Revised Proposed Rule to delist the Preble's Meadow Jumping Mouse ("Preble's") in Wyoming only under the Endangered Species Act ("ESA").

Recently, CWCD learned the FWS was relying on a paper (King et al. (in review)) for the Revised Rule that it does not even have. FWS staff informed us that we could receive a copy by FOIA. Because it would be impossible for us to receive this paper and comment on it prior to the January 22 comment deadline, we respectfully request a 90-day extension of the comment deadline.

The FWS listed Preble's as threatened in 1998 based on two basic propositions: (1) Preble's was physically distinct from other subspecies of meadow jumping mice; and (2)

[1] The CWCD was organized in 2003 by a number of rural and urban business interests including the Denver Metro Chamber of Commerce, Colorado Concern, Colorado Association of Home Builders, National Association of Industrial and Office Properties, Colorado Apartment Association and the Colorado Farm Bureau.

Holsinger Law, LLC

lands, wildlife and water law

Holsinger Law, LLC
104 Broadway, 3rd Floor
Denver, CO 80203
p: (303) 722-2828
f: (303) 496-1025
www.holsingerlaw.com

Kent Holsinger
kent@holsingerlaw.com

January 16, 2008

Ms. Susan Linner
Field Supervisor
Colorado Field Office
Ecological Services
U.S. Fish and Wildlife Service
P.O. Box 25486, MS-65412
Denver Federal Center
Denver, CO 80225

Re: Comments on Revised Proposed Rule To Amend the Listing for the Preble's Meadow Jumping Mouse ("Revised Rule")

Dear Ms. Linner:

On behalf of the Coloradans for Water Conservation and Development[1] ("CWCD"), we are writing in regards to the U.S. Fish and Wildlife Service ("FWS") Revised Proposed Rule to delist the Preble's Meadow Jumping Mouse ("Preble's") in Wyoming only under the Endangered Species Act ("ESA").

Recently, CWCD learned the FWS was relying on a paper (King et al. (in review)) for the Revised Rule that it does not even have. FWS staff informed us that we could receive a copy by FOIA. Because it would be impossible for us to receive this paper and comment on it prior to the January 22 comment deadline, we respectfully request a 90-day extension of the comment deadline.

The FWS listed Preble's as threatened in 1998 based on two basic propositions: (1) Preble's was physically distinct from other subspecies of meadow jumping mice; and (2)

[1] The CWCD was organized in 2003 by a number of rural and urban business interests including the Denver Metro Chamber of Commerce, Colorado Concern, Colorado Association of Home Builders, National Association of Industrial and Office Properties, Colorado Apartment Association and the Colorado Farm Bureau.

Preble's populations had declined over a significant portion of its range. Both of these have proven to be false. For all the reasons herein, the FWS should delist Preble's in both the states of Colorado and Wyoming.

I. Introduction

CWCD on December 17, 2003, filed its petition to delist Preble's. The State of Wyoming filed a similar petition to delist on the same day. Both states in which Preble's is alleged to occur have called for the delisting of Preble's. And the FWS must consider their input. See Interagency Policy Regarding the Role of State Agencies in ESA Activities. 59 Fed. Reg. 34275 (July 1, 1994).

After repeated irregularities in the delisting process (many of which have been highlighted in correspondence from U.S. Senator Wayne Allard to Interior Secretary Dirk Kempthorne) on March 15, 2006 the CWCD filed a Data Quality Act Challenge ("Challenge") against the FWS and the U.S. Geological Survey ("USGS"). Some 555 days after FWS acknowledged receipt of the Challenge, the FWS and USGS responded to CWCD's Challenge and refused to correct information disseminated by the FWS and USGS.

Specifically, CWCD sought to correct information that overstated threats, ignored crucial distribution, abundance and trends information, understated extensive regulatory mechanisms at the federal, state and local level and exhibited gross bias, inaccuracies and inconsistencies in the delisting process. On October 3, 2007, CWCD filed an Appeal to the Challenge. FWS Guidelines provide that a response to the Appeal would be made within 15 business days. CWCD's appeal was rejected by the agencies 93 days later, on January 4, 2008.

The FWS must consider the most recent or thorough information from stakeholders, the public and the scientific community. Accordingly, the FWS must consider not only the petition, but information available to it—including these comments. *See* 50 CFR 42414(b). Further, the FWS must ensure that the data used to make its determination is consistent with the standards of the ESA and the Data Quality Act.

In support of this comment letter, the CWCD hereby incorporates by reference its December 17, 2003 petition as well as its previous comments and correspondence to the FWS related to Preble's, including, but not limited to, its Data Quality Act Challenge and Appeal. CWCD also submits the following information for the administrative record:

Exhibit A: Cronin (2007) *The Preble's meadow jumping mouse: subjective subspecies, advocacy and management*. Animal Conservation

Exhibit B: Ramey, R.R., H.P. Liu, C. W. Epps, L. Carpenter, and J.D. Wehausen. (2005) *Genetic relatedness of the Preble's meadow jumping mouse (Zapus hudsonius preblei) to nearby subspecies of Z. hudsonius as inferred from variation in cranial*

morphology, mitochondrial DNA, and microsatellite DNA: implications for taxonomy and conservation. Animal Conservation 8:329-346.

Exhibit C: Crandall, K.A., Bininda-Emonds, O.R.P., Mace, G.M., and Wayne, R.K. (2000) *Considering evolutionary processes in conservation biology*. Trends in Ecology and Evolution 15:290–295.

Exhibit D: Crandall and Marshall (2006), *An assessment of the threatened subspecific status of the Preble's meadow jumping mouse (Zapus Hudsonius preblei) based on current molecular data sets*;

Exhibit E: Popper, K. R. (1963) *Science: conjectures and refutations. In Conjectures and Refutations: The Growth of Scientific Knowledge.* Harper and Row, New York. (Originally a lecture delivered at Peterhouse, Cambridge, in 1953 and published under the title 'Philosophy of science: a personal report' in C. A. Mace, ed., British Philosophy in Mid-Century (1957).

Exhibit F: Platt, J.R. (1964) *Strong Inference: Certain systematic methods of scientific thinking may produce much more rapid progress than others*. Science 146 (3642):347-353.

Exhibit G: Ramey testimony in the U.S. House of Representatives, Committee on Resources (2007);

Exhibit H: U.S. Senator Wayne Allard letter to Secretary Kempthorne (June 30, 2007);

Exhibit I: Preble's clips;

Exhibit J: Ramey, R.R., J.D. Wehausen, H.P. Liu, C. W. Epps, and L. Carpenter. (2006) *Response to Vignieri et a. (2006): Should hypothesis testing or selective post hoc interpretation of results guide the allocation of conservation effort?* Animal Conservation 9:244–247.

Exhibit K: Ramey, R.R., J.D. Wehausen, H.P. Liu, C. W. Epps, and L. Carpenter. (2007) *How King et al. (2006) define an "evolutionarily distinct" mouse subspecies: a response*. Molecular Ecology 16: 3518–3521.

Exhibit L: Dr. Philip Krutzsch e-mail recant re classification of Preble's;

Exhibit M: Timm, R. M., Ramey, R. R., and the Nomenclature Committee of the American Society of Mammalogists. (2005) *What constitutes a proper description?* Science 309:2163-2164.

Exhibit N: Hedrick PW (2001) *Conservation genetics: where are we now?* Trends in Ecology and Evolution 16:629–636.

Exhibit O: Quarles, S.P. (2001) *Issues Concerning the Listing of Distinct Population Segments of Vertebrates under the Endangered Species Act of 1973.* Testimony before the Subcommittee on Fish, Wildlife and Water Environment and Public Works Committee, United States Senate. May 9, 2001;

Exhibit P: Ramey, R.R., J.D. Wehausen, H.P. Liu, C. W. Epps, and L. Carpenter (2006b) Response to SEI Panel Report;

Exhibit Q: *Hail Linnaeus*, The Economist (May 17, 2007);

Exhibit R: Crandall (2006) *Advocacy dressed up as scientific critique*, Animal Conservation 9 (2006) 250–251;

Exhibit S: Animal Conservation Cover Photo;

Exhibit T: MetroStudy Development Statistics;

Exhibit U: Local Land Use Codes in Colorado;

Exhibit V: Amara Brook, Michaela Zint, Raymond De Young, *Landowners' Responses to an Endangered Species Act Listing and Implications for Encouraging Conservation,* 17 Conservation Biology 1473, 1638 (Dec. 2003)

Exhibit W: Burnham and Cade (2003) *Hands on Restoration*;

Exhibit X: MacDonald rebuttal to Inspector General Report;

Exhibit Y: GOCO record funding

II. Taxonomy and Genetics Support Delisting in Colorado and Wyoming

First and foremost, there are no quantitative standards in use by the scientific community or the FWS with which to objectively describe subspecies or DPS - these have been arbitrarily defined and their general "acceptance" in the scientific community is often nothing more than capricious opinion (*see* Cronin 1997 and 2007). Cronin (2007) is hereby attached as Exhibit A. That is the situation with the so called subspecies, *Z. h. preblei*. This is the issue that Ramey et al. (2005) (Ramey et al. 2005 is hereby attached

as Exhibit B) and others (Wehausen and Ramey 1993 and 2000; Ramey 1995; Crandall et al. 2000 (hereby attached as Exhibit C); Crandall and Marshal 2006 (hereby attached as Exhibit D) have sought to address by utilizing a hypothesis testing approach where criteria were set in advance of data collection and where hypotheses are potentially falsifiable. This is the objective scientific method (Popper 1963 (hereby attached as Exhibit E); Platt 1964 (hereby attached as Exhibit F)).

In multiple places in the Revised Rule, the FWS applied a double standard to the evidence in order to support the continued listing of *Z. h. preblei* as a subspecies. For example, the FWS selectively portrays Jones (1981) Ph.D. dissertation as "difficult to evaluate" - a conclusion not shared by the three professors on Jones's doctoral committee. Yet the FWS states: *"Jones's (1981) findings were not published in a peer reviewed journal and were not incorporated into the formal jumping mouse taxonomy, leaving his conclusions difficult to evaluate."* The FWS then proceeds to portray the unpublished study by Riggs et al. (1997) in a more favorable light. The Riggs et al. (1997) study had neither mtDNA sequence data nor specimens publicly available for additional testing. There is no record that the single reviewer (Hafner) who was allowed to *"inspect"* the Riggs et al. DNA sequence data did anything more than a casual glance at a handful of final edited DNA sequences.

Yet the Revised Rule states: *"the Preble's meadow jumping mouse specimens formed a homogenous group recognizably distinct from other nearby populations of meadow jumping mice (Riggs et al. 1997, p. 12). At the request of the Service, Hafner (1997, p. 3) reviewed the Riggs study, inspected Riggs' original sequence data, and agreed with its conclusions."* The conclusions of Riggs et al. (1997), however, were based on nothing more than the qualitative opinions of the authors about a single mtDNA tree presented in that study. In other words, the FWS discredited Jones 1981 (because it was contrary evidence to the Revised listing of *Z. h. preblei*), yet chose to use Riggs et al. (1997) to support their listing decision, even though data for the latter have been kept secret from the public for over a decade and the conclusions not independently unverifiable.

The FWS portrays the analyses of Krutzsch (1954) as if they were something more than a set of simple set of qualitative comparatives by stating: *"Additionally, Krutzsch (1954, pp. 452–453) evaluated the morphometric characteristics of 4 adult and 7 non-adult specimens. Acknowledging the small number of samples upon which his conclusion was based, Krutzsch (1954, p. 453) nonetheless concluded that the differences between Preble's meadow jumping mouse and neighboring meadow jumping mice was considerable and enough to warrant a subspecies."* The fact that Krutzsch (1954) only measured three adult skulls of *Z. h. preblei*, has been consistently ignored by the FWS, here and elsewhere. In fact, Krutzsch (1954) made no morphometric analysis beyond listing the means and ranges of 9 measurements (the same measurements that were made by Ramey et al. 2005 to retest Krutzsch's conclusions). It is clear that Krutzsch (1954) casually looked at 4 adult skins and 7 juvenile skins (the latter which he did not report on), to make qualitative comments on pelage coloration of this putative subspecies.

To illustrate how *Z. h. preblei* compares to other putative subspecies, we have attached the cover photo from the issue of Animal Conservation where Ramey et al. (2005) was published. *See* attached Exhibit S. In that photograph, the two *Z. h. preblei* specimens are indistinguishable from other subspecies. This picture graphically illustrates that *Z. h. preblei* is not morphologically or "evolutionarily distinct" from other nearby subspecies of *Z. hudsonius.* The species, *Z. hudsoniu,* is one of the most common rodents in the United States and its range covers approximately half of North America.

The FWS erroneously states in the Revised Rule that: *"Our original listing determined that Krutzsch's (1954) revision of the meadow jumping mouse species, including the description of the Preble's meadow jumping mouse, was widely supported by the scientific community as indicated by the available published literature (63 FR 26517, May 13, 1998). Our 1998 determination weighed the information in unpublished reports, such as Jones (1981), and public comments on the rule and found that they did not contain enough scientifically compelling information to suggest that revising the existing taxonomy was appropriate (63 FR 26517, May 13, 1998). Our 1998 conclusion was consistent with Service regulations that require us to rely on standard taxonomic distinctions and the biological expertise of the Department and the scientific community concerning the relevant taxonomic group (50 CFR 424.11)."*

However, the 1998 determination by the FWS to list *Z. h. preblei* was based on faulty information, selective use of information, and the opinions of a biased subset of scientists, some of whom have had vested interests in listing *Z. h. preblei* as a threatened subspecies (e.g. grant funding, consulting, or potential to serve on the recovery team).

The FWS states in the Revised Rule: *"In July 2003, we entered into a cooperative agreement with the Denver Museum of Natural Science (DMNS) to determine if the Preble's meadow jumping mouse was a unique subspecies relative to other nearby subspecies of meadow jumping mice (Service 2003a, pp. 1–2). This task was a priority of the Recovery Team (Service 2003a, pp. 1–2; Service 2003b, pp. iv, 38, 43, 76)."*

However, the Revised Rule does not accurately portray how the research by Ramey et al. was initiated and funded. Although the FWS claims that it was a Priority Task, and seeks to portray it as initiated by the FWS, the facts show otherwise. The Cooperative Agreement was the result of an investigator-initiated study proposal presented to the Recovery Team by Ramey and his collaborators in August 2002. The FWS had taken no action on this supposed "priority task" until Ramey presented his proposal. The reception to that proposal by the FWS and Recovery Team was unwelcome from the start. As documented in an article in WESTWORD (2004), *"Ramey put together a research proposal to test one simple hypothesis: Is the Preble's jumping mouse a genetically distinct sub-species? He presented his proposal to Fish and Wildlife's Preble's Mouse Recovery Team in the summer of 2002. The reception was chilly. "I could tell that my even proposing to challenge the prevailing wisdom wasn't particularly appreciated by most of the team," he remembers. "And then one member, Karen Rose, from the state of Wyoming, suddenly declared, 'This is excellent!' If looks could kill, she would have been dead on the spot.""* .

The State of Wyoming and DMNS provided initial funding and the FWS followed with funding through a Cooperative Agreement. As e-mails obtained through an investigation by Senator Allard's office clearly show, Ramey was repeatedly harassed by FWS staff, most notably, Bruce Roselund the Preble's Recovery Team leader. Rosenlund berated Ramey for late delivery when the product was actually delivered on time, under budget, and of high quality; Rosenlund wrote to his supervisors labeling Ramey as "pro-development", and left harassing telephone calls on his answering machine. This harassment and similar pressure by supposedly unbiased academics, environmental groups, and was well documented by Ramey in his recent Congressional Testimony (hereby attached as Exhibit G) (Ramey 2007b) and supported with e-mails and correspondence from U.S. Wayne Allard to Interior Secretary Kempthorne (hereby attached as Exhibit H) and highlighted in the Rocky Mountain News and Colorado Springs Gazette attached as Exhibit I.

As reported by the FWS, it is true that the results of Ramey et al. (2005): *"did not support Krutzsch's (1954, p. 452) original description of the Preble's meadow jumping mouse as ''averaging smaller in most cranial measurements'' (Ramey et al. 2005, p. 334). Ramey et al. (2005, p. 334) found that only one cranial measurement was significantly smaller, while two cranial measurements were significantly larger."*

As discussed in their extensively peer-reviewed published papers in Animal Conservation (Ramey et al. 2005, 2006 (hereby attached as Exhibit J), 2007 (hereby attached as Exhibit K) that alone should have been enough to overturn the prevailing taxonomy. However, Ramey et al. (2005) had added analyses of three other sources of information (mtDNA, microsatellites, and principal components analysis of skull measurements). Conspicuous in it absence from the Revised Rule is that fact that Dr. Krutzsch no longer accepts the taxonomy that the FWS seeks to defend. Krutzsch's retraction was entered into the Congressional Record on 28 April 2004 (hereby attached as Exhibit L).

The Revised Rule states: *"Ramey et al. examined a small number of base-pairs (346) in 1 region of the mtDNA across 5 subspecies of meadow jumping mice (205 specimens) (Ramey et al. 2005, pp. 331–332, 335). Ramey et al. (2005, p. 335, 338) found low levels of difference between the Preble's meadow jumping mouse and neighboring subspecies."*

However, the FWS fails to acknowledge that the same segment of control region DNA was used by Riggs et al. (1997) and therefore considered sufficient for the FWS to list *Z. h. preblei* as a subspecies under the ESA. An even smaller segment of DNA was recently used by one of the supposedly independent peer reviewers (Oyler-McCance) to describe a new species of grouse.

The FWS further mischaracterizes the results of Ramey et al. (2005) by stating: *"Their data demonstrated that all of the mtDNA haplotypes (alternate forms of a particular DNA sequence or gene) found in the Preble's meadow jumping mouse were also found in Z. h. campestris. The mtDNA data demonstrated evidence of recent gene flow between the Preble's meadow jumping mouse and neighboring subspecies (Ramey et al. 2005, p.*

338)."

While Ramey et al. (2005) did find shared haplotypes, however, what the FWS has consistently failed to acknowledge is that Ramey et al.'s test of uniqueness relied on a majority rule of four data sets: mtDNA, microsatellites, morphometrics, and ecological exchangeability. Ramey et al. (2005) chose that approach because multiple lines of evidence provides greater information that simply an a single data set (such as mtDNA or microsatellites). Even if the Ramey et al. (2005) mtDNA results are disregarded entirely, *Z. h. preblei*, is still fails all other tests (Ramey et al. 2006, 2007).

The FWS states in the Revised Rule that: *"Ramey et al. (2005, pp. 333–334, 338) analyzed a small number (5) of microsatellite loci (the specific position of a gene or other chromosomal marker) across 5 subspecies of meadow jumping mice (195 specimens). Ramey et al. (2005, p. 340) concluded that these results were consistent with morphometric and mtDNA results."*

However, the FWS has listed species and subspecies on far less data. For example, the Peninsular Ranges bighorn sheep were listed as a subspecies based on just three microsatellite loci although data from five MHC loci conflicted directly with that result. To call the segment of DNA "small" or "short" underscores the double standard applied to the presentation of information regarding this and other ESA-listed subspecies and DPS's.,

The FWS does not accurately report on the taxonomic revision by Ramey et al. (2005). *"Based on morphometrics, mtDNA, and microsatellites data, and a lack of recognized adaptive differences, Ramey et al. (2005, p. 340) suggested synonymizing the Preble's meadow jumping mouse and Z. h. intermedius with Z. h. campestris."*

This is a misleading and factually incorrect statement by the FWS. Ramey et al. (2005) did not "suggest" synonymy, they actually synonymized *Z. h. preblei* and *Z. h. intermedius* with *Z. h. campestris*, (the Prairie jumping mouse).

In the following section, the FWS deceives the reader and discredits the peer reviewed and published research by Ramey et al. (2005) by:

A) Selectively presenting a series criticisms of an earlier unpublished report (Ramey et al. 2004) and presenting them as if they are current criticisms of Ramey et al. 2005, 2006, and 2007). This constitutes an invalid apples-to-oranges comparison by the FWS that is both misleading and inaccurate.

B) Not acknowledging the many positive comments received on Ramey et al.'s research by numerous peer reviewers. The FWS seeks to only promote the erroneous negative perceptions of Ramey et al.'s work

C) Not acknowledging that Ramey's research team incorporated many of the suggestions from those reviews and others, including the addition of

microsatellite data analyses and ecological exchangeability, into Ramey et al. (2005).

"Prior to publication of Ramey et al. (2005) in "Animal Conservation," the CDOW and the Service solicited 16 peer reviews of the 2004 draft report provided to the Service (Ramey et al. 2004a). While many of the reviewers supported the findings of Ramey et al. (Baker and Larsen 2005; Bradley 2004, 2005; Crandall 2004, 2005; Hafner 2004; Maldonado 2005; Meaney 2004; Mitton 2004, 2005; Riddle 2004; Sites 2004; Waits 2004, 2005), the reviews raised a number of important issues. Some of the most significant issues identified included:"

Each of the ten issuesdetailed below are in the Revised Rule and we provide a critical review of each, relative to the final peer reviewed publications of Ramey et al. (2005, 2006, 2007):

"(1) Reliance upon museum specimens which can be prone to contamination (Douglas 2004, 2005; Maldonado 2005);" This is misleading because all biological specimens can be contaminated if improperly handled. Museum specimens have been used in countless studies.

Although KEA found a high level of statistical significance in their comparisons (using 27 microsatellites), the degree of differentiation among *Zapus hudsonius preblei, Z. h. campestris* and *Z. h. intermedius* were the lowest of any of the pairwise comparisons for mtDNA and microsatellites. The low degree of differentiation is illustrated by the fact that only four unique alleles were reported in *Z. h. preblei* (out of 279 in total), the lowest number of unique alleles for any subspecies sampled. KEA reported high levels of correct assignment to subspecies using the program structure. These authors attribute this to *Z. h. preblei* having 'considerable evolutionary differentiation' from other putative subspecies and to shortcomings of REA. However, KEA failed to acknowledge that this high level of correct assignment could also be an artifact of sampling design and number of loci surveyed (Rosenberg et al. 2005). KEA's findings raise a valid critique of all such studies — use of assignment tests such as structure may not be an appropriate tool for evaluating taxonomic separation because of the sensitivity of these tests to the number of loci employed. Future efforts employing these types of analyses may need to establish threshold assignment probabilities for a set number of loci with a given amount of variation per locus to allow comparability between studies.

KEA also criticized REA for using museum specimens, claiming a wide variety of problems associated with such tissue, based mostly on literature for ancient DNA samples, not museum specimens collected within the past 45 years.

In truth, Ramey *et al* . (2005) used a mixture of museum specimens and frozen tissue. However, all of the *Zapus hudsonius preblei* specimens used by KEA are subject to the same issues they raise regarding museum specimens because these ear punches were obtained without bleaching the ear punch between samples or wearing laboratory gloves to reduce cross contamination (R. Taylor, personal communication entered into the record on 7/6/06; Riggs *et al* . 1997). If only newly trapped individuals are used, as

advocated by KEA, investigations will be limited in the extent to which current patterns of variation might be parsed relative to historical natural processes vs. recent anthropogenic effects (e.g. bottlenecks).

"(2) the reliability of, and failure to validate, specimens' museum tag locality (and thus subspecies) identification (Ashley 2005; Douglas 2004, 2005; Hafner 2004; Oyler McCance 2004, 2005);"

Museum specimens are the very foundation of taxonomy. In fact the International Rules of Zoological Nomenclautre and the Nomenclature Committee of the American Society of Mammalogists require the taxonomic descriptions rely upon archived specimens (Timm et al. 2005) (hereby attached as Exhibit M). It is a double standard for the FWS to claim that on the one hand, that museum specimens are flawed yet these are used universally in taxonomic studies upon which the FWS relies. Nor is it reasonable to level the post hoc criticism that museum tags must be "validated" but neverless rely on countless studies of museum specimens without requiring the same validation. It is notable that the FWS says nothing about how such validation would be conducted.

"(3) reliance upon a small portion of mtDNA (Ashley 2004, 2005; Baker and Larsen 2005; Crandall 2004, 2005; Douglas 2004, 2005; Hafner 2005; Maldonado 2005; Oyler-McCance 2004, 2005; Riddle 2004; Sites 2004; Waits 2004, 2005);"

Ramey et al. (2005) generated multiple data sets (morphology, mtDNA, microsatellite, and ecological information) using the limited financial resources available. The study design was approved and in large measure funded by the by the FWS through a cooperative agreement with the Denver Museum of Nature & Science. For the FWS to now claim that the mtDNA data and other data were inadequate in length or number is disingenuous. For the record, we point out that the FWS has absolutely no standards whatsoever to judge how much DNA sequence is adequate for subspecies or DPS listings or delisting decisions.

"(4) the small number of microsatellite DNA loci examined (Vignieri et al. 2006, p. 241);"

As stated previously, the research study design was approved by the FWS as well as the final product report on microsatellite data. How can the FWS consider the number of loci to be "small" and therefore inadequate (even when considered in combination with morphometric, mtDNA, and data on ecological exchangeability) for delisting when fewer loci were used to list other subspecies or DPS? The bighorn sheep in the Peninsular ranges were listed based on only three microsatellite loci, even though the results were contradicted by 5 MHC loci (Boyce et al. 1997), morphometric analyses (Wehausen and Ramey 1993), and no data on ecological exchangeability?

The response of Ramey et al. (2006) to the criticisms of Vigneiri et al. (2006) regarding microsatellite data in are as follows:

> VEA [Vigneiri et al. (2006)] seek to invoke selective post hoc interpretations to explain away our microsatellite results. They equate statistical significance (in FST) with biological significance and selectively cite other mammal subspecies comparisons in support of their claim of 'strong differentiation' of Z. h. preblei. VEA incorrectly report that '95% of the northern population of Z. h. preblei' was assigned. What we did find was that 94% of the southern population could be assigned (table 6, Ramey et al., 2005), but we did not use any cut-off value for confidence of assignment (q). Therefore, some of these assignments were only slightly better than coin flips. VEA contradict themselves in stating that we 'employed too few loci' while also concluding that our microsatellite results add 'further strong support of differentiation' of Z. h. preblei.

See Exhibit J. Thus, Ramey et al. expose Vigneiri et al.'s biased use of selective post-hoc interpretations.

"(5) the criteria used and factors considered to test taxonomic validity as well as alternative interpretations of the data (Ashley 2004; Conner 2004; Douglas 2004, 2005; Hafner 2005; Oyler- McCance 2004, 2005; Vignieri et al. 2006, pp. 241–242; White 2004);"

Ramey et al. (2005) did not rely on subjective alternative, post hoc "interpretations of the data" as did the FWS and some peer reviewers. Instead, they relied on the approach multiple falsifiable hypotheses and criteria set in advance of data collection. Ramey et al. (2005) used the epistemological approach known as *strong inference* as described by Platt (1964). In contrast, the FWS in this Revised Rule, has relied on subjective post-hoc interpretation and selective use of information. As a result, the FWS's approach to evaluating information lacks an epistemological basis and is pseudoscientific in nature. The FWS seeks to promote the appearance of a measured scientific evaluation but cannot do so because they lacks any thresholds with which to test the validity of putative species, subspecies, and DPS.

"(6) whether the authors used an appropriate outgroup (a closely related group that is used as a rooting point of a phylogenetic tree) (Douglas 2004); "

This is not a valid criticism. Ramey et al. (2005) used the sister species, *Zapus princeps* as an outgroup in their multiple phylogenetic analyses (maximum likelihood, neighbor-joining, maximum parsimony methods). They also performed an unrooted network analyses that produced the similar results. It is obvious from the FWS statement above that the author of the Revised Rule did not read or understand the methodological details of Ramey et al. (2005).

The double standard that the FWS applies to evidence is also evident here. The FWS make no such criticisms of King et al. (2006a, b) who used the same species (*Zapus princeps*) as an outgroup in *their* phylogenetic analyses. Nor did they find any fault with Riggs et al. (1997) who also used *Z. princeps* as an outgroup.

"(7) the sampling regime and its impact on the analysis (Maldonado 2005; Oyler-McCance 2004);"

Ramey et al. (2005) carefully considered their choice of geographic sampling so as to capture the greatest amount of variation across putative subspecies. In that way, the sampling regime has an objective impact upon the analyses used. In contrast the sampling design by King et al. (2006a, b) involved "isolation by sampling design" - a method that left artificial gaps in the purported range.

"(8) failure to test all of the morphological characters examined by Krutzsch (1954) (Vignieri et al. 2006, p.238);"

Ramey et al. (2005) measured the same nine morphological characteristics that Krutzsch did in 1954. Krutzsch also made some qualitative statements about several vaguely defined characters but did not actually measure then. For example, Krutzsch did not actually measure the auditory bullae or the inflation of the frontal region; thus statements made about these had no objective quantitative basis. For the FWS to accept such an invalid criticism is beyond comprehension.

The lack of objectivity demonstrated by Vigneri et al. (2006) is clearly shown in the following passage from Ramey et al. (2006)):

> At the center of this debate is the separation of Z. h. preblei as a subspecies by Krutzsch (1954) based on measurements of only three skulls and comparisons of only four skins – sample sizes that no modern taxonomist would accept. In their attempt to defend this taxonomy, VEA try to discredit all of our morphometric analyses, while ignoring the work of Jones (1981) that found no morphological support for tests, and he used none; yet, VEA concluded that his finding of a smaller interorbital breadth in Z. h. preblei was a 'definitive finding'. VEA claim that interobital breadth was the only one of the morphometric variables we measured that Krutzsch (1954) found to distinguish *Z. h. preblei* and that our finding of a difference for that character confirmed Krutzsch's (1954) conclusion. What Krutzsch (1954) actually stated was that Z. h. preblei was smaller than *Zapus hudsonius campestris* in most of the nine skull dimensions measured, a hypothesis that our data clearly refuted. Such univariate tests that VEA appear to espouse were replaced decades ago in morphometric analyses by multivariate analyses of shape variation (Reyment, Blackith & Campbell, 1984), which was the approach we used.
>
> VEA criticize us for ignoring unquantified characters that Krutzsch (1954) included as the basis of his taxonomy, describing these as 'shape differences noted by a trained morpho-taxonomist'. They fail to realize that this 'trained morpho-taxonomist' (Krutzsch) does not accept his taxonomy and has publicly stated that our research 'clearly invalidates Z. h. preblei and demonstrates its relationship to Z. h. campestris'. He went on to state: 'Perhaps most significant is the model you provide to unequivocally establish the uniqueness of an organism

and its relationships before declaring it in danger of extinction. Such an analytical approach would prevent implementation of a process to support an agenda or a point of view. I can think of other listed endangered species that could have benefited for a prior, detailed, scientiifc appraisal' (P. Krutzsch in email to R. R. Ramey, entered into the U.S. Congressional Record on 28 April 2004).

See Exhibit J.

"(9) an inadequate evaluation of ecological exchangeability and habitat differences among subspecies (Ashley 2004; Conner 2004; Douglas 2004; Meaney 2004; Mitton 2004; Oyler-McCance 2004, 2005; Sites 2004; Vignieri et al. 2006, p. 238; Waits 2004, 2005);"

Contrary to the FWS assertions, Ramey et al. (2005) addressed this issue and presented a detailed rebuttal to the criticisms of Vigneri et al. (2006):

> Nor did we 'present nothing' that could be interpreted as a test of ecological exchangeability. We cited the original morphological research of Krutzsch (1954) and Jones (1981) as well as the literature reviews of Whitaker (1972, 1999), Clark & Stromberg (1987) and Cryan (2004) in support of our claim that no adaptive differences have been described between *Z. h. preblei* and other subspecies. Although it is possible that some critical adaptive difference had been 'missed' in the 106 years of study, starting with Preble (1899), none seem to have been noticed." Furthermore, we VEA make the assertion that 'the potential for ecological differentiation among these populations (putative subspecies of *Z. hudsonius*) is high'. However, the evidence and rationale they provide is speculative. VEA base their claims on Kulchler's (1964) potential natural vegetation (PNV) classifications. PNV classifications are based on hypothetical 'climax' vegetation that could potentially occupy a site without disturbance or climatic change (Zerbe, 1998). PNV classifications are not mutually exclusive categories. For example, each of the PNV classifications cited in VEA has overlap in plant species. PNV classifications are qualitative, generalized descriptions of vegetation communities that do not take into account the mosaic nature of natural landscapes, including successional stages, nor do they accurately characterize moist riparian habitat occupied by *Z. hudsonius* in the Great Plains. VEA ignore the fact that *Z. hudsonius* is a generalist species in its food habits (eating seeds, insects, fruit and fungi) and habitat preferences (Quimby, 1951; Jones, 1981), making adaptation to specific forage species less likely. VEA's assertion that the potential for ecological differentiation is high is therefore questionable. Most importantly, speculation is an inappropriate basis for definitions of subspecies or lower levels of population distinction (Ball & Avise, 1992; Crandall et al., 2000; Cronin, 2006); yet VEA declared *Z. h. preblei* a 'habitat specific subspecies group'.

Id. In summary, there is no ecological information to suggest that this population is ecologically unique in any way.

As documented by Ramey et al. (2006), Vigneiri (2006) sought to discredit and defame Ramey et al by not accurately portraying their goals, methods, results, and conclusions:

> Contrary to their stated goals, VEA did not accurately portray our goals, methods, results or conclusions. They selectively cited information and relied on speculation and post hoc interpretation of results to support their claims that Z. h. preblei is a distinct subspecies and an 'evolutionary distinct mouse'. We contend that the approach used by VEA was less than objective and, if widely applied, could result in the misallocation of conservation effort to many non-distinct local populations.

Id. Regrettably, the record to date does not show the political motivations behind criticisms of Vigneri et al. (2006) and others, including presumably independent peer reviewers and commentors (e.g. Hafner, Begstrom, Kays) working with environmental groups to discredit the research by Ramey et al. We hereby reference a series of e-mail exchanges obtained from the Internet that reveal political rather than scientific motivations to discredit the work of Ramey et al. (2005). Notably, these exchanges come from members of the supposedly impartial American Society of Mammalogists Conservation Committee, who subsequently prepared a comment letter to the FWS. This series of exchanges underscores the political interests that can compromise the objectivity of the "scientific community" that the FWS relies upon for their peer reviews and so-called scientific consensus.

To quote a few notable excerpts: 1) the President of the United States and Executive Branch is referred to as a *"Shrub Administration"*; 2) logical arguments that this group disagreed with were referred to as *"Rumsfeldian"*; 3) one participant suggested that *"Perhaps it might also be worth talking with the NGOs about legal options that they may be considering, we could likely add significant scientific evidence."* These individuals also spread misinformation amongst themselves, claiming that the Ramey et al. (2005) study was *"funded by industry"*; that *"Ramey spoke against the ESA"* when he actually testified before Congress about ways to strengthen the ESA. Other inaccuracies are too numerous to mention. It is difficult to see the American Society of Mammalogists exchange as an objective intellectual discourse on issues of scientific reasoning.

"(10) failure to consider the Preble's meadow jumping mouse's geographic isolation (Vignieri et al. 2006, pp. 237–238). Collectively, these critiques indicated that delisting based on the conclusions of Ramey et al. alone might be premature."

Ramey et al. (2005) did consider the present geographic isolation of the Colorado population of *Z. h. campestris*. As stated in Ramey et al. (2006). "Contrary to VEA's claims, we did not deny that *Z. h. preblei* seems to be currently isolated. What we questioned was how long this isolation has existed." Similarly, the SEI panel's criticisms suggest that they accept any geographic isolation, no matter how recent, to be sufficient to define a subspecies.

The Revised Rule goes on to present a false portrayal of the original delisting petitions:

"Because the Revised rule to delist the Preble's meadow jumping mouse relied solely upon an unpublished report (Ramey et al. 2004a) that had received mixed peer reviews (see above), verifying these results was a high priority of the Service (Morgenweck 2005; Williams 2004)."

The facts, well documented by e-mails obtained by Senator Allard, show that former Regional Director Morgenweck and staff instead went looking for a study to counter Ramey et al. (2005). *See* Exhibit H. The FWS also falsely reported twice in the Federal Register that the Preble's delisting petitions relied primarily on the results of Ramey et al. (2005). However, the research by Ramey et al. was only mentioned on half a page of the 106-page delisting petitions. Therefore, the FWS has erred rather than petitioners in basing its original Revised Rule to delist *Z. h. preblei* on the basis of taxonomic error.

FWS only praises the USGS study while presenting nothing but criticisms of Ramey et al. (2005). The following statement by the FWS underscores that bias: *"USGS study concluded that the Preble's meadow jumping mouse should not be synonymized with neighboring subspecies (King et al. 2006a, pp. 2, 29). An expanded version of this report was published in the journal ''Molecular Ecology'' (King et al. 2006b). This publication included an examination of microsatellite DNA, 2 regions of mtDNA, and 15 specimens critical to the conclusions of Ramey et al. (2005). King et al.'s (2006b, p. 4336) microsatellite analysis examined approximately 4 times the number of microsatellite loci (21) and 11/2 times more specimens (348 specimens) than Ramey et al. (2005) across the same 5 subspecies of meadow jumping mice.*

The FWS goes on to misrepresent the facts regarding the King et al. study:
"King et al. (2006b, p. 4337) concluded that their microsatellite data demonstrated a strong pattern of genetic differentiation between the Preble's meadow jumping mouse and neighboring subspecies. King et al. (2006b, pp. 4336–4341) also reported that multiple statistical tests of the microsatellite data verified this differentiation."

What the FWS fails to acknowledge is that the USGS equated statistical significance with biological significance. It is widely known that with larger sample sizes it is possible to find statistical significance in almost any comparison. When intervening geographical variation is ignored, as was by the USGS study, such meaningless statistical significance is exacerbated. Dr. Phil Hedrick (2001) (hereby attached as Exhibit N) published the following statement specifically about this issue: "*the statistical power for determining differentiation between groups is closely related to the number of independent alleles, so that even for a few highly variable microsatellite loci, there can be extremely high statistical power. When there is such high statistical power, extremely small molecular genetic differences between groups become statistically significant.*"

While it is true that the USGS study found statistical significance in their comparisons using 27 microsatellites, what the FWS fails to acknowledge is that the degree of differentiation among *Zapus hudsonius preblei*, *Z. h. campestris* and *Z. h. intermedius* is extremely low and can be found among local populations sampled in a similar way. The USGS results for these three subspecies were the lowest of any of the pairwise

comparisons for mtDNA and microsatellites made (Ramey et al. 2007). In their response to King et al. (2006), Ramey et al. (2007) reported that:

> This low degree of differentiation is illustrated by the fact that only four unique alleles were reported in Z. h. preblei (out of 279 in total), the lowest number of unique alleles for any subspecies sampled. KEA reported high levels of correct assignment to subspecies using the program structure. These authors attribute this to Z. h. preblei having 'considerable evolutionary differentiation' from other putative subspecies and to shortcomings of REA. However, KEA failed to acknowledge that this high level of correct assignment could alsobe an artifact of sampling design and number of loci surveyed (Rosenberget al. 2005). KEA's findings raise a valid critique of all such studies — use of assignment tests such as structure may not be an appropriate tool for evaluating taxonomic separation because of the sensitivity of these tests to the number of loci employed. Future efforts employing these types of analyses may need to establish threshold assignment probabilities for a set number of loci with a given amount of variation per locus to allow comparability between studies.

See Exhibit K. The fact that the Ramey et al. (2007) response to King et al. has been virtually ignored by the FWS is a poignant example of biased reporting of information by the FWS.

More data does not make a better study, however, the FWS would like the reader to believe that it does. In the following section of the Revised Rule, the FWS seeks to portray the USGS study as superior because it generated more data than Ramey et al. (2005).

"In their evaluation of mtDNA, King et al. (2006b, p. 4341) examined approximately 4 times the number of base-pairs across 2 regions (374 control region and 1,006 ytochrome-B region base-pairs) and 11/2 times more specimens (320 specimens for the control region analysis and 348 for the cytochrome-B analysis) than Ramey et al. (2005) across the same 5 subspecies of meadow jumping mice. King et al. (2006b, p. 4341) concluded that these data suggested strong, significant genetic differentiation among the five subspecies of meadow jumping mice surveyed."

The FWS fails to report that Crandall and Marshall (2006) reanalyzed the King et al. (2006) and Ramey et al. (2005) data in: separate analyses of microsatellite data; separate analysis of King et al.'s cytochrome B data; and a combined analysis of control region data, yet Crandall and Marshall (2006) reached the opposite conclusion of King et al. (2006) on each account. Crandall and Marshal (2006), however, reached conclusions consistent with those of Ramey et al. (2005) that these are not separate subspecies. *See* Exhibit D. The claims by King et al. (2006) that *Z. h. preblei* was evolutionarily distinct was both an artifact of their sampling design and an invalid equating of statistical significance with biological significance (Crandall and Marshall 2006; Ramey et al. 2007). None of this has been acknowledged by the FWS here in the Revised Rule or elsewhere.

Crandall and Marshall (2006) used more extensive analyses than either King et al. (2006) or Ramey et al. (2005) but used the used established thresholds for genetic uniqueness from the scientific literature. Crandall and Marshall are world-renown bioinformatics experts.

What counts however in matters of science and what distinguishes science from non-science is the criterion of potential falsifiability (Popper 1963). Yet, King et al. (2006) devised a biased geographic sampling design and used as many microsatellite loci as possible to assure they would find statistically significant differences among putative subspecies. In other words, King et al. relied on an approach and selective use of information that set the bar so low, that *Z. h. preblei* could *not* be falsified as a subspecies. That puts King et al.'s conclusions outside the realm of science.

The USFWS fails to acknowledge that King changed his mind no fewer than three times as to what constituted a subspecies. As pointed out by Ramey et al. (2007). "The publicly available record shows that KEA changed their interpretation of results at least twice, from subspecies being 'weakly differentiated' to 'evolutionarily distinct'; and the number of potential DPS's of *Zapus hudsonius preblei* changed from two, to three, to none. This subjectivity is symptomatic of an approach that lacks clearly defined thresholds, and epitomizes the problem that REA attempted to address. We believe that few if any subspecies or DPSs proposed for US-ESA listing or delisting will be falsifiable under the general approach and low level of genetic differentiation that KEA used to accept subspecies. General application of that approach may move the allocation of conservation effort outside the realm of scientific inquiry." The publicly available record unequivocally shows that King called *Z. h. preblei* 'weakly differentiated' after his initial analyses in an internet posting, where he asked for help with his analyses to 'evolutionarily distinct' in his draft and final papers. Additionally, King et al. changed the number of potential DPS's of *Z. h. preblei* from two, to three, to none in his draft paper, his SEI panel testimony, and his final paper, respectively. As Ramey et al. (2007) correctly point out " This subjectivity is symptomatic of an approach that lacks clearly defined thresholds, and epitomizes the problem that REA attempted to address." Ramey et al. (2005, 2006, 2007) are an example of a truly scientific approach to these issues.

The FWS and others misrepresent the facts in their following series of statements. This is particularly disturbing because these errors by the FWS (as well as the SEI panel and King et al. 2006) have been pointed out previously (Ramey et al. 2006a,b; 2007). The insistence of the FWS to falsely represent the research of Ramey et al. (2005) in a Federal Register Notice demonstrates egregious bias by that agency.

The Revised Rule states: *"Additionally, their[King et al. 2006] results indicated that the Preble's meadow jumping mouse did not share haplotypes with any neighboring subspecies (King et al. 2006b, p. 4341). Such haplotype sharing had led Ramey et al. to previously conclude that the Preble's meadow jumping mouse was not unique; specifically, Ramey et al. concluded that because all of the Preble's meadow jumping mouse haplotypes were found in Z. h. campestris, the Preble's meadow jumping mouse*

was a less genetically variable population of Z. h. campestris"

Contrary to the FWS assertions, the critical tests of uniqueness used by Ramey et al. (2005) did not depend upon shared mtDNA haplotypes nor results from mtDNA data alone. Instead, Ramey et al. used critical tests (thresholds) to test uniqueness based on morphology, mtDNA, microsatellites, and the literature on ecological exchangeability. Ramey et al. (2005) set criteria in advance of data collection and let the chips fall were they may. To unequivocally prove this point, we provide the excerpts Ramey et al. (2005) to tests of subspecies uniqueness:

> **MtDNA**
> The critical test of the original subspecies description was two-fold. First, the hypothesis of Z. h. preblei being a unique, smaller subspecies relative to Z. h. campestris would be rejected if the skulls of Z. h. preblei were not significantly smaller for the majority of skull measurements. Second, we used LDA to test uniqueness with the distinguishability criterion that≥90% of the specimens be correctly classified to subspecies at jackknifed posterior probabilities ≥0.95 (Wehausen & Ramey, 2000). This unambiguous criterion requires that specimens be correctly classified with a high degree of certainty using
> a multivariate analysis of shape.
>
> **Microsatellites**
> Our critical test of uniqueness for subspecies and historic genetic exchangeability (Crandall et al., 2000) was two-fold: that there be greater variation between Z. h. preblei and other subspecies than within each subspecies in pairwise comparisons (using FST and AMOVA) and that multiple private alleles be at higher frequency than shared alleles at the majority of loci. We do not claim that these criteria alone can be used to define subspecies, or that they are universally applicable, merely that they provide an unambiguous test of deeply historic genetic divergence among populations.
>
> **Morphometrics**
> Our critical test of uniqueness for Z. h. preblei using mitochondrial DNA sequence data was that there be greater molecular variance among than within subspecies (in pairwise comparisons involving Z. h. preblei) or that samples show nearly complete reciprocal monophyly with respect to other subspecies.
>
> **Ecological exchangeability**
> There is no published evidence of adaptive differences (e.g. selection for cryptic pelage on different rocky substrates as found by Hoekstra & Nachman, 2003) or ecological differences (e.g. major habitat and/or climatic differences) that would be expected to result in notable adaptive differences between Z. h. preblei and other adjacent subspecies (Krutzsch, 1954; Whitaker, 1972, 1999; Jones 1981; Clark & Stromberg, 1987; see Cryan, 2004 for an in-depth review). These animals live in a range of similar habitat types and appear to have similar life histories. While the absence of evidence does not necessarily mean there is evidence of

absence, there do not appear to be any adaptive differences that prevent the Z. hudsonius subspecies in this study from being ecologically exchangeable. We therefore cannot reject the null hypothesis of historic or recent ecological exchangeability.

See Exhibit B. The conclusions of Ramey et al. (2005) presented below and discussed further in Ramey et al. (2007) show that assertions regarding shared mtDNA haplotypes being critical to the conclusions of Ramey et al. (2005), repeatedly made by the FWS and others, are patently false:

> Based on hypothesis testing using four lines of evidence – morphometrics, mtDNA, microsatellites and a lack of recognised adaptive differences – we synonymies Z. h. preblei and Z. h. intermedius with Z. h. campestris, which was described first as the prairie jumping mouse by Preble (1899). Because we did not analyse cranial morphometric data for Z. h. luteus and Z. h. pallidus, we are cautious about their taxonomic status at this time. However, our preliminary results are consistent with Jones' (1981) findings that there do not appear to be any recognisable subspecies of Z. hudsonius in the study area.

Id. And this from Ramey et al. (2007):

> Even if the mtDNA sequences in question are excluded from analysis, it does not alter the basic conclusions of Ramey et al. (2005). That is because our critical tests did not rely on any sharing of haplotypes among putative subspecies. Instead, our subspecies test relied on: (i) morphological analyses to test the original quantitative basis of Z. h. preblei ; (ii) mtDNA reciprocal monophyly, amova ; and (iii) the proportion and frequency of unique microsatellite alleles and pairwise FST values. With the samples in question excluded, amova results just exceed our threshold but Z. h. preblei is still not even close to being reciprocally monophyletic. Similar results are obtained if all mtDNA sequences obtained by REA using nested PCR are excluded from analyses and KEA's data are substituted for REA's Z. h. campestris sequences.

See Exhibit K.

In the next section, the FWS continues with its portrayal of Ramey et al. (2005) in the worst possible light by ignoring contrary evidence: *"Because of these conflicting results, King et al. (2006b, pp. 4355– 4357) reexamined 15 specimens from the University of Kansas Museum collection relied upon by Ramey et al. in determining that neighboring subspecies shared haplotypes. Among the specimens reported to contain the Preble's meadow jumping mouse's haplotypes by Ramey et al. (2005, pp. 335–336), King et al. (2006b, p. 4357) found that the results could not be repeated. If these specimens were removed from the analysis, neither study would illustrate haplotype sharing between the Preble's meadow jumping mouse and neighboring subspecies. King et al. (2006b, p. 4357) concluded that ''these findings have identified the presence of a systemic error in the control region data reported by Ramey et al. (2005)'' and ''calls into question all of*

the results of Ramey et al. (2005) based on the mtDNA genome and prevents analysis of the combined data." King et al. (2006, p. 4357) noted that possible reasons for the difference in sequences included contamination, mislabeling of samples, or other procedural incongruity."

What the FWS fails to report is that King et al. (2006) used different DNA amplification primers and reaction conditions and buffers to obtain DNA sequences from these disputed samples (Ramey et al. 2007). This alone could have resulting in the reported differences, however, King chose to selectively report only the most negative interpretation of these results. Alternative explanations, including their use of methods designed to obtain different results, were not reported. Such omissions indicate a biased reporting of results by King et al.

Ramey et al. (2007) pointed this out succinctly:

KEA assert that the shared mtDNA haplotypes found by REA between Zapus hudsonius preblei and Z. h. campestris are the result of contamination of museum specimens, and they present a reanalysis of these specimens in support of their assertion. While this may be a point well taken, KEA did not consider any alternative explanations (e.g. nuclear paralogs, heteroplasmy, different amplification primers and conditions, and template quality). Additionally, the use by KEA of different primers, annealing temperatures (48 rather than 60 degrees) and buffers suggests conditions that could lead to different amplification success and results.

See Exhibit K.

Although King et al. (2006) and the FWS have sought to produce a wholesale portrayal of Ramey et al. (2005) as inaccurate, the FWS makes no admission of how King et al. used different reaction conditions to produce there results nor how King changed their paper at the proofs stage (last minute changes made before going to press that are made without editorial and peer reviewer knowledge). These errors in King et al. (2006) were pointed out by Ramey in detail to the SEI panel and detailed in Ramey et al. (2007). Yet the supposedly impartial SEI panel chose to selectively ignore errors by King in their report, a report that the FWS now uses as a basis to this Revised Rule. Altering a scientific paper in such a way at the proofs stage and misreporting on the methods of other scientists whom they criticize are a serious breach of professional behavior, especially when King et al. level fallacious accusations at Ramey et al. However, this is similar to another breach of professional behavior by King. In 1999, the State of Maine filed a Freedom of Information Act complaint to obtain data that King relied upon to identify DPS's in Atlantic salmon. When the UWFWS failed to release the data, Maine filed suit (*Maine v. U.S. Department of Interior*, 2298 F.3d 60, 64 (1st Cir. 2002). Following a court-ordered release of data, Maine complained that a data file had been "improperly altered" by King, a view that was shared by the Court in denying the motion by the U.S. Department of Interior to dismiss the case.[2] The alteration of a peer reviewed

[2] Available at: www.med.uscourts.gov/opinions/Carter/2000/GC_11062000_1-00cv122_Me_v_US_Dep_Interior.pdf

and accepted manuscript by King . argues strongly against the FWS promoting King et al. (2006) as best available science.

In the following section, the FWS portrays King et al. as if it has achieved a peer reviewer stamp of approval, which is far from the case in reading these reviews. Additionally, it is inconsistent and suggestive of bias that the FWS does not list specific criticisms of King et al. (2006) in the same detail as they had with Ramey (2005). And finally, why the FWS sent a genetics paper to be reviewed by someone who admittedly knows nothing about the technical details of genetic data and analysis (Armstrong) is beyond comprehension.

"Prior to its release, King et al. (2006a) underwent an internal peer review per USGS policy (U.S. Geological Survey 2003, pp. 3, 6, 12, 28–33). In an effort to provide consistent, comparable reviews, we solicited peer reviews from the same 16 reviewers asked to review Ramey et al. (2004a, 2004b). Nine of the experts provided comments (Armstrong 2006; Ashley 2006; Bradley 2006; Crandall 2006a; Douglas 2006; Hafner 2006; Maldonado 2006; Oyler-McCance 2006; Riddle 2006). Some of the most significant issues raised included the sampling regime and its impact on the analysis (Armstrong 2006; Ashley 2006; Crandall 2006a; Douglas 2006; Oyler- McCance 2006; Riddle 2006); and the criteria used and factors considered to test taxonomic validity and alternative interpretations of the data (Bradley 2006; Crandall 2006a)."

Absent from the Revised Rule is any substantive discussion of why the sampling by King et al. (2006) had an impact on their analysis and conclusions. The FWS simply states in the Revised Rule that: "*Some of the most significant issues raised included the sampling regime and its impact on the analysis.*" However, the sampling regime by King et al. (2006) did have a major impact on the analysis. By sampling many closely related mice in one or two locations per subspecies, King et al. (2006) conveniently ignored all of the intervening variation across hundreds of miles between. This created "artificial gaps" in the range of subspecies which in turn, inflated the measured genetic distance between them.

Ramey et al. (2007) illustrate the issue of King et al. (2006) biased sampling:

> KEA sampled seven populations of *Zapus hudsonius preblei* but only one or two populations from each of the other putative subspecies. In contrast, REA sampled many populations, but few individuals per population, across the range of each putative subspecies. An ideal study design with unlimited resources would incorporate both approaches, as well as sampling across the entire species; however, this is not often practical because of logistical and funding constraints. Given the choice, which strategy provides the most objective test of subspecies uniqueness? KEA claim their sampling strategy allows more appropriate statistical testing, but they do not acknowledge that their approach created artificial gaps in the distribution of genetic variation, leading to an 'isolation-by-sampling design' effect among all five of the subspecies. This sampling strategy predisposes the results to an exaggeration of genetic distances among putative

subspecies. This assertion is supported by the fact that genetic distances from KEA were strongly correlated with geographical distances for the *Z. h. preblei/campestris/intermedius* lineage (R2=0.82), including a sample from southeastern Wyoming that is intermediate between *Z. h. preblei* and *Z. h. campestris* (see KEA Fig. 3).

See Exhibit K. It is important to add that when the biased sampling scheme used by King et al. (2006) is combined with their use of a large number of microsatellite loci and their equating of statistical significance with biological significance, that subspecies status can be rationalized for countless populations of plants and animals. And finally, if the effects of population bottlenecks from very recent isolation (e.g. less than 50 years from fenced highways, clearcutting, or urban growth) on genetic variation are not taken into account, then even very recently human-isolated populations could be defined as subspecies (Ramey 2005). This was obviously not the intent of Congress in passage of the ESA.

In the following section, the FWS portrays King et al. as if it has achieved a peer reviewer stamp of approval, which is far from the case in reading these reviews. Additionally, it is inconsistent and suggestive of bias that the FWS does not list specific criticisms of King et al. (2006) in the same detail as they had with Ramey (2005). And finally, why the FWS sent a genetics paper to be reviewed by someone who admittedly knows nothing about the technical details of genetic data and analysis (Armstrong), is beyond comprehension.

"Prior to its release, King et al. (2006a) underwent an internal peer review per USGS policy (U.S. Geological Survey 2003, pp. 3, 6, 12, 28–33). In an effort to provide consistent, comparable reviews, we solicited peer reviews from the same 16 reviewers asked to review Ramey et al. (2004a, 2004b). Nine of the experts provided comments (Armstrong 2006; Ashley 2006; Bradley 2006; Crandall 2006a; Douglas 2006; Hafner 2006; Maldonado 2006; Oyler-McCance 2006; Riddle 2006). Some of the most significant issues raised included the sampling regime and its impact on the analysis (Armstrong 2006; Ashley 2006; Crandall 2006a; Douglas 2006; Oyler- McCance 2006; Riddle 2006); and the criteria used and factors considered to test taxonomic validity and alternative interpretations of the data (Bradley 2006; Crandall 2006a)."

See Exhibit D. It is important to add that when the biased sampling scheme used by King et al. (2006) is combined with their use of a large number of microsatellite loci and their equating of statistical significance with biological significance, that subspecies status can be rationalized for countless populations of plants and animals.

Absent from the Revised Rule is any substantive discussion of why the sampling by King et al. (2006) had an impact on their analysis and conclusions. The FWS simply states in the Revised Rule that: *"Some of the most significant issues raised included the sampling regime and its impact on the analysis."* However, the sampling regime by King et al. (2006) did have a major impact on the analysis. By sampling many closely related mice in one or two locations per subspecies, King et al. (2006) conveniently ignored all of the intervening variation across hundreds of miles between. This created "artificial gaps" in

the range of subspecies which in turn, inflated the measured genetic distance between them. By comparison, the approach if King et al. is similar to that used by racists to create racial or ethnic stereotypes: by ignoring the variation found across populations it is possible to create the appearance of differences when these do not otherwise exist.

Ramey et al. (2007) illustrate the issue of King et al. (2006) biased sampling:

> KEA sampled seven populations of Zapus hudsonius preblei but only one or two populations from each of the other putative subspecies. In contrast, REA sampled many populations, but few individuals per population, across the range of each putative subspecies. An ideal study design with unlimited resources would incorporate both approaches, as well as sampling across the entire species; however, this is not often practical because of logistical and funding constraints. Given the choice, which strategy provides the most objective test of subspecies uniqueness? KEA claim their sampling strategy allows more appropriate statistical testing, but they do not acknowledge that their approach created artificial gaps in the distribution of genetic variation, leading to an 'isolation-by-sampling design' effect among all five of the subspecies. This sampling strategy predisposes the results to an exaggeration of genetic distances among putative subspecies. This assertion is supported by the fact that genetic distances from KEA were strongly correlated with geographical distances for the Z. h. preblei/campestris/intermedius lineage (R2=0.82), including a sample from southeastern Wyoming that is intermediate between Z. h. preblei and Z. h. campestris (see KEA Fig. 3).

See Exhibit K. Crandall and Marshall (2006) took similar issue with the selective sampling of King et al. (2006):

> Both studies have limitations in their sampling strategies. The conclusions by King et al. of population structure are particularly suspect given the sampling design of their study. For example, King et al. fail to sample in areas most likely to show gene flow between subspecies (Figure 5). These areas include, Z. h. preblei from southern Wyoming, where you would find individuals most likely to show evidence of gene flow between Z. h. campestris, Z. h. pallidus, and even Z. princeps based on geographic proximity and previous studies (see above), and Z. h. pallidus from western Nebraska. King et al. have just a single locality sampled for Z. h. luteus and just two sites sampled for the critical Z. h. campestris and Z. h. intermedius. This is particularly problematic with the widespread distribution of Z. h. intermedius across 11 states with sampling in only the NE corner of South Dakota and an adjacent site in central Minnesota. The central problem here is a taxonomic issue relative to the entire species complex and possibly sister species within the genus, thus the entire species complex should be sampled to resolve the issue.

See Exhibit D. It is important to add that when the biased sampling scheme used by King et al. (2006) is combined with their use of a large number of microsatellite loci and their

equating of statistical significance with biological significance, that subspecies status can be rationalized for countless populations of plants and animals.

FWS states: *"Given the discrepancies between the Ramey et al. and King et al. reports, we contracted for a scientific review to analyze, assess, and weigh the reasons why the data, findings, and conclusions of the two studies differed (Service 2006, p. 14)."*

What the FWS fails to acknowledge is that it chose to contract for an outside review in large part because it has no objective standards for the determining validity of a subspecies or DPS. This issue was previously described as the use of "standardless standards" by Quarles (2002) in Senate testimony (hereby attached as Exhibit O).

The FWS portrays a misleading impression that the SEI panel performed an exhaustive reanalysis of data from Ramey et al. (2005) and King et al. (2006):*"Following an open and competitive bid process, we selected SEI as the contractor (Service 2006). SEI assembled a panel of experts with the necessary scientific expertise in genetics and systematics (SEI 2006a, p. 7). The panelists reviewed, discussed, and evaluated all of the literature relevant to this issue, including published literature, unpublished reports, third-party critiques, and other materials suggested by interested parties (SEI 2006a, pp. 48–55). Additionally, the panel examined and reanalyzed the raw data (SEI 2006a, pp. 8, 21) used by Ramey et al. and King et al., including the mtDNA data, microsatellite DNA data, and original sequence chromatograms (automated DNA sequence data output recordings) (SEI 2006a, pp. 8, 23)."*

In fact, the SEI panel review was extremely rushed and cursory analyses performed (e.g. use of simplified neighbor joining trees for mtDNA analyses without model verificaion; no simulation based analysis of migration rates using either mtDNA or microsatellite data). A truly exhaustive analysis of both data sets however, can be found in Crandall and Marsahll (2006). These two authors utilized a battery of state-of-the-art analyses to reanalyze data from both Ramey et al. (2005) and King et al. (2006). Yet the FWS makes only passing mention of this report.

It is significant to the issue attempting to be addressed by the the FWS, that the results of the Crandall and Marshall analysis were completely misrepresented by the FWS and the SEI panel. The results were practically 180 degrees opposite to that portrayed by the FWS and SEI panel. To prove this point, we provide here is an excerpt from the Crandall and Marshall (2006) report on the mtDNA phylogenetic analyses:

> Figure 6 shows monophyletic groupings with good nodal support for Z. princeps, *Z. hudsonius,* and *Napaeozapus insignis*. This is not terribly surprising due to the low numbers of individuals and localities sampled from *N. insignis* and *Z. princeps*. Figure 6 also shows a monophyletic grouping for *Z. h. pallidus* and one for a combined *Z. h. luteus* + *Z. h. pallidus* group. However, given that only a single locality from *Z. h. luteus* was sampled, only limited conclusions can be drawn. All samples from *Z. h. preblei*, *Z. h. campestris*, and *Z. h. intermedius* combined to form a single monophyletic group. However, none of these

subspecies forms a monophyletic group by themselves and thus fail this particular subspecies test and indeed fail even an evolutionarily significant unit (ESU) test of Moritz (1994). *Z. h. campestris* comes closest to forming a monophyletic group with a single *Z. h. intermedius* haplotype nested within it. As a whole this analysis, although severely restricted by the sampling design as discussed above, provides some preliminary evidence for designation of a two subspecies within the Z. hudsonius samples investigated, one including the *Z. h. pallidus* + *Z. h. luteus* samples and another including the *Z. h. preblei* + *Z. h. campestris* + *Z. h. intermedius* samples.

See Exhibit D.

In this section, the FWS promotes the impression that the SEI panel was fair, open, and unbiased: *"The scientific review panel was open to the public and allowed for interactions among panel members, Dr. King, Dr. Ramey, other scientists, and the public. In July 2006, SEI delivered a report outlining their conclusions to the Service (SEI 2006a). Although the panelists were not obligated to reach a consensus, they did not disagree on any substantive or stylistic issues (SEI 2006a, p. 9). Thus, the report represented the consensus of all three panelists, as well as the individual opinions of each panelist."*

However, as discussed herein, the record shows that the SEI panel composition and time allotted to participants was manipulated through lobbying by King, the FWS, and others interested in the listed status of Preble's.

As shown in this example, the FWS makes information that supports its position easily available to the public but does not make contrary information easily available to the public: *"The panel organized its evaluation into four sections corresponding with the different types of scientific evaluations performed, including morphology, ecological exchangeability, mtDNA, and microsatellite DNA. The panel's findings with regard to each are summarized briefly below. The full report is available for review at http://www.fws.gov/mountain-prairie/species/mammals/preble/Prebles_SEI_report.pdf."*

Although the SEI panel report is easily available on the internet at the FWS's website, information contrary to SEI's conclusions and the FWS's Revised Rule are not. Ramey provided a list of information omitted from the FWS Preble's website in his Testimony for the Committee on Natural Resources, United States House of Representatives on July 31, 2007. *See* Exhibit G.

The FWS inaccurately portrays the morphological methods and results of Ramey et al. (2005), giving a false negative impression of that study: *"Morphology: Although Ramey et al. (2005) examined two of the seven morphological characters identified by Krutzsch (1954, pp. 452–453), the panel found that all seven of these characters should have been reexamined in order to support the Revised taxonomic revision. The panel also concluded that the type specimen (a single specimen designated as the type by the original author at the time of publication of the original description of a taxon) of each taxon should have*

been included in the analysis. The panel's conclusion was that an insufficient test of the morphological definition of the Preble's meadow jumping mouse had been conducted to support the synonymy of the Preble's meadow jumping mouse with other subspecies (SEI 2006a, p. 41)."

As an initial matter, Ramey et al. (2005) measured the same nine skull measurements that Krutzsch did, not two of seven as claimed by the FWS. The panel opined that Ramey et al. (2005) should have measured additional characters; ones that the original author (Krutzsch 1954) did not actually measure but only casually commented on. Similarly, the panel's opinion that Ramey et al. (2005) should have measured type specimens is baseless. The sample sizes of Ramey et al. (2005), typically 40 per subspecies, were far more representative of the morphological variation found within subspecies that the single type specimen that was for each subspecies. The post hoc opinions by the panel are not a standard of taxonomic revision nor supported by the published literature, they are merely the unsupported opinion of the three panelists. It is inconsistent for the FWS has accepted taxonomic revision by Ramey (Wehausn and Ramey 2000) for the listing of the Sierra Nevada bighorn sheep subspecies *(Ovis canadensis sierre)* using the same methods but claim that it is not adequate in the present case, when applied to *Z. h. preblei.* This suggests a double standard when considering evidence used in an ESA listing compared to a potential ESA delisting.

To these assertions, Ramey et al. provided the following rebuttal:

> We do not agree with the panel's claim that "two of the lines of evidence presented by REA (their analyses of cranial morphometrics and ecological exchangeability) are based on insufficient data to support their suggestions for taxonomic change." The description of Z. h. preblei by Krutzsch (1954) was based on far less data and small sample sizes that precluded statistical tests. As we noted in our response to Vigneiri et al. (Ramey at al. 2006): "At the center of this debate is the separation of Z. h. preblei as a subspecies by Krutzsch (1954) based on measurements of only 3 skulls and comparisons of only 4 skins. ... Krutzch's sample sizes precluded meaningful statistical tests, and he used none." The panel's opinion that our morphometric analyses are inadequate shows bias in ignoring the weak inference and small sample size used originally to describe this subspecies in 1954.
>
> Based on their statements, the panel is willing to accept poor discriminant analysis assignments that are in many cases differ little from flips of a coin in discrimination ability (posterior probabilities of >0.50) in support of this putative subspecies. Similarly, the panel is willing to accept principal components analysis plots (an exploratory tool lacking inferential statistical capabilities that is not designed for hypothesis testing) as a means to define subspecies. The panel is unwilling to accept the fact that the only quantitative basis for Krutzsch's original description of Z. h. preblei is not supported by univariate statistical analysis using much larger sample sizes (n=40 per subspecies). Additionally, the panel does not acknowledge the fact that the scientist who originally described Z. h. preblei,

Krutzsch, no longer supports this subspecies description (Ramey et al. 2006). This should settle the issue but has been ignored by the FWS and the panel (see Ramey et al. 2006).

We do not agree with the panel that a "thorough analysis of the original characters and specimens used by Krutzsch (1954) to describe Z. h. preblei is required". Why should those three original specimens provide different results? If Z. h. preblei is a good subspecies, then any adequate sampling of its population should show statistically meaningful differences from other subspecies. We performed such an analysis and obtained clear results to the contrary. Relative to characters we did not measure, the following points are pertinent.

1) Krutzsch did not actually measure the auditory bullae or the inflation of the frontal region; those characters were only described qualitatively, thus conclusions had no objective basis. The panel's opinion that these could somehow be "coded for systematic analysis", ignores the fact that Krutzsch never measured them in the first place. Furthermore, the panel did not provide specifics as to how this could be done, nor thresholds that could be applied to such data to test (and potentially falsify) the hypothesis of uniqueness for Z. h. preblei.
2) Although Krutzsch's (1954) methods indicated that he measured incisive foramina width and length, no values for those measurements were reported.
3) We measured and analyzed the same nine measurements for which Krutzsch (1954) reported actual measurement data (mean, ranges, and sample sizes in Table 5, Krutzsch 1954), and we did not measure those qualitative skull characteristics (discussed above) for which he did not report measurements.
4) After examining pelage in several hundred museum specimens with the pelage descriptions in hand (sides duller, less black-tipped hair on dorsal stripe), we concluded that these were subjective qualitative assessments that would not be repeatable. (The attached photograph gives some idea of the variation within, and overlap among, putative subspecies.) In fact, the species, Z. princeps and Z. hudsonius, cannot be reliably distinguished on pelage alone, which was why Conner and Shenk (2003) developed their cranial morphometric test to distinguish them and noted the following : "Furthermore, the taxa [the species: Z. princeps and Z. hudsonius] are ecologically and physically similar and no reliable technique exists to distinguish live specimens in the field." In other words, those species are not reliably distinguishable using pelage color or pattern. Based on our examination of several hundred museum skins, the subspecies are even less so.

The best available science shows that morphologically, Z. h. preblei is not reliably distinguishable from Z. h. campestris and Z. h. intermedius.

Hereby attached as Exhibit P.

The FWS discussion of "Ecological Exchangeability" was similarly biased: *"The panel concluded that no persuasive evidence was presented regarding ecological*

exchangeability, and that the ecological exchangeability of the subspecies remains unknown (SEI 2006a, p. 41)."

By this same reverse logic, the FWS could ESA list subspecies and DPSs based on speculation only. This speculative approach is neither scientifically defensible nor legally defensible.

As discussed in their *Response to the report: Evaluation of Scientific Information Regarding Preble's Meadow Jumping Mouse prepared by the Sustainable Ecosystems Institute,* Ramey et al. (2006b) highlighted these failings of the SEI panel report:

> We do not agree with the panel's claim that ecological exchangeability is inadequate. The panel mistakenly reports that we used tests of ecological exchangeability upon which to base subspecies synonymy. In fact we used this as one of the tests to determine whether Z. h. preblei could be considered a DPS (Ramey et al. 2005). By their own logic, the panel could not find our evidence to be inadequate because we did not use it to specifically test this subspecies in the first place. However, as we have found in our review of the literature, in 106 years of study no one has noticed any adaptations that would preclude ecological exchangeability among the putative subspecies Z. h. preblei and nearby subspecies (Please see Ramey et al. 2005 and 2006 for a more extensive treatment). Relative to what the U.S. Fish and Wildlife Service is supposed to consider in making its findings on the listing of Z. h. preblei, the question is: "What do we know?", not: "What do we not know?" If the panel's approach is extended to other cases, it would mean that speculation about as yet undescribed or hypothetical uniqueness is adequate justification to create or maintain an ESA-listed subspecies. This amounts to proposing that science not be the basis of such decisions, however, the ESA clearly requires the FWS to make listing determinations "solely on the basis of the best scientific and commercial data available" 16 U.S.C. § 1533(b)(1)(A).

See Exhibit P.

The Revised Rule ignored the alternative explanations for differences in results for a handful of mtDNA sequences between Ramey et al. (2005) and King et al. (2006). The SEI panel erroneously insist that these shared haplotypes were somehow critical to the conclusions of Ramey et al. (2005), an assertion that is not supported by reading the methods, results, and conclusions of Ramey et al. (2005): *"MtDNA: The panel noted that data provided by Ramey et al. (2005) and King et al. (2006b) differed in geographic sampling strategy, amount of sequence data examined, aspects of the analysis, and quality (SEI 2006a, p. 41). All of these could help explain why the two studies came to differing conclusions. However, the panel noted that the most significant difference between the two studies in terms of mtDNA was whether the Preble's meadow jumping mouse shared any mtDNA haplotypes with other subspecies of meadow jumping mice. Upon review of the raw data, the panel found evidence of contamination within some of the key sequences reported by Ramey et al. The panel concluded that there was no reliable evidence of any haplotype sharing (SEI 2006a, p. 42). The panel further*

determined that if these conflicting mtDNA sequences were removed from consideration, the two studies' mtDNA data would largely agree (SEI 2006a, p. 32). The panel also suggested that because the western jumping mouse and the meadow jumping mouse are distantly related, western jumping mouse may perform poorly as an outgroup, leading to poor resolution of relationships among meadow jumping mouse subspecies. While both Ramey et al. and King et al. used this outgroup, unrooted analysis showed clearer structuring between the subspecies (SEI 2006a, p. 42)."

As noted in the response to the SEI panel by Ramey et al. (2006b):

> However, the panel failed to notice that our critical tests did not rely on any sharing of haplotypes among putative subspecies; instead, our subspecies test relied on: morphological analyses to test the original quantitative basis of Z. h. preblei, mtDNA reciprocal monophyly and Analysis of Molecular Variance, and the proportion and frequency of unique microsatellite alleles and pairwise FST. Our tests for distinct populations used the approach of Crandall et al. (2000) and did not rely on sharing of mtDNA haplotypes among putative subspecies.
>
> The panel presents a neighbor-joining tree but fails to tell the reader that this is one of a number of potential graphical representations of evolutionary relationships among
> mtDNA haplotypes. Many other trees that are equally parsimonius can be found (as discussed in Ramey et al. 2005). The reliability of the branches of the single tree presented by the panel was not indicated by any bootstrap support values. As both Ramey at al. (2005) and KEA both showed, the bootstrap support for all but the two major mtDNA lineages representing Z. h pallidus/luteus and Z. h. preblei/intermedius/campestris are not reliable. Therefore, none of the phylogenetic analysis to date support Z. h. preblei as being reciprocally monophyletic or even close to being so. This result was confirmed by Crandall and Marshall (2006) in their analysis of KEA's data.

Id. Thus, the conclusions of the SEI panel involved: the selective use of explanations; an inaccurate portrayal of the methods, results, and conclusions of Ramey et al. (2005); and relied upon a subjective post hoc interpretation of results from a single mtDNA tree (a level of analysis that is unacceptable in the contemporary phylogenetics literature).

The FWS present a subjective rationale for accepting microsatellite data as evidence of subspecies uniqueness. What is especially disturbing here is that the panel misreport the conclusions of Crandall and Marshall (2006) and Ramey et al. (2005): *"Microsatellite DNA: The panel found that the two microsatellite datasets contain similar information. The panel pointed out that both the Ramey et al. (2005) and King et al. (2006) microsatellite data, as well as Crandall and Marshall's (2006) reanalysis of these data, strongly support a statistically significant independent cluster that corresponds to the Preble's meadow jumping mouse, providing support for a distinct subspecies (SEI 2006a, pp. 42– 43). The panel indicated that while the microsatellite data alone did not make a strong case for evolutionary significance, in concert with the mtDNA data (discussed*

above), the two datasets corroborate the distinctness of the Preble's meadow jumping mouse (SEI 2006a, pp. 43)."

As documented in the response to the SEI panel by Ramey et al. (2006b):

> The panel and KEA equate statistical significance with biological significance in their evaluation of microsatellite data. This is the same pitfall that basic statistic textbooks urge students to avoid: statistical significance should not be blindly equated with practical significance. In other words, while a difference among populations might be statistically significant, the actual difference in means can be small in magnitude and with substantial overlap in range of values. KEA's data shows this pattern with extensive shared microsatellite alleles with other subspecies, especially Z. h. campestris and Z. h. intermedius. Statistical significance can also be an artifact of sampling design. As several authors have shown that when a large number of individuals are sampled for a large number of microsatellite loci, and modern statistical tests are applied (as in KEA), it is very likely that statistically significant differences will be found among populations even with high levels of interbreeding and there will be a high level of correct assignment of individuals to populations (Cornuet et al. 1999; Pritchard et al. 2000; Cegelski et al. 2003; Baudouin et al. 2004; Waples and Gaggiotti 2006). Application of such an approach to defining subspecies and DPSs, means that population-level differences could qualify as ESA-listable units. This would lead to an unlimited number of potential ESAlistable units. It also means that these designations (and subsequent ESA listings) could be acceptable even if they were an artifact of sampling design. This was clearly pointed out in Crandall and Marshall (2006), and by Crandall and Ramey to the panel. Please refer to our discussion of statistical significance vs. biological significance in our comments on KEA submitted to the FWS and the panel: Is the Preble's meadow jumping mouse an evolutionarily distinct subspecies? Comments on the report by King et al. (2006) and to the technical report by Crandall and Marshall (2006).
>
> Additional problems with equating statistical significance in genetic results with biological significance were pointed out to the panel but were ignored. These included: 1) the fact that very recent human induced genetic bottlenecks and isolation can increase apparent genetic divergence among populations (such as Z. h. preblei north and south of Denver as discussed in Ramey et al. 2005 but not acknowledged by KEA, Vigneiri et al. (2006), or the panel); 2) a low level of female dispersal relative to males can result in higher levels of mtDNA divergence than nuclear markers (trapping data show that females disperse less often and over shorter distances); 3) a faster mutation rate in small mammals (due to small body size and short generation time) can result in higher levels of genetic divergence over the same period of time than in larger mammals. If genetic results are accepted without taking these factors into account, then ESA listings could be based on statistical significance that is of little or no biological significance.

See Exhibit P. The use of the term "broadly consistent" in the following section of the

Revised Rule underscores the arbitrary and capricious nature of the SEI panel's evaluation.:*'The panel's overall conclusion was that the available data are broadly consistent with the current taxonomic status of the Preble's meadow jumping mouse as a valid subspecies and that no evidence was presented that critically challenged its status (SEI 2006a, p. 4)."*

In the following statement, the FWS seeks to dismiss the substantive information contained in response of Ramey et al (2006) to the SEI panel report.

"In August 2006, Ramey et al. (2006c) submitted a statement to the Service disputing the findings and conclusions of the SEI report. No new data or analyses were presented in this statement, and the panel previously considered most of the contentions (Ramey et al. 2003, 2004a, 2004b, 2005, 2006a, 2006b; SEI 2006a, 2006b, 2006c)."

The response by Ramey et al. (2006b) to the SEI panel report contains substantial information that clearly demonstrates the arbitrary and capricious nature of the SEI panel's conclusions regarding *Z. h. preblei* as a subspecies. In that report, Ramey and his colleagues systematically addressed each issue raised by the panel. Most importantly, the Ramey et al. (2006b) clearly lay out the epistemological issues with the SEI panel report. In matters of science the criterion of potential falsifiability is what distinguishes science from non-science and the SEI panel clearly used an approach that would prevent the potential falsification of (Popper 1963). This is what distinguishes science from non-science. However that report and other contrary information to the continued recognition of *Z. h. preblei* as a subspecies remains conspicuously misrepresented by the FWS. The rebuttal of Ramey et al. seriously undermines the FWS use of the SEI panel report as "best available science".

As discussed on the opening page of Ramey et al. (2006b):

> The panel opined that the lines of evidence in Ramey et al. (2005) are insufficient to overturn Z. h. preblei as a subspecies and that additional data need to be gathered to "clarify" the issue. Yet no thresholds were advanced by the panel that could be used to objectively test this subspecies with additional data. It is our contention that the stance taken by the panel amounts to support for an approach that precludes falsification of virtually any ESA-listed subspecies or DPS.
>
> Instead of reviewing all of the available science, the panel arbitrarily created its own burden of proof, which it then unilaterally applied only to Ramey et al. (2005). Rather than critically analyze the underlying data upon which Z. h. preblei became a subspecies and was ESA-listed (e.g. Krutzsch 1954; Riggs et al. 1997), the panel only examined the most recent papers and correspondence while ignoring much contrary information. If all available scientific information are considered, the U.S. Fish and Wildlife Service currently has sufficient scientific information to conclude that Z. h. preblei does not qualify as a valid subspecies or DPS.

See Exhibit P.

The FWS suggests that they have considered other evidence but do not present any analysis of it in the Revised Rule: *"Other evaluations of the available literature and data include Ramey et al. (in press), King et al. (in review), Crandall and Marshall (2006), Spencer (2006b), and Cronin (2007)."* Why does the FWS not list any of the substantive issues brought up in these papers about King et al. (2006) but promotes a long list of criticisms of Ramey et al. (2005)?

In its section titled "Taxonomic Conclusions", the FWS presents no analysis upon which to base this conclusion. *"Specifically, the Preble's meadow jumping mouse's geographic isolation from other subspecies of meadow jumping mice (Krutzsch 1954, pp. 452– 453; Long 1965, pp. 664–665; SEI 2006a, p. 34) has resulted in the accretion of considerable genetic differentiation (King et al. 2006b, pp. 4336–4348; SEI 2006a, pp. 41–43). The available data suggest that the Preble's meadow jumping mouse meets or exceeds numerous, widely accepted subspecies definitions (Mayr and Ashlock 1991, pp. 43–45; Patten and Unitt 2002, pp. 26– 34; SEI 2006a, p. 44). In reaching this conclusion, we do not use a presumption that we must rely on the established taxonomy in the absence of conclusive data to the contrary (see SEI report at p. 39). In Therefore, after a review of all available information, we have determined that the taxonomic revision for the Preble's meadow jumping mouse suggested in our Reviseddelisting rule (70 FR 5404, February 2, 2005) is no longer appropriate."*

As this statement clearly shows, the FWS should admit that it uses no objective, quantifiable, definitional basis for subspecies or DPS. Instead, the FWS relies on a non-scientific approach where subjectivity is an acceptable substitute for objective hypothesis testing and the potential for falsification. In seeking to discredit the measured scientific reasoning and epistemological grounding found in Ramey et al. (2005, 2006a,b, 2007) the FWS seeks to avoid the real issues regarding subjectivity in defining subspecies and DPS listed under the ESA. *See, e.g.* attached Exhibit Q. In effect, the FWS in this Revised Rule has demonstrated a willingness to abandon scientific reasoning and rationalism, that could result in an arbitrary and capricious ESA final decision regarding the continued listing of *Z. h. preblei* as a subspecies. See, e.g. attached Exhibit R.

III. Threats Overstated

A. FWS Cannot Rely on Significant Portion of the Range Opinion to Delist in Wyoming but not Colorado

FWS seems to rely entirely on the Significant Portion of the Range ("SPR") opinion of the Solicitor of the U.S. Department of the Interior for its revision to delist in Wyoming only. Revised Rule 63017. But the FWS has yet to issue implementing rules for this opinion. More importantly, the SPR opinion does not apply to subspecies.

FWS characterizes Colorado as a significant portion of the range of the purported subspecies. Yet a significant portion of the range is characterized by "particularly high-

quality habitat or it its location or characteristics make it less susceptible to certain threatens than other portions of the range." Revised Rule at 63018. If Colorado habitat is much better than Wyoming habitat, how can FWS delist where there is poor habitat and maintain a listing where there is high quality habitat?

If immediacy of threats varies geographically across the range, how can FWS draw a distinction between Wyoming and Colorado where geography varies little, if any, at the state line? Revised Rule at 63019. If Preble's have persisted in areas that have been hayed for many years in Wyoming, can they not persist in areas that have been hayed for many years in Colorado? *See* Revised Rule at 63010.

How can the FWS characterize the existence of Preble's in four drainages in Wyoming as "widespread," yet consider its existence in Colorado in 13 drainages as anything other than widespread? Even the lack of trapping success in Wyoming is characterized differently than Colorado. FWS avers Preble's "may simply be uncommon" in drainages in Wyoming. Revised Rule at 63018.

How can FWS support its characterization of reported increases in Preble's sites in Wyoming as increases in population and range, yet refer to increases in sites reported in Colorado as an "improvement in our understanding?" *See* 72 Fed. Reg. 62992, 62998.

In the Revised Rule, the FWS takes pains to reference the number of sites now documented in Wyoming, but omits any reference to the number of sites now documented in Colorado. Why has FWS omitted reference to the number of sites in Colorado?

Why does FWS state that 80% of trapping efforts in Colorado fail, but omit any reference to what percent of trapping efforts in Wyoming fail?

Could what the FWS labels as a "shy, timid rodent" be averse to live-trapping? Indeed, there is documented literature that such is the case.

The FWS fails to state if, or how, Preble's habitat in Wyoming is markedly different than Preble's habitat in Colorado. Are Preble's different in Wyoming than Colorado? If so, how? How does the FWS distinguish Preble's from other purported subspecies of meadow jumping mice? How does the FWS distinguish meadow jumping mice from other species of jumping mice? Whom is going to inform Preble's about this dramatic change of course? Whom is going to tell Preble's mice that they are "safe" in Colorado, but not Wyoming, under the Revised Rule?

If FWS can differentiate Preble's listed status between Colorado and Wyoming, then surely FWS can differentiate listed status between counties, between towns, between neighborhoods, between drainages, between ditches and between sites. Perhaps each individual mouse should be assessed separately for its status as threatened or endangered?

What the FWS proposes in this Revised Rule is directly equivalent the what the General

Account Office (GAO) warned against in 1978: trivializing the ESA by listing insignificant populations such as "squirrels in city parks". *Endangered Species – A Controversial Issue Needing Resolution*, 52, 58 (GAO Rep. CED 79-65, 1979). According to Senate Report No. 96-151, the rationale for the listing of subspecies and populations was to prevent full species from going extinct: *"The committee agrees that there may be instances in which the FWS should provide for different levels of protection for populations of the same species. For instance, the U.S. population of an animal should not necessarily be permitted to become extinct simply because the animal is more abundant elsewhere in the world. Similarly, listing of populations may be necessary when the preponderance of evidence indicates that a species faces a widespread threat, but conclusive data is available with regard to only certain populations."* This is followed by the frequently quoted expectation of congress: *"Nonetheless, the committee is aware of the great potential for abuse of this authority and expects the FWS to use the ability to list populations sparingly and only when the biological evidence indicates such action is warranted."* Senate Report No. 96-151 - Endangered Species Act Authorization, Report from Committee on Environment and Public Works (May 15, 1979).

In the present case, the FWS trivializes the ESA by proposing to list meadow jumping mice along streams in Colorado, even though: 1) meadow jumping mice are more widespread and abundant that previously thought in Colorado and Wyoming; and 2) there are no "preponderance of evidence that the species (or even the subspecies) faces a widespread threat". In fact, the species is found across half of North America (Krutzsch 1954; Ramey et al. 2005) - from Alaska to Georgia.

Why does FWS cite Colorado's Comprehensive Wildlife Conservation Strategy (a public-input driven process) yet fail to mention that the State of Colorado has called for delisting Preble's? *See* Revised Rule at 63006.

B. Distribution Abundance and Trends Support Delisting in Colorado and Wyoming

According to the FWS, at the time of listing, "the primary threat to the Preble's was habitat loss and degradation caused by agricultural, residential, commercial and industrial development." 70 Fed. Reg. 5404, 5405 (Feb. 2, 2005). But the distribution, abundance and trends data alone merits delisting in both Colorado and Wyoming. Historically, the Preble's was found in 14 hydrologic units in eastern Colorado and southeastern Wyoming. When it was listed, the FWS could find the mouse in only nine hydrologic units. Since the listing, the mouse has been found in 17 hydrologic units, including all that were historically occupied and three where it had never been known to occur.

More populations of the mouse are now known to exist than at any time before. And, as the USGS has stated, "it is likely that habitat suitable for *Z. hudsonius* (meadow jumping mice) is becoming increasingly available across western parts of the Great Plains with the westward expansion of riparian forests and mixed-grass prairie." (Cryan 2005) (Emphasis added). At the time of listing, the mouse was documented at only 29 sites. Today, it has been found at no fewer than 132 sites.

Additional survey work on the mouse resulted in the discovery of significant additional populations and has greatly expanded the documented distribution of the Preble's.[3] FWS goes to lengths to characterize activities in Colorado as threats. That so many section 7 consultations for Preble's have taken place in Colorado is evidence of their expansive range and increasing populations. *See* Revised Rule 63007 to 63012.

What empirical evidence does FWS have that "[A]t some sites, researchers have seen dramatic changes in estimated populations from season to season and year to year?" Revised Rule at 63020. What, specifically, do changes in "estimated" populations refer to?

Distribution, abundance and trends data on the mouse demonstrates ample populations throughout an expansive range. Even if the mouse was a valid subspecies, which it is not, the alleged threats to the species do not occur over a significant portion of its range.

FWS avers that intensive trapping studies designed to estimate populations of the Preble's are limited and, as a result, no regional, statewide or rangewide population estimates have been developed. *Id.* at 63003. Yet Figure 1 to the Revised Rule demonstrates hundreds of confirmed locations and efforts to trap Preble's in Colorado and Wyoming.

FWS also stated that little trend information exists for Colorado populations and that short-term studies may not accurately characterize abundance. *Id.* But FWS's own trapping records show sizeable increases in population and range. As has been demonstrated time and time again, additional survey work on the Preble's has resulted in, and will continue to result in, the discovery of additional populations.[4] For many other species, additional survey work has indeed identified additional populations:

- The FWS delisted the pine barrens tree frog where a considerable amount of potential habitat within the range had not been investigated, and results from surveys indicated much of that habitat was likely to harbor the species.[5]

- The FWS recognized that estimates of black-tailed prairie dog ("BTPD") density varied depending upon the season, region, and climatic conditions and removed

---

[3] Even if Preble's numbers were in decline, annual fluctuations in population (up to 50%) do not necessarily equate to the need to list a species. *See* 69 Fed. Reg. 64889, 64892 (Nov. 9, 2004) (Where the FWS issued a negative 90-day Finding on the petition to list the white-tailed prairie dog). 72 Fed. Reg. 62992, 62998 (Nov. 7, 2007).

[4] Even if Preble's numbers were in a cyclic decline, annual fluctuations in population (up to 50%) do not necessarily equate to the need to list a species. *See* 69 Fed. Reg. 64889, 64892 (Nov. 9, 2004) (Where the FWS issued a negative 90-day Finding on the petition to list the white-tailed prairie dog).

[5] 48 Fed. Reg. 52740 (Nov. 22, 1983).

the species from the candidate list after additional surveys provided more accurate estimates of occupied habitat.[6]

- The discovery of additional populations and higher population estimates of the Rydberg milk-vetch were sufficient to subsequently delist that species. 54 Fed. Reg. 37941 (Sept. 14, 1989). Only three previously unknown populations sufficed to delist the least chub. 64 Fed. Reg. 41061 (July 29, 1999).

- Finding the Chiricahua dock, a 3- to 6-foot-tall perennial, to be more abundant and widespread than originally documented, the U.S. Fish and Wildlife Service withdrew its proposal to list the plant as threatened under the Endangered Species Act on August 9, 1999.[7]

- When studies were completed, researchers concluded that the Dismal Swamp southeastern shrew is more widespread than originally thought and is found in a wide variety of habitats throughout southeastern Virginia and the coastal plain of North Carolina.[8]

- FWS biologists found listing of the sicklefin and sturgeon chub unwarranted due to new information that indicated populations are more abundant and better distributed throughout their range than previously believed.[9]
- The spineless hedgehog cactus was found to be more abundant than previously believed.[10]

- The Tumamoc globeberry was found to be more abundant than previously believed.[11]

- Eggert's Sunflower was proposed for delisting when populations increased from 34 known sites to 279 known sites.[12]

Lastly, FWS states that without comprehensive population estimates for the Preble's, the only basis for trend assessment is the presence or absence surveys in historical habitat. 72 Fed. Reg. 62992, 63003 (Nov. 7, 2007). With not other support, FWS then comes to divergent conclusions for the Preble's in Wyoming and Colorado.

---

[6] 69 Fed. Reg. 51217 (Aug. 18, 2004); see also Press Release, U.S. Fish and Wildlife Service, Black-tailed Prairie Dog Removed from Candidate Species List (Aug. 12, 2004).
[7] Press Release, U.S. Fish and Wildlife Service, U.S. Fish and Wildlife Service Concludes that a Southwestern Plant is not Threatened (Aug. 9, 1999).
[8] Press Release, U.S. Fish and Wildlife Service, Dismal Swamp Southeastern Shrew No Longer Needs Endangered Species Act Protection, (Mar. 3, 2000).
[9] Press Release, U.S. Fish and Wildlife Service, U.S. Fish and Wildlife Service Determines Sicklefin and Sturgeon Chub Do Not Warrant Listing as Threatened or Endangered (Apr. 19, 2001).
[10] 58 Fed. Reg. 49242 (Sept. 22, 1993).
[11] 58 Fed. Reg. 33562 (June 18, 1993).
[12] 69 Fed. Reg. 17627 (April 5, 2004).

C. FWS Cites No Authority that Other Threats to Preble's Merit Listing

FWS cites invasive weeds and fires as potential threats to the mouse. Ironically, the Preble's listing, as FWS seems to concede, may negatively impact the ability to manage lands to avoid the presence of invasive weeds and to mitigate against catastrophic forest fires. *See* Revised Rule at 63015. FWS concedes disease is not a "substantive issue" for Preble's. Revised Rule at 63012.

FWS cites the potential for additional water storage and development in Colorado as a threat to Preble's, yet cites few specific projects planned which may impact Preble's habitat. Revised Rule at 63009. FWS states runoff-related impacts to riparian habitat may increase and that restoration of riparian systems would be less likely to occur. Revised Rule at 63009. How does the FWS substantiate this assertion on its part? What authority does the FWS cite for the proposition that "secondary impacts to riparian habitats have likely diminished the areas that are capable of sustaining Preble's populations? See Revised Rule at 63012. What are negative indirect effects from upstream development? Revised Rule at 63017. What authority does the FWS cite for such alleged impacts? Where does the FWS document that such impacts will occur in Colorado, but not Wyoming?

How can FWS concede "threats to the Preble's meadow jumping mouse from these [overutilization, disease, predation, fire, flooding, invasive weeds, weed control programs, pesticides, herbicides, non-point source pollution, secondary impacts associated with human-caused development, scarcity, the potential for competition between [Preble's] and the western jumping mouse, and the future effects of climate change] are generally poorly understood and difficult to predict," yet recommend delisting in Wyoming only? *See* Revised Rule at 63017.

D. Development in Colorado is Vastly Overstated as a Threat

FWS relies heavily upon population projections for its distinction between listed status in Colorado and Wyoming. FWS also relies upon 2005 projections for development in Colorado published by the Center for the American West. Revised Rule at 63007. But that very publication qualifies:

> Our model for projecting land development has its virtues, but . . . it is not very smart. It assumes that all private land that is technically buildable in the West is on the real estate market. It does not know that some owners have placed conservation easements on their land, nor that many ranchers and farmers are not interested in selling, even if the price is right. And it does not know where state or local governments have enacted effective growth limits.

Center for the American West, *Western Futures* (2005) at 2.

FWS cites Miller et al. 2003 for the proposition that human development affects riparian habitat needed by Preble's. But this paper, as titled, examines the "Effects of Human

Settlement on Bird Communities in Lowland Riparian Areas of Colorado (USA)") (Miller et al. 2003) (Emphasis added).

FWS relies heavily on population forecasts in its discussion of threats to Preble's. But population forecasts can be unreliable. "Actual population change is likely to differ from projected change because it is unlikely that any statistical model can completely anticipate the future." Population Projections Program of the Colorado Division of Local Government (August, 2002) at 8. Discrepancies between assumptions, changes in migration patterns, and changes in labor force participation rates all affect the accuracy of such projections. *Id.* Moreover, many demographic variables have not been updated since the 1990 Census. *Id.* at 9.

Indeed, the housing market is experiencing its worst slump in 12 years, prompting fears of recession. Jeannine Aversa, *New-home sales plummet in Nov.*, Rocky Mountain News, Wall Street West 3 (Dec. 29, 2007); see also attached Exhibit T. And FWS admits it has "an incomplete understanding of the mechanisms by which the breadth of human-caused development impacts Preble's meadow jumping mouse populations." Revised Rule at 63016.

IV. Existing Regulatory Mechanisms are more than Adequate

Habitat for Preble's is more than adequately protected. In its lengthy June 1, 2004 comments to the FWS in favor of delisting the mouse, the State of Colorado outlined extensive habitat conservation efforts underway, and ongoing, on the Front Range. The State of Colorado cited Great Outdoors Colorado (GOCO) estimates that over one-quarter of a billion dollars has been spent on land acquisition and preservation on Colorado's Front Range during the last decade. And GOCO recently announced a record $100 million for funding of open space in 2008. *See* attached Exhibit Y. The Interagency Policy for the Ecosystem Approach to the ESA requires FWS to consider these important conservation efforts. 59 Fed. Reg. 34274 (July 1, 1994).

As previously stated, the USGS indicates habitat suitable for meadow jumping mice is becoming increasingly available. (Cryan 2005) (Emphasis added). Given the wide distribution of the species and large blocks of habitat, the present or threatened destruction, modification, or curtailment of the species' habitat or range due to agricultural, residential, commercial and industrial development is not a factor that threatens or endangers the mouse over all or a significant portion of its range. Even if the mouse was a valid subspecies, all U.S. cities and towns occupy only 3 percent of the nation's land. U.S.D.A. 1997 Natural Resources Inventory. And alleged localized threats are insufficient to merit listing.

Preble's is said to inhabit riparian areas after ice retreated from the last ice age. Revised Rule at 63000. At the same time FWS states riparian areas are but a small part of the landscape within the species range. *Id.* at 63007. If riparian areas are, as FWS now claims, but a small portion of Preble's habitat, how can FWS allege impacts to riparian

areas result in such drastic impacts to Preble's? The authority FWS cites for the range of Preble's refers to riparian habitat—not upland habitat. Revised Rule at 63019.

FWS goes to great lengths to dismiss the regulatory effectiveness of the Clean Water Act, yet cites only one creek in Elbert County that may not be subject to Clean Water Act jurisdiction. Revised Rule at 63013. The Environmental Protection Agency, which is charged by statute with implementing the Clean Water Act, appears to disagree:

> The Clean Water Act (CWA) is the cornerstone of surface water quality protection in the United States. (The Act does not deal directly with ground water nor with water quantity issues.) The statute employs a variety of regulatory and nonregulatory tools to sharply reduce direct pollutant discharges into waterways, finance municipal wastewater treatment facilities, and manage polluted runoff. These tools are employed to achieve the broader goal of restoring and maintaining the chemical, physical, and biological integrity of the nation's waters so that they can support "the protection and propagation of fish, shellfish, and wildlife and recreation in and on the water."

Available at: http://www.epa.gov/watertrain/cwa/. Interestingly, the FWS has relied upon the regulatory effectiveness of the Clean Water Act in other delisting decisions.[13]

In addition, numerous city and county land use codes and development standards prohibit development of habitat which includes wetlands, riparian areas or even grasslands. See attached Exhibit U. Preble's generally inhabits riparian areas (Jones 1981) that are often unsuitable for development. Extensive regulatory mechanisms (irrespective of the listed status of Preble's) continue to prohibit residential, commercial and industrial development in riparian habitat. *Id.* In addition, Section 404 of the Clean Water Act ("CWA"), requires avoidance, minimization, and mitigation of adverse impacts to riparian habitats within jurisdictional wetlands and water of the U.S. 33 U.S.C. 1251 et seq.

V. Listings Often Hinder Conservation Efforts

The FWS must focus its efforts on listing actions that will provide the greatest conservation benefits to imperiled species in the most expeditious and biologically sound manner. See Final Listing Priority Guidance for Fiscal Year 2000. 64 Fed. Reg. 57114 (Oct. 22, 1999). The FWS expressed that it "supports voluntary conservation as the most effective method to protect species and their habitats." 70 Fed. Reg. 2245. The FWS does "recognize that listing may affect local planning efforts, due to its effect on voluntary conservation efforts." *Id.* at 2246.

Unfortunately, there have been virtually no successes under the ESA: less than one percent of all listed species have ever recovered. Because the regulatory straightjacket of

---

[13] Press Release, U.S. Fish and Wildlife Service, Dismal Swamp Southeastern Shrew No Longer Needs Endangered Species Act Protection, at http://news.fws.gov/NewsReleases/r4/01B3D1E7-F31D-11D4-A17F009027B6B5D3.html (March 3, 2000).

the ESA creates a disincentive to landowners, a federal listing, would do little to help (and could even harm) Preble's.

Listings often restrict the ability to manage for species and could even result in harm to the species. *See* Amara Brook, Michaela Zint, Raymond De Young, *Landowners' Responses to an Endangered Species Act Listing and Implications for Encouraging Conservation,* 17 Conservation Biology 1473, 1638 (Dec. 2003) (Where an extensive survey of landowners showed that many managed their land so as to avoid the presence of a listed species) hereby attached as Exhibit V.

Many landowners managed their forest lands to avoid the nesting of federally-listed red-cockaded woodpeckers. For example:

> Ben Cone of North Carolina managed 7,200 acres of timberland with 70-80 year harvest rotations, small cuts, and controlled burns, which . . . created habitat for the red-cockaded woodpecker. When the endangered woodpecker took up residence on Cone's land, more than 1,500 acres were placed under the control of the U.S. Fish and Wildlife Service (see Stroup 1997). In response, Cone began a harvest rotation of 40 years on the rest of his land in order to eliminate the mature pines favored by the woodpecker and also remove any possibility that the federal government would take control of his remaining land.
>
> Ben Cone's experience is not an isolated incident, as a study by economists Dean Lueck and Jeffrey Michael (1999) confirms. Using data from hundreds of forest plots in North Carolina, they found that the more red-cockaded woodpeckers in the vicinity, the more likely the landowners were to harvest younger trees. . . . (Lueck and Michael 1999, 36). The landowners' incentive for using this shorter rotation was to ensure the birds did not move onto their property, possibly leading to land-use restrictions. Clearly, the ESA is creating perverse incentives.[14]

According to Bureau of Land Management ("BLM") and U.S. Forest Service officials, the ESA creates " . . . a complex maze of processes and procedures, which field biologists and managers must attempt to negotiate on a daily basis in order to implement on-the-ground projects." USFS and BLM, Improving the Efficiency and Effectiveness of the Endangered Species Act, (Dec. 15, 2003). In regards to the peregrine falcon, leading experts concluded, "despite having the authority for implementing the ESA, and a number of their biologists contributing importantly to the recovery program, as an agency the FWS had a limited role, and its law enforcement division, which was in charge of issuing permits as well as enforcing regulation, was regularly an obstacle to recovery actions." (Burnham and Cade 2003b) (emphasis added) hereby attached as Exhibit W.

FWS appears to concede the ESA is ineffective, but on other grounds. Preble's has been listed since 1998, yet FWS states that its habitat has been destroyed, degraded, and fragmented. 72 Fed. Reg. 62992, 623012 (Nov. 7, 2007).

---

[14] Holly Lippke Fretwell, Forests: Do we get what we pay for? Available at http://www.perc.org/publications/landreports/report2.php#tale.

VI. PECE Policy Supports Delisting in Colorado and Wyoming

The FWS' Policy for Evaluation of Conservation Efforts When Making Listing Decisions ("PECE") establishes a consistent set of criteria to evaluate whether formalized conservation efforts, that have yet to be implemented or to show effectiveness, will improve the status of the species such that listing is unnecessary. Final Policy for Evaluation of Conservation Efforts When Making Listing Decisions. 68 Fed. Reg. 15100 (Mar. 28, 2003).

There are mulitudes of conservation strategies and conservation efforts in place that benefit Preble's. Consistent with the FWS Ecosystem Approach, conservation efforts that protect open space and riparian habitat should also be considered to benefit Preble's.

Here, there is a high level of certainty that the resources necessary to carry out the conservation efforts are available and numerous federal, state and local parties have the authority to carry them out. Moreover, there are ample and extensive regulatory and procedural mechanisms already in place.

There is a high level of certainty that the legal procedural requirements will be met; that adequate regulatory mechanisms are in place to eliminate or adequately reduce the threats to the Preble's; that conservation efforts will be implemented (and effective); and that the parties to the conservation efforts have demonstrated commitments to the efforts and a history of receiving funding for conservation.

VII. FWS Improperly Relies on Elevation as a Means to Differentiate Species and Subspecies

Due to the practical, if not legal, impossibility of identifying Preble's, FWS relies upon elevation to distinguish between Preble's and the Western jumping mouse (a species separate from meadow jumping mice). According to the FWS, mice found about 6700 feet in elevation are western jumping mice. Mice found below 6700 feet are deemed Preble's. FWS claims to have positively identified Preble's. But the entire controversy between Ramey and King demonstrate there is no accepted means to identify Preble's. And the FWS concedes "field differentiation between the [Preble's] and the western jumping mouse is difficult." Revised Rule at 63000. "In Colorado, jumping mice are considered Preble's meadow jumping mice when identification is confirmed or if they occur in areas where western jumping mice are not known." *Id.* How is identification of Preble's "confirmed" or "verified?"

VIII. Serious Flaws in the Delisting Process

A. Senator Wayne Allard Letter to Secretary Kempthorne

Evidence released in a July 30, 2007 letter from Senator Allard to Interior Secretary Kempthorne suggests a pattern of collusion among agency staff, environmental groups, academics and others with vested interests in the listing. *See* Exhibit H. The dramatic change in course in the Revised Rule is symptomatic of this.

FWS based its proposed delisting on the genetic and taxonomic review of Dr. Rob Roy Ramey, II and then launched an aggressive campaign to discredit the scientist. Service staff are now on record: "Since the Preble's [Ramey's work] has now published and the reality of what we need to accomplish is now coming into focus, we're starting to think more seriously about this USGS study." Service staff hustled to reallocate funds so that Dr. Tim King could provide "greater clarity" than Ramey. The cost of King's review was then estimated to be $15,000. "[I]f needed, we'll find a way to get the money." This was "priority research."

All the while, the Service ignored the population and range data that supported delisting in the first place. When CWCD filed a Data Quality Challenge on these issues, the Service ignored that too.

Senator Allard showed that FWS staff had inside contact with the panel selected to review Ramey's work. Staff didn't want to get their "contact" within the Sustainable Ecosystems Institute (SEI) "in trouble" for spreading the word that Interior was having high-level discussions about genetic and taxonomic standards in listing decisions. When an independent review clearly sided with Ramey over King, academic Andrew Martin wrote to King and copied other listing proponents, "[T]he Crandall report [the independent review] apparently commissioned by the State of Wyoming is interesting and contradicts, in very specific terms, the King et al. study. I have two questions: First, why was the Crandall report commissioned? And what the @#$%$#@ is going on?"

B. Bias in SEI Panel and Process

As discussed previously, there were serious flaws with the SEI panel review of genetic issues. For example:

- The Panel report likely exceeded its scope of work. The scope of work for the panel mandated they avoid discussions on policy, management and that they make no recommendations to the FWS on such issues. The panel referred to *Z. h. preblei* as a *"valid, formally recognized subspecies"*; however, it is important to recognize that there was no standard for describing subspecies in 1954, nor is there currently.

- The Panel exhibited bias by changing it's agenda from equal time on the agenda to almost 2:1 in favor of the critics of Ramey *et al.*

- A double standard was applied as to whom could participate in the review with more deference to the critics of Ramey *et al.*

- The panel claimed that *"two of the lines of evidence presented by REA* (Ramey *et al.* 2005) *(their analyses of cranial morphometrics and ecological exchangeability) are based on insufficient data to support their suggestions for taxonomic change,"* yet ignored the weak inference and small sample size used originally by Krutzsch (1954) to describe this subspecies (measurements of only 3 skulls and comparisons of only 4 skins). The panel also failed to acknowledge that Krutzsch, no longer supports his original subspecies description. See Attached Exhibit J.

- The Panel also ignored that a genus-wide study across the entire *Zapus* genus has already been conducted by Jones (1981) and that a copy was personally handed to the panel. That study involved morphological, ecological, and evolutionary information (including the fossil record), and examined specimens from 123 collections, totaling almost 9,900 individuals and concluded that: *"There is no evidence of any population of Zapus hudsonius being sufficiently isolated or distinct to warrant subspecific status"* pages V and 303 from Jones (1981)).

The panel's agenda changed in response to lobbying by environmental groups, their scientific supporters, and the USGS. For example, the schedule went from approximately equal time on the agenda sent out on 6/30/06, to almost 2:1 in favor of the critics of Ramey *et al.* (on the agenda sent out on 7/4/06 and after the call-in from Patton was added). And a double standard was applied as to who could participate in the panel review. USGS objected to one panelist, Routman, because he trained in the same laboratory as Keith Crandall (Crandall agreed with the conclusions of Ramey *et al.*). Yet another panelist, Scott Stephan, whom collaborates with one of Ramey's most vociferous critics (Jim Patton) was not recused.[15]

The panel was willing to accept poor discriminant analysis assignments in support of subspecies status for Preble's as well as principal components analysis plots (an exploratory tool lacking inferential statistical capabilities that is not designed for hypothesis testing) as a means to define subspecies. On the other hand, the panel was unwilling to accept the fact that the only quantitative basis for Krutzsch's original description of *Z. h. preblei* is not supported by univariate statistical analysis using much larger sample sizes (n=40 per subspecies) and showed little concern over the fact that King cherry-picked samples to maximize divergence.

The panel concluded, *"thorough analysis of the original characters and specimens used by Krutzsch (1954) to describe Z. h. preblei is required"*. But why would those original specimens provide different results? If *Z. h. preblei* is a subspecies, then any adequate sampling of its population should show statistically meaningful differences from other subspecies. In fact, Ramey *et al.* (2005) performed such an analysis and obtained clear results to the contrary.

---

[15] Stephan has a paper in press with Patton and has a chapter in a book edited by Patton. At the request of environmental groups, Patton called in as a participant to the panel review, yet had not contributed any scientific work to the issue.

Ramey *et al.* measured and analyzed the same nine measurements for which Krutzsch (1954) reported actual measurement data (mean, ranges, and sample sizes in Table 5, Krutzsch 1954), and did not measure those qualitative skull characteristics (discussed above) for which he did not report measurements. After examining pelage in several hundred museum collections with the pelage descriptions in hand (sides duller, less black-tipped hair on dorsal stripe), Ramey *et al.* concluded that these were subjective qualitative assessments that would not be repeatable. The photograph attached as Exhibit K gives some idea of the variation within, and overlap among, putative subspecies.
In fact, the species, *Z. princeps* and *Z. hudsonius*, cannot be reliably distinguished on pelage alone, which was why Conner and Shenk (2003) developed their cranial morphometric test to distinguish them and noted the following : *"Furthermore, the taxa* [the species: *Z. princeps* and *Z. hudsonius*] *are ecologically and physically similar and no reliable technique exists to distinguish live specimens in the field."* In other words, *those species* are not reliably distinguishable using pelage color or pattern. Based on Ramey *et al.*'s examination of several hundred museum skins, *the subspecies* are even less so.

In their discussion of differences in mtDNA results of the two studies, the panel stated: "In this section we discuss how the mtDNA sampling, quantity and quality issues outlined above may have lead REA and KEA to come to different conclusions regarding the taxonomic status of *Z. h. preblei*." However, the panel failed to notice that Ramey *et al.*'s critical tests did not rely on any sharing of haplotypes among putative subspecies; instead, their subspecies test relied on: morphological analyses to test the original quantitative basis of *Z. h. preblei*, mtDNA reciprocal monophyly and Analysis of Molecular Variance, and the proportion and frequency of unique microsatellite alleles and pairwise F_{ST}. Their tests for distinct populations used the approach of Crandall *et al.* (2000). Such an approach does not rely on sharing of mtDNA haplotypes among putative subspecies.

In review of the literature, in 106 years of study no one has noticed any adaptations that would preclude ecological exchangeability among the putative subspecies *Z. h. preblei* and nearby subspecies (Please see Ramey *et al.* 2005 and 2006 for a more extensive treatment).

If the panel's suggestion is extended to other cases, it would mean that speculation about as yet undescribed or hypothetical uniqueness is adequate justification to create or maintain an ESA-listed subspecies. This amounts to proposing that science not be the basis of such decisions.

The panel noted that *"However, we also note that Z. h. preblei appears to be at a stage in its evolution in which clearly determining taxonomic rank will not be easy to do [our emphasis], and that large groups of scientists are unlikely to reach a unanimous consensus concerning its status."* The panel goes on to recommend an extensive research program to *"further clarify this issue,"*; however, the panel failed to provide any critical tests that could be used to potentially falsify *Z. h. preblei* as a taxon. Such lax standards would preclude scientifically evaluating the classification of any subspecies, and

therefore, makes the whole inquiry into its taxonomic status a moot point.

While the panel identified a handful of samples used by Ramey *et al.* that led to unexpected results, there are multiple explanations for differences. Should these mtDNA sequences be excluded from the analyses, the same basic result is obtained. In fact, even If Tim King's mtDNA data from *Z. h. campestris* was substituted for Ramey's, the same basic results are obtained.

The panel's conclusions suggest that they accept any geographic isolation (no matter how recent) and genetic divergence (no matter how minor) to be sufficient to defend a subspecies (no matter how weak the evidence was to describe the subspecies in the first place). This does not serve conservation or the public interest when applied to local populations of common and widespread species, like meadow jumping mice which range over *half of North America* (see map in Ramey *et al.* 2005).

C. Flawed Peer Reviews

FWS violated its own peer review process in contracting with Dr. Tim King outside of the comment period after Ramey et al. had already been peer reviewed. *See* Interagency Policy for Peer Review in ESA Activities. 59 Fed. Reg. 34270 (July 1, 1994). In addition, many reviewers selected by FWS had vested interests in the Preble's listing.

IX. Preble's Invalidated Under International Code of Zoological Nomenclature

The International Code of Zoological Nomenclature ("The Code") sets forth procedural guidelines for taxonomic description and not thresholds of uniqueness, data quality, or evolutionary basis. For example, *The Code* requires a type specimen (a body or parts thereof, Timm *et al.* 2005) as well as publication of the taxonomic revision and its distribution to at least four libraries. *See* Exhibit M. The Code does not require that the publication of taxonomic revision be peer-reviewed. The Preble's subspecies description in Krutzsch (1954) is a half page in length. The repeated citation of it in books on mammal taxonomy is simply a restatement (or summary) of the original description. **Under The Code, *Z. h. preblei* and *Z. h. intermedius* are officially synonymous with *Z. h. campestris* with the publication of Ramey *et al.* (2005).**

If this panel's recommendation is followed and applied to other cases, it would mean that the classification of subspecies would be above scientific question or reproach.
In sum, the panel abrogated their responsibility and, by default, has advocated that listings under the ESA need no scientific basis.

We urge FWS to issue a final rule delisting Preble's based on its distribution, abundance and trends. FWS should also use the disagreement on Preble's genetics as an opportunity to revise its DPS Policy so that questionable subspecies with little or no quantifiable physical differences cannot be listed under the ESA.

IX. Appropriate Involvement of Deputy Assistant Secretary Julie MacDonald

Contrary to the assertions of some in the FWS and environmental community, the involvement of former Deputy Assistant Secretary Julie MacDonald in the Preble's delisting process and critical habitat process was perfectly appropriate. Justifiably, the record indicates Ms. MacDonald simply questioned how purported subspecies of rodents that could not be physically nor ecologically distinguished should be classified separately and/or listed under the ESA. Her inquiry and review was consistent with the statutory role of the Deputy Assistant Secretary. That a questionable Inspector General Report ("IG Report") criticized MacDonald for requiring FWS staff to back their assertions with data and scientific literature is irrelevant, at best. In fact, Ms. MacDonald had no opportunity to review the IG Report before it was issued. Oddly, she had no opportunity to submit a rebuttal (which is standard practice) to this IG Report. Her rebuttal is attached as Exhibit X; see also Exhibit I.

X. Conclusion

Should FWS stand by its determination that the Preble's is a valid subspecies, nearly any population of any common creature could be listed under the ESA without scientific inquiry or recourse. Preble's presents an opportunity for the FWS to set some meaningful standard against which such classifications can be made. The alternative, rampant and politically-motivated subspecies and population listings, does a disservice to conservation and wastes resources that could be allocated to species that truly need protection. FWS should issue a rule delisting Preble's in both Colorado and Wyoming based upon its positive distribution, abundance and trends and should thereby recognize that questionable subspecies with little or no quantifiable physical differences cannot be listed under the ESA.

The FWS spends more resources on Preble's than the blue whale, greenback cutthroat trout and snail darter combined. Where are the FWS's conservation priorities?

While the FWS has a poor record in regards to listing improperly classified species, subspecies and population segments,[16] it has declined to list, withdrawn proposed listings, and delisted other species, subspecies and population segments based upon far less data and analyses than that supporting the petitions to delist Preble's. Here, distribution, abundance and trends data alone merits delisting.

Continued listing of Preble's and delay violates the best available science standard under the ESA, the FWS Policy on Information Standards,[17] the FWS Peer Review Policy and the Data Quality Act. There is no legitimate disagreement between Dr. King and (Ramey *et al.* 2005). It is Dr. King's puzzling conclusions that differ markedly from (Ramey *et*

[16] For example, cuneate bidens, the Mexican duck, the purple-spined hedgehog cactus, the Truckee barberry,[16] the coastal cutthroat trout, and the Bahama swallowtail were all determined not to be unique species. Similar issues have arisen with the California gnatcatcher, the California tiger salamander and the cactus ferruginous pygmy owl, among others.

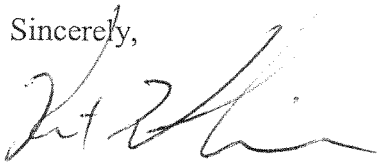
[17] 59 Fed. Reg. 34271 (July 1, 1994).

al. 2005). And the ESA's implementing regulations disqualify Dr. King, an aquatic ecologist, from review (Ramey *et al.* 2005) in the context of rodents, let alone purported subspecies of prairie jumping mice.

Dr. King's work, and previous criticisms of his work, raise serious questions about its reliability and objectivity. That Dr. King would label one-half of one percent of variance "strong genetic differentiation" sufficient to conclude not only that Preble's is unique, but that it should be split into additional subspecies is unconscionable. Taken with, among other things, clear sampling bias, questionable protocols and the lack of meaningful thresholds, reliance on Dr. King's work on Preble's would clearly violate the Peer Review Policy and the Data Quality Act.

The FWS should focus on species that do not have the benefit of the distribution, abundance and trends of Preble's nor the extensive regulatory protections already in place. In sum, it is time for the FWS to issue a final rule to delist the Preble's in both Colorado and Wyoming so resources can go to species that are truly threatened and endangered.

Sincerely,



Kent Holsinger

Encl.

Animal Conservation. Print ISSN 1367-9430

CORRESPONDENCE

Exhibit A

The Preble's meadow jumping mouse: subjective subspecies, advocacy and management

M. A. Cronin

School of Natural Resources and Agricultural Sciences, University of Alaska Fairbanks, AK, USA

Correspondence
Matthew A. Cronin, School of Natural Resources and Agricultural Sciences, University of Alaska Fairbanks, 533 East Fireweed Ave, Palmer, AK, 99645, USA. Email: ffmac1@uaf.edu

Received 25 October 2006; accepted 6 February 2007

doi:10.1111/j.1469-1795.2007.00103.x

I read with concern the letters to the editor regarding the Preble's meadow jumping mouse *Zapus hudsonius preblei* in which Martin (2006) criticized Ramey *et al.* (2005) for questioning the subspecies designation and the editor for a failed peer review, and Crandall (2006) defended his editorship.

However, the debate over the subspecies status of the Preble's meadow jumping mouse does not properly acknowledge the subjectivity of the subspecies category. Designation of subspecies status is inherently subjective and this should be openly admitted by both sides of the debate. Accusations of advocacy in this issue are spurious because applied fields such as wildlife conservation or agriculture have inherent advocacy for management objectives. As discussed below, I suggest management units of intraspecific groups should be based on geography, not subjective judgements of subspecies status or genetic differentiation.

The subspecies status of this mouse has been discussed extensively (Ramey *et al.*, 2005, 2006; Crandall, 2006; Martin, 2006; Vignieri *et al.*, 2006) because it has been listed as threatened under the Endangered Species Act (ESA). Briefly, the Preble's mouse was designated a subspecies with limited descriptive morphological data. There are no diagnostic characters that unequivocally distinguish it from conspecifics. It does not have monophyletic mitochondrial DNA. It may be geographically isolated from, and have different allele frequencies than, con-specific populations. Sample sizes and locations studied are probably small relative to population numbers. The allele frequency differences are for DNA loci that are usually considered selectively neutral. There are no data documenting local adaptation, but it is possible. Given the lack of quantitative criteria for naming subspecies the Preble's mouse could be considered a legitimate subspecies, or not a legitimate subspecies. My concerns center on the lack of appreciation of the subjectivity of subspecies and on misunderstanding of the nature of advocacy and management in the context of the Preble's mouse.

It is well established that the subspecies category is subjective (reviewed by Cronin, 1993, 2006; Geist, O'Gara & Hoffmann, 2000; Zink, 2004). This includes other cases involving the ESA (e.g. Cronin, 1997; Zink *et al.*, 2000) and recognition of this could have avoided much of the debate over the Preble's mouse. The following quotes are telling:

'Most of the prominent commentators on the theory of speciation have been careful to emphasize the inherently subjective and even arbitrary nature of racial limits.' '. . . we are convinced that the subspecies concept is the most critical and disorderly area of modern systematic theory . . .' (Wilson & Brown, 1953). Wilson and Brown note the synonymy of the terms subspecies and geographical race. It is noteworthy that Krutzsch (1954) named the Preble's subspecies in the year following Wilson & Brown's (1953) classic critique of subspecies. Krutzsch's analysis was typical of many at the time, with few specimens and a qualitative subspecies designation.

'. . . because so many characters show independent patterns of geographic variation, and because so many loci are polymorphic and vary in allele frequency from one population to another, some combination of characters will distinguish every population from all others so there is no clear limit to the number of subspecies that can be recognized.' (Futuyma, 1986, pp. 108–109).

'. . . present applications of the subspecies concept are uneven, frequently undocumented and lead to no improvement of either evolutionary theory or practical taxonomy.' (Vanzolini, 1992, p. 189).

'Widespread species thus can be divided into any number of different sets of 'subspecies' simply by selecting different characteristics on which to base them.' 'As . . . with other species, geographic variation . . . does not allow *Homo sapiens* to be divided into natural evolutionary units. That basic point . . . has subsequently been demonstrated in a variety of organisms . . . and use of the subspecies (or race) concept has essentially disappeared from the mainstream evolutionary literature.' (Ehrlich, 2000: 49, 291).

 © 2007 The Author. Journal compilation © 2007 The Zoological Society of London

The subjectivity of subspecies designation is exemplified by the Preble's mouse. Ramey *et al.* (2005) used a hypothesis-testing approach for genetic, ecological and morphological data and concluded that the subspecies designation was not warranted. Vignieri *et al.* (2006) presented criteria (no or significantly reduced gene flow), acknowledged subspecies are not well defined, and then concluded the Preble's mouse is a legitimate subspecies. The ensuing critiques (Crandall, 2006; Martin, 2006; Ramey *et al.*, 2006; Vignieri *et al.*, 2006) demonstrate neither was an absolute result. It is important to recognize that other intra-specific groups that can be listed under the ESA, distinct population segments-DPS and evolutionarily significant units-ESU, are also subjectively defined (Cronin, 2006).

Despite these problems, subspecies can still be used in scientific discourse. For example, a subspecies has been defined as an aggregate of populations in a geographic subdivision of the species' range that differs taxonomically from other populations (Mayr, 1963) or groups phylogenetically distinguishable from, but reproductively compatible with, other groups (Avise & Ball, 1990). Wilson (1994) notes that his criticism of subspecies (Wilson & Brown, 1953) was overstated and traits may vary concordantly over geography. However, what constitutes enough differentiation to warrant subspecies status remains subjective.

The subjectivity of subspecies should result in scientists agreeing to disagree on designations. However, discussion of the Preble's mouse subspecies status has degenerated to accusations of 'advocacy dressed up as science' (Martin, 2006). Crandall (2006) pointed out that Martin himself was practicing advocacy while accusing Ramey *et al.* (2005) of practicing advocacy. It is important to note the applicable definition of advocacy: defending or maintaining a cause or proposal (Webster's Ninth New Collegiate Dictionary). I hope the reader recognizes that conservation biology, conservation genetics, animal conservation and related fields can be construed as advocacy. After all, conservation is a specific cause (or management objective), not a science. One may use science to achieve wildlife conservation, as one would use science to achieve a management objective of agricultural crop production. It is important to recognize that The Wildlife Society and Society for Conservation Biology are composed primarily of scientists, but openly practice advocacy for conservation. Indeed, an issue of *The Wildlife Society Bulletin* featured policy and advocacy (The Wildlife Society, 1995). To avoid unproductive accusations, we need to recognize that advocating for different management objectives is inherent in applied fields such as in conservation, agriculture, forestry and medicine.

It is also important to recognize the distinction between science and management. Science can be considered as knowledge obtained from observations, measurements and data analyses of the physical world. In the case of the Preble's mouse, this includes analyses of morphology, range and genetics. Management is what one does to achieve objectives. In this case, some people have management objectives to maximize conservation of the mouse. Others may have objectives that include agriculture, building or other land uses. In either case, science may be used to achieve management objectives. Conservationists may use science to enhance habitat, control predators or implement other measures to manage the mouse population. Others may use science to enhance crop or livestock production. The point is that science is value-neutral and does not necessarily support either conservation or development.

The Preble's mouse exemplifies what I perceive as a state of confusion in resource management because of redundant terminology, artificial classifications and a shift of focus from practical applications to vague discussions of conservation of biological diversity (Cronin, 2006). For example, Vignieri *et al.* (2006) stated '... in our efforts to preserve *biodiversity* ...', and that *distinct evolutionary lineages* are the units of concern, while they support designation of the Preble's mouse as a *subspecies* (my italics). It seems that intra-specific classification is becoming typological, and variable groups are being put into artificial classes rather than being acknowledged as variable populations (Mayr, 2001; Cronin, 2006). This phenomenon is also recognized by Dawkins (2004) who coined the term 'tyranny of the discontinuous mind' for the tendency to force discrete names and classifications on organisms rather than acknowledge the inherent temporal and spatial variation within and among populations and species.

It is my contention that we should continue the rigorous sciences of systematics, phylogenetics and population genetics, but we should focus management on geography (i.e. management units based on geography; Cronin, 1993, 1997, 2003). That is, for most practical fish and wildlife management applications, the geographic area and the species on it are the primary issue. If *Z. hudsonius* is desired in a geographic area, then manage for it regardless of its subspecies or genetic status. Although native populations may be favored if they are adapted to local conditions, mixing stocks of different geographic origins may also be advantageous because this can enhance genetic variation (Paabo, 2000), and use of non-native stock may be successful if they are 'ecologically exchangeable' (Crandall *et al.*, 2000). In the case of *Z. hudsonius*, there is no indication that populations from different areas are not ecologically exchangeable (Ramey *et al.*, 2005). Consider three other prominent examples. The lynx *Lynx lynx* (Federal Register, 2000) and wolf *Canis lupus* (Fritts *et al.*, 1997) in the US Rocky Mountain States have been reintroduced or augmented with Canadian sources of animals. In Florida, the panther *Felis concolor* (Pimm, Dollar & Bass, 2006) has been successfully augmented with the introduction of animals from Texas. The primary management objective was to have these species in specific geographic areas, and using the native source was secondary.

As these examples show, we regularly identify fish and wildlife management units based on geography, although it is perhaps taken for granted. In some cases, populations may be identified as separate management units because they are geographically separate from other populations. In other cases, a contiguous population may be comprised of different management units because of geographic subdivision by state or international boundaries, land ownership, topographic features or other criteria. The point is that geographically based units are *necessary* because of these

real factors that affect management. Designation of criteria for genetic or subspecies units are not necessary for management and will rely on subjective judgment, as evidenced by the exchange of papers on the Preble's mouse.

Interestingly, my proposal to base fish and wildlife management on geography was preceded by Wilson & Brown's (1953) common sense taxonomic suggestion of simply using the Latin binomial species name with the locality or geographic range instead of subjective trinomial subspecies names. That is, for management and intra-specific classification, the species and geographic location are the primary units of concern and they can usually be defined in an objective, empirical manner. I recognize that some species designations are questionable (see Baker & Bradley, 2006), but for most management applications species identity is not a problem. From this point of view, if the time and money spent on *Z. hudsonius* subspecies, genetic studies, debate, meetings, committees, travel, phone calls and lawyers was spent on purchasing habitat, giving landowners incentives to enhance or maintain habitat, and trans-locating mice to vacant habitat, there probably would not be a Preble's mouse problem at all.

References

Avise, J.C. & Ball, R.M. Jr. (1990). Principles of genealogical concordance in species concepts and biological taxonomy. *Oxford Survey Evol. Biol.* **7**, 45–67.

Baker, R.J. & Bradley, R.D. (2006). Speciation in mammals and the genetic species concept. *J. Mammal.* **87**, 643–662.

Crandall, K.A. (2006). Advocacy dressed up as scientific critique. *Anim. Conserv.* **9**, 250–251.

Crandall, K.A., Bininda-Emonds, O.R.P., Mace, G.M. & Wayne, R.K. (2000). Considering evolutionary processes in conservation biology. *Trends Ecol. Evol.* **15**, 290–295.

Cronin, M.A. (1993). Mitochondrial DNA in wildlife taxonomy and conservation biology: cautionary notes. *Wildl. Soc. Bull.* **21**, 339–348.

Cronin, M.A. (1997). Systematics, taxonomy, and the Endangered Species Act: the example of the California gnatcatcher (*Polioptila californica*). *Wildl. Soc. Bull.* **25**, 661–666.

Cronin, M.A. (2003). Research on deer taxonomy and its relevance to management. *Ecoscience* **10**, 333–343.

Cronin, M.A. (2006). A Proposal to eliminate redundant terminology for intra-species groups. *Wildl. Soc. Bull.* **34**, 237–241.

Dawkins, R. (2004). *The Ancestor's Tale*. New York: Houghton Mifflin Company.

Ehrlich, P.R. (2000). *Human natures*. Washington, DC: Island Press, Shearwater Books.

Federal Register (2000). Determination of threatened status for the contiguous U.S. district population segment of the Canada lynx and related rule. 24 March 2000, Vol. 65, No. 58, 16052–16086.

Fritts, S.H., Bangs, E.E., Fontaine, J.A., Johnson, M.R., Phillips, M.K., Koch, E.D. & Gunson, J.R. (1997). Planning and implementing a reintroduction of wolves to Yellowstone National Park and central Idaho. *Restor. Ecol.* **5**, 7–27.

Futuyma, D.J. (1986). *Evolutionary biology*. Sunderland, MA: Sinauer Associates.

Geist, V., O'Gara, B. & Hoffmann, R.S. (2000). Taxonomy and the conservation of biodiversity. In *Ecology and management of large mammals in North America*: 1–26. Demarais, S. & Krausman, P.R. (Eds). Upper Saddle River, NJ: Prentice-Hall.

Krutzsch, P.H. (1954). North American jumping mice (genus *Zapus*). *Univ. Kansas Publ. Mus. Nat. Hist.* **4**, 349–472.

Martin, A. (2006). Advocacy dressed up as science: response to Ramey *et al.* (2005). *Anim. Conserv.* **9**, 248–249.

Mayr, E. (1963). *Populations, species, and evolution*. Cambridge, MA: Belknap Press, Harvard University Press.

Mayr, E. (2001). *What evolution is*. New York: Basic Books.

Paabo, S. (2000). Of bears, conservation genetics, and the value of time travel. *Proc. Natl. Acad. Sci. USA* **97**, 1320–1321.

Pimm, S.L., Dollar, L. & Bass, O.L. Jr. (2006). The genetic rescue of the Florida panther. *Anim. Conserv.* **9**, 115–122.

Ramey, R.R., Liu, H.-P., Epps, C.W., Carpenter, L.M. & Wehausen, J.D. (2005). Genetic relatedness of the Preble's meadow jumping mouse (*Zapus hudsonius preblei*) to nearby subspecies of *Z. hudsonius* as inferred from variation in cranial morphology, mitochondrial DNA and microsatellite DNA: implications for taxonomy and conservation. *Anim. Conserv.* **8**, 329–346.

Ramey, R.R., Wehausen, J.D., Liu, H.-P., Epps, C.W. & Carpenter, L.H. (2006). Response to Vignieri *et al.* (2006): should hypothesis testing or selective *post hoc* interpretation of results guide the allocation of conservation effort. *Anim. Conserv.* **9**, 244–247.

The Wildlife Society (1995). Policy features, reflections on policy. *Wildl. Soc. Bull.* **23**, 317–370.

Vanzolini, P.E. (1992). Third world museums and biodiversity. In *Systematics, ecology, and the biodiversity crisis*: 185–198. Eldredge, N. (Ed.). New York: Columbia University Press.

Vignieri, S.N., Hallerman, E.M., Bergstron, B.J., Hafner, D.J., Martin, A.P., Devers, P., Grobler, P. & Hitt, N. (2006). Mistaken view of taxonomic validity undermines conservation of an evolutionarily distinct mouse: a response to Ramey *et al.* (2005). *Anim. Conserv.* **9**, 237–243.

Wilson, E.O. (1994). *Naturalist*. Washington, DC: Island Press, Shearwater Books.

Wilson, E.O. & Brown, W.L. (1953). The subspecies concept and its taxonomic applications. *Syst. Zool.* **2**, 97–122.

Zink, R.M. (2004). The role of subspecies in obscuring avian biological diversity and misleading conservation policy. *Proc. Roy. Soc. Lond. Ser. B* **271**, 561–564.

Zink, R.M., Barrowclough, G.F., Atwood, J.L. & Blackwell-Rago, R.C. (2000). Genetics, taxonomy, and conservation of the threatened California gnatcatcher. *Conserv. Biol.* **14**, 1394–1405.

Animal Conservation (2005) 8, 329–346 © 2005 The Zoological Society of London. Printed in the United Kingdom doi:10.1017/S1367943005002313

Genetic relatedness of the Preble's meadow jumping mouse (*Zapus hudsonius preblei*) to nearby subspecies of *Z. hudsonius* as inferred from variation in cranial morphology, mitochondrial DNA and microsatellite DNA: implications for taxonomy and conservation

Exhibit B

Rob Roy Ramey II[1,†], Hsiu-Ping Liu[1,2], Clinton W. Epps[3], Lance M. Carpenter[1] and John D. Wehausen[4]

[1] Denver Museum of Nature and Science, Department of Zoology, 2001 Colorado Blvd., Denver, Colorado 80205-5798, USA
[2] University of Denver, Department of Biological Sciences, 2190 E. Iliff, Denver, CO 80208, USA
[3] University of California, Department of ESPM, 137 Mulford Hall, Berkeley, CA 94720-3114, USA
[4] University of California, White Mountain Research Station, 3000 East Line Street, Bishop, CA 93514, USA

(First received 5 August 2004; Resubmitted 9 March 2005; accepted 11 May 2005)

Abstract

The Preble's meadow jumping mouse (*Zapus hudsonius preblei*) is listed as a threatened subspecies under the United States Endangered Species Act (US-ESA). The quantitative description of this subspecies was based on cranial measurements of only three adult specimens. It is one of twelve subspecies of *Z. hudsonius* and is a peripheral population at the western edge of its range. We tested the uniqueness of *Z. h. preblei* relative to other nearby subspecies of *Z. hudsonius* using a hypothesis testing approach and analyses of cranial morphometric, mtDNA sequence and nuclear microsatellite data obtained from museum specimens and archived tissues. Morphometric analysis of variance did not support the original description of *Z. h. preblei* as a subspecies. Principal component analysis of these data showed *Z. h. preblei* within the range of variation found in *Z. h. campestris* and *Z. h. intermedius*. Discriminant analysis correctly classified only 42% of *Z. h. preblei* skulls at jackknifed posterior probabilities >0.95 relative to *Z. h. campestris*. All mtDNA haplotypes found in *Z. h. preblei* were also found in *Z. h. campestris*. Simulation based estimates of current and historical gene flow (MDIV) revealed low, but non-zero, mtDNA gene flow among *Z. h. preblei* and several nearby subspecies. Analyses of five nuclear microsatellite loci using population pairwise F_{ST}, BAPS and STRUCTURE were consistent with morphometric and mtDNA results. These revealed low levels of genetic structure and evidence of recent gene flow and bottlenecks in *Z. h. preblei*. Due to a lack of clearly recognisable genetic, morphological, or adaptive differences, we synonymise *Z. h. preblei* and *Z. h. intermedius* with *Z. h. campestris*. We suggest that candidates for listing under the US-ESA, or similar biodiversity laws, be evaluated for genetic and/or morphological uniqueness to prevent the misallocation of resources to non-distinct taxa like *Z. h. preblei*.

INTRODUCTION

The United States Endangered Species Act (US-ESA) is intended to protect organisms that are threatened with extinction and promote their recovery. Organisms 'listed' for protection can include species, subspecies and distinct vertebrate population segments. Since the enactment of the US-ESA in 1973, 1851 organisms have been listed as threatened or endangered. Thirty-five organisms have since been removed from the list. Seven 'delistings' resulted from correction of taxonomic errors and six from recognition of other types of errors, while 14 organisms recovered and eight went extinct (http://ecos.fws.gov/tess_public/TESSWebpage). One of the criticisms of the US-ESA is that listings are sometimes based on antiquated taxonomy or weak inference (National Research Council, 1995; Cronin, 1997; Gordon, Lacy & Streeter, 1997). It is in the best interest of biodiversity conservation to evaluate the systematics and taxonomy of candidates for listing and delisting. If defensible data are lacking and a protected organism is not distinguishable with a high degree of certainty from neighbouring, non-threatened relatives, considerable financial and logistical conservation effort may be misallocated at the expense of other endangered organisms. This applies to biodiversity laws globally.

The Preble's meadow jumping mouse (*Zapus hudsonius preblei*) was listed as a threatened subspecies under the US-ESA in 1998 (US Fish & Wildlife Service, 1998). It is one of 12 subspecies of the meadow jumping mouse (*Z. hudsonius*), a species whose range covers approximately half of North America (Fig. 1). *Zapus hudsonius* are hibernators and generalists in their food and

[†]All correspondence to: Rob Roy Ramey II. Tel: +1 303 370 6443; Tel/Fax: +1 303 258 9535; E-mail: ramey@spot.colorado.edu

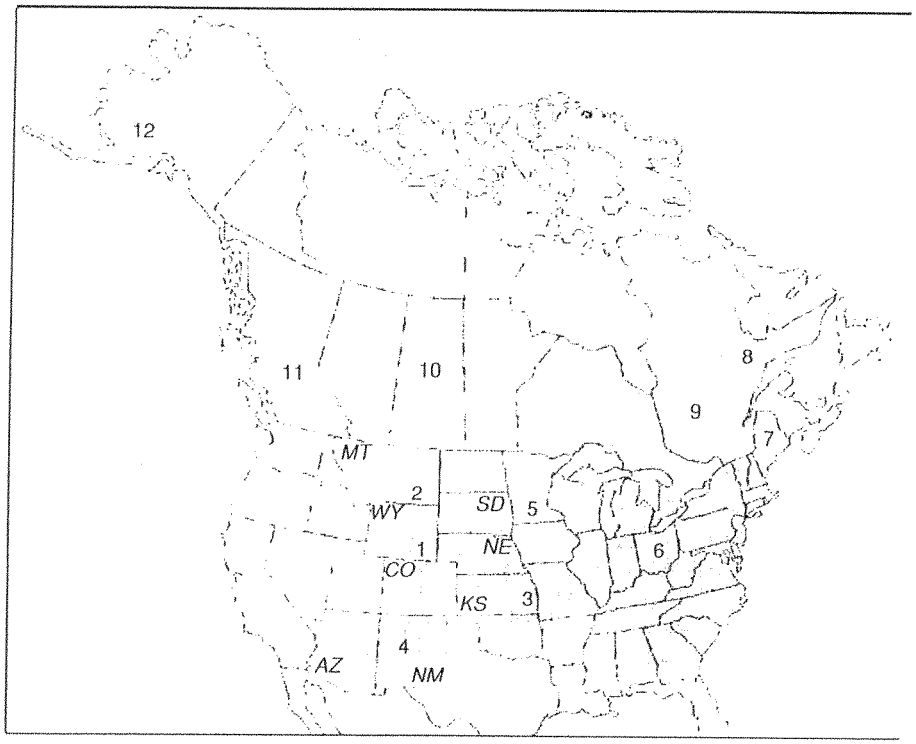


Fig. 1. Map of North America showing distribution and subspecies of *Zapus hudsonius* (Krutzsch, 1954; Hafner *et al.*, 1981). (1) *Z. h. preblei*, (2) *Z. h. campestris*, (3) *Z. h. pallidus*, (4) *Z. h. luteus*, (5) *Z. h. intermedius*, (6) *Z. h. americanus*, (7) *Z. h. acadicus*, (8) *Z. h. ladas*, (9) *Z. h. canadensis*, (10) *Z. h. hudsonius*, (11) *Z. h. tenellus* and (12) *Z. h. alascensis*.

habitat preferences. They typically occupy moist habitats (e.g. meadows, marshes, bogs, streams and irrigation ditches) and adjacent drier areas including coniferous and hardwood forests, sand dunes, strip-mined land and tundra (Jones 1981). They are vagile compared to other small rodents (Quimby, 1951; Whitaker, 1972). Krutzsch (1954) described *Z. h. preblei* as a separate subspecies from the prairie jumping mouse (*Z. h. campestris*) based on skull measurements of three adult specimens and a qualitative description of four adult and seven juvenile skins. In contrast, Jones (1981) concluded that there were no valid subspecies of *Z. hudsonius* based on univariate morphometric analyses, a lack of distinguishing pelage differences, or plausible isolating mechanisms. Despite the weakness of Krutzsch's taxonomic inference by modern standards and the subsequent opposite conclusions reached by Jones (1981), the presumed uniqueness of *Z. h. preblei* based on morphological characters and geographical isolation was an important part of the decision to list it under the US-ESA. Less than 160 km of short grass prairie and agricultural land are presumed to separate *Z. h. preblei* from *Z. h. campestris* to the north, from *Z. h. pallidus* to the east and from *Z. h. luteus* to the south (Fig. 1).

Here, we test the uniqueness of *Z. h. preblei* relative to other nearby subspecies of *Z. hudsonius* using tests for multiple, genetically-based traits (Wehausen & Ramey, 2000; Pearse & Crandall, 2004). We treated taxonomic categories as testable hypotheses and used critical tests laid out in advance of data collection to provide an objective evaluation of the genetic distinctiveness of *Z. h. preblei* from nearby subspecies of *Z. hudsonius*. First, we retested the original quantitative basis of Krutsch's (1954) conclusions to split *Z. h. campestris* into three subspecies (*Z. h. preblei*, *Z. h. campestris* and *Z. h. intermedius*) using univariate and multivariate statistical analyses of skull measurements. Second, we used the conceptual approaches of Ball & Avise (1992), Avise & Johns (1999) and Hendry *et al.* (2000) as the basis of additional tests of *Z. h. preblei* as a subspecies. These authors and others (Crandall *et al.*, 2000; Zink, 2004) have sought consistency by suggesting that taxa or distinct populations be defined by congruence of multiple genetically-based traits. This is also important because phenotypic variation can reflect both genetic and environmental influences (Keita *et al.*, 2004). Third, we tested genetic and ecological exchangeability (Crandall *et al.*, 2000) of *Z. h. preblei* relative to other subspecies to determine if it should be considered a distinct population and, therefore, a conservation priority.

Although it has long been recognised that many named subspecies are questionable (Wilson & Brown, 1953), it has also been recognised that subspecies classification can have some conservation utility if it has an evolutionary basis (Avise & Ball, 1990). Ball & Avise (1992) proposed that subspecies represent a major division in the gene pool diversity of a species based on concordant distributions of multiple genetically-based traits and have a plausible evolutionary mechanism for differentiation. These criteria are similar to those suggested for Evolutionary Significant Units by some authors (Fraser & Bernatchez, 2001). Hendry *et al.* (2000) proposed that conservation priority be afforded to populations that show greater genetic diversity among, relative to within, populations. We satisfied these

requirements using tests of uniqueness for multiple genetically-based traits including cranial morphometric data, mtDNA sequences and microsatellite markers. We required that at least two of the three data sets be considered corroborating evidence.

Crandall *et al.* (2000) proposed a hypothesis testing approach for recognising distinct populations at several levels, using the criteria of genetic and ecological exchangeability on recent and historic time scales. They proposed that ecological differences among populations could reflect adaptive differences that would not be detected by molecular markers alone. Therefore, we examined the literature for evidence of adaptive differences (e.g. life history, morphology) between subspecies and tested for potential shape differences in cranial morphology using principal components analysis (PCA) and linear discriminant analysis (LDA). We estimated the extent of current gene flow for mtDNA (using MDIV) and divergence at presumably neutral microsatellite loci (using pairwise genetic distances, F_{ST} and assignment tests).

We attempted to use threshold levels for various tests (AMOVA, LDA, etc) that have some conventional history below the level of species (e.g. Worley *et al.*'s 2004 use of $q > 0.90$ as a standard in assignment tests; Wehausen & Ramey's 2000 use of >0.90 correct assignment using posterior probabilities of $P > 0.95$ in LDA on morphometric data). Any such threshold level (such as the $P < 0.05$ test for significance commonly employed in frequentist statistics) can be seen as arbitrary; however, we hope to establish reasonable threshold levels for these sorts of tests where they have often been absent. Systematic decisions rely on distinguishability among groups at hierarchical levels (Avise & Johns, 1999). In the case of endangered taxa or populations, a higher certainty of correct assignment and congruence among data sets suggests a higher degree of genetic uniqueness and conservation priority. Appropriate thresholds can be debated and revised, but we feel that the first step in establishing standards and objectively applying them is to state them explicitly.

MATERIALS AND METHODS

Cranial morphometrics

We retested the quantitative basis of Krutsch's (1954) conclusions regarding cranial differences between *Z. h. preblei* and *Z. h. campestris* using the same nine skull measurements: occipitonasal length, condylobasal length, palatal length, zygomatic length, zygomatic breadth, mastoidal breadth, braincase breadth, interorbital breadth and upper tooth row length. Skulls were from collections at the Denver Museum of Nature & Science (DMNS) and the University of Kansas Museum of Natural History (KU) (Appendix 1). Identity of samples was from museum tags, which relied upon geographical area from which a sample was collected and the current subspecies classification (Krutzsch, 1954; Hafner, Peterson & Yates, 1981). For each variable, four repeated measurements were taken using digital calipers and recorded to the nearest hundredth of a millimetre. Only adult skulls were measured, as determined by all molars being completely erupted and having slight wear on M^3 (Krutzsch, 1954). Fewer measurements were taken for some specimens due to incomplete material. Calipers were moved away from the skull and reset for each measurement. One person (L.M.C.) measured all skulls (Palneirim, 1998). We used the means of the repeated measurements for 40 *Z. h. preblei* and 40 *Z. h. campestris* in ANOVA, PCA and LDA (Conner & Shenk, 2003). Those two subspecies were then combined for comparisons with 37 *Z. h. intermedius*.

The critical test of the original subspecies description was two-fold. First, the hypothesis of *Z. h. preblei* being a unique, smaller subspecies relative to *Z. h. campestris* would be rejected if the skulls of *Z. h. preblei* were not significantly smaller for the majority of skull measurements. Second, we used LDA to test uniqueness with the distinguishability criterion that $\geq 90\%$ of the specimens be correctly classified to subspecies at jackknifed posterior probabilities ≥ 0.95 (Wehausen & Ramey, 2000). This unambiguous criterion requires that specimens be correctly classified with a high degree of certainty using a multivariate analysis of shape. Outliers were removed using Grubb's and Dixon's tests (Sokal & Rohlf, 1981) and stepwise procedures were used to limit the model to discriminating variables for 33 *Z. h. preblei* and 39 *Z. h. campestris* that had complete measurements. We also used the combined sample of *Z. h. preblei* and *Z. h. campestris* for comparison with *Z. h. intermedius* ($n = 37$). Males and females were pooled because of an apparent lack of sexual dimorphism (Jones, 1981; Conner & Shenk, 2003). Incomplete specimens could not be used in LDA if any variable used in the model was missing for that specimen.

Krutzsch's qualitative descriptions of skull shape and pelage, that presumably distinguished *Z. h. preblei* from *Z. h. campestris*, included: incisive foramina not truncate posteriorly; auditory bullae smaller and less well inflated; frontal region usually more inflated; upper parts generally dull, averaging lighter; sides duller; less black tipped hair. These subjective criteria and those describing *Z. h. campestris* relative to *Z. h. intermedius* (coloration more tawny and ochraceous, less yellow; auditory bullae averaging larger, more inflated; incisive foramina not truncate posteriorly), were not readily quantifiable and were not used in subsequent analyses.

We used PCA as an exploratory tool to look for geographical patterns in cranial size and shape variation across the study area and to identify variables that contributed strongly to any components that showed geographical separation (Reyment, 1990). We ran PCA on the nine cranial characteristics. PCA was performed on covariance matrices derived from pairwise analyses of natural-log-transformed variables (Reyment, Blackith & Campbell, 1984).

MtDNA sequencing

We analysed a segment of highly variable mitochondrial DNA control region sequence for 205 museum skins or liver tissues of *Z. hudsonius* (58 *Z. hudsonius preblei*, 33 *Z. h. campestris*, 32 *Z. h. luteus*, 35 *Z. h. pallidus* and 47 *Z. h. intermedius*) (Appendix 2). For outgroup comparison

we used 17 specimens of western jumping mouse (*Z. princeps*) (Appendix 3). Specimens were obtained for genetic analysis from museum collections at DMNS, KU, the Nebraska State Museum (NSU) and the University of New Mexico Museum of Southwestern Biology (MSB). We sampled across the range of each putative subspecies in order to assess the amount of genetic variation within a subspecies. Thus, we sampled more locations but fewer individuals per location.

Genomic DNA was extracted from frozen liver tissue, museum skin samples (5–10 mg) and ear punch specimens using Qiagen DNeasy Tissue kit (Qiagen Inc.). Approximately 460 base-pairs (bp) of the mitochondrial control region were amplified via polymerase chain reaction (PCR) using the primers L15320 (5'ATAAACATTACTCTGGTCTTGTAAACC3') and ZAP5P1r (5'ATGGCCCTGAAGTAAGAACCAG3'). Amplifications were conducted in a 25 μl total volume, containing 5 μl of Invitrogen optimiser buffer D (17.5 mM $MgCl_2$, pH 8.5) (Invitrogen, Inc.), 2.5 μl of dNTPs (2.5 mM each), 1.25 μl of each primer (10 μM), one unit *Taq* polymerase, one μl of template (*ca.* 50–100 ng double-stranded DNA) and 13.8 μl of sterile water. Thermal cycling was performed with an initial denaturation for 2 min at 94 °C, followed by 30 cycles of 1 min at 94 °C, 1 min at 58 °C, 2 min at 72 °C, with a final extension of 10 min at 72 °C. Some museum specimens were amplified using nested PCR. We designed the nested primers, L15398 (5'ATCAGCACCCAAAGCTGATATTC3') and H16498 (5'CCTGAAGTAAGAACCAGATG3'), which amplified roughly 385bp within the first amplicon. Nested PCR was performed by using 1.0 μl of the PCR product from the first reaction mixture as a template and reamplifying it with the nested pairs of primers. The remaining procedure was identical to the first PCR. Multiple negative controls were run with every PCR batch. The amplified PCR product was cleaned using the Exo/SAP method. Double-stranded DNA templates were incubated at 37 °C for 30 min and then at 85 °C for another 15 min with five units of Exonuclease I (ExoI, Amersham) and 0.5 unit Shrimp Alkaline Phosphatase (SAP, Amersham). For cycle sequencing reactions we used 1–5 μl (20ng) of the cleaned PCR product as a template in a 10 μl total volume with the CEQ DTCS Quick Start Kit (Beckman Coulter, Inc.). The following cycling conditions were used: 96 °C for 2 min, then 30 cycles of 96 °C for 20s, 50 °C for 20s and 60 °C for 2 min. The cycle-sequenced products were purified using an ethanol precipitation method following the Beckman Coulter protocol and separated by electrophoresis using a Beckman Coulter CEQ8000 sequencer.

Consensus sequences were aligned using Sequencher 3.1.1 (Gene Codes Corp., Ann Arbor, MI) and verified manually. Phylogenetic analyses based on distance, parsimony and maximum-likelihood methods were conducted using PAUP* 4.0b10 (Swofford, 2002). Modeltest 3.06 (Posada & Crandall, 1998) was used to evaluate 56 models of evolution in order to obtain an appropriate substitution model and parameter values for distance and maximum-likelihood analyses. Appropriate genetic distance (based on Modeltest results) was used to generate neighbour-joining (NJ) trees based on the clustering method of Saitou & Nei (1987). Node support was assessed by completion of 10 000 bootstrap replications (Felsenstein, 1985) in PAUP*, using the fast-search option. Maximum-parsimony (MP) analyses were conducted with equal weighting, using the heuristic search option with tree bisection reconnection branch-swapping, 100 replications of random stepwise additions, gaps treated as missing, and MAXTREES set to 10 000. Bootstrapping with 10 000 replications (as implemented in PAUP*) was used to evaluate node support. The most likely model selected by Modeltest was used for maximum likelihood (ML) analyses. A neighbour-joining tree with appropriate genetic distance was used as the initial topology for branch-swapping. Node support was evaluated by 100 bootstrap pseudoreplicates. Split decomposition (SD) was calculated using SplitsTree version 2.4 (Huson, 1998) for all mtDNA data and for *Z. hudsonius* mtDNA data alone. Branch support was evaluated using 50 bootstrap replications.

Four *Z. hudsonius* specimens from Wyoming, one from Kansas, one from Montana and one from South Dakota had mtDNA haplotypes nearly identical to the highly divergent haplotypes found in *Z. princeps*. These seven specimens were presumed to be misidentified and were excluded (Appendix 3). *Zapus hudsonius* and *Z. princeps* are difficult to distinguish from pelage alone, although the latter tend to be larger. In order to provide a reasonable tree size, one sequence from each haplotype of *Z. hudsonius* and one representative sequence from each *Z. princeps* subspecies were used in all phylogenetic analyses.

ARLEQUIN 2.0 was used to perform an analysis of molecular variance (AMOVA) to partition the amount of genetic variation in a hierarchical fashion within and between the subspecies (Excoffier, Smouse & Quattro, 1992). MEGA 2 (Kumar *et al.*, 2002) was also used to estimate mtDNA nucleotide diversity. Tajima's *D* was used as a test of selective neutrality for mtDNA using ARLEQUIN 2.0.

Our critical test of uniqueness for *Z. h. preblei* using mitochondrial DNA sequence data was that there be greater molecular variance among than within subspecies (in pairwise comparisons involving *Z. h. preblei*) or that samples show nearly complete reciprocal monophyly with respect to other subspecies.

MtDNA MDIV

Fixation indices such as F_{ST} (Wright, 1921) are calculated under assumptions of equilibrium; any shared genetic variation is therefore assumed to be the result of current gene flow. Thus F_{ST} cannot distinguish between recently-isolated populations with no gene flow and populations isolated for a longer period of time but with continuing low levels of gene flow. As an alternative, we used the maximum-likelihood based program MDIV (Nielsen & Wakeley, 2001) to evaluate whether shared mtDNA variation between *Z. h. preblei*, *Z. h. campestris*, *Z. h. intermedius*, *Z. h. pallidus* and *Z. h. luteus* reflected very

Table 1. Dinucleotide microsatellite primers used in this study

Locus	GenBank accession no.	Primer sequence (5′ to 3′)	Annealing temp (°C)	Repeat of cloned allele	No. of alleles	Allele size range
Z.20	DQ063596	F:TCTTCCTCCCCCAGACCTAC R:TCCCAAGGCCTAAACAGTGA	60	$(CA)_9$	20	109–149
Z.48	DQ063597	F:GCTCATCTGCAATGGAGGA R:TTGTCTTTAGAAACAAGATTTACT	60	$(CA)_{23}$	18	182–210
Z.52	DQ063598	F:CCTCCCAGCTCTGTCTTTGA R:TGGACAAGGCTACTGCTTCC	60	$(GT)_{21}$	13	155–181
Z.7	DQ063599	F:CTTAGGCCTTGCAGTCAAGC R:TTAGCACTTCCAGCACATGG	60	$(GT)_7$	20	154–190
Z.26	DQ063600	F:CATTTTACACCAGCAAACAGG R:TATTGGCTGCACATTCTTGC	60	$(CA)_{16}$	19	141–171
Z.47	DQ063601	F:TGAAAAGAGCTAAATACTTGGGTAGA R:TGTCATTGCTCACTGTTTCCA	60	$(CA)_{24}$	15	121–149

recent (including current) gene flow or complete, but recent, isolation.

MDIV uses Markov-chain Monte Carlo simulations to estimate for two populations the likelihood of the parameters *theta* ($4N_e\mu$) and *M* ($2N_em$), where N_e is the effective population size, m is the migration rate and μ is the mutation rate. MDIV assumes that N_e and m are the same for both populations. We used MDIV to estimate migration (m) between *Z. h. preblei* and *Z. h. campestris* and to compare this estimate of gene flow to estimates of gene flow between other pairs of populations. We ran 5 000 000 chains for each simulation with burn-in of 500 000 chains, set T_{MAX} and M_{MAX} to 10 and used the HKY model of sequence evolution (a software constraint). Parallel simulations gave similar results, suggesting that this number of chains was adequate. MDIV tests a wide range of values for each parameter and calculates the likelihood of each tested value. We calculated confidence intervals around the parameter estimates using Akaike's Information Criterion (AIC: Burnham & Anderson, 1998) to determine the range of parameter values that were not significantly less likely than the best estimated value (Nielsen & Wakeley, 2001). Because the number of parameters was fixed, we calculated AIC as:

$$\mathrm{AIC} = -2^{*}\log\ (\text{likelihood})$$

We accepted parameter values within 2 AIC units on either side of the most likely estimated parameter value. However, due to the assumptions made by MDIV about N_e and m, these confidence intervals may be understated. We calculated N_e from the estimate of theta using $\mu = 2.5{*}10^{-5}$ over 346 bp (estimated from divergence in vole mtDNA control region sequences by Matson & Baker (2001)). We converted M to m using the most likely estimate of N_e.

Microsatellites

Six dinucleotide-repeat microsatellite loci (Table 1) were isolated and sequenced using methods described previously (Oyler-McCance *et al.*, 2005). The amplification, electrophoresis and scoring methods used were as previously described (Wehausen, Ramey & Epps, 2004).

We estimated allelic richness (A) using FSTAT (Goudet, 1995) to correct for variation in sample size, as recommended by Leberg (2002). Allelic richness can be a sensitive comparative indicator of population bottlenecks or founder effects (Leberg, 2002). We also used FSTAT to calculate F_{IS} values within populations and to test for linkage disequilibrium within populations within loci and within loci across populations. Population pairwise F_{ST} values (Weir & Cockerham, 1984) were calculated by GENEPOP (Raymond & Rousset, 1995). Our critical test of uniqueness for subspecies and historic genetic exchangeability (Crandall *et al.*, 2000) was two-fold: that there be greater variation between *Z. h. preblei* and other subspecies than within each subspecies in pairwise comparisons (using F_{ST} and AMOVA) and that multiple private alleles be at higher frequency than shared alleles at the majority of loci. We do not claim that these criteria alone can be used to define subspecies, or that they are universally applicable, merely that they provide an unambiguous test of deeply historic genetic divergence among populations.

We used BAPS (Corander, Waldmann & Sillanpaa, 2003) to examine genetic clustering of putative subspecies and 'populations'. We also used it to estimate Nei's genetic distance (D) between putative subspecies and between populations. We used the 'population' analyses to compare variation between biogeographically-relevant groupings within putative subspecies with variation between putative subspecies. We divided *Z. h. preblei* into 'North' and 'South' populations, based on a suspected biogeographical split imposed by the Denver metropolitan area. We divided *Z. h. intermedius* into 'West' (North Dakota, South Dakota, western Iowa) and 'East' populations (central and eastern Iowa, Illinois and Indiana). This divided the range approximately in half. We treated *Z. h. campestris*, *Z. h. pallidus* and *Z. h. luteus* as discrete populations in this analysis. We estimated D using the multiple-chain McMC approach. We set burn-in time to 15 000, chain length to 50 000, ran five chains, set thinning to 5 and checked to ensure that these values were sufficient to

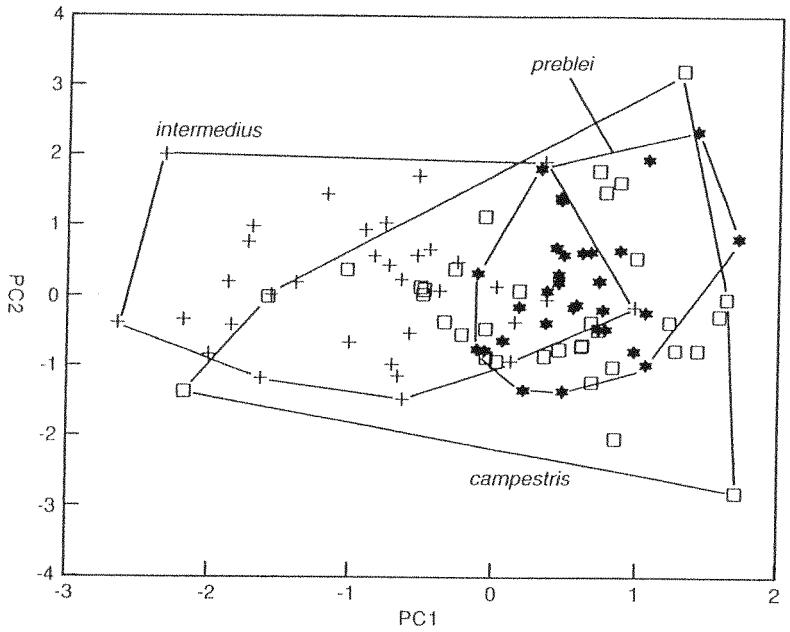


Fig. 2. Plot of Principal Component scores for PC1 and PC2. Subspecies are indicated by polygons.

achieve convergence. We used an initialisation of $k = 7$ clusters and, in the results state, set the minimum partition to 0.05.

We used STRUCTURE (Pritchard, Stephens & Donnelly, 2000) to attempt to determine how many clusters (k) were diagnosable in the combined data set of all 195 specimens. For each cluster number examined, STRUCTURE generates a likelihood value; the maximum value indicates the most likely cluster number. We tested $k = 1$ through $k = 15$, using a burn-in of 15 000 followed by 100 000 replications. Using the cutoff value of $q = 0.90$ (where q is the likelihood of assignment of an individual to a given cluster), as suggested by Worley *et al.* (2004), we examined how many specimens of each population (with *Z. h. preblei* samples divided into southern and northern populations and *Z. h. intermedius* divided into eastern and western populations) were assigned with confidence greater than or equal to this cut-off value of q. Our critical test of recent genetic exchangeability using STRUCTURE was that clusters correspond to subspecies or populations – with a high level of correct assignment of individuals (>90%) using $q = 0.90$. This criterion rules out weakly differentiated populations as conservation priorities.

RESULTS

Testing the original quantitative basis of taxonomic categories

Krutzsch (1954) stated that *Z. h. preblei* was smaller than *Z. h. campestris* in 'most skull dimensions measured.' However, our results revealed that *Z. h. preblei* was significantly ($P < 0.05$) smaller for only one measurement (interorbital breadth), but larger for two measurements (zygomatic and mastoid breadth) and insignificantly different for the six others. The significant differences between subspecies were very small and of questionable biological significance relative to measurement resolution. The classification of *Z. h. preblei* as a separate subspecies therefore failed the test of uniqueness using the original criteria. When a combined sample of *Z. h. campestris* and *Z. h. preblei* was compared to *Z. h. intermedius*, they were significantly larger in all skull measurements. This is consistent with Krutzsch's description of *Z. h. intermedius* being slightly smaller, although Krutzsch noted substantial intergradation with *Z. h. campestris* and *Z. h. pallidus*. Measurement data used in morphometric analyses were deposited with the Archivist at DMNS and are available online at www.dmns.org.

PC1 explained 67.1% of the variation with positive loadings on all variables, suggesting that this is a general size component. PC2 accounted for 11% of the variance, mostly in tooth row length. PC3 accounted for 10.3% of the variance, mostly in interorbital breadth. When PC1 is plotted against PC2 on a pooled sample of *Z. h. preblei*, *Z. h. campestris* and *Z. h. intermedius*, *Z. h. preblei* specimens fall entirely within *Z. h. campestris* along the PC1 axis. *Zapus h. intermedius* however, is somewhat separable as smaller (Fig. 2). There is no subspecies separation on the PC2 axis or when PC3 and PC4 were plotted. While PCA on cranial measurements has limitations for inferring shape differences that are independent of size, there appears to be almost no difference between *Z. h. preblei* and *Z. h. campestris*. About half of the *Z. h. intermedius* specimens, however, appear to be smaller than *Z. h. preblei* and *Z. h. campestris*.

Four variables were determined to have the greatest discriminating power between *Z. h. campestris* and *Z. h. preblei*, using forward and backward stepwise procedures in LDA. These were zygomatic breadth, mastoidal breadth, breadth of skull and condylobasal length. Only 42% of the specimens could be classified correctly at

posterior probabilities ≥ 0.95, further indicating a lack of morphometric distinguishability of these two subspecies. We therefore rejected the hypothesis that *Z. h. preblei* is unique in cranial shape from *Z. h. campestris*.

Only mastoidal breadth and interorbital breadth contributed significantly to the discriminant function for the combined sample of *Z. h. preblei* and *Z. h. campestris* ($n = 73$) against *Z. h. intermedius* ($n = 35$). That function had poor discrimination ability, with only 31.5% of specimens being correctly classified at a jackknifed posterior probability of ≥ 0.95. We therefore rejected the hypothesis of uniqueness for *Z. h. campestris* and *Z. h. preblei* combined from *Z. h. intermedius*. In comparison, Conner & Shenk (2002) had found a high degree of classification certainty between species of jumping mice (*Z. princeps* and *Z. hudsonius*) in Colorado and Wyoming with >96% of specimens correctly classified at a posterior probability > 0.95.

Testing putative subspecies: MtDNA analyses

DNA sequences were deposited in GenBank with accession numbers AY598142 – AY598316 and AY971529 – AY971575. The final aligned data matrix for mtDNA analyses, including indels, was 346 bp, of which 68 (19.7%) sites were variable and 47 (13.6%) were parsimony informative. Values of Tajima's *D* were not significant ($P > 0.05$) for subspecies considered individually or pooled together. Therefore, the null hypothesis of selective neutrality for mtDNA could not be rejected. Nucleotide diversity ranged from 0.0027 in *Z. h. preblei* to 0.0215 in *Z. h. campestris* (Table 2). Forty-three haplotypes were observed for *Z. hudsonius*. Modeltest (version 3.06, Posada & Crandall, 1998) selected the TVM model (Transversional model, a variation of the General Time Reversible model (GTR)), with some sites assumed to be invariable and with variable sites assumed to follow a discrete gamma distribution (e.g. TVM + I + G: Tavare, 1986; Posada & Crandall, 1998) as the best fit for the dataset using AIC. The optimised parameters were base frequencies of A = 0.2919, C = 0.2629, G = 0.0957, T = 0.3495; Rmat = {3.2955 24.2634 7.5746 0.8175 24.2634}; shape of gamma distribution = 0.6499; and proportion of invariant sites = 0.6174. GTR distance was used to generate NJ trees and the TVM + I + G model was used for ML analyses. Distance analysis (neighbour-joining tree), MP, ML and SD resolved haplotypes into two strongly supported *Z. hudsonius* lineages. These included a *Z. h. preblei/Z. h. campestris/Z. h. intermedius* lineage and a *Z. h. luteus/Z. h. pallidus* lineage (Fig. 3). The MP, ML and SD (not figured) topologies were congruent with the NJ tree (Fig. 3) and differed in the positioning of terminal taxa. SD analysis of *Z. hudsonius* mtDNA data supported the two lineages (100% bootstrap support) and unresolved polytomies for terminal branches with low bootstrap support (< 66%).

The number of variable nucleotides and haplotypes and nucleotide diversity for each subspecies are presented in Table 2. The four haplotypes that occurred in *Z. h. preblei* also occurred within the range of *Z. h. campestris*.

Table 2. MtDNA control region sequence diversity found in subspecies of *Zapus hudsonius*

Taxa	*N*	Variable sites	Haplotypes	Nucleotide diversity
Z. h. preblei	54	3	4	0.0027
Z. h. campestris	31	29	15	0.0215
Z. h. intermedius	47	31	16	0.0068
Z. h. luteus	32	6	8	0.0042
Z. h. pallidus	34	30	12	0.0138

These shared haplotypes span a range of up to 700 km, from central Colorado to western South Dakota and southeastern Montana. Four sequences (two haplotypes) of *Z. h. campestris* were grouped in the *Z. h. luteus/Z. h. pallidus* lineage. Nearly all of the *Z. h. intermedius* haplotypes (except one) were found in the *Z. h. preblei/Z. h. campestris/Z. h. intermedius* lineage (Figs 3 & 4). Four of the *Z. h. intermedius* haplotypes were identical to those found in *Z. h. campestris* (Fig. 3). *Zapus h. preblei* was not reciprocally monophyletic with respect to any other subspecies. Two sequences of *Z. h. pallidus* from Clay Co., South Dakota were more similar to sequences of *Z. h. campestris* and *Z. h. preblei* than to other sequences of *Z. h. pallidus*.

Analysis of molecular variance between *Z. h. preblei* and *Z. h. campestris* revealed that most of the genetic variation was within (63%) rather than between (37%) these putative subspecies. In the case of *Z. h. luteus* and *Z. h. pallidus* (separated by ~500 km), each has several unique haplotypes (6 and 9, respectively) but, as with *Z. h. preblei* and *Z. h. campestris*, most of the molecular variance was within (72%) rather than between (28%) these putative subspecies. In combination with the absence of any genetic structure that even approached reciprocal monophyly, these results led us again to reject the hypothesis of uniqueness of *Z. h. preblei* relative to *Z. h. campestris*.

When *Z. h. intermedius, Z. h. campestris* and *Z. h. preblei* were considered separately from *Z. h. pallidus* and *Z. h. luteus*, greater variation was found within (69.3%) than between (30.7%) the subspecies. When only *Z. h. intermedius* and *Z. h. campestris* were compared, considerably greater variation was found within (96.2%) than between those two subspecies (3.8%). When *Z. h. campestris* and *Z. h. preblei* were combined as a single subspecies and compared with *Z. h. intermedius*, only 18.5% of the variation was found between subspecies (81.5% within subspecies). Based on these analyses, we reject the hypothesis of uniqueness for *Z. h. intermedius* relative to *Z. h. campestris*.

Testing putative subspecies: microsatellites

Six microsatellite loci genotypes were recorded for 195 *Z. hudsonius* specimens for which mtDNA was sequenced (Appendix 2). One locus, .47, was not considered a reliable neutral genetic marker because it had very high

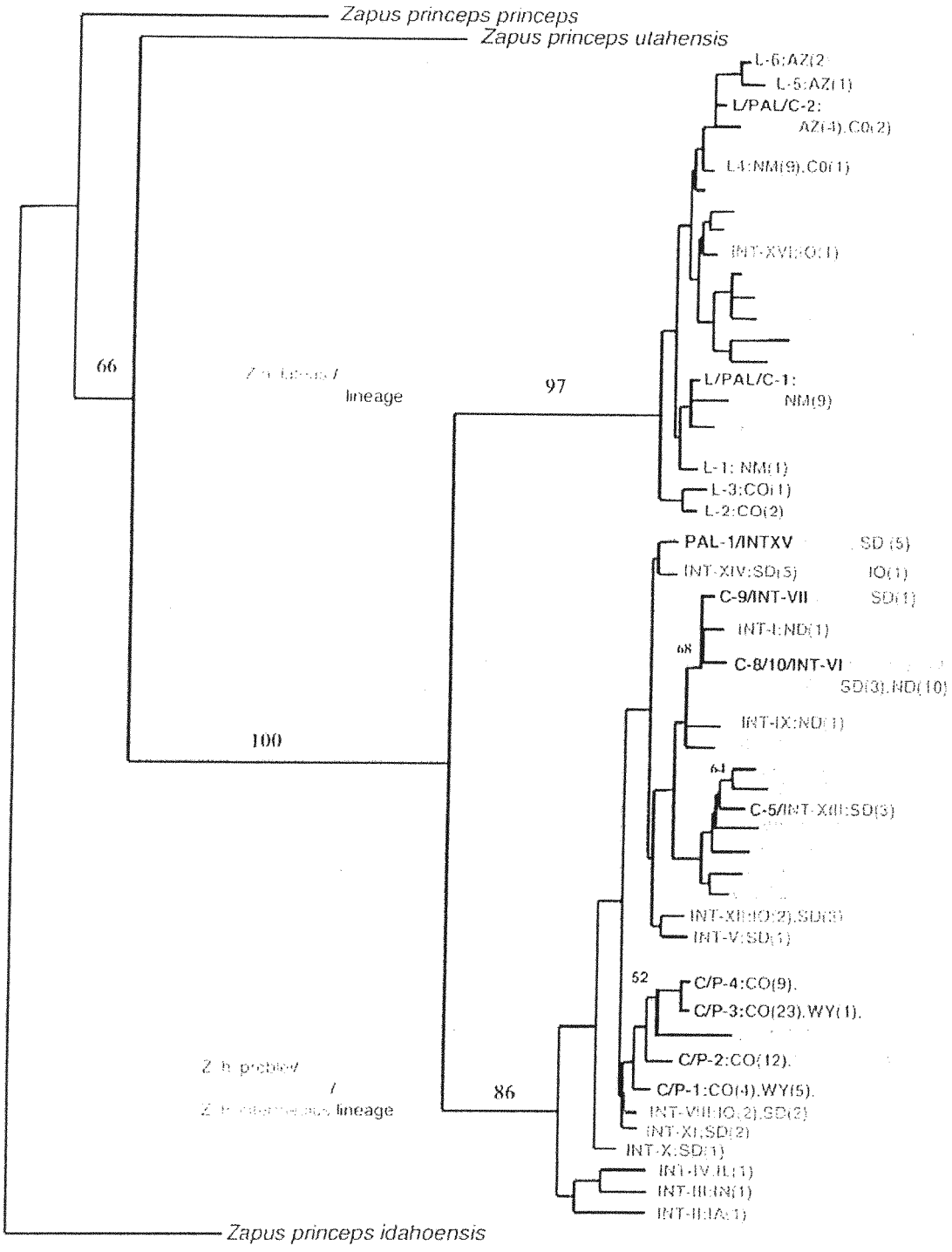


Fig. 3. Neighbour-joining phylogram inferred from partial mitochondrial DNA control region, depicting phylogenetic relationships between haplotypes of *Zapus hudsonius*. Bootstrap percentages are given when ≥ 50%. State and number of individuals with identical haplotypes are listed. Colours indicate subspecies (Krutzsch, 1954; Hafner *et al.*, 1981) as follows: *Z. h. preblei* (blue), *Z. h. campestris* (pink), *Z. h. intermedius* (red), *Z. h. luteus* (dark green) and *Z. h. pallidus* (light green).

values of F_{IS} (0.69–0.94) and consistently violated Hardy–Weinberg equilibrium, suggesting the presence of null alleles or selection at closely linked loci. It was dropped from subsequent analyses. When the data set was divided into five putative subspecies or into seven populations, no significant linkage disequilibrium ($P < 0.05$, after correcting for multiple comparisons) was found in any population at any locus, or by locus across populations. F_{IS} for all populations was positive, with a pattern of heterozygote deficiency across most loci (Table 3). A probable explanation for the observed heterozygote deficiency is a Wahlund effect due to sampling of only one,

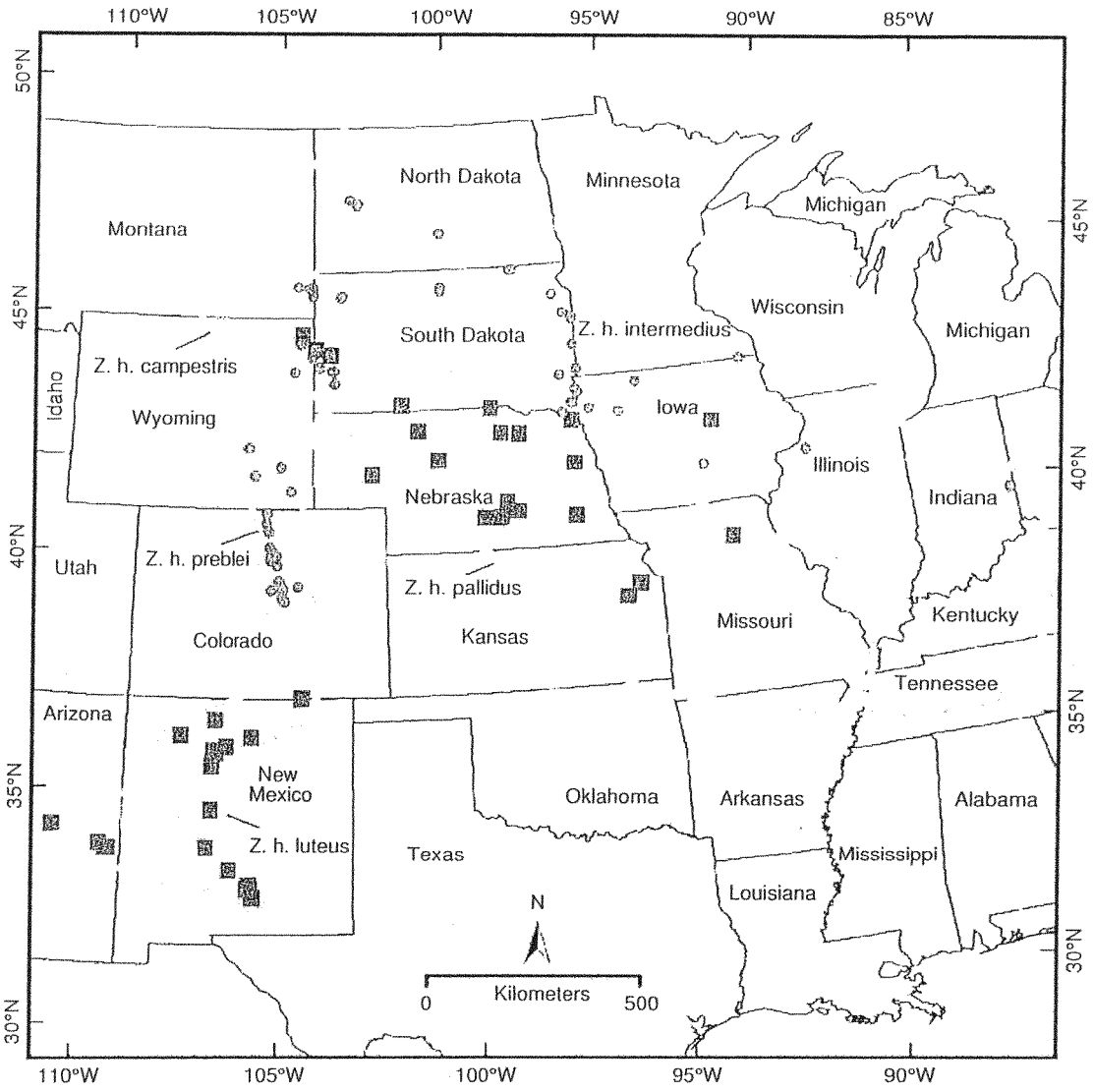


Fig. 4. Map showing collection locations of specimens used in mtDNA analyses. Multiple samples were taken from some locations. Grey outlines indicate subspecies ranges (Krutzsch, 1954; Hafner *et al.*, 1981). Pink circles indicate specimens on the *Z. h. preblei*/ *Z. h. campestris* mtDNA lineage, green squares indicate specimens on the *Z. h. luteus*/*Z. h. pallidus* mtDNA lineage.

or few, individuals per site across a broad geographical area (Hartl & Clark, 1999). This is supported by the result that when *Z. h. preblei* and *Z. h. intermedius* were further subdivided in biogeographically meaningful ways for analysis, the number of loci violating conditions of Hardy–Weinberg equilibrium dropped sharply (Table 3). Non-random mating (inbreeding) or extensive substructuring in local populations could also potentially contribute to heterozygote deficiency (Wilson, Naish & Boulding, 1999; Yu, Liao & Kao, 2001). A low rate of missing data (2%) suggests that null alleles and allelic drop-out were not likely explanations.

Allelic richness estimates based on putative subspecies designations showed that *Z. h. preblei* had much lower allelic richness than any of the other putative subspecies, suggestive of a strong bottleneck, founder effect, or low effective population size (Table 3). For analyses based on the seven populations, the northern and southern populations of *Z. h. preblei* both had lower allelic richness than any of the remaining subpopulations.

AMOVA tests of the five putative subspecies showed that only 7.5% of the variance was between populations, while 92.5% of the variance was within populations. For the seven population division, only 8.9% of the variance was between populations, while 91.1% of the variance was within populations. When *Z. h. preblei* and *Z. h. campestris* were compared using AMOVA (which provides an estimate of F_{ST} using pairwise distances among alleles), 9.0% of the variance was between populations and 91.0% was within populations.

Three unique alleles were found in *Z. h. preblei* in three loci and these were all at low frequency (<0.05). (The locus dropped because of strong heterozygote deficiency

Table 3. Genetic variability estimates for microsatellite loci used in this study

Comparison	*N*	H_o	H_E	Loci not in *HE*	Private alleles	*A*	F_{IS}
Z. h. preblei	54	0.58	0.74	20, 7	3	6.89	0.212
(*Z. h. preblei* – South)	(33)	0.62	0.69	–	2	(4.49)	(0.087)
(*Z. h. preblei* – North)	(21)	0.51	0.73	–	1	(4.46)	(0.288)
Z. h. campestris	29	0.52	0.78	7, 26	2	9.28 (6.18)	0.333
Z. h. intermedius	46	0.66	0.83	20, 48, 52, 26	8	11.69	0.209
(*Z. h. intermedius* – West)	(38)	0.67	0.81	–	4	(6.75)	(0.159)
(*Z. h. intermedius* – East)	(8)	0.58	0.94	52	4	(9.00)	(0.385)
Z. h. pallidus	34	0.74	0.89	26	7	13.42 (8.58)	0.174
Z. h. luteus	32	0.68	0.85	20, 26	2	10.21 (7.00)	0.189

Allelic richness (*A*) is averaged across loci; *A* values were sub-sampled with FSTAT using a minimum sample size of 29, *A* values in parentheses were sub-sampled using a minimum sample size of 8. Hardy–Weinberg Equilibrium (*HE*) tests were preformed using GENEPOP for five and seven subpopulations and were corrected for multiple comparisons. The mean frequency of private alleles was 0.029 (range 0.013–0.125), as calculated by GENEPOP (Raymond & Rousset, 1995).

Table 4. Maximum likelihood (MDIV) estimates of very recent gene flow between populations of *Zapus hudsonius*

Comparison	*theta*	N_e	*m* (range)	*M* (range)
Z. h. preblei – *Z. h. campestris*	2.7	27,409	3.3×10^{-6}–3.2×10^{-5}	0.18–1.74
Z. h. campestris – *Z. h. intermedius*	23.0	230,924	1.3×10^{-6}–1.3×10^{-5}	0.58–5.86
Z. h. pallidus – *Z. h. intermedius*	10.5	105,622	1.9×10^{-7}–2.3×10^{-6}	0.04–0.48
Z. h. preblei – *Z. h. luteus*	5.6	56,124	0–1.0×10^{-6}	0.0–0.14
Z. h. preblei – *Z. h. pallidus*	6.4	64,558	0–2.2×10^{-6}	0.0–0.28
Z. h. preblei – *Z. h. intermedius*	19.1	191,767	0–2.2×10^{-6}	0.0–0.84

Theta $= 4N_e\mu$, N_e is the estimated effective population size, *m* is the migration rate between populations and *M* is the scaled migration rate. The range of *m* was defined as within 2 Akaike's Information Criterion (AIC) units of the most likely parameter value.

had one private allele at a frequency of 0.55 in the southern population of *Z. h. preblei* and 0.048 in the northern population.) On the basis of these microsatellite analyses, we again reject the hypothesis of uniqueness for *Z. h. preblei* relative to *Z. h. campestris*.

Testing genetic exchangeability

After correcting for N_e, the range of migration estimates (using MDIV) between *Z. h. preblei* – *Z. h. luteus*, *Z. h. preblei* – *Z. h. pallidus* and *Z. h. preblei* – *Z. h. intermedius* included zero, suggesting that little or no very recent mtDNA gene flow has occurred between *Z. h. preblei* and these other subspecies. *Z. h. preblei* and *Z. h. campestris* showed low, but non-zero, levels of very recent gene flow (*m* and *M*) (Table 4). Thus, the null hypothesis of no very recent gene flow between these putative subspecies can be rejected. Gene flow between *Z. h. campestris* and *Z. h. intermedius* was also greater than zero, therefore the null hypothesis of no recent gene flow can also be rejected for those putative subspecies. The null hypothesis of historic genetic exchangeability cannot be rejected using the results of the subspecies tests above.

For microsatellite data, Nei's genetic distance between the seven subpopulations reflected a general pattern of gene flow between adjacent subpopulations that is consistent with isolation by distance (Table 5, Fig. 5): more distant comparisons had larger *D* values, as expected. An exception to this pattern was the high *D* value between *Z. h. luteus* and the southern population of *Z. h. preblei*. While both of these populations showed evidence of gene flow with *Z. h. pallidus* to the east, high genetic distance suggests little or no current gene flow between them. Notably, *D* between the northern and southern populations of *Z. h. preblei* was comparable with *D* between the northern *Z. h. preblei* and *Z. h. pallidus* populations (Fig. 5). Pairwise F_{ST} estimates between *Z. h. preblei* populations (0.10) and adjacent subspecies (0.07–0.16) suggest that the number of migrants per generation is above the cutoff ($Nm < 1$) suggested by Crandall *et al.* (2000) as evidence for rejecting the hypothesis of recent genetic exchangeability, where $F_{ST} = 1/(1 + 4\,Nm)$.

BAPS population clustering suggested a greater degree of genetic structure of the southwestern populations: both the north and south populations of *Z. h. preblei* and *Z. h. luteus* had a high posterior probability (> 0.95) of different allele frequencies, while *Z. h. campestris*, both the eastern

Table 5. Matrix of pairwise genetic distances (Nei's D) as calculated by BAPS (Corander *et al.*, 2003) above the diagonal and pairwise F_{ST} values (Weir & Cockerham, 1984) as calculated by GENEPOP (Raymond & Rousset, 1995) below the diagonal

Comparison	*preblei* North	*preblei* South	*campestris*	*intermedius* West	*intermedius* East	*pallidus*	*luteus*
preblei – North	–	0.36	0.53	0.49	0.87	0.40	0.66
preblei – South	0.10	–	0.46	0.48	0.90	0.48	0.96
campestris	0.11	0.11	–	0.11	0.43	0.49	0.86
intermedius – West	0.10	0.11	0.01	–	0.43	0.38	0.84
intermedius – East	0.11	0.14	0.03	0.03	–	0.47	0.59
pallidus	0.07	0.10	0.07	0.05	0.01	–	0.34
luteus	0.11	0.16	0.11	0.10	0.03	0.03	–

All pairwise F_{ST} values were significant at $P < 0.05$.

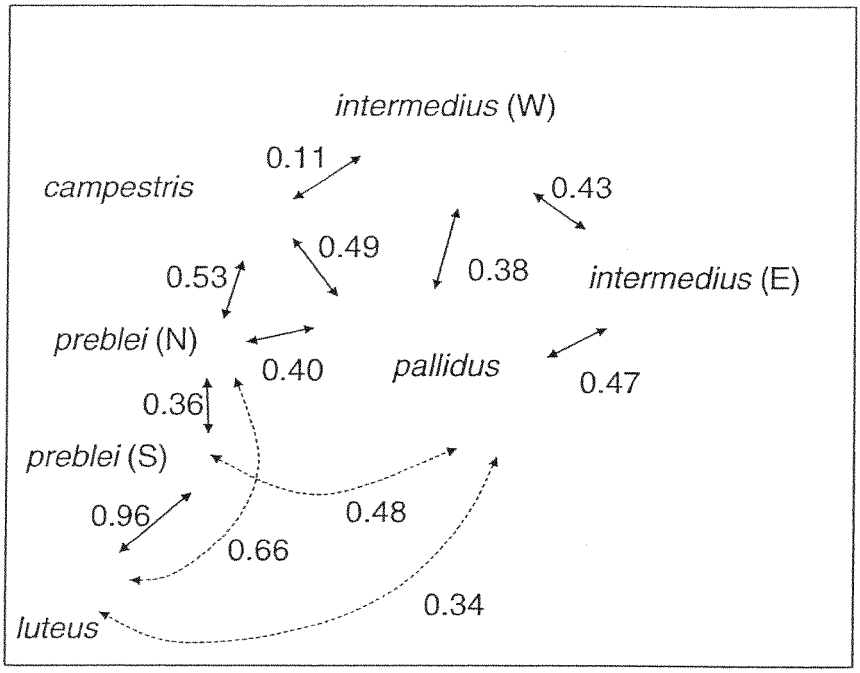


Fig. 5. Schematic diagram of seven subpopulations of *Z. hudsonius* showing Nei's genetic distances (D) between subpopulations as determined from five microsatellite loci. Comparisons between non-adjacent subpopulations are denoted by dotted arrows; comparisons between adjacent subpopulations are denoted by arrows. N, north, S, south, E, east, W, west.

and western populations of *Z. h. intermedius* and *Z. h. pallidus* were indistinguishable at this level of posterior probability.

STRUCTURE analyses indicated that $k = 8$ was the most likely cluster number and this was used for assignment analyses, but the variability of the likelihood estimates was high, suggesting that clusters were poorly defined. This result is typical of situations reflecting isolation by distance (Worley *et al.*, 2004). STRUCTURE analyses supported a potential, very recent biogeographical separation of northern and southern populations of *Z. h. preblei*. Most individuals in each of these populations were assigned to unique clusters, although 24% of the northern samples were assigned to the cluster 5 (to which all but two of the southern samples were assigned; Table 6). Overall, our analyses suggested a pattern of greater genetic structure in the southwestern populations of *Z. hudsonius*: average values of q_{MAX} were highest in the southern population of *Z. h. preblei*, followed by the northern population of *Z. h. preblei*, then *Z. h. luteus* (Table 7). Likewise, roughly 55% of individuals were assignable at $q > 0.90$ to the southern population of *Z. h. preblei*, whereas the northern population of *Z. h. preblei* had 42% and *Z. h. luteus* had 22% of individuals assignable at this level of q (Table 7). Other groups had few or no individuals assignable at $q > 0.90$ (Table 7). Thus, we could reliably assign only 55% of individuals to the most clearly defined population (the southern population of *Z. h. preblei*). We therefore cannot reject the hypothesis of *recent* genetic exchangeability, or the null hypothesis of *historic* genetic exchangeability (using results of the subspecies tests on mtDNA or microsatellite data).

Testing ecological exchangeability

There is no published evidence of adaptive differences (e.g. selection for cryptic pelage on different rocky

Table 6. Results of STRUCTURE individual-level clustering, with proportion of each subpopulation assigned to each of $k = 8$ clusters, on the basis of the highest value of q (no cut-off value of q was used)

Subpopulation	1	2	3	4	5	6	7	8
Z. h. preblei (North)		0.71	0.05		0.24			
Z. h. preblei (South)		0.03			0.94	0.03		
Z. h. campestris	0.03	0.07	0.03	0.03	0.1	0.17		0.55
Z. h. pallidus	0.21		0.32	0.09		0.03	0.32	0.03
Z. h. luteus	0.44		0.22	0.31			0.03	
Z. h. intermedius (East)	0.25		0.38		0.13		0.13	0.13
Z. h. intermedius (West)	0.03	0.03	0.05	0.05	0.03	0.34	0.03	0.45

'Zero' values are omitted for clarity.

Table 7. Results of STRUCTURE analyses of seven sub-populations of *Zapus hudsonius*, for $k = 8$ clusters, reflecting the trend of greater genetic structure at the south-western extent of the range

Sub-Population	Average value of q_{MAX}	Percentage (and No.) of samples assigned at $q > 0.90$
Z. h. luteus	0.67	21.9% (7)
Z. h. preblei (South)	0.86	54.5% (18)
Z. h. preblei (North)	0.85	42.9% (9)
Z. h. campestris	0.61	0
Z. h. pallidus	0.47	0
Z. h. intermedius (West)	0.64	2.6% (1)
Z. h. intermedius (East)	0.44	0

Sub-populations are listed generally from south to north and from west to east.

substrates as found by Hoekstra & Nachman, 2003) or ecological differences (e.g. major habitat and/or climatic differences) that would be expected to result in notable adaptive differences between *Z. h. preblei* and other adjacent subspecies (Krutzsch, 1954; Whitaker, 1972, 1999; Jones 1981; Clark & Stromberg, 1987; see Cryan, 2004 for an in-depth review). These animals live in a range of similar habitat types and appear to have similar life histories. While the absence of evidence does not necessarily mean there is evidence of absence, there do not appear to be any adaptive differences that prevent the *Z. hudsonius* subspecies in this study from being ecologically exchangeable. We therefore cannot reject the null hypothesis of historic or recent ecological exchangeability.

While PCA and LDA on cranial measurements have limitations for inferring adaptive divergence, there appears to be almost no difference between *Z. h. preblei* and *Z. h. campestris* from a multivariate perspective. While *Z. h. intermedius* appears to be smaller than *Z. h. preblei* and *Z. h. campestris*, there is substantial overlap and no reliable multivariate distinguishability using the more powerful LDA. Because size alone (represented by PC1 in PCA) can be due to ecophenotypic and/or genetic differences, these results do not provide an adequate basis for rejecting the null hypothesis of ecological exchangeability.

DISCUSSION

Putative subspecies and taxonomic conclusions

Our morphometric results refuted the univariate quantitative basis for the description of *Z. h. preblei* as a subspecies. Distinguishability between groups is the key to valid systematic divisions, which for morphometric data is a multivariate question that should be investigated as such. Our multivariate analyses also refuted the distinguishability of *Z. h. preblei*, as well as *Z. h. intermedius*. We found that microsatellite and mtDNA analyses also did not support *Z. h. preblei* as a separate subspecies. *Zapus h. preblei* appears instead to be a population of *Z. h. campestris* with lower genetic variability. If *Z. h. preblei* had evolved in long-term isolation from *Z. h. campestris*, it should at least approach reciprocal monophyly of mtDNA with strong bootstrap support. This was not the case and the amount of molecular variance found between populations was below that required in our critical tests. The same conclusion was found for *Z. h. intermedius*. Additional sequence data would undoubtedly reveal additional structure, but would be unlikely to change the basic conclusions.

Although there are limitations to the applicability of microsatellites to phylogeographic questions (Paetkau *et al.*, 1997; Balloux *et al.*, 2000; Zink, 2004), analysis of microsatellite data also leads us to reject the hypothesis of uniqueness for *Z. h. preblei*, *Z. h. campestris* and *Z. h. intermedius*. These results were concordant with those obtained from morphometrics and mtDNA, except that *Z. h. pallidus* is largely fixed for one lineage of mtDNA relative to adjacent populations of *Z. h. campestris* and *Z. h. intermedius* and it shows low levels of differentiation for microsatellite loci (Table 5, Fig. 5). Lineage sorting is one possible explanation for the greater genetic structure in mtDNA among these subspecies. Sex-biased dispersal, with males moving nuclear genetic material over longer distances, is also a possibility. A selective sweep appears to have been ruled out by neutrality tests.

Based on hypothesis testing using four lines of evidence – morphometrics, mtDNA, microsatellites and a lack of recognised adaptive differences – we synonymise *Z. h. preblei* and *Z. h. intermedius* with *Z. h. campestris*, which was described first as the prairie jumping mouse by Preble (1899). Because we did not analyse cranial

morphometric data for *Z. h. luteus* and *Z. h. pallidus*, we are cautious about their taxonomic status at this time. However, our preliminary results are consistent with Jones' (1981) findings that there do not appear to be any recognisable subspecies of *Z. hudsonius* in the study area.

Testing genetic and ecological exchangeability

Assignment test results reflected a general pattern of gene flow between populations, with lower gene flow to isolated populations at the margins of the range. While *Z. h. preblei* had a higher proportion of individuals assignable at high confidence ($q > 0.90$) in STRUCTURE analyses (Table 7), both as a combined group or split into northern and southern populations, *Z. h. preblei* also showed much lower allelic richness than the other groups (Table 3). This implies that the genetic structure observed in the BAPS and STRUCTURE analyses for this region may stem from repeated population bottlenecks or founder effects and recent isolation, which reduced the microsatellite alleles to a subset of those present in neighbouring populations, rather than long-term divergence. This interpretation is consistent with mtDNA analyses, which show fewer haplotypes and lower nucleotide diversity in *Z. h. preblei*.

Estimates of *D* imply that gene flow between the northern population of *Z. h. preblei* and the adjoining populations of *Z. h. pallidus* and *Z. h. campestris* has occurred more recently than between *Z. h. preblei* and *Z. h. luteus* and that the level of isolation between the northern and southern populations of *Z. h. preblei* is comparable with that between the northern population of *Z. h. preblei* and *Z. h. pallidus*, as well as between the eastern and western populations of *Z. h. intermedius*. Isolation of southwestern populations of *Z. hudsonius* therefore appears to be a recent phenomenon that has accompanied the Holocene drying of the Great Plains as well as more recent agriculture and development (Hafner *et al.*, 1981; Jones, 1981). Population densities of *Z. hudsonius* are limited by competition with *Microtus* (Boonstra & Hoyle, 1986) as well as by anthropogenic causes.

Although some degree of population discrimination can be achieved for *Z. h. preblei* using discriminant analysis and assignment tests, classification of individuals to this putative subspecies with a high degree of confidence (as determined by posterior probabilities) is low. While our ability to quantitatively assess ecological exchangeability was limited, as is often the case (Crandall *et al.*, 2000), the morphometric analyses address at least some aspects of ecological interchangeability. In lieu of better options, such analyses can provide evidence suggestive of consistent physical differences that may be attributable to different selective environments.

In summary, we found no convincing evidence that would result in our rejection of the hypotheses of genetic or ecological exchangeability on recent or historic timescales for *Z. h. preblei*, *Z. h. campestris* and *Z. h. intermedius*. Therefore, these putative subspecies do not appear to be distinct populations (Crandall *et al.*, 2000). The results are consistent with the fact that *Z. h. preblei*, in particular the southern population, is a peripheral population at the edge of the species range and subject to founder effects. *Zapus h. luteus* does not appear to have had much current or historic gene flow with *Z. h. preblei* based on mtDNA and microsatellite analyses. The extent to which very recent human development (e.g. the past 100 years) may have contributed to additional isolation and bottlenecks is unknown. Both of these would be expected to increase the degree of genetic distance from other populations (Hedrick, Gutierrez-Espeleta & Lee, 2001). Regardless of whether more relaxed criteria are used for testing recent genetic exchangeability, or if trapping studies confirm isolation, a rejection of *recent* genetic exchangeability would be insufficient to treat *Z. h. preblei* as a distinct population using the criteria proposed by Crandall *et al.* (2000). The results also suggest that *Z. hudsonius* from healthy nearby populations could be used to augment or re-establish populations within the range of *Z. h. preblei*, should this become a management objective.

Although there may be genetically-based differences that are currently unknown, the majority of the evidence suggests that neutral genetic divergence among these putative subspecies is low and adaptive genetic divergence is non-existent. Therefore, based on the evidence examined here, *Z. h. preblei* does not appear to qualify as a distinct population using the approach of Crandall *et al.* (2000).

Currently, the US-ESA requires that a Distinct Vertebrate Population Segment (DPS) be 'discrete' and 'of significance' (US Fish and Wildlife Service, US National Oceanic and Atmospheric Administration, 1996). Discrete is defined as 'markedly separated from other populations of the same taxon by physical, physiological, ecological, or behavioral factors' using evidence from 'quantitative measures of genetic or morphological discontinuity' (US Fish and Wildlife Service, US National Oceanic and Atmospheric Administration, 1996). Significance is defined as 'evidence that loss of the discrete population segment would result in a significant gap in the range of a taxon.' While both of these criteria are vague, our results for *Z. h. preblei* and its neighbouring populations do not appear to support the discrete requirement and the broad distribution of *Z. hudsonius* does not appear to support the significance requirement.

Evaluating the genetic basis of taxa and populations proposed for listing or delisting under the US-ESA

Two types of error are inherent in the process of listing taxa or populations as endangered or threatened and both can have negative effects on conservation (National Research Council, 1995). The first, as illustrated by *Z. h. preblei*, occurs where an invalid taxon or non-distinct population is listed. This affects other species because limited conservation resources are then misallocated. It can also have negative socioeconomic consequences, including the restriction of some benign human activities and can undermine public support for the US-ESA. The other type of error occurs when a valid taxon is not listed because its unique properties were not identified and it goes extinct – an irreversible loss of biodiversity. Like

Type I and II statistical errors, criteria set relative to one of the ESA listing errors will influence the rate of the other type of error. Well-defined criteria and regulations are needed for US-ESA listing procedures that minimise both errors to the maximum extent possible.

Criteria for genetic uniqueness need to adequately identify natural discontinuities in gene pool variation and distinguish these from recent (e.g. last 100 years) differences that may be due to genetic drift from human-induced population bottlenecks or isolation (Hedrick *et al.*, 2001; Brown *et al.*, 2004). These criteria should not be so stringent that unique organisms fail to be listed.

Recognising the problem of using only genetic data, Crandall *et al.* (2000) proposed that populations be recognised as distinct if they show evidence of recent genetic isolation (not genetically exchangeable) and adaptive differences (not ecologically exchangeable), or both historic and recent adaptation (not ecologically exchangeable). However, these authors did not fully address the question of how much genetic difference is sufficient for each of these distinctions.

In our study, we used a three-step approach to test the validity of subspecies and the validity of distinct populations. This process could be reduced to two steps if candidates for listing met a minimum standard of genetic uniqueness within the conceptual framework of Crandall *et al.* (2000). First, test the original taxonomic or DPS description. This is especially important below the level of species, because original descriptions often relied on poorly-quantified traits that have an unknown genetic basis (Hendry *et al.*, 2000; Wehausen & Ramey, 2000; Zink, 2004). Second, apply critical tests (like the ones used in this study) to the hypotheses of genetic and ecological exchangeability as proposed by Crandall *et al.* (2000). Establishing a conceptually sound and consistent methodological approach for listings is imperative because there are currently no uniform criteria among taxonomic groups (or investigators) as to what constitutes a species, subspecies, or DPS (Avise & Johns, 1999; Crandall *et al.*, 2000). This approach applies equally to taxa being considered for listing or delisting under the US-ESA and could also be applied to biodiversity laws in other countries. Because 561 out of the 1855 species listed under the US-ESA occur outside the USA, the basis of US-ESA listings is also an international scientific issue.

Acknowledgements

We thank the KU, MSB, NSM and DMNS for access to zoological and genomic research collections. Pioneer Environmental Services and the City of Fort Collins provided two ear punch specimens. T. Quinn and S. Oyler-McCance provided access to the DNA sequencing facility at Rocky Mountain Center for Conservation Genetics and Systematics. We thank four anonymous reviewers and P. Krutzsch for useful comments and. L. M. Brown for keen editing. Funding was provided by the State of Wyoming, U.S. Fish & Wildlife Service, Department of Energy, Department of Defense, U.S. Forest Service and the DMNS.

REFERENCES

Avise, J. C. & R. M. Ball Jr. (1990). Principles of genealogical concordance in species concepts and biological taxonomy. *Oxf Surv Evol Biol* 7: 45–67.

Avise, J. C. & Johns, G. C. (1999). Proposal for a standardized temporal scheme of biological classification for extant species. *Proc. Natl. Acad. Sci. USA* **96**: 7358–7363.

Ball, M. R. & Avise, J. C. (1992). Mitochondrial DNA phylogeographic differentiation among avian populations and the evolutionary significance of subspecies. *Auk* **109**: 626–636.

Balloux, F., Brunner, H., Lugon-Moulin, N., Hausser, J. & Goudet, J. (2000). Microsatellites can be misleading: an empirical and simulation study. *Evolution* **54**: 1414–1422.

Boonstra, R. & Hoyle, J. A. (1986). Rarity and coexistence of a small hibernator, *Zapus hudsonius*, with fluctuating populations of *Microtus pennsylvanicus* in the grasslands of southern Ontario. *J. Anim. Ecol.* 55: 773–784.

Brown, L. M., Ramey, R. R., Tamburini, B. & Gavin, T. A. (2004). Population structure and mitochondrial DNA variation in sedentary Neotropical birds isolated by forest fragmentation. *Conserv. Genet.* 5: 743–757.

Burnham, K. P. & Anderson, D. R. (1998). *Model selection and inference.* New York: Springer-Verlag.

Clark, T. W. & Stromberg, M. R. (1987). *Mammals in Wyoming.* Lawrence, KA: University of Kansas Museum.

Conner, M. M. & Shenk, T. M. (2003). Distinguishing *Zapus hudsonius preblei* from *Zapus princeps princeps* by using repeated cranial measurements. *J. Mamm.* **84**: 1456–1463.

Corander, J., Waldmann, P. & Sillanpaa, M. J. (2003). Bayesian analysis of genetic differentiation between populations. *Genetics* **163**: 367–374.

Crandall, K. A., Bininda-Emonds, O. R. P., Mace, G. M. & Wayne, R. K. (2000). Considering evolutionary processes in conservation biology. *Trends Ecol. Evol.* **15**: 290–295.

Cronin, M. A. (1997). Systematics, taxonomy and the Endangered Species Act: the example of the California gnatcatcher (*Polioptila californica*). *Wildl. Soc. Bull.* **25**: 661–666.

Cryan, P. M. (2004). *Synthesis of existing information on meadow jumping mice (**Zapus hudsonius**) in the northern Great Plains.* An information review compiled for the U.S. Fish and Wildlife Service, Region 6, Denver, Colorado. USGS Fort Collins Science Center, 2150 Centre Ave., Building C, Fort Collins, CO 80526.27 pp.

Excoffier, L., Smouse, P. E. & Quattro, J. M. (1992). Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial restriction data. *Genetics* **131**: 479–491.

Felsenstein, J. (1985). Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* **39**: 783–791.

Fraser, D. J. & Bernatchez, L. (2001). Adaptive evolutionary conservation: towards a unified concept for defining conservation units. *Mol. Ecol.* **10**: 2741–2752.

Goldstein, P. Z., DeSalle, R., Amato, G. & Vogler, A. P. (2000). Conservation genetics at the species boundary. *Conserv. Biol.* **14**: 120–131.

Gordon, R. E., Lacy, J. K. & Streeter, J. R. (1997). Conservation under the Endangered Species Act. *Environ. Int.* 23: 359–419.

Goudet, J. (1995). FSTAT (Version 1.2): a computer program to calculate F-statistics. *J. Heredity* **86**: 485–486.

Hafner, D. J., Petersen, K. E. & Yates, T. L. (1981). Evolutionary relationships of jumping mice (Genus *Zapus*) of the southwestern United States. *J. Mamm.* **62**: 501–512.

Hartl, D. L. & Clark, A. G. (1989). *Principles of population genetics.* Sunderland, MA: Sinauer Assoc.

Hedrick, P. W., Gutierrez-Espeleta, G. A. & Lee, R. N. (2001). Founder effect in an island population of bighorn sheep. *Mol. Ecol.* **10**: 851–857.

Hendry, A. P., Varnosi, S. M., Latham, S. J., Heibuth, J. C. & Day, T. (2000). Questioning species realities. *Conserv. Gen.* **1**: 67–76.

Hoekstra, H. E. & Nachman, M. W. (2003). Different genes underlie adaptive melanism in different populations of rock mice. *Mol. Ecol.* **12**: 1185–1194.

Huson, D. H. (1998). SplitsTree: analyzing and visualizing evolutionary data. *Bioinformatics* **14**: 68–73.

Jones, G.S. (1981). *The systematics and biology of the genus Zapus (Mammalia, Rodentia, Zapodidae)*. Unpublished Ph.D. thesis: Indiana State University, Terre Haute.

Keita, S. O. Y., Kittles, R. A., Royal, C. D., Bonney, G. E., Furbert-Harris, P., Dunston, G. M. & Rotimi, C. N. (2004). Conceptualizing human variation. *Nature Genet. Suppl.* **36**:17–20.

Krutzsch, P. H. (1954). North American jumping mice (genus *Zapus*). *Univ. Kansas Publ., Mus. Nat. Hist.* **4**: 349–472.

Kumar, S., Koichiro, K., Jakobsen, I. & Nei, M. (2002). *MEGA: molecular evolutionary genetics analysis, Version 2.0*. Pennsylvania State University, University Park and Arizona State University, Tempe.

Leberg, P. L. (2002). Estimating allelic richness: effects of sample size and bottlenecks. *Mol. Ecol.* **11**: 2445–2449.

Matson, C. W. & Baker, R. J. (2001). DNA sequence variation in the mitochondrial control region of red-backed voles (*Clethrionomys*). *Mol. Biol. Evol.* **18**: 1494–1501.

National Research Council. (1995). *Science and the Endangered Species Act*. Washington, DC: National Academy Press.

Nielsen, R. & Wakeley, J. (2001). Distinguishing migration from isolation: a Markov chain Monte Carlo approach. *Genetics* **158**: 885–896.

Oyler-McCance, S. J., St. John, J., Parker, J. M. & Anderson, S. H. (2005). Characterization of microsatellite loci isolated in midget-faded rattlesnakes (*Crotalus viridis concolor*). *Mol. Ecol. Notes* **5**: 455–458.

Paetkau, D., Waits, L. P., Clarkson, P. L., Craighead, L. & Strobeck, C. (1997). An empirical evaluation of genetic distance statistics using microsatellite data from bear (Ursidae) populations. *Genetics* **147**: 1943–1957.

Palneirim, J. M. (1998). Analysis of skull measurements and measurers: can we use data obtained by various observers? *J. Mamm.* **79**: 1021–1028.

Pearse, D. E. & Crandall, K. A. (2004). Beyond Fst: analysis of population genetic data for conservation. *Conserv. Genet.* **5**: 585–602.

Posada, D. & Crandall, K. A. (1998). Modeltest: testing the model of DNA substitution. *Bioinformatics* **14**: 817–818.

Preble, E. A. (1899). Revision of the jumping mice of the genus *Zapus*. *N. Am. Fauna* **15**: 1–41.

Pritchard, J. K., Stephens, M. & Donnelly, P. (2000). Inference of population structure using multilocus genotype data. *Genetics* **155**: 945–959.

Quimby, D. C. (1951). The life history and ecology of the jumping mouse, *Zapus hudsonius*. *Ecol. Monogr.* **21**: 61–95.

Raymond, M. & Rousset, F. (1995). An exact test for population differentiation. *Evolution* **49**: 1280–1283.

Reyment, R. (1990). Reification of classical multivariate statistical analysis in morphometry. In *Proceedings of the Michigan morphometrics workshop*: 123–144.

Reyment, R. A., Blackith, R. E. & Campbell, N. A. (1984). *Multivariate morphometrics*. Orlando, FL: Academic Press.

Saitou, N. & Nei, M. (1987). The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol. Biol. Evol.* **4**: 406–425.

Sokal, R. R. & Rohlf, F. J. (1981). *Biometry*. San Francisco: W. H. Freeman and Company.

Swofford, D. L. (2002). *PAUP*: Phylogenetic Analysis Using Parsimony (and other methods)*. Version 4.0b10. Sunderland, MA: Sinauer Associates.

Tavare, S. (1986). Some probabilistic and statistical problems in the analysis of DNA sequences. *Lect. Math. Life Sci.* **17**: 57–86.

US Fish and Wildlife Service, US National Oceanic and Atmospheric Administration (1996). Policy regarding the recognition of distinct population segments under the Endangered Species Act. *The Federal Register* **61**: 4722–4725.

U.S. Fish & Wildlife Service (1998). Final rule to list the Preble's meadow jumping mouse as a threatened species. *The Federal Register* **53**: 26517–26530.

Wehausen, J. D. & Ramey, R. R. (2000). Cranial morphometric and evolutionary relationships in the northern range of *Ovis canadensis*. *J. Mamm.* **81**: 145–161.

Wehausen, J. D., Ramey, R. R. & Epps, C. W. (2004). Experiments in DNA extraction and PCR amplification from bighorn sheep feces: the importance of DNA extraction method. *J. Hered.* **95**: 503–509.

Weir, B. S. & Cockerham, C. C. (1984). Estimating F-statistics for the analysis of population structure. *Evolution* **38**: 1358–1370.

Whitaker, J. O. (1972). *Zapus hudsonius*. *Mamm. Spec.* **11**: 1–7.

Whitaker, J. O. (1999). Meadow jumping mouse (*Zapus hudsonius*). In *The Smithsonian book of North American Mammals*: 666–667. Wilson, D. E. & Ruff, S. (eds). Washington, DC: Smithsonian Institution Press.

Wilson, E. O. & Brown, W. L. (1953). The subspecies concept and its taxonomic applications. *Syst. Zool.* **2**: 97–122.

Wilson, A. B., Naish, K.-A. & Boulding, E. G. (1999). Multiple dispersal strategies of the invasive quagga mussel (*Dreissena bugensis*) as revealed by microsatellite analysis. *Can. J. Fish. Aquat. Sci.* **56**: 2248–2261.

Worley, K., Strobeck, C., Arthur, S., Carey, J., Schwantje, H., Veitch, A. & Coltman, D. W. (2004). Population genetic structure of North American thinhorn sheep (*Ovis dalli*). *Mol. Ecol.* **13**: 2545–2556.

Wright, S. (1921). Systems of mating. *Genetics* **6**: 111–178.

Yu, H., Liao, Y. & Kao, C. (2001). Relatedness structure and individual identification in a semi-fossorial shrew (Soricidae: *Anourosorex squamipes*) – an application of microsatellite DNA. *Zool. Stud.* **40**: 226–232.

Zink, R. M. (2004). The role of subspecies in obscuring avian biological diversity and misleading conservation policy. *Proc. Roy. Soc. Lond.* **271**: 561–564.

APPENDIX 1. Catalog numbers of specimens used for cranial measurements.

Specimens are listed in the order they were examined. DMNS, Denver Museum of Nature & Science; KU, University of Kansas Museum of Natural History.

DMNS: *Z. h. preblei* 9572, 9864, 10380, 9843, 9853, 9570, 9569, 9562, 9561, 9315, 9205, 9204, 9868, 9862, 10355, 10404, 10269, 10354, 10169, 10265, 10267, 2822, 10604, 9876, 10618, 10630, 10621, 9564, 9312, 10635, 9877, 10620, 10611, 9571, 10266, 10610, 9579, 10613 and 10615.

DMNS: *Z. h. campestris* 8512.

KU: *Z. h.campestris* 101551, 101552, 101554, 101555, 101558, 101560, 87040, 87041, 87042, 87034, 87035, 87036, 87037, 112664, 112657, 20835, 20836, 20837, 20838, 20839, 20840, 20842, 20843, 20844, 20845, 20846, 20847, 20848, 20849, 20851, 20850, 20852, 41450, 41451, 42467, 42468, 42469, 42471, 42517 and 42518.

KU: *Z. h. intermedius* 153184, 153186, 153187, 153188, 153189, 159186, 141254, 141255, 159188, 123023, 123026, 123028, 123029, 123031, 123032, 123033, 108589, 123035, 116266, 116267, 116262, 116263, 116264, 116268, 108068, 116265, 104062, 37275, 154080, 47773, 47774, 47775, 47776, 47777, 47779, 47781 and 47784.

APPENDIX 2. Specimens of *Zapus hudsonius* used in phylogenetic and population genetic analyses.

These are listed by museum or tissue archive catalog number.

DMNH, Denver Museum of Nature & Science; TK, Texas Tech; KU, University of Kansas; UNSM, University of Nebraska State Museum; MSB and NK, Museum of Southwestern Biology; PIONEER, Pioneer Environmental Services.

Abbreviations for states are as follows: AZ, Arizona; CO, Colorado; IL, Illinois; IN, Indiana; IA, Iowa; KS, Kansas; MO, Missouri; MT, Montana; NM, New Mexico; NE, Nebraska; SD, South Dakota; WY, Wyoming. State abbreviations are followed by counties. The location of haplotypes in the table approximately corresponds to the location of the haplotypes in the neighbour-joining tree. Representative individuals used in phylogenetic analysis are indicated with an asterisk

Additional specimens with identical mtDNA haplotype: ID, state and county	Subspecies	Haplotype	Additional specimens with identical mtDNA haplotype: ID, state and county	Subspecies	Haplotype
MSB40951, AZ:Apache*	*Z. h. luteus*	L6	NK871, NM:Otero	*Z. h. luteus*	
MSB40994, AZ:Apache	*Z. h. luteus*		NK884, NM: Socorro	*Z. h. luteus*	
MSB89194, AZ:Navajo*	*Z. h. luteus*	L5	DMNH8630: CO:Las Animas	*Z. h. luteus*	
MSB86344, AZ:Apache*	*Z. h. luteus*	L/PAL/C2	DMNH8631, CO:Las Animas*	*Z. h. luteus*	L3
MSB91627, AZ:Navajo	*Z. h. luteus*		DMNH8632, CO:Las Animas*	*Z. h. luteus*	L2
MSB91675, AZ:Apache	*Z. h. luteus*		DMNH8634, CO:Las Animas	*Z. h. luteus*	
NK1584, AZ:Apache	*Z. h. luteus*		NK9976, NM:Bernalillo*	*Z. h. luteus*	L1
DMNH8635, CO:Las Animas	*Z. h. luteus*		MSB62103, NM:Valencia	*Z. h. luteus*	
DMNH8633, CO:Las Animas	*Z. h. luteus*		MSB58370, NM:Rio Arriba*	*Z. h. luteus*	L/PAL/C1
KU41451, WY:Crook	*Z. h. campestris*		MSB56980, NM:Sandoval	*Z. h. luteus*	
KU153706, KS:Leavenworth	*Z. h. pallidus*		MSB56986, NM:Sandoval	*Z. h. luteus*	
KU112661, SD: Lawrence	*Z. h. campestris*		MSB56987, NM:Sandoval	*Z. h. luteus*	
UNSM20596, NE:Buffalo*	*Z. h. pallidus*	PAL10	MSB56991, NM:Sandoval	*Z. h. luteus*	
UNSM26492, NE:Buffalo*	*Z. h. pallidus*	PAL9	MSB56993, NM:Sandoval	*Z. h. luteus*	
UNSM20879, NE:Buffalo	*Z. h. pallidus*		MSB62096, NM:Sandoval	*Z. h. luteus*	
UNSM13217, NE:Cherry*	*Z. h. pallidus*	PAL8	NK856, NM:Sandavol	*Z. h. luteus*	
UNSM12980, NE:Garden	*Z. h. pallidus*		KU112665, SD:Lawrence	*Z. h. campestris*	
UNSM12991, NE:Garden	*Z. h. pallidus*		KU109963, SD:Lawrence	*Z. h. campestris*	
UNSM26316, NE:Hall	*Z. h. pallidus*		KU110033, SD:Bennett	*Z. h. pallidus*	
UNSM20744, NE:Hall	*Z. h. pallidus*		KU110022, SD:Bennett*	*Z. h. pallidus*	PAL2
UNSM20747, NE:Hall	*Z. h. pallidus*		UNSM27388, SD:Clay*	*Z. h. pallidus*	PAL1/
UNSM26462, NE:Merrick	*Z. h. pallidus*		UNSM27389, SD:Clay	*Z. h. pallidus*	INT-XV
UNSM13067, NE:Thomas	*Z. h. pallidus*		KU116266, IO:Buena Vista	*Z. h. intermedius*	
KU116269, IO:Tama*	*Z. h. intermedius*	INT-XVI	KU140721, SD:Brown	*Z. h. intermedius*	
UNSM17482, NE:Antelope*	*Z. h. pallidus*	PAL7	KU153190, SD:Walworth	*Z. h. intermedius*	
UNSM17495, NE:Antelope	*Z. h. pallidus*		KU153209, SD:Minnehaha	*Z. h. intermedius*	
UNSM17498, NE:Antelope	*Z. h. pallidus*		KU153212, SD:Minnehaha	*Z. h. intermedius*	
UNSM17499, NE:Antelope	*Z. h. pallidus*		KU153221, SD:Moody	*Z. h. intermedius*	
UNSM13084, NE:Dixon	*Z. h. pallidus*		KU147020, SD:Brown*	*Z. h. intermedius*	INT-XIV
UNSM14008, NE:Dodge	*Z. h. pallidus*		KU153176, SD:Brown	*Z. h. intermedius*	
UNSM13118, NE:Holt	*Z. h. pallidus*		KU153177, SD:Brown	*Z. h. intermedius*	
UNSM13343, NE:Lancaster	*Z. h. pallidus*		KU153180, SD:Brown	*Z. h. intermedius*	
UNSM13119, NE:Holt*	*Z. h. pallidus*	PAL6	KU153181, SD:Brown	*Z. h. intermedius*	
UNSM13065, NE:Thomas	*Z. h. pallidus*		KU101564, SD:Pennington*	*Z. h. campestris*	C8/10/
UNSM17727, NE:Boyd*	*Z. h. pallidus*	PAL5	DMNH10638/TK86190, WY:Weston	*Z. h. campestris*	INT-VI
UNSM20600, NE:Buffalo*	*Z. h. pallidus*	PAL4	DMNH10639/TK86191, WY:Weston	*Z. h. campestris*	
KU109633, KS:Osage	*Z. h. pallidus*		KU101558, SD:Pennington	*Z. h. campestris*	
KU109634, KS:Osage	*Z. h. pallidus*		KU123593, MT:Carter	*Z. h. campestris*	
KU153597, MO:Macon*	*Z. h. pallidus*	PAL3	KU123598, MT:Carter	*Z. h. campestris*	
KU153598, MO:Macon	*Z. h. pallidus*		KU123599, MT:Carter	*Z. h. campestris*	
KU153784, KS:Douglas	*Z. h. pallidus*		KU115700, ND:Burleigh	*Z. h. intermedius*	
KU153707, KS:Leavenworth	*Z. h. pallidus*		KU115702, ND:Burleigh	*Z. h. intermedius*	
MSB37154, NM:Otero*	*Z. h. luteus*	L4	KU115710, ND:Burleigh	*Z. h. intermedius*	
MSB61696, NM:Otero	*Z. h. luteus*		KU115731, SD:Walworth	*Z. h. intermedius*	
MSB61684, NM:Otero	*Z. h. luteus*		KU115732, SD:Walworth	*Z. h. intermedius*	
MSB61690, NM:Otero	*Z. h. luteus*		KU120018, ND:Burleigh	*Z. h. intermedius*	
MSB61693, NM:Otero	*Z. h. luteus*		KU120019, ND:Burleigh	*Z. h. intermedius*	
MSB61712, NM:Otero	*Z. h. luteus*		KU123021, ND:Dunn	*Z. h. intermedius*	
MSB58369, NM:Rio Arriba	*Z. h. luteus*		KU123022, ND:Dunn	*Z. h. intermedius*	

APPENDIX 2. Continued

Additional specimens with identical mtDNA haplotype: ID, state and county	Subspecies	Haplotype	Additional specimens with identical mtDNA haplotype: ID, state and county	Subspecies	Haplotype
KU123031, ND:Dunn	*Z. h. intermedius*		DMNH9868/TK86032, CO:Douglas	*Z. h. preblei*	
KU123032, ND:Dunn	*Z. h. intermedius*		DMNH9843/TK86034, CO:Boulder	*Z. h. preblei*	
KU159190, SD:Walworth	*Z. h. intermedius*		DMNH10169/TK86048, CO:Boulder	*Z. h. preblei*	
DMNS7764, ND: Mercer	*Z. h. intermedius*		DMNH10266/TK86080,CO:Douglas	*Z. h. preblei*	
KU123033, ND:Dunn*	*Z. h. intermedius*	INT-I	DMNH10269/TK86083,CO:Douglas	*Z. h. preblei*	
KU112663, SD:Lawrence*	*Z. h. campestris*	C9/INT-VII	DMNH10354/TK86090, CO:Boulder	*Z. h. preblei*	
KU115730, SD:Walworth	*Z. h. intermedius*		DMNH10408/TK86098, WY:Albany	*Z. h. preblei*	
KU20839, WY:Crook*	*Z. h. campestris*	C7	DMNH9564/TK86105, CO:Boulder	*Z. h. preblei*	
KU83559, SD:Harding*	*Z. h. campestris*	C6	DMNH9561/TK86109, CO:Larimer	*Z. h. preblei*	
KU20844, WY:Crook*	*Z. h. campestris*	C5/INT-XIII	DMNH9576/TK86115, CO:Douglas	*Z. h. preblei*	
KU42471, WY:Weston	*Z. h. campestris*		DMNH9574/TK86116, CO:Douglas	*Z. h. preblei*	
KU87040, SD:Harding	*Z. h. campestris*		DMNH10520/TK86124,CO:Jefferson	*Z. h. preblei*	
KU83557, SD:Harding	*Z. h. campestris*		DMNH10602/TK86163, CO:Elbert	*Z. h. preblei*	
KU87042, SD:Harding	*Z. h. campestris*		KU110013, SD:Custer	*Z. h. campestris*	
KU112660, SD:Lawrence	*Z. h. campestris*		KU123597, MT:Carter	*Z. h. campestris*	
KU115895, SD:Harding	*Z. h. intermedius*		DMNH9579/XM1166, CO:El Paso*	*Z. h. preblei*	C/P2
KU115896, SD:Harding	*Z. h. intermedius*		DMNH9313/XM875, CO:El Paso	*Z. h. preblei*	
KU115897, SD:Harding	*Z. h. intermedius*		DMNH9315/XM879, CO:El Paso	*Z. h. preblei*	
KU20843, WY:Crook*	*Z. h. campestris*	C4	DMNH10380/TK86093, CO:El Paso	*Z. h. preblei*	
KU109970, SD:Lawrence*	*Z. h. campestris*	C3	DMNH9565/TK86106, CO:El Paso	*Z. h. preblei*	
KU120017, ND:Burleigh*	*Z. h. intermedius*	INT-IX	DMNH9563/TK86107, CO:El Paso	*Z. h. preblei*	
KU42469, WY:Weston*	*Z. h. campestris*	C2	DMNH9566/TK86118, CO:El Paso	*Z. h. preblei*	
KU101552, SD:Pennington*	*Z. h. campestris*	C1	DMNH9573/TK86120, CO:Douglas	*Z. h. preblei*	
KU116263, IO:Emmet*	*Z. h. intermedius*	INT-XII	DMNH9572/TK86121, CO:Douglas	*Z. h. preblei*	
KU116265, IO:Plymouth	*Z. h. intermedius*		DMNH9571/TK86122, CO:Douglas	*Z. h. preblei*	
KU147018, SD:Deuel	*Z. h. intermedius*		DMNH9574/TK86166, CO:El Paso	*Z. h. preblei*	
KU153196, SD:Deuel	*Z. h. intermedius*		DMNH10607/TK86167, CO:El Paso	*Z. h. preblei*	
KU153203, SD:Lincon	*Z. h. intermedius*		KU109978, SD:Custer	*Z. h. campestris*	
KU153201, SD:Deuel*	*Z. h. intermedius*	INT-V	KU123592, MT:Carter	*Z. h. campestris*	
DMNH10614/TK86183,CO:El Paso*	*Z. h. preblei*	C/P4	DMNH10405/TK86095,WY:Albany*	*Z. h. preblei*	C/P1
DMNH10331/TK86088,CO:Teller	*Z. h. preblei*		DMNH10258/TK86074,WY:Laramie	*Z. h. preblei*	
DMNH10606/TK86165, CO:El Paso	*Z. h. preblei*		DMNH10270/TK86081, CO:Larimer	*Z. h. preblei*	
DMNH10604/TK86169, CO:El Paso	*Z. h. preblei*		DMNH10404/TK86094, WY:Platte	*Z. h. preblei*	
DMNH10612/TK86170, CO:El Paso	*Z. h. preblei*		DMNH10406/TK86096, WY:Albany	*Z. h. preblei*	
DMNH10605/TK86173, CO:El Paso	*Z. h. preblei*		DMNH10407/TK86097, WY:Albany	*Z. h. preblei*	
DMNH10618/TK86182, CO:El Paso	*Z. h. preblei*		DMNH9568/TK86117, CO:Larimer	*Z. h. preblei*	
DMNH10611/TK86185, CO:El Paso	*Z. h. preblei*		PIONEER9A43, CO: Larimer	*Z. h. preblei*	
DMNH10635/TK86196,CO:Douglas	*Z. h. preblei*		PIONEER9B89, CO:Larimer	*Z. h. preblei*	
KU109972, SD:Custer	*Z. h. campestris*		KU109984, SD:Custer	*Z. h. campestris*	
DMNH9204/XM871, CO:Boulder*	*Z. h. preblei*	C/P3	KU109985, SD:Custer	*Z. h. campestris*	
DMNH9205/XM872, CO:Boulder	*Z. h. preblei*		KU104062, IO:Winneshiek*	*Z. h. intermedius*	INT-VIII
DMNH9312/XM874, CO:Gilpin	*Z. h. preblei*		KU116264, IO:Emmet	*Z. h. intermedius*	
DMNH9046/XM876, CO:Boulder	*Z. h. preblei*		KU153229, SD:Union	*Z. h. intermedius*	
DMNH9314/XM877, CO:Boulder	*Z. h. preblei*		KU153203, SD:Lincon	*Z. h. intermedius*	
DMNH9203/TK51406, CO:Jefferson	*Z. h. preblei*		KU140722, SD:Brown*	*Z. h. intermedius*	INT-X
DMNH9880/TK86021, CO:Boulder	*Z. h. preblei*		KU153215, SD:Minnehaha*	*Z. h. intermedius*	INT-XI
DMNH9854/TK86026, CO:Douglas	*Z. h. preblei*		KU153205, SD:Lincon	*Z. h. intermedius*	
DMNH9876/TK86029, CO:Douglas	*Z. h. preblei*		KU127252, IL:Henry*	*Z. h. intermedius*	INT-IV
DMNH9857/TK86030, CO:Douglas	*Z. h. preblei*		KU112830, IN:Wayne*	*Z. h. intermedius*	INT-III
DMNH9865/TK86031, CO:Douglas	*Z. h. preblei*		KU108068, IA:Marion*	*Z. h. intermedius*	INT-II

APPENDIX 3. Specimens of *Zapus princeps* used as outgroups in the phylogenetic analysis and specimens that have an identical mtDNA haplotype or are on the same lineage as the mtDNA haplotypes of representative individuals.

Only the mtDNA haplotypes of the three represenative *Z. princeps* individuals were used in the phylogenetic analysis. Note that some individuals previously identified as *Z. hudsonious* have mtDNA haplotypes that are identical to *Z. princeps*. These individuals were presumed to be misidentified and were excluded from any analyses. Abbreviations are the same as those given in Appendix 2. Representative individuals of *Z. princeps* used in the phylogenetic analysis are indicated with an asterisk

Additional specimens with identical mtDNA haplotype or mtDNA on the same lineage with strong bootstrap support: ID, state and county	Subspecies as per museum tag
DMNH9316, WY:Laramie	*Z. p. princeps*
DMNH10327/TK86085, CO:Teller*	*Z. p. princeps*
DMNH10328/TK86086, CO:Douglas	*Z. p. princeps*
DMNH10330/TK86089, CO:Douglas	*Z. p. princeps*
DMNH10873/TK103545, CO:Conejos	*Z. p. princeps*
DMNH10875/TK103589, CO:Las Animas	*Z. p. princeps*
DMNH10874/TK103593, CO:Las Animas	*Z. p. princeps*
DMNH10257/TK86070, WY:Albany	*Z. h. preblei*
DMNH9567/TK86123, WY:Albany	*Z. h. preblei*
DMNH9569/TK86113, WY:Albany	*Z. h. preblei*
DMNH10698/TK86202, WY:Albany	*Z. h. preblei*
DMNH10274/TK86075, WY:Teton*	*Z. p. utahensis*
DMNH10559/TK86135, WY:Teton	*Z. p. utahensis*
DMNH10535/TK86155, WY:Teton	*Z. p. utahensis*
DMNH10542/TK86175, WY:Teton	*Z. p. utahensis*
DMNH9921/TK86039, WY:Park	*Z. p. idahoensis*
DMNH9923/TK86040, WY:Park	*Z. p. idahoensis*
DMNH9925/TK86041, WY:Park	*Z. p. idahoensis*
KU109994, SD:Custer	*Z. h. campestris*
KU123595, MT:Carter	*Z. h. campestris*
KU30814, KS:Douglas	*Z. h. pallidus*
DMNH9595/TK86112, WY:Fremont*	*Z. p. idahoensis*
DMNH9837/TK86028, WY:Fremont	*Z. p. idahoensis*
DMNH9839/TK86037, WY:Fremont	*Z. p. idahoensis*

Exhibit C

Considering evolutionary processes in conservation biology

Keith A. Crandall, Olaf R.P. Bininda-Emonds, Georgina M. Mace and Robert K. Wayne

Conservation biologists assign population distinctiveness by classifying populations as evolutionarily significant units (ESUs). Historically, this classification has included ecological and genetic data. However, recent ESU concepts, coupled with increasing availability of data on neutral genetic variation, have led to criteria based exclusively on molecular phylogenies. We argue that the earlier definitions of ESUs, which incorporated ecological data and genetic variation of adaptive significance, are more relevant for conservation. Furthermore, this dichotomous summary (ESU or not) of a continuum of population differentiation is not adequate for determining appropriate management actions. We argue for a broader categorization of population distinctiveness based on concepts of ecological and genetic exchangeability (*sensu* Templeton).

Keith Crandall is at the Dept of Zoology and M.L. Bean Museum, Brigham Young University, Provo, UT 84602-5255, USA (keith_crandall@byu.edu); Olaf Bininda-Emonds is at the Section of Evolution and Ecology, University of California at Davis, Davis, CA 95616, USA (orbininda@ucdavis.edu); Georgina Mace is at the Institute of Zoology, Zoological Society of London, Regent's Park, London, UK NW1 4RY (georgina.mace@ioz.ac.uk); and Robert Wayne is at the Dept of Organismic Biology, Ecology and Evolution, University of California, 621 Charles E. Young Drive, Los Angeles, CA 90095-1606, USA (rwayne@ucla.edu).

The evolutionarily significant unit (ESU) is a population unit that merits separate management and has a high priority for conservation[1]. The ESU has been associated with distinct population segments that receive protection under the US Endangered Species Act and is also used in a variety of less formal contexts around the world. However, the general concept of ESUs has evolved over time (Box 1) and the implementation of the concept has been inconsistent across different cases. ESUs were first described as population units that 'actually represent significant adaptive variation' based on 'concordance between sets of data derived by different techniques'[1]. Later, Waples[2] described them as 'reproductively separate from other populations and have unique or different adaptations'. Thus, the concept has two components: reproductive and historical isolation, and adaptive distinctiveness. These components are not necessarily correlated. Adaptive differences can arise under gene flow and might depend upon it for continued variability, but gene flow is prevented under reproductive isolation. Therefore, within the ESU concept an implicit tension exists between maintaining isolation between independently evolving units and maintaining adaptive variability within such units.

Operationally, efforts to document ESUs have emphasized reproductive isolation rather than the maintenance of adaptive differences. This focus is apparent in the progression of definitions since the concept was first developed in 1986 (Box 1). Moritz's[3] more recent rationale for a focus on the evolutionary past is that long isolated populations have distinct potential, presumably to develop into populations, population groups or ones that are uniquely adapted to existing environmental conditions. Therefore, recognizing ESUs as reciprocally monophyletic groups ensures that populations will be managed separately and that the evolutionary heritage within species will be maintained. We agree that the maintenance of historical population structure, as defined by molecular genetic techniques, should be one goal of species management. However, there are several conceptual and practical problems with this approach.

First, although populations with a history of reproductive isolation should perhaps be managed separately, this goal is overemphasized. In general, the potential for species evolutionary success is maximized through the maintenance of adaptive diversity[4–9], by preserving the maximum diversity of functionally divergent gene copies across the geographic range of a species. Moritz[3] asserts that maintaining adaptive variants is not only difficult in practice but also negates the evolutionary process, because preserving variants adapted to previous conditions might retard the response to contemporary selective pressures. However, as long as maintenance of evolutionary processes in populations is a goal of management, rather than the maintenance of variants alone, natural selection should eliminate those adapted to previous conditions. Functionally divergent populations might not be those with a long history of isolation; in fact, substantial functional divergence and reproductive isolation can occur despite high levels of gene flow[10].

Second, the widespread use of molecular genetic markers in the context of Moritz's ESU concept (Table 1) has led many investigators to regard these data as essential to conservation management, often to the exclusion of other data. However, ESUs, as defined by Moritz, are unlikely to be found in species characterized by high levels of gene flow (e.g. many species of birds and large mammals). Furthermore, the reciprocal monophyly criterion presents difficulties for populations with paraphyletic histories (Box 2)[11]. Conversely, populations designated as ESUs might not be functionally distinct from one another. With the increasing resolution of molecular techniques, significant differentiation can be found at very small scales, even down to the individual, and this can lead to inappropriate diagnosis of ESUs within functionally equivalent populations.

Finally, the conceptual framework of the ESU designation forces just two categories (ESU or not) from the continuous distribution of genetic diversity, habitat types and selective pressures across populations. In practice, it would be more useful for conservation management if multiple categories were used to represent these continua. Furthermore, both genetic and ecological information should be used, with an emphasis placed on exchangeability instead of genetic distinctiveness. This approach will provide better insights into the conservation units that can best maintain evolutionary processes and the potential for evolutionary change in the future.

Diagnosing distinct populations

To become useful in conserving species, theoretical concepts must be practical in application. Similar to species concepts[12], ESU concepts are often troubled by a lack of applicability to the real world[3,13,14]. The Moritz definition is appealing in this regard because the ESU is defined primarily by reciprocal monophyly, a characteristic that can be deduced using established phylogenetic techniques[15]. However, within species, reciprocal monophyly is a common characteristic only of animal mitochondrial genes, because they are rapidly evolving

 0169-5347/00/$ – see front matter © 2000 Elsevier Science Ltd. All rights reserved. PII: S0169-5347(00)01876-0

Box 1. Definitions of evolutionarily significant units over time

Concepts of population distinctiveness have changed over the past 15 years, with the latest definition emphasizing genetic distinctiveness to the point of reciprocal monophyly. We argue that the previous definitions of evolutionarily significant units (ESUs) appropriately incorporated adaptive differences, as evidenced by genetic and ecological data. Here, we present operational criteria for determining the genetic and ecological distinctiveness of populations, in terms of rejection criteria for genetic and ecological exchangeability. In the past, ESUs have been defined in the following ways:

Ryder 1986: populations that actually represent significant adaptive variation based on concordance between sets of data derived by different techniques[1].

Waples 1991: populations that are reproductively separate from other populations and have unique or different adaptations[2].

Moritz 1994: populations that are reciprocally monophyletic for mtDNA alleles and show significant divergence of allele frequencies at nuclear loci[3].

Rejection criteria for different classes of exchangeability

Ecological exchangeability: the factors that define the fundamental niche and the limits of spread of new genetic variants through genetic drift and natural selection[17].

- Exchangeability is rejected when there is evidence for population differentiation owing to genetic drift or natural selection.
- Representative evidence includes differences in life history traits, morphology, habitat, QTLs and allozymes under selection – such differences would ideally be demonstrably heritable.

Genetic exchangeability: the factors that define the limits of spread of new genetic variants through gene flow[17].

- Exchangeability is rejected when there is evidence of restricted gene flow between populations.
- Representative evidence could be obtained from microsatellites, nucleotide sequences (mtDNA, cpDNA and nDNA) and allozymes.

and are presumed to be nonrecombining (but see Ref. 16). Thus, plants and other life forms will be proportionately less well represented as ESUs under Moritz's concept. Our diagnosis of distinct populations avoids this problem because it emphasizes variation in phenotypes, thus allowing preservation of important adaptive characters and their associated underlying genetic variation. This variation can be shaped by gene flow, genetic drift and natural selection, with the effects of gene flow falling under genetic exchangeability, and the effects of genetic drift and natural selection falling under ecological exchangeability[17,18]. Exchangeability serves as a null hypothesis[19,20]. From both recent and historical perspectives, the rejection of, or failure to reject, exchangeability (Box 1) forms the foundation of population distinctiveness and management practices (Fig. 1).

Ecological exchangeability

The central idea of ecological exchangeability is that individuals can be moved between populations and can occupy the same ecological niche or selective regime. Ecological exchangeability arises from the shared fundamental adaptations of a population[18], such as similar life history traits, ecological requirements, morphologies and demographic characteristics. Ecologically exchangeable individuals perform similar functions in each ecological community. Characters used to demonstrate ecological exchangeability should be demonstrably heritable to avoid confusing environmentally and genetically based differences. The null hypothesis of ecological exchangeability is tested by overlaying the ecological data (either categorical or continuous) on the underlying genealogy of the population, and by using the statistical procedures outlined in Templeton and Sing[21]. Such tests of ecological exchangeability are straightforward to perform and examples are given by Templeton[19,20]. In the absence of genealogical data only current ecological exchangeability can be tested, but in a straightforward fashion, using standard statistical tests for morphological variation (t-tests and principle components analyses, etc.).

Genetic exchangeability

Individuals from different populations are genetically exchangeable if there is ample

Table 1. Example studies surveyed for evolutionarily significant units (ESU) usage[a]

Taxon	Data source	Management conclusion	Crosshair classification[b]	Refs
Puritan tiger beetle (*Cicindela puritana*)	mtDNA (RFLP haplotypes)	Connecticut River and Chesapeake Bay are independent units	+ + + –	40
	Ecology (habitat parameters)	Chesapeake Bay is a single unit	+ – + –	
African elephants (*Loxodonta africana*)	mtDNA (ND5-6 restriction fragment haplotypes)	Single, albeit subdivided population	+ – – –	42
Black rhinoceros (*Diceros bicornis*)	mtDNA (restriction maps)	Maintain current conservation units (equivalent to subspecies), although mixing could be permissible	– – – –	38
Cryan's buckmoth (*Hemiluca* spp.)	Allozymes	ESU based on ecology (despite no reciprocal monophyly)	– + – –	39
	mtDNA (CO-II sequence phylogeny) ecology (larval–host plant performance)			
Coho salmon (*Oncorhynchus kisutch*)	Microsatellites	Two populations form an ESU with a third	+ + – –	43
	Heritable characters (morphology, swimming ability and age at maturation)			

[a]Full details of all references and a summary of the studies can be found at http://www.nceas.ucsb.edu

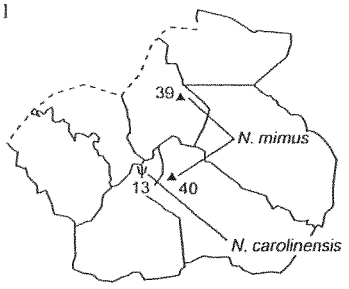
[b]Details of crosshair classification can be found in Fig. 1. Upper versus lower rows correspond to a temporal comparison with upper being recent and lower being historical. Left and right correspond to genetic versus ecological exchangeability, respectively. A plus sign indicates a rejection of that null hypothesis (e.g. populations are not genetically exchangeable) and a minus sign is a failure to reject the null hypothesis.

Box 2. Nonmonophyletic evolutionarily significant units

The reciprocal monophyly criterion of Moritz is problematic because it does not incorporate important information about adaptive differences between populations and because these differences (and populations) might not be partitioned in a monophyletic fashion. Monophyly implies that all the members of that population share a more recent common ancestor with each other than with individuals from outside the population. However, the process of population subdivision and speciation is known to produce polyphyletic relationships that slowly progress over time to paraphyletic and then to monophyletic relationships[44–46]. Thus, the criterion of reciprocal monophyly is overly stringent.

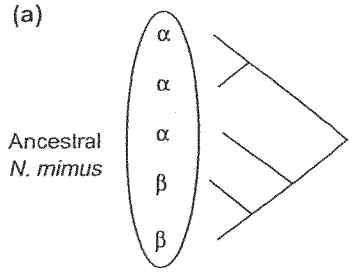
We demonstrate this idea with an example (Fig. I) from cave and surface spiders studied by Hedin[47]. Our example consists of three populations of spider (*Nesticidae: Nesticus*), two sampled from mountain tops (*Nesticus mimus*) and one from a cave (*Nesticus carolinensis*) in North Carolina, USA (populations 13 and 40 are found in the vicinity of Linville Mountain, whereas population 39 is from the vicinity of Grandfather Mountain, North Carolina). During the Pleistocene, these cryophilic spiders apparently lived at lower elevations and were more continuously distributed; however, climatic changes led to their current fragmented distribution in mountain top populations. By contrast, the cave population was able to survive by entering the cave habitat. This population shows evidence for morphological change consistent with a cave-limited existence, including a reduction in pigmentation and eyes, and an elongation of legs.

I

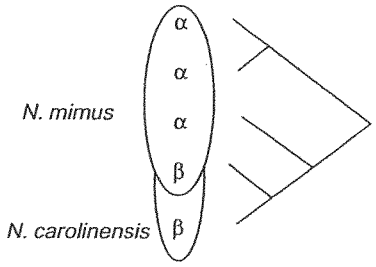


Trends in Ecology & Evolution

II (a)



(b) Microvicariance



Trends in Ecology & Evolution

The evolutionary history of these three populations has been estimated using both mtDNA and nDNA (Fig. II; alpha indicates the geographic region around population 39, whereas beta represents the region around populations 13 and 40), and it has been shown that the mountain top populations are paraphyletic with respect to the cave population. However, as far as we know, the surface forms are ecologically exchangeable, whereas the cave form, restricted to deep cave environments, is not. Thus, the surface forms are genetically nonexchangeable but ecologically exchangeable (Case 7, Fig. 1), which leads to the conclusion that they should be treated as a single population. Compared with the surface forms, the cave form leads to a rejection of both genetical and ecological exchangeability (Case 1, Fig. 1) (here, we are considering the significant mtDNA divergence as evidence for historical separation), which leads to the conclusion that they are separate species (indeed, this is how they have been treated with the cave form called *N. carolinensis* and the surface populations called *N. mimus*). Fig. IIa shows the ancestral geographic distribution (over regions alpha and beta) of ecologically similar spiders, whereas Fig. IIb indicates the ecological and morphological divergence of the cave populations through microvicariance. However, the relationship of the cave form to the two surface lineages is one of paraphyly, not reciprocal monophyly. Thus, populations classified under our categorization as distinct species (owing to reproductive isolation and a suite of adaptive differences) would not even be classified as ESUs under the Moritz criterion of reciprocal monophyly. *Figures reproduced, with permission, from Ref. 47.*

gene flow between populations. Unique alleles, low gene flow estimates ($Nm < 1$, where Nm is the effective number of migrants per generation)[22] or phylogenetic divergence concordant with geographic barriers[23] provide criteria for rejecting genetic exchangeability. Levels of gene flow can be estimated from microsatellites, allozymes, nucleotide sequences (mtDNA, nDNA and cpDNA), AFLPs, RAPDS and so on[24,25]. Various molecular techniques have differential strengths and weaknesses depending upon the historical time frame involved[13,24,25].

Historical versus recent exchangeability

Both ecological and genetic exchangeability can be partitioned along a timescale. We consider three approaches for deducing such temporal divisions. The first is to evaluate historical data directly. For many plant species, historical community structure, geographic distribution and associated ecological variables (e.g. climate) can be reconstructed through pollen analyses[26]. Genetic data have been sampled directly from historical samples[27]. Historical data on distributions of species and habitat types can also be obtained from expedition notes and museum collections[28,29].

The second approach uses different molecular markers to obtain information from different timescales. For example, microsatellite data can identify sibling relationships within a pedigree, whereas mtDNA sequences typically have a more distant range of inference on the order of thousands to millions of years. Thus, the combination of different types of data with different underlying mutation rates allows partitioning of historical from recent phenomena.

The third approach examines phylogenetic relationships. A phylogenetic tree allows temporal partitioning by the relative position on the topology[30]. Both nuclear and mitochondrial data tend to form nonbifurcating relationships at the population genetic level[31]; thus, network approaches are more applicable to population genetic data. Inherent in such an approach is a nested statistical design that corresponds to partitions in evolutionary time. This nested design can be effectively used to partition historical from recent genetic[32,33] and ecological[18] events.

Although these approaches offer only operational criteria for establishing different time frames, the dichotomy of historical versus recent (current) must be made in the context of a conservation assessment of a particular species and a particular set of molecular markers. What is historical for microsatellite data can be recent for mtDNA and what is historical for *Escherichia coli* (after 5000 generations) is recent to a mammal species. Thus, the division must be made relative to the question and data at hand.

Sampling strategy

Central to population-based analyses is an appropriate sampling strategy, in terms of both numbers of individuals and the geographic coverage. Inferences about ecological and genetic exchangeability can change drastically depending on the sampling scheme. For example, limited sampling can lead to the erroneous diagnosis of distinct populations when sampling intermediate populations would show ongoing gene flow. Conversely, limited sampling can have the opposite effect if data are inadequate to reject the null hypothesis of exchangeability. Guidelines can be obtained from coalescent theory; for biologically relevant levels of genetic diversity, samples of between 20 and 50 individuals (for low to high levels of diversity, respectively) will sample 95% or more of the existing haplotypes in a population[34].

Management and restoration of populations

Three general principles should govern management actions. First, management should aim to preserve adaptive diversity and evolutionary processes across the geographic range of a species[9]. A lack of evidence for nonexchangeability should not be used to promote homogenization or other alterations of the gene pool, or to allow contraction or changes in the geographic distribution of populations. Failure to reject the null hypothesis does not imply that the null hypothesis is true, but could simply be a result of the lack of relevant data. To preserve evolutionary processes, the goal of management should be to preserve the natural network of genetic connections between populations, rather than just distinct populations within that network. This ensures that the processes that maintain adaptive diversity and evolutionary potential are conserved. To preserve adaptive diversity, the population network that best samples functional diversity within the species should be given high priority for conservation.

Second, management actions might depend on the severity and nature of recent disturbance. In general, restoration (either of separate or single populations) should be attempted more often for populations that have become disjunct or mixed as a result of recent anthropogenic activities (e.g. habitat degradation, unnatural barriers to dispersal and introductions of nonnative forms). Attempts at restoration should rarely be considered if isolation or mixing result from more natural processes, such as postglaciation environmental change[35].

Finally, when possible, management recommendations should be made on the basis of adequate sampling and appropriate analyses[12]. We suggest that managers use techniques that explicitly consider the statistical power of alternative hypotheses[36]. In practice, managers tend to assume that maintaining separate management units is always the conservative action because it is potentially reversible, whereas mixing distinct units is not. However, this action is not conservative in the sense that continued isolation could compromise the future viability and evolutionary potential of a population[37].

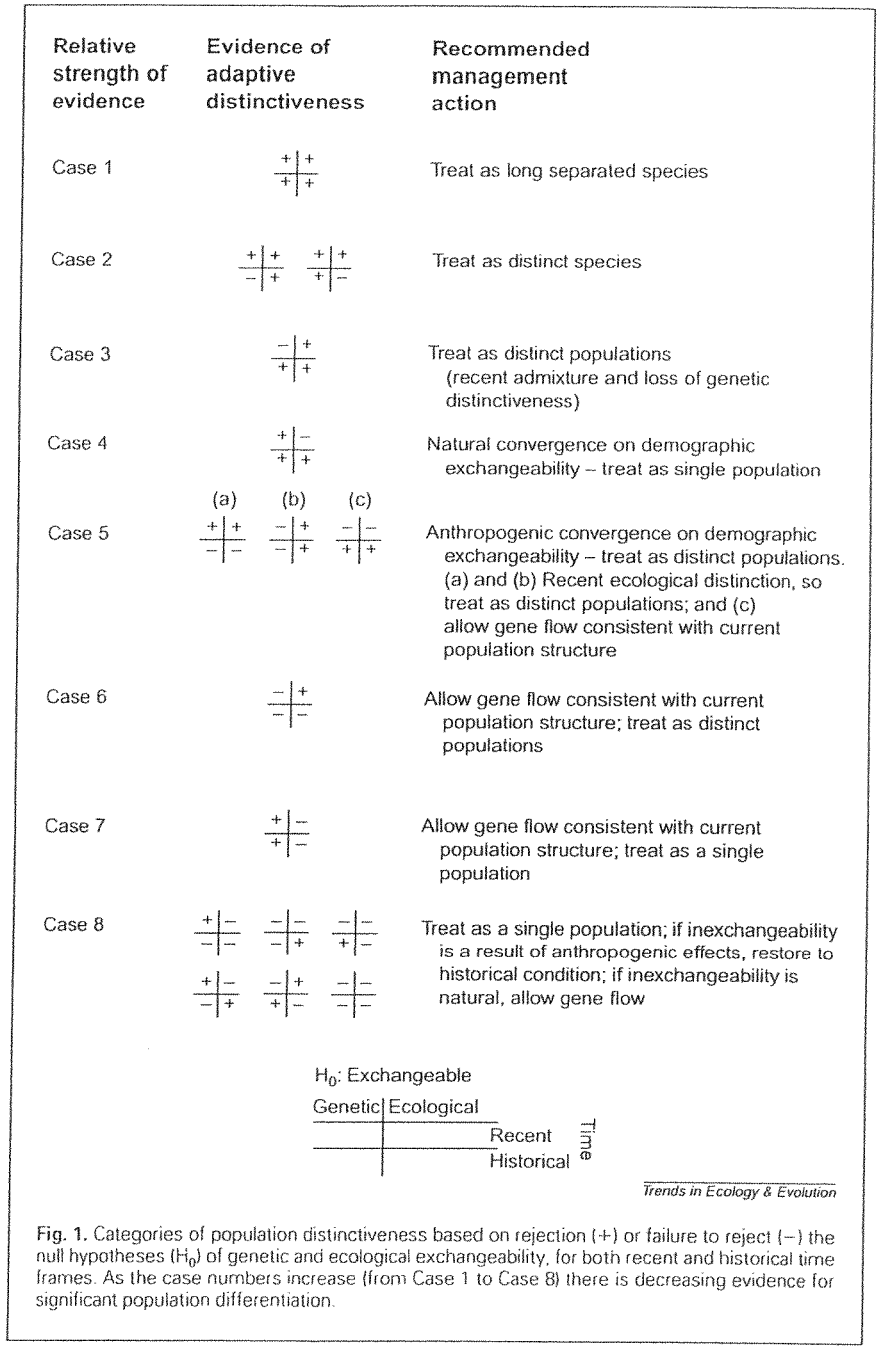


Fig. 1. Categories of population distinctiveness based on rejection (+) or failure to reject (−) the null hypotheses (H_0) of genetic and ecological exchangeability, for both recent and historical time frames. As the case numbers increase (from Case 1 to Case 8) there is decreasing evidence for significant population differentiation.

Applications

Methodology and management implications

Null hypotheses of exchangeability are scored as rejected (+) or not rejected (−) in each of four categories: genetic, ecological, recent and historical (Fig. 1). Sixteen outcomes are, therefore, possible; however, some are biologically implausible or unlikely. For example, rejection of only recent genetic and historical ecological exchangeability (or the converse) is implausible and would cast doubt on the accuracy of information.

Management implications follow from our concern to preserve adaptive diversity and evolutionary processes within species (Fig. 1). In general, we stress evidence for recent ecological nonexchangeability because it is indicative of the adaptive divergence necessary for population persistence. In Case 1 (Fig. 1), exchangeability is rejected in all four categories; consequently, the units under comparison represent separate species. Where only historical information for either genetic or ecological exchangeability is not rejected (Case 2, Fig. 1), the evidence is still strong that these are distinct units and we suggest that they should be managed as separate species.

Evidence for historical adaptive divergence only indicates divergence that is now not essential for population persistence, although it might have been in the past. Similarly, evidence for rejection of recent or historical genetic exchangeability alone is not sufficient to warrant separate priority conservation unless it is accompanied by adaptive divergence (Cases 4 to 7, Fig. 1). In these

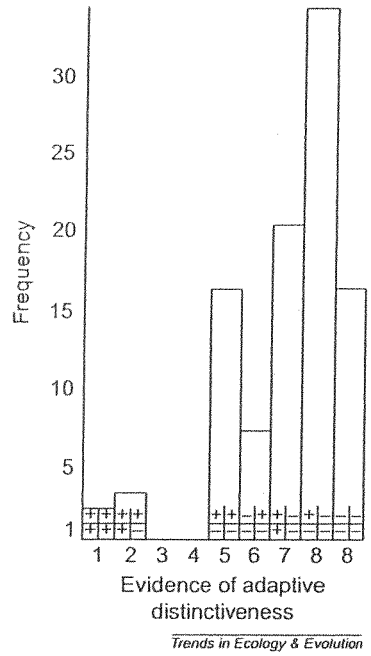


Fig. 2. A survey of the use of the evolutionarily significant units (ESU) concept from 98 population genetic studies. Each study was categorized using the exchangeability rejection criteria discussed in the text, with + for rejection of exchangeability and – for failure to reject exchangeability. Based on the classification of each study using the scheme in Fig. 1, we tallied the occurrence of each of the eight cases presented. ESUs were claimed under each of the eight cases.

instances, as well as in the straightforward result where there is no evidence to reject exchangeability (Case 8, Fig. 1), we recommend that the units be treated as populations connected by various degrees of gene flow. The goal of management should be to maintain levels of gene flow, as inferred from molecular data. Thus, reciprocally monophyletic populations would represent one end of a continuum in a network of populations connected by different degrees of gene flow. However, they would not receive special conservation concern unless there was evidence for adaptive divergence. Furthermore, if recent genetic divergence is a result of anthropogenic causes, we recommend that restoration to the natural condition of interchange be attempted through habitat restoration, translocation or other appropriate methods.

To explore the empirical distribution of cases in this continuum, we surveyed 98 studies from the literature and scored their results according to our four tests of exchangeability. We present a representative set of studies in Table 1 (references and a summary of all studies surveyed can be found at http://www.nceas.ucsb.edu/). In many cases, failure to reject the null hypothesis arises because the evidence for some categories was not presented or was inadequate to constitute an effective test. Indeed, ecological data was frequently lacking. Our survey shows that the overwhelming majority of analyses fall within Case 8 (Fig. 1), either rejecting just recent genetic exchangeability or failing to reject any exchangeability (Fig. 2). Interestingly, the authors assigned the term ESU to every category represented in Fig. 2, even when there was no evidence against exchangeability.

A few examples highlight the weaknesses of the ESU as it is currently applied. First, researchers often hesitate to consider geographically distinct populations as indistinguishable for fear that their endangered status be lessened. For example, ecological and genetic exchangeability could not be rejected for the black rhino (*Diceros bicornis*); however, the management recommendation by O'Ryan *et al.*[38] was for two distinct subspecies. Second, reciprocally monophyletic relationships might not always indicate historical isolation. Significant adaptive differences might be partitioned in a nonmonophyletic fashion (Box 2).

Two studies exemplify the joint application of phenotypic and genetic data. Legge *et al.*[39] found no evidence to reject the null hypothesis of genetic exchangeability among populations of Cryan's buckmoth (*Hemiluca* spp.); however, there was significant evidence to reject ecological exchangeability. Thus, these populations fell under Case 6 (Fig. 1) in our scheme with recent ecological nonexchangeability. Here, the authors argue, as we do in general, for the adaptive significance of ecological differentiation and that this differentiation provides better evidence for divergence relevant to population persistence and conservation. Finally, a study of tiger beetles (*Cicindela puritana*) shows how both ecological and genetic data can be used to determine population differentiation. Populations were found to be genetically nonexchangeable both recently and historically, and were ecologically nonexchangeable[40]. Thus, this example falls into our Case 2 (Fig. 1), showing strong evidence for adaptive distinctiveness.

However, studies using both adaptive characteristics and molecular markers are rare. In some cases, especially in the plant literature, information on life history variation and morphology are available to be incorporated in evaluation of population status. Recently, quantitative genetic techniques have been introduced for the study of phenotypic variation in the wild, thus suggesting that the genetic component of variation can be precisely defined[7,9,41]. Lynch[8] offers an approach that uses relatedness data based on molecular markers to estimate the heritable component of character variation in populations. Such new approaches highlight the importance of molecular genetic techniques to assess relatedness and population history. By contrast, measurement of adaptive diversity requires analysis of ecologically relevant heritable traits.

Prospects

The ESU concept has evolved over the past 15 years, and with the increasing sophistication of molecular techniques and genetic data analysis, ESUs now rely on measures that reflect genetic isolation rather than adaptive diversity. This is not appropriate for the overall goal of maintaining evolutionary potential, and, in certain cases, might limit the options for gene flow and adaptation via natural selection. Population units for conservation should be explicitly defined on traits that enhance the potential for species survival. Thus, there needs to be a focus on preservation of functional diversity rather than of historical legacy. Genetic techniques are also essential because they provide estimates of gene flow between populations and thus guide efforts to maintain historical levels of genetic exchange between populations. The network of genetic connections among populations is the web that maintains the potential for evolution. Rather than a focus on long-isolated populations, efforts should be directed at maintaining networks that capture the adaptive diversity within species. The necessity for genetic and phenotypic data provides a mandate for collaboration and consensus between molecular and ecologically oriented biologists.

In this context, the application of a specific terminology (ESU) hardly seems necessary or appropriate. The removal of this dichotomous terminology from its conceptual foundations in evolutionary biology might have been responsible for the frequent misdiagnosis of conservation units (Fig. 2). We suggest that the terminology is abandoned and replaced with a more holistic concept of species, consisting of populations with varying levels of gene flow evolving through drift and natural selection.

Acknowledgements

This work was conducted as part of the Phylogeny and Conservation Working Group supported by the National Center for Ecological Analysis and Synthesis, a Center funded by NSF (Grant #DEB-94-21535), the University of California at Santa Barbara, and the State of California. We thank the organizers, J. Gittleman and M. McKinney, and the workshop participants for excellent discussions. We thank M. Hedin for helpful discussion relative to his cave spider example

used here. We also thank A. Templeton, two anonymous reviewers, M. Hedin and S. Mazer for helpful comments on this article. O.B-E. was supported by an NSERC Postdoctoral Fellowship, G.M.M. by the NERC and K.A.C. by the Alfred P. Sloan Foundation.

References

1 Ryder, O.A. (1986) Species conservation and systematics: the dilemma of subspecies. *Trends Ecol. Evol.* 1, 9–10

2 Waples, R.S. (1991) Pacific salmon, *Oncorhynchus* spp., and the definition of 'species' under the endangered species act. *Mar. Fish. Rev.* 53, 11–22

3 Moritz, C. (1994) Defining 'evolutionary significant units' for conservation. *Trends Ecol. Evol.* 9, 373–375

4 Lande, R. and Shannon, S. (1996) The role of genetic variation in adaptation and population persistence in a changing environment. *Evolution* 50, 434–437

5 Frankel, O.H. (1974) Genetic conservation: our evolutionary responsibility. *Genetics* 78, 53–65

6 Bowen, B.W. (1998) What is wrong with ESUs? The gap between evolutionary theory and conservation principles. *J. Shellfish. Res.* 17, 1355–1358

7 Lynch, M. *et al.* (1999) The quantitative and molecular genetic architecture of a subdivided species. *Evolution* 53, 100–110

8 Lynch, M. and Ritland, K. (1999) Estimation of pairwise relatedness with molecular markers. *Genetics* 152, 1753–1766

9 Storfer, A. (1996) Quantitative genetics: a promising approach for the assessment of genetic variation in endangered species. *Trends Ecol. Evol.* 11, 343–348

10 Dieckmann, U. and Doebeli, M. (1999) On the origin of species by sympatric speciation. *Nature* 400, 354–357

11 Paetkau, D. (1999) Using genetics to identify intraspecific conservation units: a critique of current methods. *Conserv. Biol.* 13, 1507–1509

12 Sites, J.W., Jr and Crandall, K.A. (1997) Testing species boundaries in biodiversity studies. *Conserv. Biol.* 11, 1289–1297

13 Newton, A.C. *et al.* (1999) Molecular phylogeography, intraspecific variation and the conservation of tree species. *Trends Ecol. Evol.* 14, 140–145

14 Karl, S.A. and Bowen, B.W. (1999) Evolutionary significant units versus geopolitical taxonomy: molecular systematics of an endangered species. *Conserv. Biol.* 13, 990–999

15 Swofford, D.L. *et al.* (1996) Phylogenetic inference. In *Molecular Systematics* (Hillis, D.M. *et al.*, eds), pp. 407–514, Sinauer

16 Awadalla, P. *et al.* (1999) Linkage disequilibrium and recombination in hominid mitochondrial DNA. *Science* 286, 2524–2525

17 Templeton, A.R. (1989) The meaning of species and speciation: a genetic perspective. In *Speciation and its Consequences* (Otte, D. and Endler, J.A., eds), pp. 3–27, Sinauer

18 Templeton, A.R. (1994) The role of molecular genetics in speciation studies. In *Molecular Ecology and Evolution: Approaches and Applications* (Schierwater, B. *et al.*, eds), pp. 455–477, Birkhäuser

19 Templeton, A.R. (1999) Using gene trees to infer species from testable null hypotheses: cohesion species in the *Spalax ehrenbergi* complex. In *Evolutionary Theory and Processes: Modern Perspectives, Papers in Honour of Eviatar Nevo* (Wasser, S.P., ed.), pp. 171–192, Kluwer Academic Publishers

20 Templeton, A.R. (1998) Species and speciation: geography, population structure, ecology, and gene trees. In *Endless Forms: Species and Speciation* (Howard, D.J. and Berlocher, S.H., eds), pp. 32–43, Oxford University Press

21 Templeton, A.R. and Sing, C.F. (1993) A cladistic analysis of phenotypic associations with haplotypes inferred from restriction endonuclease mapping. IV. Nested analyses with cladogram uncertainty and recombination. *Genetics* 134, 659–669

22 Slatkin, M. (1985) Gene flow in natural populations. *Annu. Rev. Ecol. Syst.* 16, 393–430

23 Avise, J.C. and Ball, M.R., Jr (1990) Principles of geneological concordance in species concepts and biological taxonomy. In *Oxford Surveys in Evolutionary Biology* (Futuyma, D. and Antonovics, J., eds), pp. 45–67, Oxford University Press

24 Avise, J.C. (1994) *Molecular Markers, Natural History and Evolution*, Chapman & Hall

25 Smith, T.B. and Wayne, R.K., eds (1996) *Molecular Genetic Approaches in Conservation*, Oxford University Press

26 Graham, R.W. and Grimm, E.C. (1990) Effects of global climate change on the patterns of terrestrial biological communities. *Trends Ecol. Evol.* 5, 289–292

27 Rosenbaum, H.C. *et al.* (1997) An effective method for isolating DNA from historical specimens of baleen. *Mol. Ecol.* 6, 677–681

28 Anonymous (1998) 101 uses for a dead bird. *Nature* 394, 105

29 Anonymous (1998) Museum research comes off list of endangered species. *Nature* 394, 115–118

30 Baum, D.A. and Larson, A. (1991) Adaptation reviewed: a phylogenetic methodology for studying character macroevolution. *Syst. Zool.* 40, 1–18

31 Crandall, K.A. and Templeton, A.R. (1996) Applications of intraspecific phylogenetics. In *New Uses for New Phylogenies* (Harvey, P.H. *et al.*, eds), pp. 81–99, Oxford University Press

32 Templeton, A.R. *et al.* (1995) Separating population structure from population history: a cladistic analysis of geographical distribution of mitochondrial DNA haplotypes in the tiger salamander, *Ambystoma tigrinum*. *Genetics* 140, 767–782

33 Templeton, A.R. (1998) Nested clade analyses of phylogeographic data: testing hypotheses about gene flow and population history. *Mol. Ecol.* 7, 381–397

34 Crandall, K.A. and Templeton, A.R. (1993) Empirical tests of some predictions from coalescent theory with applications to intraspecific phylogeny reconstruction. *Genetics* 134, 959–969

35 Hewitt, G.M. (1996) Some genetic consequences of ice ages, and their role in divergence and speciation. *Biol. J. Linn. Soc.* 58, 247–276

36 Taylor, B.L. and Dizon, A.E. (1996) The need to estimate power to link genetics and demography for conservation. *Conserv. Biol.* 10, 661–664

37 Hedrick, P.W. (1995) Gene flow and genetic restoration: the Florida panther as a case study. *Conserv. Biol.* 9, 996–1007

38 O'Ryan, C. *et al.* (1994) Mitochondrial DNA variation in black rhinoceros (*Diceros bicornis*): conservation management implications. *Conserv. Biol.* 8, 495–500

39 Legge, J.T. *et al.* (1996) Genetic criteria for establishing evolutionarily significant units in Cryan's buckmoth. *Conserv. Biol.* 10, 85–98

40 Vogler, A.P. *et al.* (1993) Using molecular and ecological data to diagnose endangered populations of the puritan tiger beetle *Cicindela puritana*. *Mol. Ecol.* 2, 375–383

41 Lynch, M. (1996) A quantitative-genetic perspective on conservation issues. In *Conservation Genetics: Case Histories from Nature* (Avise, J.C. and Hamrick, J.L., eds), pp. 471–501, Chapman & Hall

42 Georgiadis, N. *et al.* (1994) Structure and history of African elephant populations: I. Eastern and Southern Africa. *J. Hered.* 85, 100–104

43 Small, M.P. *et al.* (1998) Discriminating coho salmon (*Oncorhynchus kisutch*) populations within the Fraser River, British Columbia, using microsatellite DNA markers. *Mol. Ecol.* 7, 141–155

44 Takahata, N. and Slatkin, M. (1990) Genealogy of neutral genes in two partially isolated populations. *Theor. Popul. Biol.* 38, 331–350

45 Neigel, J.E. and Avise, J.C. (1986) Phylogenetic relationships of mitochondrial DNA under various demographic models of speciation. In *Evolutionary Processes and Theory* (Karlin, S. and Nevo, E., eds), pp. 515–534, Academic Press

46 Powell, J.R. (1991) Monophyly/paraphyly/polyphyly and gene/species trees: an example from *Drosophila*. *Mol. Biol. Evol.* 8, 892–896

47 Hedin, M.C. (1997) Speciational history in a diverse clade of habitat-specialized spiders (Araneae: Nesticidae: *Nesticus*): inferences from geographic-based sampling. *Evolution* 51, 1929–1945

Do you want to reproduce material from *TREE*?

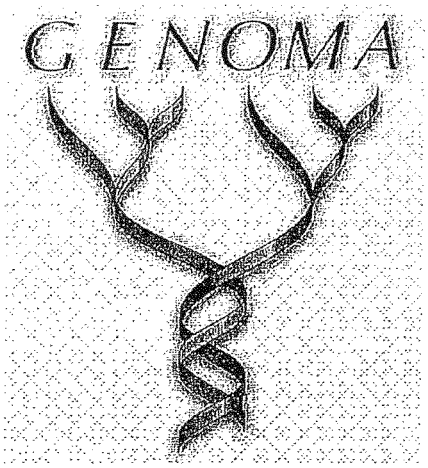
This publication and the individual publications contained in *TREE* are protected by the copyright of Elsevier Science. Except as outlined in the terms and conditions (see p. iv), no part of *Trends in Ecology & Evolution* may be reproduced, either in print or in electronic form, without written permission from Elsevier Science. Please send any permission requests to:

Elsevier Science Ltd
PO Box 800
Oxford
UK OX5 1DX

Exhibit D

REPORT

Prepared by Genoma LLC – Keith A. Crandall, Ph.D.[1] and Jonathon C. Marshall, Ph.D.



For the State of Wyoming, Office of the Attorney General

An assessment of the threatened subspecific status of the Preble's meadow jumping mouse (*Zapus hudsonius preblei*) based on current molecular data sets

[1]Address correspondence to:
Dr. Keith A. Crandall
50 East Maple Drive
Woodland Hills, UT 84653-2052
(801) 423-3340
Mobile: (801) 885-0284
kcrandall@genoma-llc.com

I Introduction

Preble's meadow jumping mouse (*Zapus hudsonius preblei*) is one of twelve proposed subspecies within the *Zapus hudsonius* (meadow jumping mouse) species complex. *Zapus hudsonius* is found throughout North America ranging from West to East coast and as far north as Alaska and as far south as central New Mexico, Mississippi, and Alabama (see distribution map from Ramey et al. 2005, Figure 1). The distribution of the Preble's meadow jumping mouse (PMJM) does not overlap with other meadow jumping mouse subspecies and corresponds to the Front Range corridor running from Colorado Springs, Colorado to Cheyenne, Wyoming (Ramey et al. 2005, Figure 1).

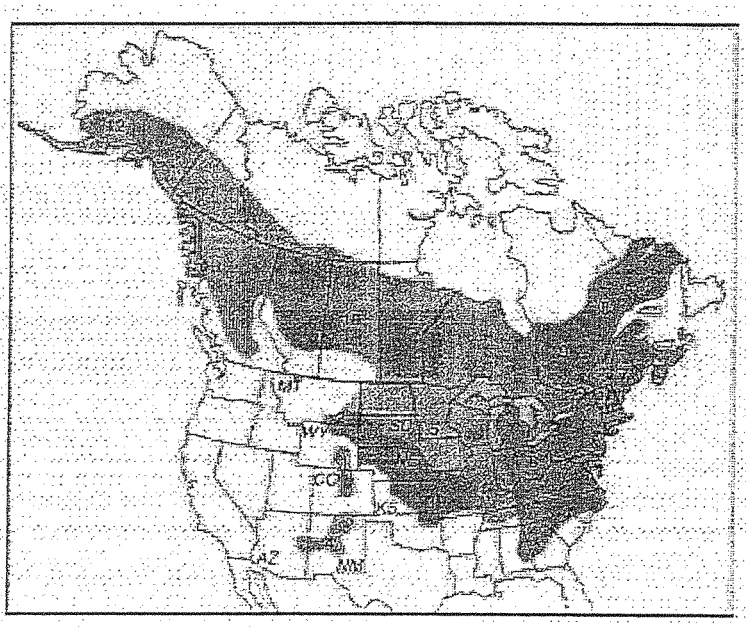


Fig. 1. Map of North America showing distribution and subspecies of *Zapus hudsonius* (Krutzsch, 1954; Hafner *et al.*, 1981). (1) *Z. h. preblei*, (2) *Z. h. campestris*, (3) *Z. h. pallidus*, (4) *Z. h. luteus*, (5) *Z. h. intermedius*, (6) *Z. h. americanus*, (7) *Z. h. acadicus*, (8) *Z. h. hudsonius*, (9) *Z. h. canadensis*, (10) *Z. h. hudsonius*, (11) *Z. h. tenellus* and (12) *Z. h. alascensis*.

On May 13, 1998 the PMJM was designated as a threatened subspecies by the U.S. Fish and Wildlife service (http://mountain-prairie.fws.gov/preble/). However, a recent study has called into question the appropriateness of such a designation (Ramey et al. 2005). The Ramey et al. (2005) study reanalyzed and expanded a previous morphological study (Krutch 1954) used in the listing process but found: 1) that Krutzsch's (1954) conclusions were not supported, and 2) there was no reliable multivariate morphometric discrimination between PMJM and other nearby *Z. hudsonius* subspecies. Additionally, Ramey et al. (2005) was unable to find significant underlying

genetic differentiation between PMJM and the other subspecies using microsatellite and mitochondrial DNA sequence data. Due primarily to the findings of Ramey et al. in February 2005 the U.S.F.W. service issued a 12-Month Finding on a petition to delist the PMJM as a threatened subspecies under the U.S. Endangered Species Act. In early 2006 a new study was released (King et al. 2006) that questioned the conclusions of Ramey et al. 2005 and called for a continuation of the threatened subspecies status for the PMJM. In their study, King et al. also analyzed microsatellite and mitochondrial DNA sequences but approached their study from a drastically different sampling scheme as compared to the Ramey et al. 2005 study. The Ramey et al. study had widespread and fairly dense sampling but few individuals were taken from each locality, on the other end of the spectrum, the King et al. study sampled few localities but large numbers of individuals from each locality.

In this study, we investigate the seemingly different conclusions of these two studies and consider them in light of the sampling schemes employed. We also combine the data sets where possible and extend the analytical approaches used to determine, according to the current data, if the PMJM represents a distinct subspecies within the *Z. hudsonius* species.

II Ramey et al. 2005

A. Microsatellite Data

We reanalyzed the Ramey et al. 2005 microsatellite data using all six microsatellite loci from five *Zapus hudsonius* subspecies, *Zapus hudsonius preblei*, *Zapus hudsonius campestris*, *Zapus hudsonius intermedius*, *Zapus hudsonius pallidus*, and *Zapus hudsonius luteus*. Figure 2 shows the localities of the samples taken in both the Ramey et al. and King et al. studies. Table 1 also provides the state and county names for each locality, the numbers of samples taken, and GPS coordinates for a central locality within the county. We converted the Ramey et al. microsatellite data into a format (Appendix 1) appropriate to run on the computer program STRUCTURE (Pritchard et al. 2000).

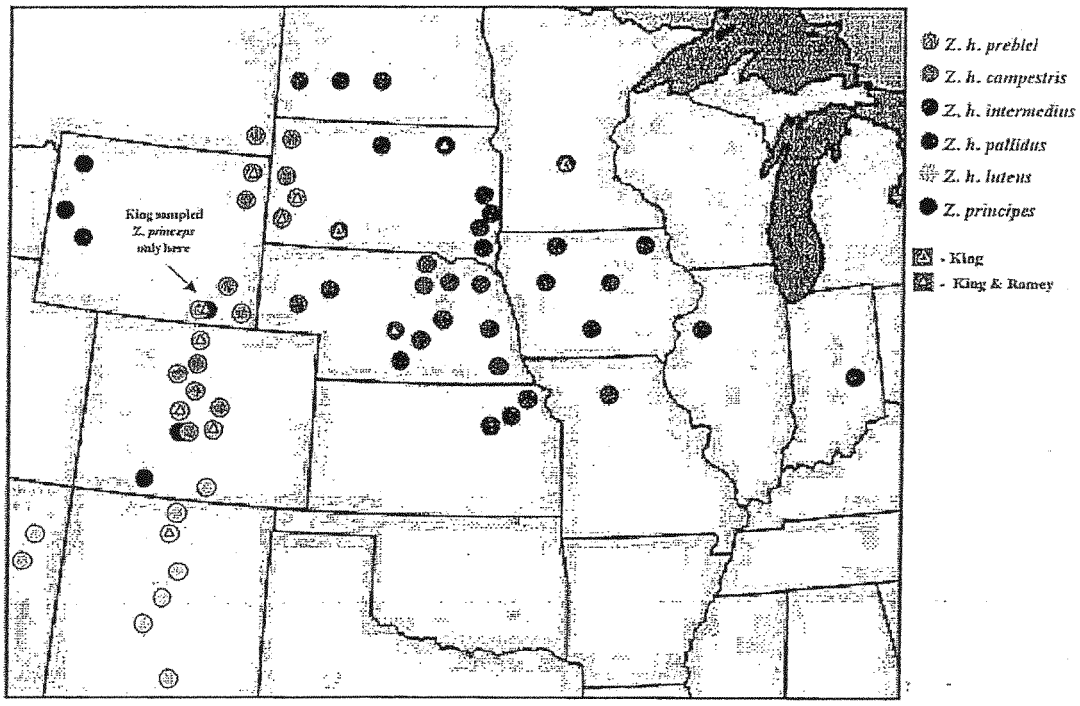
STRUCTURE organizes individuals into clusters or populations that minimize Hardy-Weinberg and Linkage disequilibria. In this way researchers let the data determine the population boundaries rather than assigning individuals to populations based solely on geographic location. Our strategy was to allow for an ancestral admixture model in selecting the optimal number of population clusters (K) for the entire data set regardless of *a priori* subspecies determination. We selected an optimal K by adhering to the following suggestions from the STRUCTURE help files

"There are a couple of informal pointers which might be helpful in selecting K. The first is that it's often the situation that Pr(K) is very small for K less than the appropriate value (effectively zero), and then more-or-less plateaus for larger K … In this sort of situation where several values of K give similar estimates of log Pr(X|K), it seems that the smallest of these is often ``correct". It is a bit difficult to provide a firm rule for what we mean by a ``more-or-less plateaus". I think that a sensible way to think about this is in terms of model choice. That is, we may not always be able to know the TRUE value of K, but we should aim for the smallest value of K that captures the major structure in the data. … A corollary of this is that when there is no population structure, you will typically see that the proportion of the sample assigned to each population is roughly symmetric (~1/K in each population), and most individuals will be fairly admixed. If some individuals are strongly assigned to one population or another, and if the proportions assigned to each group are asymmetric, then this is a strong indication that you have real population structure. … In summary, you should be skeptical about population structure inferred on the basis of small differences in K if (1) there is no clear biological interpretation for the assignments, and (2) the assignments are roughly symmetric to all populations and no individuals are strongly assigned."

After an optimal K was selected, we then looked for evidence of admixture between clusters by identifying individuals that have similar assignment probabilities (inferred ancestry) to more than one cluster or no assignment probability greater than 0.80 to any cluster. We also looked for evidence of admixture between subspecies by identifying individuals from a subspecies that were assigned to clusters with individuals

predominately from other subspecies. Like Ramey et al. 2005 and King et al. 2006, we used a burn-in of 15,000 followed by 100,000 replicates and tested K = 1 through K = 10. We performed these analyses 10 separate times in order to adequately search the likelihood space.

Figure 2. Distribution of all localities used in this study. Colored dots with no symbol indicate localities that were sampled only by Ramey et al. Color dots and symbols are explained in key below.



Extended results for all runs are given in support files (Supporting Documents/Data & Result Files/ Ramey MS Structure Results). Table 2 shows a summary of the likelihood scores for each of the 10 runs and the average likelihood scores for all runs at each K. A visual representation of average likelihood scores reveals a leveling-off of scores after K = 3 (Figure 3). We also noted possible leveling-off points at K = 5 and K = 9. In order to select a preferred K value we compared assignment probabilities for best score values at each alternative (see Supporting Documents/Data & Result Files/ Ramey MS Structure Results/Ramey Structure K = 3/5/9) and determined that K = 3 not only had the least admixed population assignments but also was the most concordant with "a clear biological interpretation for the assignments" or sets of subspecies designations. This result was also similar to the ΔK ad hoc statistic result of the King et al. 2006 study discussed below. Unfortunately a statistical test for selecting

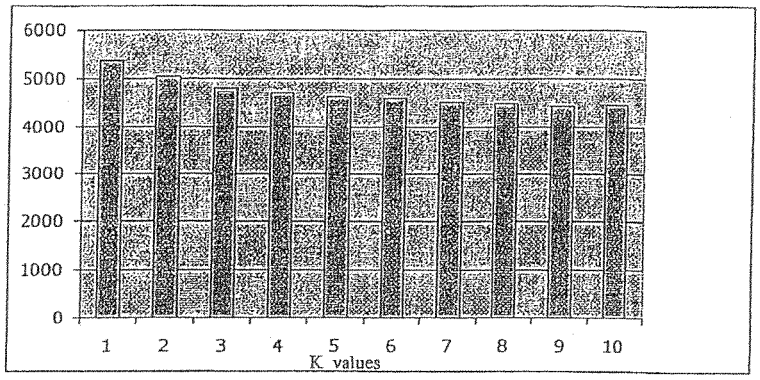


Figure 3. A visual summary average likelihoods scores for each K value estimated in STRUCTURE based on the Ramey et al. data, Purple bars represent absolute values of these scores, where the lower the bar the better the score. The optimal K value is the lowest value of K with a 'good' score or one that divides the individuals into populations that explain most of the variation.

K is not available in STRUCTURE. Table 3 shows the inferred ancestry for each sample for the best score at K=3. Population boundaries between clusters appeared to be semi-permeable as a number of individuals (13% of total) showed assignment probabilities < 0.80. Additionally a number of individuals (11% of total) had their highest probability of assignment to clusters of non-subspecific individuals (cluster 1 = *Z. h. preblei*, cluster 2 = *Z. h. pallidus* + *Z. h. luteus*, and cluster 3 = *Z. h. campestris* + *Z. h. intermedius*). These cases occurred most frequently in *Z. h. intermedius*, followed by *Z. h. campestris*, *Z. h. pallidus*, *Z. h. preblei,* and *Z. h. luteus*, in that order (Table 3). These results demonstrate that there is indeed limited gene flow among the three populations identified by the STRUCTURE analysis.

Our STRUCTURE results indicated that the five subspecies samples by the Ramey et al. study can be divided into three populations roughly equivalent to the three clusters identified above. To quantify the degree of admixture between *Z. h. preblei, Z. h. campestris* + *Z. h. intermedius*, and *Z. h. pallidus* + *Z. h. luteus*, we used coalescent-based methods to estimate relative measures of Θ ($4N_e\mu$, a measure of effective population size and mutation rate) and interpopulation migration rates (Nm) using the program MIGRATE (Beerli and Felsenstein 2001) based on the Brownian motion model. Appendix 2 shows the formatted infile. A summary of the results of this analysis is given in Table 4. Conditions of analysis and extended results for each run are given on accompanying disk (Supporting Documents/Data & Result Files/ Ramey MS Migrate Results). Table 4 (B) converts Migrate output into migration rates (Nm) that can be compared across studies. One advantage that these likelihood-based estimates have over traditional estimates of gene flow via Fst statistics is that asymmetrical migration rates

can be estimated between populations. When considering gene flow into and out of *Z. h. preblei,* we see that the most restricted migration is from the *Z. h. campestris* + *Z. h. intermedius* cluster into the *Z. h. preblei* cluster (0.46) and the highest migration from the *Z. h. preblei* cluster into the *Z. h. campestris* + *Z. h. intermedius* cluster (2.14). The immigration and emigration into and away from the all clusters ranged from 0.46 to 5.76; comparing this to other migration rates in rodents shows that comparatively high rates are found in these *Z. hudsonius* 'subspecies'. For instance in the African ground squirrel (*Xerus inauris*) migration rates (Nm) between populations within the species were estimated to be 0.64 to less than 0.001 (Herron et al. 2005), between population of common voles (*Microtus arvalis*) estimates ranged from 3.3 to 0.15 (Hamilton et al. 2006), between population of Tuco-tuco (*Ctenomys rionegrensis*) estimates ranged from 0.17 to less than 0.001 (Wlasiuk et al. 2003), between population of two deer mice species (*Peromyscus keeni* and *Peromyscus maniculatus*) estimates ranged from 1.00 to less than 0.001 and 4.74 to less than 0.001 respectively (Zheng et al. 2003). In all of these cases, we find migration rates lower than the lowest estimate between any of the *Z. hudsonius* populations and only a few higher, however, only in one of the above cases (*Peromyscus maniculatus*) have subspecies based on molecular data been described. This calls into question support of subspecific designation based on these microsatellite data.

B. Mitochondrial DNA Sequence Data

Ramey et al. 2005 sequenced a 346 bp piece of the mitochondrial control region (CR) gene to test for reciprocal monophyly between *Z. h. preblei* and its neighboring subspecies. King et al. 2006 also sequenced this same region of the mtDNA and we have combined these data sets and performed various analyses with them below. Here we will only mention briefly a couple of interesting points noted when comparing Ramey et al.'s CR data with their microsatellite data.

In their study, Ramey et al. found that *Z. h. preblei* contained few unique CR haplotypes and most haplotypes were also found in low frequencies within the range of *Z. campestris* (Table 5). The low frequencies of these shared haplotypes within *Z. h.*

campestris caused King et al. to question the quality of these data. King et al. 2006 (p22, line 666) states:

"For example, Ramey et al. (2005) reported the presence of *Z. h. preblei* haplotypes in DNA extracted from five dried museum skins of Z. *h. campestris* collected from Custer County, SD. The authors suggested this finding indicated recent gene flow and alluded to the presence of these haplotypes as a critical element in the decision to recommend synonymy of these subspecies. In the present study, 31 *Z. h. campestris* sampled recently from the same site in Custer County, SD used by Ramey et al. (2005), along with 30 additional specimens from neighboring Crook County, WY were subjected to mtDNA CR and CytB sequence analysis. All 61 individuals were determined to posses *Z. h. campestris*-specific mtDNA haplotypes. Moreover, the same conclusion was reached with the microsatellite loci, as no *Z. h. campestris* individual from either of these collections was assigned to *Z. h. preblei*. Given the prominent role the haplotypes obtained for the five museum skins from Custer County, SD and two additional specimens from Carter County, MT have played in the conclusions drawn by Ramey et al. (2005), it is unsatisfactory that an *a posteriori* analysis was not considered as part of a routine quality assurance/quality control effort. Since no attempts were made to reproduce the previous CR results, to confirm the findings with another region of mtDNA, or to apply an additional finer resolution technique such as microsatellite DNA analysis, combined with our failure to detect *Z. h. preblei* haplotypes among 61 *Z. h. campestris* from the same and an adjacent location, the conclusions drawn by Ramey et al. (2005) should be considered questionable."

The point made above by King et al. is well taken and when much is dependent on these few samples assurances should be taken that these samples were not misidentified or that the DNA isolated from these samples has not been cross contaminated. One way to control against this is to look at the microsatellite profiles for each of the *Z. h. campestris* individuals that have a *'Z. h. preblei'* CR haplotype. If these individuals were misidentified before DNA extraction or contaminated with *Z. h. preblei* DNA after extraction then their microsatellite genotypes should also show a '*Z. h.*

preblei' profile and have a high probability assignment to the *Z. h. preblei* cluster. Table 5 shows the groupings of all identical CR haplotypes from both studies. From Table 5 we see that ZhcaK110013, ZhcaK109984, ZhcaK109985, ZhcaK123592, ZhcaK109978, and ZhcaK109972, all have '*Z. h. preblei*' CR haplotypes. However, assignment probabilities from Table 3 show that ZhcaK110013 is assigned to the *Z. h. campestris/intermedius* cluster with a probability of 0.988. Also, all the others samples in question have their highest probability assignment to the *Z. h. campestris/intermedius* cluster, ZhcaK109985 (0.969), ZhcaK109978 (0.937), ZhcaK109972 (0.694), with the exception of ZhcaK109982 which shows similar assignment probabilities to all three clusters and ZhcaK123592 for which no microsatellite data are given. This indicates that these samples were neither misidentified, miscataloged, nor cross contaminated. Conflicting nuclear and mtDNA signals could be the products of different levels of resolution targeted by the different markers and types of analysis. The fast evolving microsatellite markers coupled with the **population**-level STRUCTURE analysis illustrate the current interactions of these populations whereas the slower evolving shared CR haplotypes may be indicative of historical interactions or mitochondrial introgression. If the subspecific category is to represent historical isolation in addition to current population structure, high levels of concordance between these analyses should be required. If simple allele frequency differences (highly dependent on sampling scheme) were allowed to fill this requirement most if not all colonization and bottleneck events would also instantaneously spawn new subspecies, something many scientists would find discomforting. An alternative explanation for different assignments based on mtDNA versus nuclear (microsatellite) markers is the potential for sex biased dispersal of mtDNA alleles (maternally inherited) given the different and asymmetric migration rates for the diagnosed populations.

A number of the CR mtDNA haplotypes from *Z. h. preblei*, *Z. h. campestris*, and *Z. h. pallidus*, individuals from the Ramey et al. study were identical or nearly identical to *Z. princeps* haplotypes (see Network 3 and 4 in Figure 10). Ramey et al. interpreted these results as cases of misidentification. Unfortunately, no microsatellite data were generated to test these in the same way the *Z. h. campestris* individuals with *Z. h. preblei* haplotypes were tested above. The four *Z. h. preblei* individuals that were 'misidentified'

all came from Albany County, Wyoming. *Z. princeps* were also sampled from this county, which merits consideration of a different interpretation than 'misidentification'. It is possible that some gene flow is occurring at this much deeper interspecific level. If so, this may be indicative of a tradition of 'over-splitting' taxa by biologist within the *Zapus* genus. Results from previous studies indicate that gene flow between *Z. princeps* and *Z. h. preblei* and other subspecies is not only likely but probable. As summarized in Beauvais 2001:

"The relatively large zone of co-occurrence in southeast Wyoming raises the issue of potential hybridization between the 2 species [*Z. h. preblei* and *Z. princeps*]. Hybridization between related species in areas of co-occurrence is known to occur in several other free-ranging vertebrates (see examples in Pague and Grunau 2000). Hybridization between *Z. hudsonius* and *Z. princeps* in Wyoming is suggested by recent analyses of variation in mitochondrial DNA. Although these analyses can distinguish the 2 species in other parts of their ranges (e.g., the South Platte basin in Colorado), they are unable to reliably assign species identity to *Zapus* specimens from southeast Wyoming. **The general consensus among regional mammalogists is that *Z. hudsonius X Z. princeps* hybridization is the most parsimonious explanation for such results** (Riggs et al. 1997, Pague and Grunau 2000, Schorr 2001)."

It may be that the genus *Zapus* may be suffering not only from a tendency to split taxa but also from non-rigorous delimitation of species boundaries. This makes any discussion of subspecies dubious. Biologists may be better served by preceding debate on subspecific classification with substantial and meticulous examinations of species boundaries. In other words 'you can't have cupboards if you ain't got walls' *Neil Young-Old Laughing Lady*. A detailed analysis of this potential hybrid zone that incorporates both nuclear (microsatellite) and mitochondrial markers would contribute substantially to clarification of our current issue.

III King et al. 2006

A. Microsatellite Data

King et al. 2006 screened 320 samples for 21 microsatellite loci across the same five *Zapus* subspecies as above. We reanalyzed the King et al. microsatellite to verify their results and also to observe patterns in assignment probabilities for the optimal number of populations (K). Again, Figure 2 shows the localities of the samples taken in both the Ramey et al. and King et al. studies. Table 1 also provides the state and county names for each locality, the numbers of samples taken, and GPS coordinates. We converted the King et al. microsatellite data into a format (Appendix 3) appropriate to run on the computer program STRUCTURE (Pritchard et al. 2000). Conditions for the King et al. STRUCTURE analysis and method for selecting the optimal K were identical to the analysis of the Ramey et al. data and are given above. Resulting output files for all runs are given in support files (Supporting Documents/Data & Result Files/ King MS Structure Results).

We confirm the findings of King et al. 2006 and select an optimal K value of three (Table 6, Figure 4). The result is identical to the K value selected with the Ramey et al. data set only in the current analysis we see a more profound leveling of likelihood

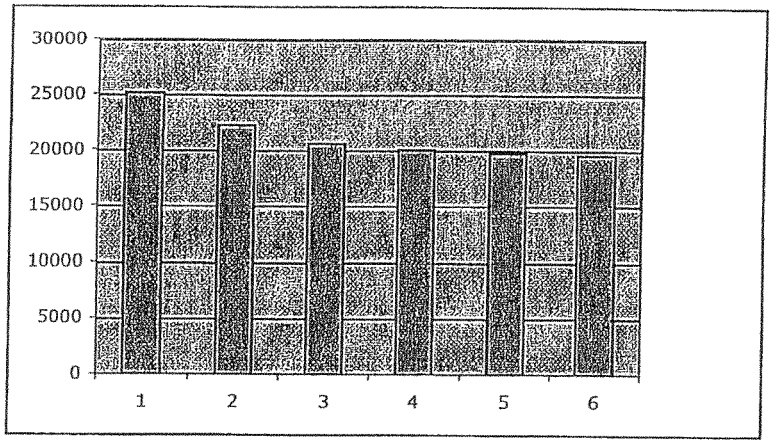


Figure 4. A visual representation of the absolute values of likelihood scores from ten separate STRUCTURE runs for King et al. data set. K values range from 1 to 6. Little improvement of likelihood scores is evident after K = 3.

scores at K = 3. The composition of resultant clusters was also very similar to our reanalysis of the Ramey et al. data (*Z. h. preblei, Z. h. pallidus* + *Z. h. luteus*, and *Z. h. campestris* + *Z. h. intermedius*). Table 7 shows the inferred ancestry for each sample. Population boundaries between clusters appeared to be much more distinct than in the STRUCTURE analysis of the Ramey et al. data. For instance, few individuals showed assignment probabilities < 0.80 and all of these occurred in the *Z. h campestris/intermedius* cluster. Additionally no individuals had their highest probability of assignment to clusters of non-subspecific individuals.

The differences in results and conclusion of these studies seem to be largely due to the sampling schemes employed by each study. King et al. rightly point out that sampling is critical in intraspecific studies and is distinct from systematic studies. King et al. argue for dense sampling at specific locations with sparse sampling across locations throughout the distribution of the subspecies. King et al. correctly point out that the basis of inference by Ramey et al. (frequency differences instead of evolutionary relationships) is highly dependent upon sampling individuals at a given location with the Ramey et al.

sampling design lacking in terms of individuals per site. Yet the conclusions reached by King et al. are also highly suspect in that leaving large geographic gaps between sampling sites when the taxon is known to range within those gaps leads to artificial inferences of population structure when, in fact, a gradient of variation may exist with gene flow across the gradient. Thus the optimal sampling strategy for such studies is a combination of the two approaches; broad geographic sampling with multiple individuals sampled per sampling location (Templeton et al. 1995; Sites & Crandall 1997; Templeton 2004; Morando et al. 2003). Below we attempt an approximation of this scheme by combining the CR data sets of Ramey et al. and King et al. Unfortunately, the scoring of microsatellite allele size on different machines can be tricky and the lack of generalized size standards run by these lab groups made it is impossible to combine the microsatellite data into a single analysis. Ideally some of the samples scored in the first study (Ramey et al al. 2005) should have been run in the second (King et al. 2006) to allow the combining and calibration of results.

Both studies have limitations in their sampling strategies. The conclusions by King et al. of population structure are particularly suspect given the sampling design of their study. For example, King et al. fail to sample in areas most likely to show gene flow between subspecies (Figure 5). These areas include, *Z. h. preblei* from southern Wyoming, where you would find individuals most likely to show evidence of gene flow between *Z. h. campestris*, *Z. h. pallidus,* and even *Z. princeps* based on geographic proximity and previous studies (see above), and *Z. h. pallidus* from western Nebraska. King et al. have just a single locality sampled for *Z. h. luteus* and just two sites sampled for the critical *Z. h. campestris* and *Z. h. intermedius*. This is particularly problematic with the widespread distribution of *Z. h. intermedius* across 11 states with sampling in only the NE corner of South Dakota and an adjacent site in central Minnesota. The central problem here is a taxonomic issue relative to the entire species complex and possibly sister species within the genus, thus the entire species complex should be sampled to resolve the issue.

Like our STRUCTURE analysis of the Ramey et al. microsatellite data set our analysis of the King et al. data indicated that the five subspecies samples can be divided into three populations equivalent to the three clusters identified above. Again to quantify

the degree of admixture between *Z. h. preblei, Z. h. campestris* + *Z. h. intermedius*, and *Z. h. pallidus* + *Z. h. luteus*, we used MIGRATE (Beerli and Felsenstein 2001) to estimate migration between these clusters. Conditions of analysis and extended results for each run are given in the supporting material (Supporting Documents/Data & Result Files/ King MS Migrate Results). Table 8 shows the estimated genetic diversity (Θs) for each cluster as well as the migration rates (Mxy) between all clusters (x and y). Table 8 (B) shows estimates of population migration rates (Nm) between clusters. The results of Table 8 (B) are similar to those estimated from the Ramey et al. microsatellite data (Table 4). We see nearly equal rates of migration out of *Z. h. preblei* but interestingly an increase of migration into *Z. h. preblei* from the other two clusters (1.21 and 2.45 as opposed to 0.46 and 0.47). Conversely lower migration rate estimates between the *Z. h. campestris* + *Z. h. intermedius*, and *Z. h. pallidus* + *Z. h. luteus* clusters resulted in the analysis of the King et al. data. As a whole, we draw similar conclusions here as with the analysis of the Ramey et al. data with migration rates again on par with and even in a little excess of other within species comparisons where subspecies are not recognized (see above).

B. Mitochondrial DNA Sequence Data

The King et al. studies generated sequence data from two mtDNA genes. Like Ramey et al. they sequenced a piece of the control region only slightly larger. We will discuss this below in the section on the combined analysis. King et al. also generated a ~1 Kb piece of the cytochrome b (CytB) mitochondrial gene for 292 individuals from 13 localities (Figure 2) representing the subspecies *Z. h. preblei*, *Z. h. campestris*, *Z. h. intermedius*, *Z. h. pallidus*, and *Z. h. luteus* as well as a single *Z. princeps* sample. In order to test the monophyly of the King et al. subspecies samples, we combined them with 27 outgroup plus one *Z. h. luteus* sequences provided by R. Ramey and J. Cook. The outgroup samples included 21 *Z. princeps* (ZP), two *Z. trinotatus* (ZT), three *Napaeozapus insignis* (Ntinsig), one *Ratus ratus* (Rratus), and one *Mus musculus* (Mmus). In order to combine these data, the total length of sequence had to be trimmed to 518 bp. The high throughput multiple sequence alignment program MUSCLE (Edgar 2004) was used to align sequences. From the 320 individual sequences 48 distinct haplotypes were found

(Appendix 4). No haplotypes were shared between subspecies groups. Maximum parsimony (MP) trees were generated in PAUP* (Swofford 1999) by heuristic searches with 100 random additions and using the TBR branch swapping method (see Appendix 5 for PAUP* haplotype data file). Figure 6 shows the resulting 50% majority rule consensus tree for the 48 MP trees. We also generated Bayesian tree topologies with MRBAYES (Huelsenbeck and Ronquist 2001) using 1,000,000 iterations and a burn-in of 47,000. We used Modeltest (Posada and Crandall 1998) to select the GTR + G + I model as the optimal model of evolution. Because of the short length of the DNA fragment used, separate models for each codon position were not estimated. Tree topologies from the two methods were identical in all major divisions and differed only slightly by levels of resolution, for this reason, only the 50% consensus MP tree is shown and bootstrap and posterior probability values from both analyses combined and placed on the tree (Figure 6).

Figure 6 shows monophyletic groupings with good nodal support for *Z. princeps*, *Z. hudsonius*, and *Napaeozapus insignis*. This is not terribly surprising due to the low numbers of individuals and localities sampled from *N. insignis* and *Z. princeps*. Figure 6 also shows a monophyletic grouping for *Z. h. pallidus* and one for a combined *Z. h. luteus* + *Z. h. pallidus* group. However, given that only a single locality from *Z. h. luteus* was sampled, only limited conclusions can be drawn. All samples from *Z. h. preblei, Z. h. campestris*, and *Z. h. intermedius* combined to form a single monophyletic group.

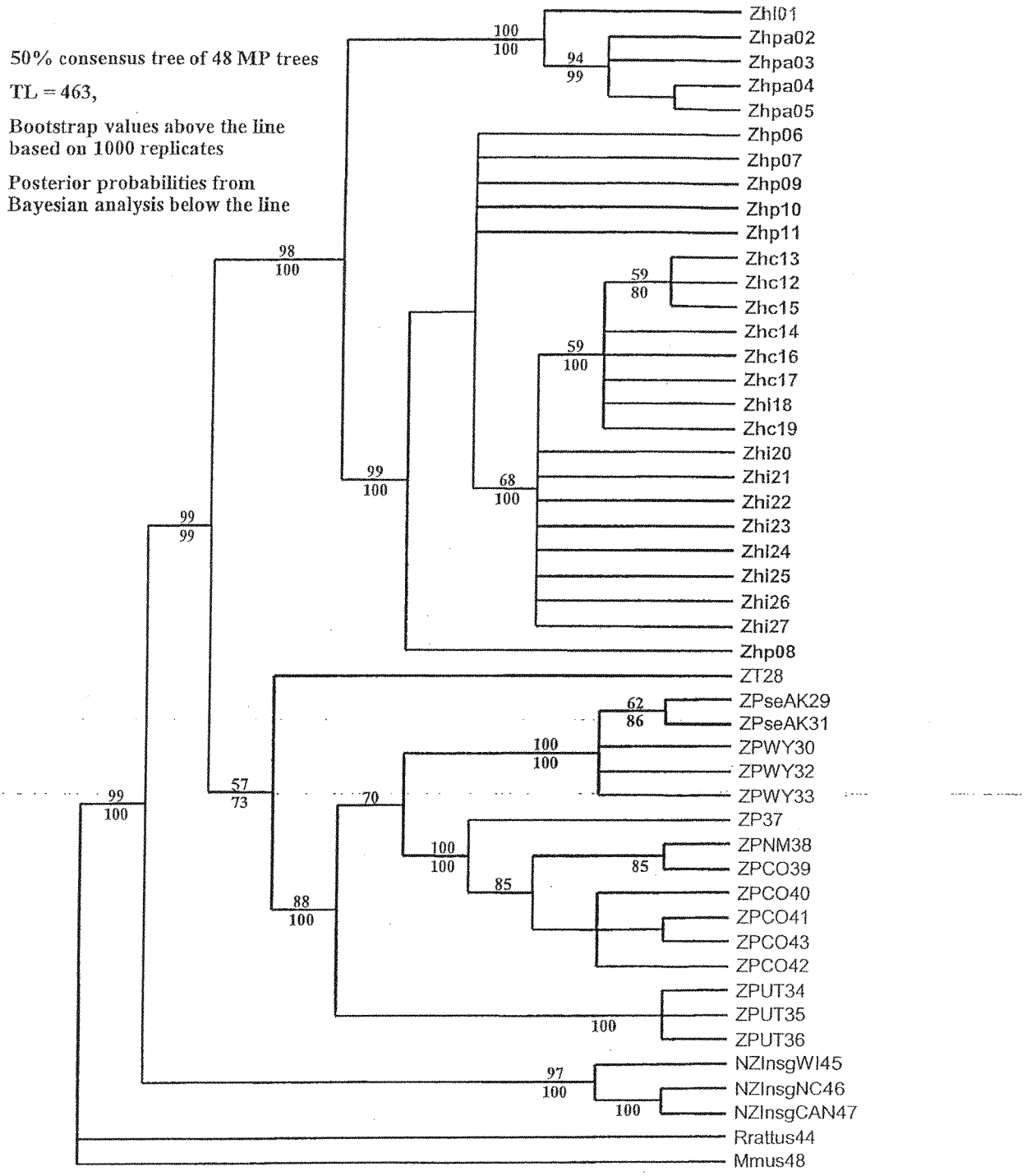


Figure 6. Phylogenetic tree based on part of the CytB mtDNA gene. Numbers at taxa represent haplotype numbers listed in Appendix 4.

However, none of these subspecies forms a monophyletic group by themselves and thus fail this particular subspecies test and indeed fail even an evolutionarily significant unit (ESU) test of Moritz (1994). *Z. h. campestris* comes closest to forming a monophyletic group with a single *Z. h. intermedius* haplotype nested within it. As a whole this analysis, although severely restricted by the sampling design as discussed above, provides some preliminary evidence for designation of a two subspecies within the *Z. hudsonius* samples investigated, one including the *Z. h. pallidus* + *Z. h. luteus* samples and another including the *Z. h. preblei* + *Z. h. campestris* + *Z. h. intermedius* samples.

IV Combined Data Analysis

A. Combined control region phylogenetics

As mentioned above the only data we were able to combine between the Ramey et al. 2005 and King et al 2006 studies were the sequences generated from the mitochondrial control region. Total sample size for the combined analysis was 520 individuals (including several *Z. princeps* samples) from 14 states. Individuals were pooled by their county and state or origin because exact GPS coordinates were not available for all samples (Table 1). GPS coordinates for all localities were taken from roughly the geographic center of the county. Sequences were aligned in MUSCLE (Edgar 2004) and then trimmed to a total base pair length of 347. Sequences collapsed into 63 distinct haplotypes. Nine of the haplotypes were shared either between our five focal *Z. hudsonius* subspecies and/or *Z. hudsonius* and *Z. princeps* (Table 5).

We estimated phylogenetic relationships based on Bayesian, maximum likelihood (ML), and maximum parsimony (MP) criteria. Topologies and well-supported nodes were similar for all three optimality criteria used. Figure 7 shows a 50% majority rule consensus tree of 23,329 most parsimonious trees with a tree length of 146. Trees were

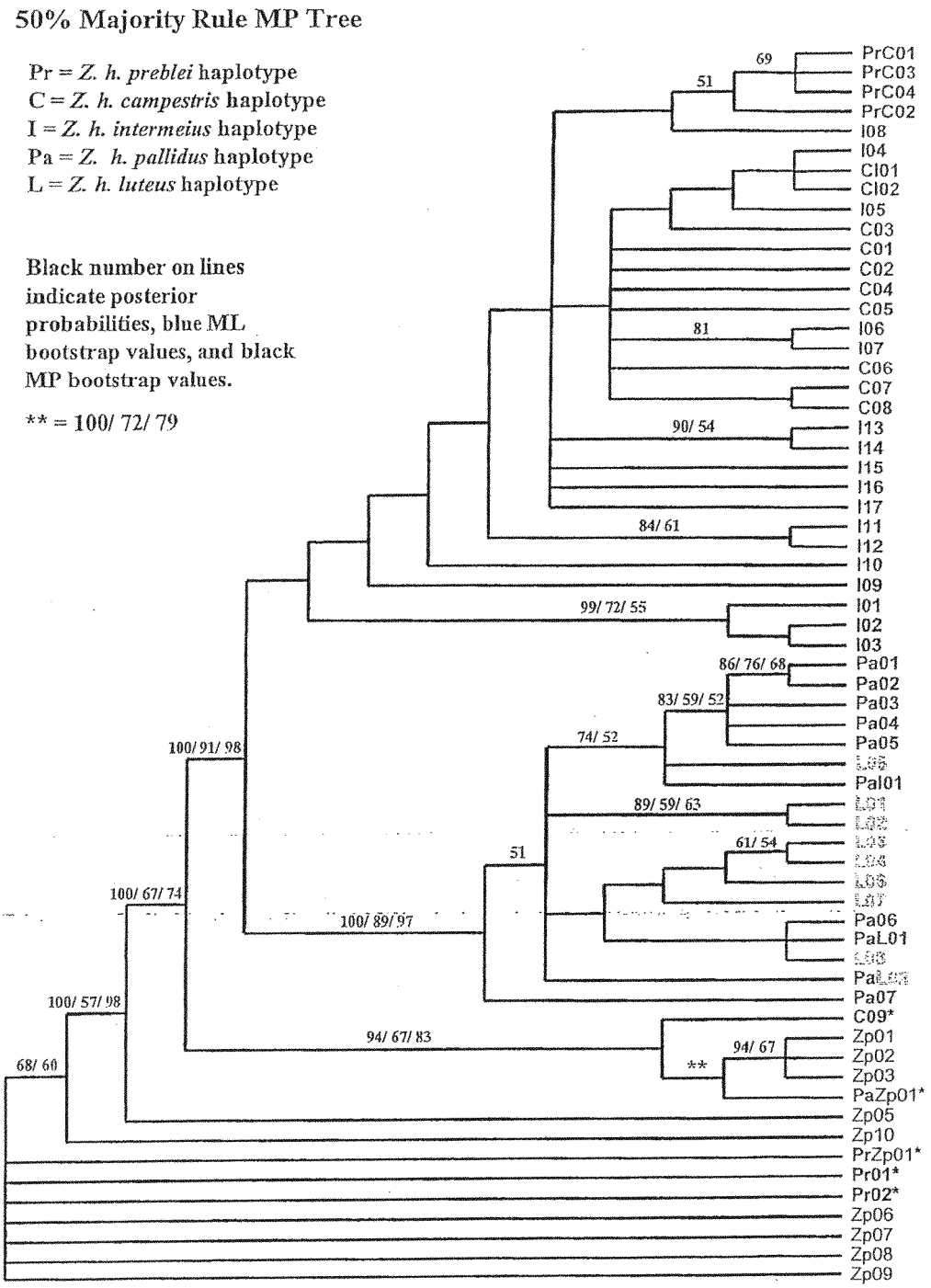


Figure 7. Phylogenetic tree based on the combined CR mtDNA data sets from Ramey et al. 2005 and King et al. 2006 studies. Lists of haplotypes represented by each haplotype label are found in Table 5. Colors correspond to different *Z. hudsonius* subspecies. * = *Z. hudsonius haplotypes* associated with *Z. princeps* haplotypes.

generated using 100 random additions and the TBR branch swapping method. Bootstrap values were based on 1000 replicates where a maximum of 2 x 10^7 rearrangements was set for each replicate. We generated our Bayesian topologies with MRBAYES (Huelsenbeck and Ronquist 2001) using 1,000,000 iterations and a burn-in of 46,000. We used Modeltest (Posada and Crandall 1998) to select the TVM + I + G model as the optimal model of molecular evolution. Maximum likelihood analysis was performed with GARLI v0.94 (Zwickl 2006, http://www.bio.utexas.edu/grad/zwickl/web/garli.html) under the TVM + I + G model and 100 replicates used for ML bootstrap values.

Results from the CR phylogenetic analysis are similar to the results from the CytB phylogenetic analysis except with less distinct clustering of subspecies and groups of subspecies. This is not surprising because the current analysis incorporated samples from regions avoided by the King et al. study that were located in area most likely to see gene flow between different subspecies/populations. Noticeably widespread across the tree topology are the *Z. h. preblei* samples. Our analysis included some samples of *Z. h. preblei* dropped from the Ramey et al. study on the basis of their similarity to *Z. princeps* sequences. As mentioned above, gene flow between these species is suspected in southeastern Wyoming and these samples seem equally likely to be a result of interspecific gene flow as a result of misidentification. Some haplotypes found both in *Z. h. pallidus* and *Z. h. campestris* also clustered with *Z. princeps* haplotypes (C09, PaZp01) and together formed a moderately supported (posterior probabilities and bootstrap values = 100/67/74, Figure 7) sister group to most *Z. hudsonius* populations. These results at a minimum merit further study on species boundaries between *Z. hudsonius* and *Z. princeps*. Quantification of levels gene flow between these species could then serve to add a base line level of gene flow between proposed *Z. hudsonius* subspecies and aid in the identification of proper subspecific boundaries if such boundaries exist.

Although many of the nodes in Figure 7 are either unresolved or poorly supported we can draw limited conclusions based on some of the moderately support ones. We see that most *Z. hudsonius* samples cluster into a well-supported (100/91/98) monophyletic group. Within this monophyletic group, we see most *Z. h. luteus* and *Z .h. pallidus* samples clustering into a well-supported (100/89/97) group nested within the larger Z. *hudsonius* clade. However, no support for exclusive clustering of any of the *Z. hudsonius*

subspecies is evident anywhere in the tree. In fact, even combining most *Z. h. campestris*, *Z. h. preblei*, and *Z. h. intermedius* into a single group did not result in a well-supported clade (as opposed to the CytB result in Figure 6). These results could be indicative of different marker resolution, different sampling schemes, or a combination of both. If exclusivity or near-exclusivity of taxa based on mtDNA markers is to be taken as evidence of historical isolation between populations and thus incorporated into subspecific designation then the results in Figure 6 and Figure 7 question subspecific status for any of the individual *Z. hudsonius* subspecies. However the formation of two subspecies by combining *Z. h. pallidus* with *Z. h. luteus*, and *Z. h. campestris* with both *Z. h. preblei* and *Z. h. intermedius* would merit subspecific status under the near-exclusivity criterion.

B. Nested clade analysis (NCA) on control region

When assessing patterns of genetic variation at the intraspecific level, it is often difficult to distinguish current population structure from population history using traditional population genetic estimates such as Fst (Templeton et al. 1995). For instance, two population sharing similar alleles at similar frequencies could be the product of ongoing gene flow (current population structure) or a past range expansion of the organism (population history). NCA uses haplotype frequencies in conjunction with the genealogical relationships and geographic distribution of the haplotypes in a novel methodology that allows the researcher to distinguish between structure and different historical events (Templeton et al. 1995). Such an analysis was lacking from both the King et al. and the Ramey et al. studies. Thus their studies possibly confound population history and population structure.

To implement the NCA, a parsimony haplotype network was first constructed for the mitochondrial control region sequences using the program TCS (version 1.21, Clement et al. 2000). Haplotypes were connected using a 95% parsimony limit that imposed a maximum of seven mutational steps between connections. Four separate networks plus and unconnected single haplotype (Zp05) resulted (Figures 8-10).

The independent networks were then connected into a total network using TCS by relaxing the 95% parsimony criterion (Figure 10). A number of ambiguous connections or loops in the resulting haplotype networks were resolved using the criteria set forth in Crandall & Templeton (1993). The total network was then nested (Templeton 1998) and input into GEODIS (version 2.4, Posada et al. 2000) together with geographic sampling information (Appendix 7, input file). We then performed permutation tests (1000) to determine association between phylogeny and geographic distribution. Clade distances (Dc) and nested clade distances (Dn) were measured and interior and tip clade differences estimated. Templeton's revised (2004) inference key (Modified 11 November 2005) was then applied to the clades with significant results from GEODIS to determine the outcome of the NCA.

Appendix 8 provides the extended GEODIS results and Table 9 summarized the general conclusions from the NCA inference key. A total of 33 clades with both geographic and genetic variation from various nesting levels were input into GEODIS; of these clades only 17 resulted in significant results that lent themselves to interpretation. This demonstrates the necessity of the need for a sampling scheme that employs both large numbers of localities (as in Ramey et al. 2005) and large numbers of individuals per locality (as in King et al. 2006) (Sites & Crandall 1997; Morando et al. 2003). However, even with our limited sampling scheme a number of important conclusions can be drawn. Of the 63 distinct haplotypes eleven were shared between species and subspecies. Noting the distribution of these haplotypes on our networks (Figs. 7-9), we see most of the shared haplotypes are interior clades indicating ancestral types.

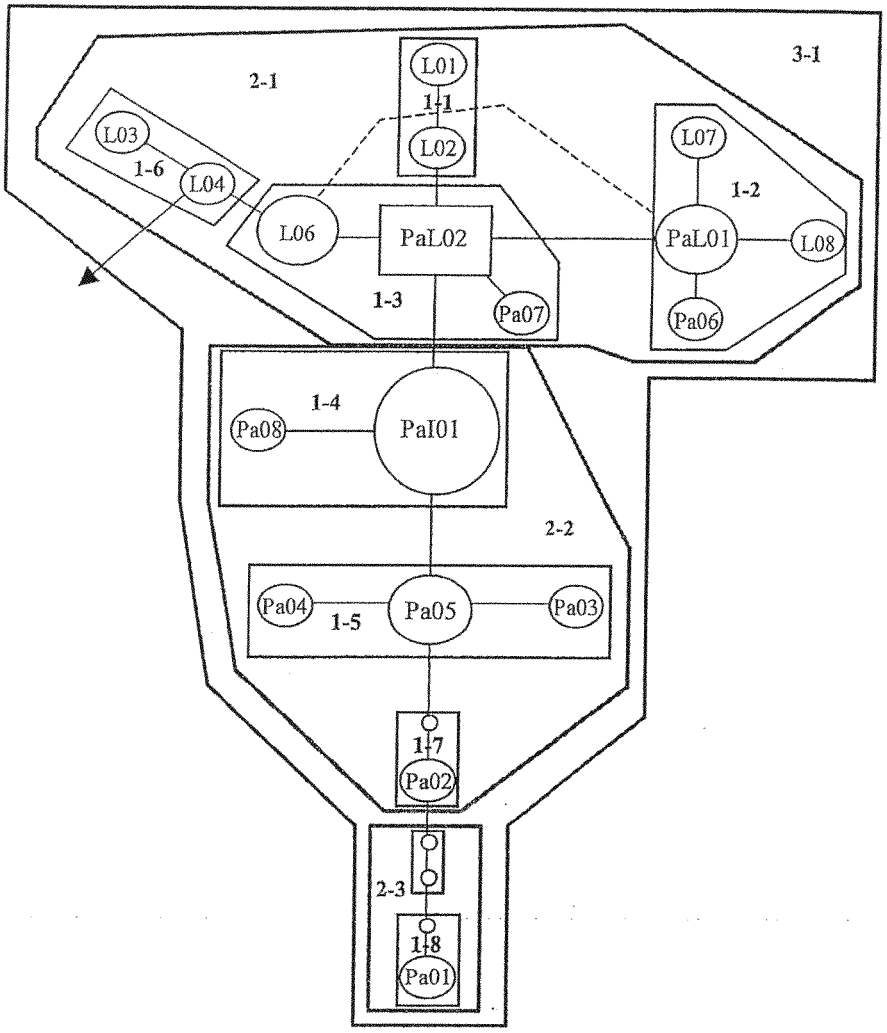


Figure 8. Haplotype network 1 estimated in TCS with 1, 2, and 3 step nesting groups shown. Ovals and squares represent haplotypes where labels correspond to labels in Table 5 and size roughly correlated with frequency of haplotype. Lines separating haplotypes and empty circles represent single mutational steps. Arrows indicate connections to other networks. Dashed lines represent broken loops. Colored boxes correspond to different nesting levels. Network consists of haplotypes mostly from *Z. h. pallidus* and *Z. h. luteus* individuals.

Network 1 (Figure 8) consisted of mostly haplotypes from *Z. h. pallidus* and *Z. h. luteus* individuals, with a single haplotype (PAI01) also being found in *Z. h. intermedius*. The distribution of these haplotypes between subspecies showed non-exclusive clustering within the network. Also clades 1-3 and 2-1 both spanned the geographic divide between *Z. h. luteus* and *Z. h. pallidus* populations (Figure 11), thus including non-subspecific populations. However, the NCA inference for clade 1-3 (Table 9) indicated possible allopatric fragmentation across this geographic divide. NCA inferences for clades 2-1

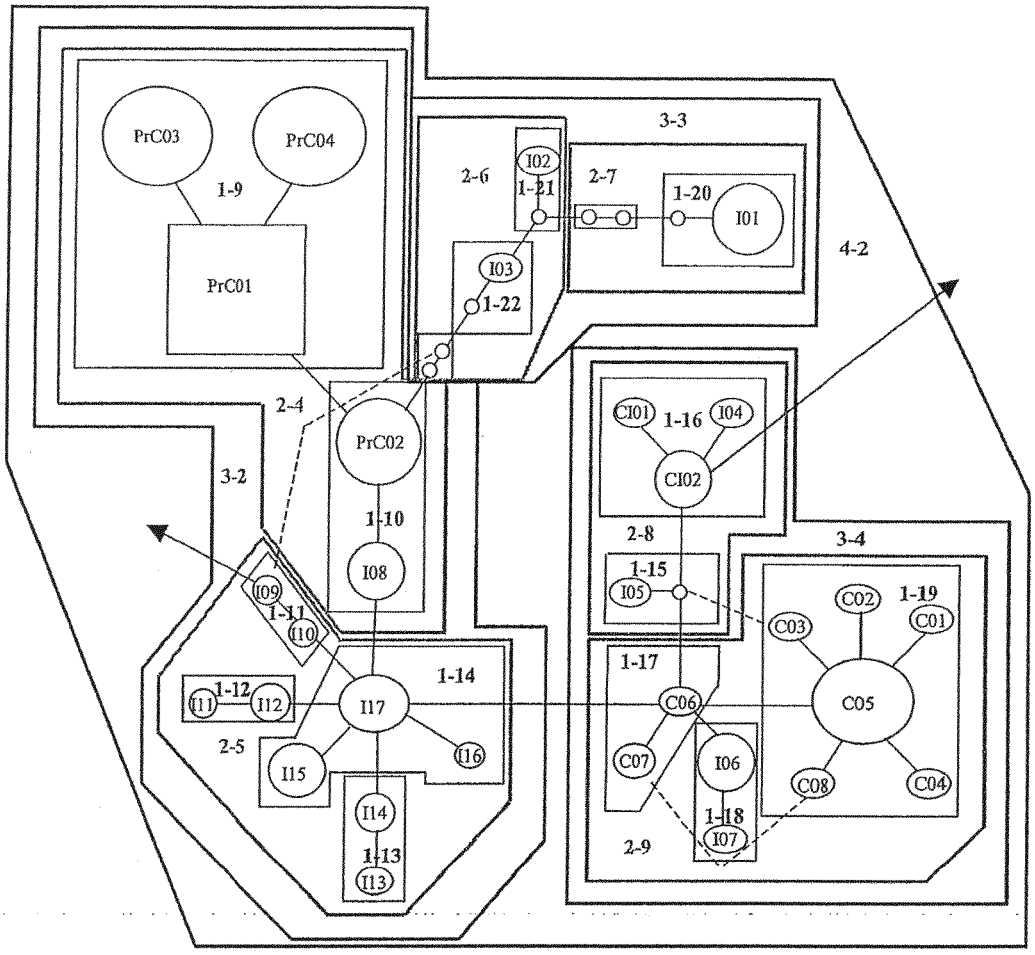
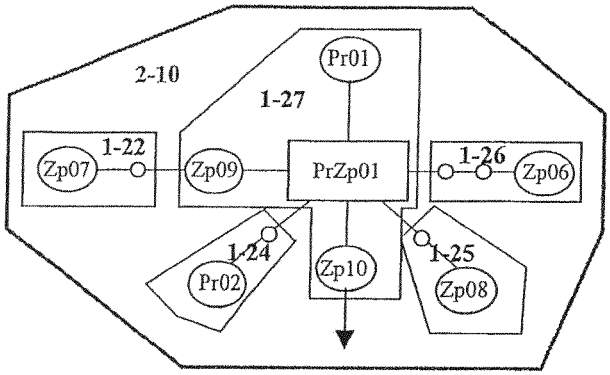
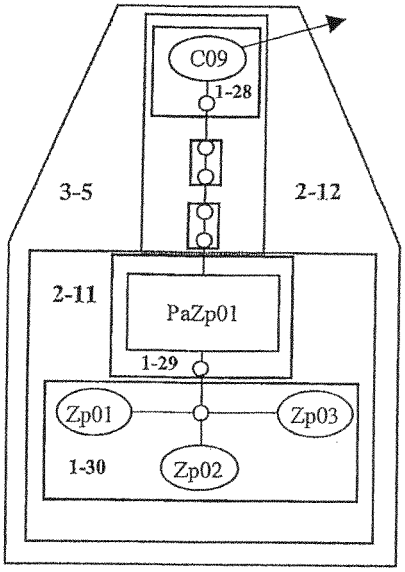


Figure 9. Haplotype network 2 estimated in TCS with 1, 2, 3, and 4 step nesting groups shown. Schematics are the same as in Figure 7. Network consists of haplotypes mostly from *Z. h. preblei*, *Z. h. campestris* and *Z. h. intermedius* individuals.

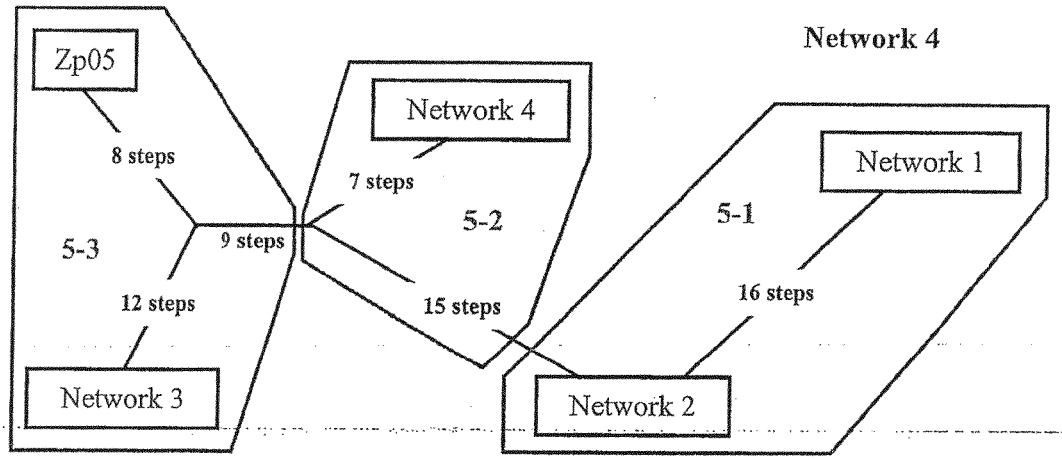
and 3-1 indicate restricted gene flow with isolation by distance and a contiguous range expansion but these results depend on adequate sampling for *Z. hudsonius* populations in eastern Colorado and Kansas. Although further investigation via more dense sampling in New Mexico, Kansas and eastern Colorado is merited to illuminate the extent of separation between the *Z. h. luteus* populations of New Mexico and various *Z. h. pallidus* populations of Kansas and Nebraska, taken as a single unit (*Z h. pallidus* + *Z. h. luteus*) evidence based on the CR sequence data seems to indicate some separation from the other *Z. hudsonius* populations sampled in these studies. Evidence for this includes, clustering into a single network separated from all other networks by a minimum of 16 mutational steps (Figure 10) and the inference of possible fragmentation within clade 5-1 (Figure 12, Table 5) between clades 4-1 (network 1) and 4-2 (network 2).



Network 3



Network 4



Total Network

Figure 10. Haplotype networks 3, 4 and the total network. The total network represent connections above the 95% parsimony cut-off. Schematics are the same as in Figure 7. Networks 3 and 4 consist of haplotypes mostly from *Z. princeps* individuals but contain some individuals from *Z. hudsonius* subspecies indicating possibly low levels of gene flow.

Network 2 (Figure 9) consists of haplotypes primarily derived from *Z. h. preblei*, *Z. h. campestris*, and *Z. h. intermedius* individuals. Like network 1 haplotypes from different subspecies show some clustering but no subspecies form exclusive groups. Further, all the *Z. h. preblei* haplotypes found in this network are shared with *Z. h. campestris* haplotypes (although in every case the group is dominated by *Z. h. preblei* samples). Clades 1-9 (Figure 11) and 3-2 (figure 12) illustrate the geographical

connection between these haplotypes. At no clade level do the *Z. h. preblei* haplotypes separate out. Taking frequency differences into account the NCA inferences for these clades indicate that contiguous range expansion is the best-supported conclusion in both cases (Table 9). At the deeper clade level (Clade 4-2) NCA indicates that restricted gene flow occurs within the *preblei/campestris/intermedius* cluster but is best explained by

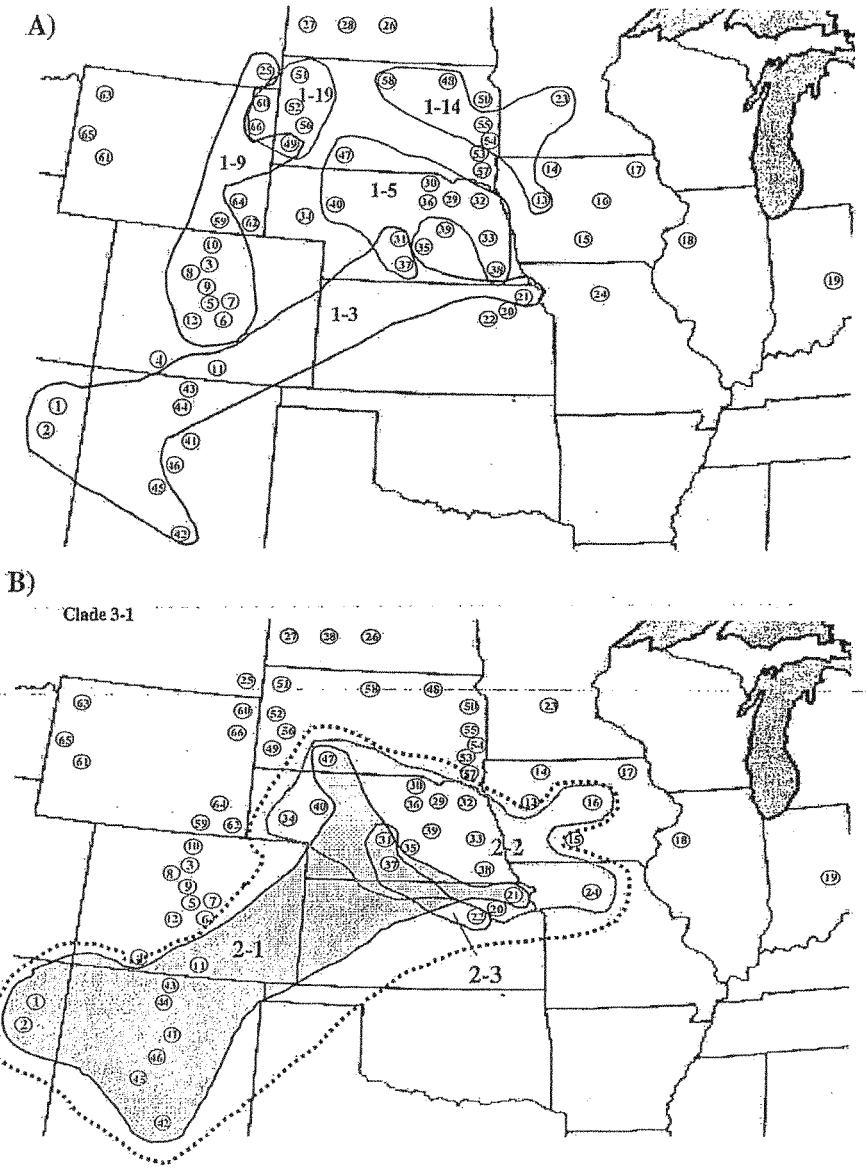


Figure 11. Geographic spread of selected clades used in NCA. Population numbers correspond to those in Table 1.

long distance dispersal for the populations 15, 18, and 19 (Table 1 and Figure 12) and isolation by distance for the vast majority of all other *preblei, campestris,* and *intermedius* populations.

Networks 3 and 4 and haplotype Zp05 were comprised primarily of *Z. princeps* individuals with the exception of a few *Z. hudsonius* individuals. These divergent *Z. hudsonius* samples were discarded by Ramey et al. 2005 under the assumption that they were misclassified. This may very well be the case but as mentioned above the possible introgression of *Z. princeps* haplotypes into *Z. hudsonius* populations should be

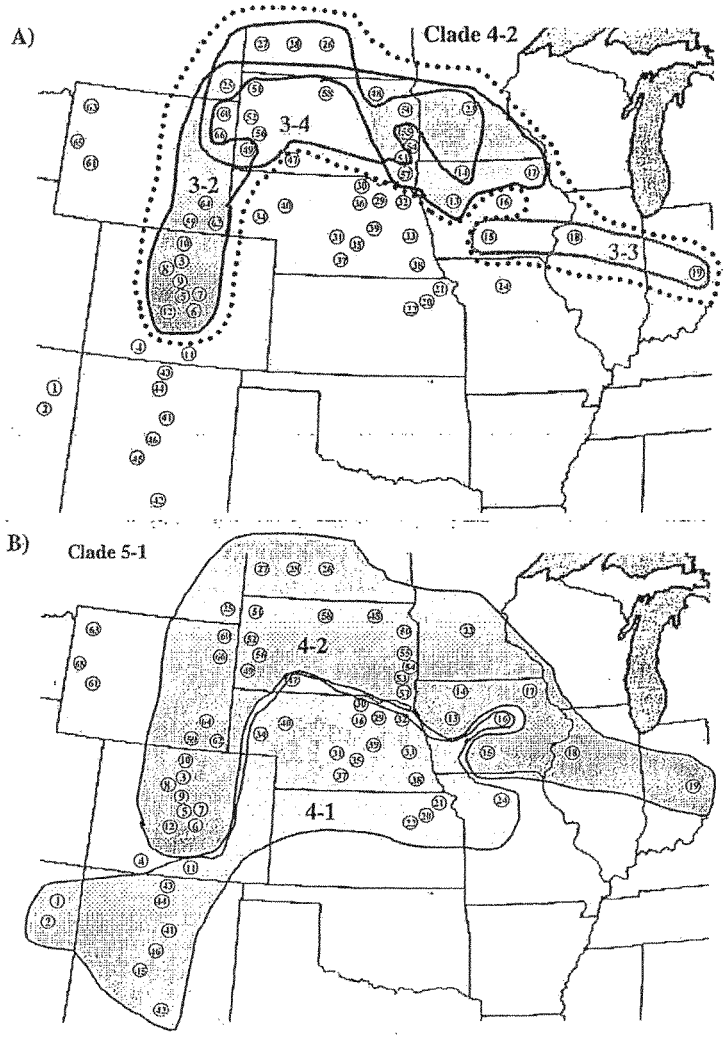


Figure 12. Geographic spread of selected clades used in NCA. Population numbers correspond to those in Table 1.

considered further. Few *Z. princeps* were sampled and thus few conclusions can be drawn from NCA aside from inadequate geographic sampling in Clade 5-1 (Table 9).

V. Subspecies designation

Much debate has centered around diagnosing units below the species level (Green 2005). At this time no established universally accepted criteria exist for diagnosing subspecies. Other units such as populations (Waples and Gaggiotti 2006) and evolutionary significant units have received more attention but still a number of competing criteria are used (Moritz 2002, Waples 2005). Crandall et al. 2000 established a methodology for rejecting or accepting evidence of distinctiveness based on genetic, ecological, recent, and historical categories. Crandall et al. established recommended management actions based on the relative strength of evidence for 8 separate cases. A criticism of the King et al. 2006 study is that no criteria are offered. However, Ramey et al. 2005, on the other hand, used the robust criteria from Crandall et al. 2000 to diagnose Distinct Vertebrate Population Segments (DPS) and concluded that 'our results for *Z. h. preblei* and its neighboring populations [*Z. h. campestris* and *Z. h. intermedius*] do not appear to support the discrete requirement'. Cases such as that of the *Z. h. preblei* illustrate the need for explicit criteria to be established at the species, subspecies, and DPS level (Sites & Crandall 1997) as were detailed and followed in Ramey et al., but not in King et al. The 'fuzziness' of boundaries at each of these levels makes this a challenge but a diversity of data from ecologists, taxonomists, population geneticists, and phylogeneticists coupled with explicit criteria evaluated in a robust hypothesis testing framework make it attainable.

VI. Conclusion

A number of general conclusions can be drawn from our reanalysis of the Ramey et al . 2005 and King et al. 2006 data sets. First, the highest resolution analysis performed in this study was the STRUCTURE analysis using microsatellite data. With both the King

et al. and the Ramey et al. data sets, we saw clusters of subspecies into three groups consisting of *Z. h. pallidus* + *Z h. luteus, Z. h. intermedius* + *Z. h. campestris,* and *Z. h. preblei*, best accounted for the genetic variation. Although some admixture was evident in the STRUCTURE analysis and moderate levels of migration rates were estimated between these clusters, we still believe relatively good population boundaries exist between these three groups. This is consistent with general population structure within a species and similar structure is found in, for example, human populations (Rosenberg et al. 2005). Such structure does not warrant conservation action and is recent in evolutionary origin.

Analysis of the mtDNA data revealed two more inclusive groupings. Phylogenetic analysis of the CytB and CR data sets fairly consistently revealed two major clades. One consisted of a combined *Z. h. intermedius* + *Z. h. campestris* + *Z. h. preblei* group and the other a combined *Z. h. pallidus* + *Z h. luteus* group. In most analysis these groups were monophyletic or nearly so but almost without exception none of the subspecies ever formed monophyletic groups within these distinct clades. The same 'non-exclusive' pattern was evident in the haplotype networks. Add to this the NCA results where range expansion or restricted gene flow with isolation by distance were inferred for most clades at most level and preponderance of evidence indicates extensive gene flow within the groups. The most parsimonious conclusion based on the current available data suggests that *Z. h. pallidus* + *Z. h. luteus* may represent a distinct 'subspecies' or population segment or ESU and *Z. h. intermedius* + *Z. h. campestris* + *Z. h. preblei* form another. This conclusion is consistent with that drawn in Ramey et al. 2005.

When taken as a whole, the preponderance of evidence (mtDNA [both control region and cytb] and morphometric data indicate that the taxon *Z. h. preblei* is not a valid taxonomic unit. The microsatellite data suggest that there may be recent population subdivision consistent with subdivision within, for example, humans (Rosenberg at al. 2005). The data clearly show a grouping of *Z. h. pallidus* + *Z. h. luteus* relative to *Z. h. intermedius* + *Z. h. campestris* + *Z. h. preblei*. These groupings form what we would consider ESUs and demonstrate the invalidity of the current taxonomy for these subspecies and throws questions on the taxonomy of the species complex as a whole. If

we employ the Crandall et al. (2000) criteria for ESU designation on the "subspecies" *Z. h. preblei*, the subspecies shows, at most, recent genetic inexchangeability, with historical exchangeability and ecological exchangeability both recently and historically. As such, it would fall under Case 8 with a management recommendation to "treat as a single population" (*Z. h. intermedius* + *Z. h. campestris* + *Z. h. preblei*). Note that the "subspecies" *Z. h. preblei* would also not be found to be an ESU under the more stringent criteria of Mortiz (1994), the criterion used by King et al. in their assessment, due to the lack of reciprocal monophyly as required by the Moritz (1994) definition. Thus using an explicit framework for testing taxonomic boundaries (Crandall et al. 2000 & Mortiz 1994), we find that the "subspecies" *Z. h. preblei* does not even warrant ESU status, let alone subspecific status and therefore recommend it be delisted as an endangered entity under the Endangered Species Act.

Literature Cited

Beauvais GP. Preble's meadow jumping mouse (*Zapus hudsonius preblei*) in Wyoming: status report, July 2001. Laramie, Wyoming.

Beerli P, and Felsenstein J. 2001. Maximum likelihood estimation of a migration matrix and effective population sizes in n subpopulations by using a coalescent approach. PNAS 98: 4563-4568.

Clement M, Posada D, and Crandall KA. 2000. TCS: a computer program to estimate gene genealogies. Mol. Ecol. (: 1657-1659.

Crandall KA, templeton AR. 1993. Empirical tests of some predictions of coalescent theory with applications to intraspecific phylogeny reconstruction. Genetics 134: 959-969.

Edgar RC. 2004. MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Research 32: 1792-1979.

Green DM. 2005. Designatable units for status assessment of endangered species. Conserv. Biol. 1813-1820.

Hamilton G, Currat M, Ray N, Heckel G, Beaumont M, and Excoffier L. 2005. Bayesian estimation of recent migration rates after a spatial expansion. Genetics 170: 409-417.

Herron MD, Waterman JM, and Parkinson CL. 2005. Phylogeny and historical biogeography of African ground squirrels; the role of climate change in the evolution of *Xerus*. Mol. Ecol. 14: 2773-2788.

Huelsenbeck JP, Ronquist F, 2001. MrBayes: Bayesian inference of phylogeny. Bioinformatics 17: 754--755.

King TL, Switzer JF, Morrison CL, Eackles MS, Young CC, Lubinski B, and Cryan P. 2006. Comprehensive analysis of molecular phylogeographic structure among meadow jumping mice (*Zapus hudsonius*) reveals evolutionarily distinct subspecies. A report submitted to U.S. Fish and Wildlife Service. January 27, 2006.

Krutzsch PH. 1954. North American jumping mice (genus *Zapus*) Univ. Kansas Publ. Mus. Nat. Hist. 4: 349-472.

Morando M, Avila LJ, and Sites JW. 2003 Sampling strategies for delimiting species: Genes, individuals, and populations in the *Liolaemus elongates-kriegi* complex

(Squamata: Liolaemidae) in Andean-Patagonian South America. Systematic Biology 52:159-185.

Moritz C. 1994. Defining 'evolutionary significant units' for conservation. Trends Ecol Evol. 9: 373-375.

Moritz C. 2002. Strategies to protect biological diversity and the evolutionary processes that sustain it. Syst. Biol. 51: 238-254.

Pague C, and Grunau L. 200. Factbook on the Preble's meadow jumping mouse (*Zapus hudsonius preblei*): 9 january 2000 draft. Preble's Meadow Jumping Mouse Science Team, Boulder, Colorado.

Pritchard JK, Stephens M, and Donnelly P. 2000. Inference of population structure using multilocus genotype data. Genetics 155: 945-959.

Posada D, Crandall KA, 1998. ModelTest: testing the model of DNA substitution. Bioinformatics 14: 817--818.

Posada D, Crandall KA, Templeton AR. 2000. GEODIS: a program for the cladistic nested analysis of the geographical distribution of genetic haplotypes. Mol. Ecol. (: 487-488.

Ramey RR II, Liu H, Epps CW, Carpenter LM, and Wehausen JD. 2005. Genetic relatedness of the Preble's meadow jumping (*Zapus hudsonius preblei*) to nearby subspecies of *Z. hudsonius* as inferred from variation in cranial morphology, mitochondrial DNA and microsatellite DNA: implications for taxonomy and conservation. Animal Cons. 8: 329-346.

Riggs LA, Dempcy JM, and Orrego C. 1997. Evaluating distinctiveness and evolutionary significance of Preble's meadow jumping mouse: phylogeography of mitochondrial DNA non-coding region variation. Colorado Division of Wildlife, Denver, Colorado.

Schorr RA. 2001 Meadow jumping mouse (*Zapus hudsonius preblei*) on the U. S. Air Force Academy El Paso County, Colorado. USDOD Air Force Academy, Colordo.

Sites, JW, Jr. and Crandall KA. 1997 Testing species boundaries in biodiversity studies. Conservation Biology 11:1289-1297.

Swofford DL. 1999. PAUP*: phylogenetic analysis using parsimony (*and other methods). Ver. 4. Sinauer, Sutherland, MA

Templeton AR. 1998. Nested clade analysis of phylogeographic data: testing hypotheses about gene flow and population history. Mol. Ecol. 7: 381-397.

Templeton AR. 2004. Statistical phylogeography: methods of evaluating and minimizing inference errors. Mol. Ecol. 13: 789-809.

Templeton AR, Routman E, and Phillips CA. 1995. Separating population structure from population history: a cladistic analysis of the geographical distribution of mitochondrial DNA haplotypes in the Tiger Salamander, *Ambystoma tigrinum*. Genetics 140: 767-782.

Waples, RS. 2005. Identifying conservation units of Pacific salmon using alternative ESU concepts. In Press in DD Goble, JM Scott and EW Davis, editors. The endangered species act at thirty: renewing the conservation promise. Island Press, Washington, D.C.

Waples RS, and Gaggliotti O. 2006. What is a population? An empirical evaluation of some genetic methods for identifying the number of gene pools and their degree of connectivity. Mol. Ecol. 15: 1419-1439.

Wlasiuk G, Carlos JC, and Lessa EP. 2003. Genetic and geographic differentiation in the Rio Negro Tuco-Tuco (*Ctenomys Rionegrensis*): Inferring the roles of migration and drift from multiple markers. Evolution 57: 913-926.

Zheng X, Arbogast BS, and Kenagy GJ. 2003. Historical demography and genetic structure of sister species: deermice (*Peromyscus*) in the North American temperate rain forest. Mol. Ecol. 12: 711-724.

Table 1. Localities for all samples the King et al. & Ramey et al. studies. Abbr = abbreviations used in combined data set to indicate county and state of sample. Zhl = *Zapus hudsonius luteus*, Zhpr = *Zapus hudsonius preblei*, Zhi = *Zapus hudsonius intermedius*, Zhpa = *Zapus hudsonius pallidus*, Zhc = *Zapus hudsonius campestris*, ZPp = *Zapus princeps princeps*, ZPid = *Zapus princeps idahoensis*, ZPut = *Zapus princeps utahensis*. Samples were pooled by county and GPS coordinates taken from geographical center of county.

	State	County	Abbr	N	Species	GPS coordinates
1	Arizona	Apache	ApAZ	3	Zhl	N 35° 50' W 109° 32'
2		Navajo	NCAZ	4	Zhl	N 35° 42' W 110° 37'
3	Colorado	Boulder	BCCO	9	Zhpr	N 40° 07' W 105° 29'
4		Conejo	CCCO	1	Zhpr	N 37° 15' W 105° 58'
5		Douglas	DCCO	74	Zhpr	N 39° 18' W 105° 12'
6		El Paso	ECCO	61	Zhpr	N 39° 04' W 104° 15'
7		Elbert	EbCO	1	Zhpr	N 39° 16' W 103° 34'
8		Gilpin	GCCO	1	Zhpr	N 39° 52' W 105° 40'
9		Jefferson	JCCO	1	Zhpr	N 39° 30' W 105° 18'
10		Larimer	LCCO	33	Zhpr	N 40° 40' W 105° 38'
11		Las Animas	LACO	8	Zhl	N 37° 13' W 103° 23'
12		Teller	TCCO	2	Zhpr/ZPp	N 39° 46' W 105° 18'
13	Iowa	Buena Vista	BVIA	1	Zhi	N 42° 38' W 95° 12'
14		Emmet	ECIA	3	Zhi	N 43° 24' W 94° 50'
15		Marion	MCIA	1	Zhi	N 41° 19' W 93° 06'
16		Tama	TCIA	1	Zhi	N 42° 04' W 92° 24'
17		Winneshiek	WCIA	1	Zhi	N 43° 18' W 91° 47'
18	Illinois	Henry	HCIL	1	Zhi	N 41° 06' W 90° 12'
19	Indiana	Wayne	WCIN	1	Zhi	N 39° 36' W 85° 02'
20	Kansas	Douglas	DCKS	2	Zhpa	N 38° 57' W 95° 23'
21		Leavenworth	LCKS	2	Zhpa	N 39° 20' W 94° 59'
22		Osage	OCKS	2	Zhpa	N 38° 38' W 95° 48'
23	Minnesota	Morrison	MCMN	21	Zhi	N 46° 13' W 94° 34'
24	Missouri	Macon	MAMO	2	Zhpa	N 39° 45' W 92° 52'
25	Montana	Carter	CCMT	5	Zhc	N 45° 23' W 104° 42'
26	North Dakota	Burleigh	BCND	6	Zhi	N 47° 14' W 100° 12'
27		Dunn	DCND	5	Zhi	N 47° 23' W 102° 52'
28		Mercer	MCND	1	Zhi	N 47° 20' W 102° 01'
29	Nebraska	Antelope	ACNE	4	Zhpa	N 42° 04' W 97° 58'
30		Boyd	BONE	1	Zhpa	N 42° 52' W 98° 42'
31		Buffalo	BCNE	25	Zhpa	N 40° 47' W 99° 09'
32		Dixon	DCNE	1	Zhpa	N 42° 41' W 97° 02'
33		Dodge	DGNE	1	Zhpa	N 41° 42' W 96° 50'
34		Garden	GCNE	2	Zhpa	N 41° 41' W 102° 20'
35		Hall	HCNE	3	Zhpa	N 40° 55' W 98° 22'
36		Holt	HONE	2	Zhpa	N 42° 27' W 98° 39'
37		Kearney	KCNE	11	Zhpa	N 40° 30' W 98° 57'
38		Lancaster	LCNE	1	Zhpa	N 40° 51' W 96° 43'

39		Merrick	MCNE	1	Zhpa	N 41° 07' W 97° 60'
40		Thomas	TCNE	1	Zhpa	N 41° 58' W 100° 33'
41	New Mexico	Bernalillo	BCNM	1	Zhl	N 35° 30' W 106° 46'
42		Otero	OCNM	7	Zhl	N 32° 48' W 105° 45'
43		Rio Arriba	RANM	2	Zhl	N 36° 32' W 106° 47'
44		Sandoval	SCNM	26	Zhl	N 35° 52' W 106° 48'
45		Socorro	SONM	1	Zhl	N 33° 50' W 107° 11'
46		Valencia	VCNM	1	Zhl	N 34° 46' W 106° 58'
47	South Dakota	Bennett	BeCSD	18	Zhpa	N 43° 10' W 101° 50'
48		Brown	BrCSD	33	Zhi	N 45° 37' W 98° 26'
49		Custer	CCSD	29	Zhc	N 43° 43' W 103° 01'
50		Deuel	DCSD	3	Zhi	N 44° 40' W 96° 49'
51		Harding	HCSD	3	Zhc	N 45° 27' W 103° 33'
52		Lawrence	LaSD	3	Zhc	N 44° 29' W 103° 44'
53		Lincoln	LCSD	2	Zhi	N 43° 15' W 96° 48'
54		Minnehaha	MCSD	3	Zhi	N 43° 42' W 96° 44'
55		Moody	MOSD	1	Zhi	N 43° 50' W 96° 40'
56		Pennington	PCSD	9	Zhc	N 44° 13' W 102° 30'
57		Union	UCSD	1	Zhi	N 42° 59' W 96° 42'
58		Walworth	WCSD	5	Zhi	N 45° 22' W 100° 03'
59	Wyoming	Albany	AbWY	16	Zhp/ZPp	N 41° 18' W 105° 32'
60		Crook	CCWY	33	Zhc	N 44° 38' W 104° 46'
61		Fremont	FCWY	3	ZPid	N 43° 04' W 108° 14'
62		Larimae	LCWY	2	Zhpr	N 41° 09' W 104° 33'
63		Park	PaWY	3	Zpid	N 44° 31' W 109° 25'
64		Platte	PCWY	1	Zhpr	N 41° 58' W 104° 46'
65		Teton	TCWY	4	ZPut	N 43° 59' W 110° 12'
66		Weston	WCWY	4	Zhc	N 43° 52' W 104° 35'

Table 2- A summary of likelihoods scores for each STRUCTURE run for Ramey et al. data, average likelihood scores for each K for all runs, and a visual representation of the absolute values of these scores.

	K									
	1	2	3	4	5	6	7	8	9	10
Run 01	-5366.8	-5057.1	-4802.5	-4695.5	-4590.1	-4582	-4508.1	-4471.6	-4420.6	-4725.8
	-5364.7	-5049.4	-4799.6	-4694.9	-4585	-4530.1	-4517.5	-4507.9	-4463	-4402.4
	-5365.7	-5055.5	-4805.7	-4697.2	-4593.3	-4596.5	-4502.8	-4460.6	-4425	-4409.8
	-5365.9	-5065.1	-4794.3	-4726.5	-4815.9	-4533.3	-4505.8	-4520	-4421.9	-4411.1
	-5360.4	-5075.1	-4801.5	-4717.4	-4586.6	-4527.1	-4509.3	-4461.3	-4413.6	-4452.7
	-5361.2	-5077.3	-4803.9	-4814.8	-4672	-4603.4	-4504	-4445.3	-4421.6	-4434.7
	-5367.2	-5054.1	-4795.4	-4703	-4585	-4802.7	-4508	-4467.9	-4403.9	-4412.6
	-5364.6	-5085.8	-4801.4	-4759.2	-4582.6	-4571.2	-4516.7	-4468.3	-4417.4	-4415.1
	-5365.4	-5058.8	-4801	-4707	-4585.5	-4523.2	-4515.8	-4479.8	-4425.5	-4411
Run 10	-5359.7	-5084.7	-4805.5	-4702.4	-4582.5	-4576.3	-4504.3	-4449.3	-4409.2	-4428.9
Aver. -Ln	5364.16	-5066.29	-4801.08	-4721.79	-4617.85	-4584.58	-4509.23	-4473.2	-4422.17	-4450.41

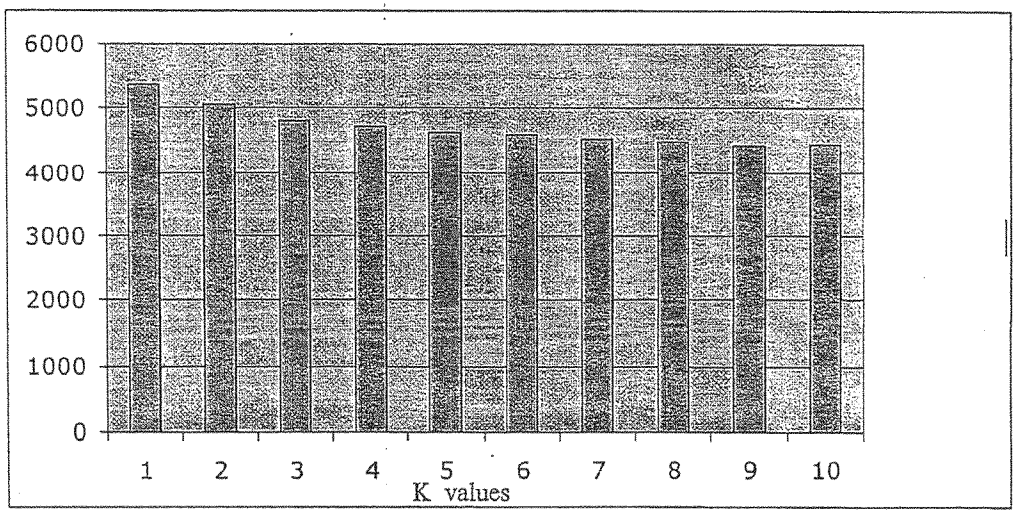


Table 3. Inferred ancestry of all individuals based on the run with the best likelihood score (-4794.3, run 4, Table 2) at K = 3. Probabilities in bold text indicate cluster with highest assignment. Individuals in bold red text indicate individuals with mixed ancestry (no probability > 0.80) and individuals that belong to one subspecies but have highest probability ancestry assigned to a cluster with predominately individuals from a different subspecies are indicated in green. Importantly, individuals indicated in blue text are ones that are *Z.h. campestris* who have a high probability assignment to cluster 3 but have *Z.h. preblei* control region mitochondrial DNA. Cluster 1 consists of predominately *Z.h. preblei* individuals, Cluster 2 consists of predominately *Z.h. luteus* and *Z.h. pallidus* individuals, and, Cluster 3 consists of predominately *Z.h. campestris* and *Z.h. intermedius* individuals.

Inferred ancestry of individuals:

Label	(%Miss)	: Inferred clusters		
		: Clstr 1	Clstr 2	Clstr 3
1 ZhprNM871	(0)	: **0.983**	0.010	0.008
2 M872Zhpr	(0)	: **0.860**	0.035	0.105
3 M876Zhpr	(0)	: **0.970**	0.013	0.018
4 **M877Zhpr**	(0)	: **0.763**	**0.107**	**0.130**
5 TK86021Zhpr	(0)	: **0.840**	0.039	0.121
6 TK86034Zhpr	(0)	: **0.899**	0.026	0.076
7 **TK86048Zhpr**	(0)	: **0.182**	**0.792**	0.027
8 TK86090Zhpr	(0)	: **0.873**	0.018	0.108
9 TK86105Zhpr	(0)	: **0.973**	0.013	0.014
10 **TK86074Zhpr**	(0)	: **0.477**	**0.476**	0.047
11 TK86094Zhpr	(0)	: **0.895**	0.092	0.013
12 9A34Zhpr	(0)	: **0.977**	0.011	0.012
13 9B89Zhpr	(0)	: **0.948**	0.024	0.028
14 M874Zhpr	(0)	: **0.976**	0.010	0.014
15 TK86081Zhpr	(0)	: **0.980**	0.010	0.011
16 TK86109Zhpr	(0)	: **0.962**	0.024	0.014
17 TK86117Zhpr	(0)	: **0.942**	0.023	0.036
18 TK86095Zhpr	(0)	: **0.952**	0.019	0.029
19 TK86096Zhpr	(0)	: **0.970**	0.014	0.016
20 TK86097Zhpr	(0)	: **0.965**	0.028	0.007
21 TK86098Zhpr	(0)	: **0.973**	0.010	0.017
22 TK86026Zhpr	(0)	: **0.986**	0.006	0.008
23 TK86029Zhpr	(0)	: **0.959**	0.030	0.011
24 TK86030Zhpr	(0)	: **0.918**	0.072	0.009
25 TK86031Zhpr	(0)	: **0.979**	0.009	0.012
26 TK86032Zhpr	(0)	: **0.958**	0.020	0.022
27 TK86080Zhpr	(0)	: **0.971**	0.007	0.022
28 TK86083Zhpr	(0)	: **0.978**	0.014	0.008
29 TK86115Zhpr	(0)	: **0.977**	0.010	0.013
30 TK86116Zhpr	(0)	: **0.977**	0.013	0.011
31 TK86120Zhpr	(0)	: **0.981**	0.008	0.011

32 TK86121Zhpr	(0)	: **0.983** 0.008 0.009
33 TK86122Zhpr	(0)	: **0.986** 0.006 0.008
34 TK86196Zhpr	(0)	: **0.946** 0.038 0.016
35 TK86163Zhpr	(0)	: **0.977** 0.009 0.014
36 M875Zhpr	(0)	: **0.987** 0.007 0.006
37 TK51406Zhpr	(0)	: **0.893** 0.016 0.090
38 TK86124Zhpr	(0)	: **0.980** 0.012 0.008
39 TK86088Zhpr	(0)	: **0.944** 0.045 0.011
40 M879Zhpr	(0)	: **0.988** 0.006 0.006
41 M1166Zhpr	(0)	: **0.951** 0.008 0.041
42 TK86093Zhpr	(0)	: **0.978** 0.009 0.013
43 TK86106Zhpr	(0)	: **0.985** 0.009 0.006
44 TK86107Zhpr	(0)	: **0.974** 0.012 0.013
45 TK86118Zhpr	(0)	: **0.971** 0.009 0.019
46 TK86165Zhpr	(0)	: **0.982** 0.007 0.011
47 TK86166Zhpr	(0)	: **0.981** 0.011 0.008
48 TK86167Zhpr	(0)	: **0.954** 0.035 0.011
49 TK86169Zhpr	(0)	: **0.986** 0.006 0.008
50 TK86170Zhpr	(0)	: **0.983** 0.008 0.009
51 TK86173Zhpr	(0)	: **0.976** 0.010 0.014
52 TK86182Zhpr	(0)	: **0.988** 0.006 0.006
53 TK86183Zhpr	(0)	: **0.982** 0.008 0.010
54 TK86185Zhpr	(0)	: **0.983** 0.008 0.009
55 **ZhcTK86190**	(0)	: 0.100 **0.229 0.671**
56 **TK86191Zhc**	(0)	: **0.755** 0.015 **0.230**
57 KU123597Zhc	(0)	: 0.028 0.046 **0.926**
58 KU123598Zhc	(0)	: 0.014 0.009 **0.977**
59 **KU123599Zhc**	(0)	: 0.095 **0.535 0.370**
60 KU101558Zhc	(0)	: 0.148 0.026 **0.827**
61 KU109972Zhc	(0)	: 0.023 **0.283 0.694** --- Zhp mtDNA
62 KU109978Zhc	(0)	: 0.053 0.010 **0.937** --- Zhp mtDNA
63 **KU109984Zhc**	(0)	: **0.382 0.411** 0.207 --- Zhp mtDNA
64 KU109985Zhc	(0)	: 0.014 0.017 **0.969** --- Zhp mtDNA
65 KU110013Zhc	(0)	: 0.005 0.007 **0.988** --- Zhp mtDNA
66 **KU7Zhc**	(0)	: **0.466 0.054** 0.480
67 KU8Zhc	(0)	: 0.026 0.015 **0.959**
68 KU13Zhc	(0)	: 0.036 0.017 **0.947**
69 KU14Zhc	(33)	: 0.016 0.011 **0.973**
70 KU18Zhc	(0)	: 0.008 0.023 **0.969**
71 KU19Zhc	(0)	: 0.007 0.012 **0.980**
72 KU20Zhc	(0)	: 0.007 0.098 **0.895**
73 KU23Zhc	(16)	: 0.013 0.023 **0.964**
74 **KU24Zhc**	(0)	: **0.718** 0.068 0.215
75 **KU25Zhc**	(0)	: **0.261** 0.028 **0.711**
76 **KU26Zhc**	(0)	: **0.225** 0.022 **0.753**

77	KU1235Zhc	(0)	: 0.007 0.008 **0.985**
78	KU123593Zhc	(0)	: 0.006 0.008 **0.986**
79	KU27Zhc	(16)	: 0.012 0.015 **0.972**
80	KU28Zhc	(16)	: 0.007 0.010 **0.983**
81	KU29Zhc	(0)	: 0.067 0.008 **0.925**
82	**KU30Zhc**	(0)	: **0.228** 0.046 **0.726**
83	KU34Zhc	(0)	: 0.013 0.008 **0.979**
188	**K127252Zhia**	(0)	: 0.008 **0.953** 0.040
189	**K112830Zhi**	(0)	: 0.005 **0.980** 0.014
190	**K116263Zhi**	(0)	: **0.389 0.315 0.295**
191	K116264Zhi	(0)	: 0.027 0.090 **0.883**
192	**K116266Zhi**	(0)	: 0.034 **0.481 0.485**
193	**K116269Zhi**	(0)	: 0.006 **0.958** 0.035
194	**K104062Zhi**	(0)	: 0.165 **0.410 0.425**
195	**K108068Zhi**	(0)	: 0.025 **0.728 0.247**
84	DMNS7764Zhi	(0)	: 0.013 0.008 **0.978**
85	K115895Zhi	(0)	: 0.008 0.040 **0.952**
86	K115896Zhi	(0)	: 0.007 0.009 **0.984**
87	K115897Zhi	(0)	: 0.010 0.031 **0.960**
88	K123021Zhi	(0)	: 0.013 0.020 **0.967**
89	K123022Zhi	(0)	: 0.014 0.034 **0.952**
90	K123031Zhi	(0)	: 0.007 0.133 **0.860**
91	K123032Zhi	(0)	: 0.023 0.013 **0.964**
92	K123033Zhi	(0)	: 0.008 0.015 **0.976**
155	**K140721Zhi**	(0)	: **0.521** 0.132 **0.347**
156	**K140722Zhi**	(0)	: **0.199 0.385 0.416**
157	**K153176Zhi**	(0)	: 0.010 **0.202 0.788**
158	K153177Zhi	(0)	: 0.017 0.030 **0.953**
159	K153180Zhi	(0)	: 0.039 0.014 **0.947**
160	K153181Zhi	(0)	: 0.040 0.043 **0.917**
161	**K153190Zhi**	(0)	: **0.156** 0.059 **0.785**
162	K153196Zhi	(0)	: 0.013 0.028 **0.960**
163	K147018Zhi	(0)	: 0.017 0.011 **0.972**
164	K147020Zhi	(0)	: 0.019 0.017 **0.964**
165	K153201Zhi	(0)	: 0.119 0.017 **0.864**
166	K153203Zhi	(8)	: 0.091 0.013 **0.897**
167	**K153205Zhi**	(0)	: 0.044 **0.919** 0.038
168	K153212Zhi	(0)	: 0.021 0.062 **0.918**
169	K115700Zhi	(0)	: 0.013 0.026 **0.962**
170	K115702Zhi	(0)	: 0.010 0.040 **0.950**
171	**K115710Zhi**	(0)	: 0.026 **0.600 0.374**
172	**K120017Zhi**	(0)	: 0.042 **0.701 0.256**
173	K120018Zhi	(0)	: 0.026 0.012 **0.962**
174	K120019Zhi	(0)	: 0.018 0.050 **0.931**
175	**K153215Zhi**	(0)	: 0.027 **0.197 0.775**

176	**K153221Zhi**	(0)	: 0.088 **0.792** **0.120**
177	K115730Zhi	(0)	: 0.008 0.012 **0.980**
178	K115731Zhi	(0)	: 0.006 0.052 **0.942**
179	K115732Zhi	(0)	: 0.023 0.018 **0.958**
180	K116265Zhi	(0)	: 0.025 0.012 **0.963**
181	K159190Zhi	(0)	: 0.094 0.053 **0.853**
182	K153230Zhi	(0)	: 0.099 0.035 **0.865**
183	K153229Zhi	(0)	: 0.016 0.007 **0.977**
93	**ZhpaUNL1UNS**	(0)	: 0.054 **0.655** **0.292**
94	ZhpaUNL2UNS	(0)	: 0.015 **0.916** 0.069
95	ZhpaUNL3UNS	(0)	: 0.017 **0.957** 0.026
96	**ZhpaUNL4UNS**	(0)	: 0.010 **0.372** **0.617**
97	**ZhpaUNL5UNS**	(0)	: 0.107 **0.652** **0.241**
98	ZhpaUNL7UNS	(0)	: 0.016 **0.971** 0.013
99	ZhpaUNL8UNS	(0)	: 0.010 **0.946** 0.044
100	ZhpaUNL9UNS	(0)	: 0.031 **0.817** 0.152
101	ZhpaUNL12UN	(0)	: 0.068 **0.865** 0.067
102	ZhpaUNL16UN	(0)	: 0.015 **0.945** 0.040
103	**ZhpaUNL23UN**	(0)	: 0.015 **0.355** **0.630**
104	ZhpaUNL26UN	(0)	: 0.019 **0.892** 0.090
105	ZhpaUNL27UN	(0)	: 0.077 **0.913** 0.010
106	ZhpaUNL28UN	(0)	: 0.022 **0.953** 0.025
107	ZhpaUNL35UN	(0)	: 0.006 **0.979** 0.015
108	ZhpaUNL36UN	(0)	: 0.030 **0.860** 0.111
109	**ZhpaUNL37UN**	(0)	: 0.009 **0.738** **0.253**
110	ZhpaUNL41UN	(0)	: 0.013 **0.962** 0.025
111	ZhpaUNL42UN	(0)	: 0.023 **0.952** 0.025
112	ZhpaUNL46UN	(0)	: 0.023 **0.953** 0.024
113	ZhpaUNL51UN	(0)	: 0.010 **0.961** 0.029
114	ZhpaUNL55UN	(0)	: 0.035 **0.896** 0.070
115	ZhpaUNL56UN	(0)	: 0.023 **0.970** 0.007
116	KU40Zhpa	(0)	: 0.155 **0.801** 0.044
117	KU44Zhpa	(0)	: 0.028 **0.961** 0.011
118	KU45Zhpa	(0)	: 0.080 **0.867** 0.053
119	KU47Zhpa	(0)	: 0.008 **0.970** 0.023
120	**KU48Zhpa**	(0)	: 0.031 **0.716** **0.252**
121	KU51Zhpa	(0)	: 0.022 **0.964** 0.014
122	KU52Zhpa	(0)	: 0.014 **0.976** 0.011
184	**UNL60Zhpa**	(0)	: 0.087 0.018 **0.896**
185	**UNL61Zhpa**	(0)	: 0.126 0.040 **0.834**
186	KU53Zhpa	(0)	: 0.018 **0.975** 0.007
187	KU54Zhpa	(0)	: 0.010 **0.982** 0.008
123	DMNH8630Zhl	(0)	: 0.009 **0.978** 0.013
124	DMNH8631Zhl	(0)	: 0.016 **0.964** 0.019

125 DMNH8632Zhl	(0)	: 0.010 **0.982** 0.008
126 DMNH8633Zhl	(0)	: 0.008 **0.978** 0.014
127 DMNH8634Zhl	(0)	: 0.009 **0.975** 0.016
128 DMNH8635Zhl	(0)	: 0.008 **0.985** 0.007
129 NK856Zhl	(0)	: 0.011 **0.979** 0.010
130 NK871Zhl	(0)	: 0.008 **0.986** 0.007
131 NK884Zhl	(0)	: 0.034 **0.942** 0.023
132 NK1584Zhl	(0)	: 0.019 **0.951** 0.031
133 NK9976Zhl	(0)	: 0.014 **0.977** 0.009
134 MSB2Zhl	(0)	: 0.021 **0.960** 0.019
135 MSB4Zhl	(0)	: 0.019 **0.964** 0.017
136 MSB5Zhl	(0)	: 0.056 **0.923** 0.021
137 MSB6Zhl	(0)	: 0.014 **0.954** 0.032
138 MSB7Zhl	(0)	: 0.091 **0.882** 0.026
139 MSB8Zhl	(0)	: 0.038 **0.947** 0.015
140 MSB9Zhl	(0)	: 0.022 **0.969** 0.009
141 MSB11Zhl	(0)	: 0.006 **0.985** 0.009
142 MSB12Zhl	(0)	: 0.028 **0.963** 0.009
143 MSB14Zhl	(0)	: 0.010 **0.984** 0.006
144 MSB16Zhl	(0)	: 0.009 **0.953** 0.038
145 MSB18Zhl	(0)	: 0.012 **0.974** 0.014
146 MSB19Zhl	(0)	: 0.022 **0.858** 0.121
147 MSB20Zhl	(0)	: 0.072 **0.914** 0.013
148 MSB21Zhl	(0)	: 0.008 **0.978** 0.014
149 MSB23Zhl	(0)	: 0.013 **0.977** 0.011
150 MSB24Zhl	(0)	: 0.014 **0.975** 0.011
151 MSB25Zhl	(0)	: 0.009 **0.854** 0.137
152 MSB26Zhl	(0)	: 0.011 **0.859** 0.130
153 MSB27Zhl	(0)	: 0.026 **0.959** 0.015
154 MSB30Zhl	(0)	: 0.008 **0.982** 0.009

Table 4. Summary of results for MIGRATE analysis of Ramey et al. microsatellite data between three hypothesized populations based on three separate runs. A) Theta (Θ) is equal to the estimated effective population size and Mxy is equal to the relative importance of migration from cluster on 'x' axis into cluster on 'y' axis relative to mutation rate in introducing new variants into the population. B) Nm estimates based on Migrate results run 2.

A)

Runs	Clusters	Θ	Mxy(m/μ)			Chains
			Zhpr	Zhc/Zhi	Zhpa/Zhl	
Run 1	Zhpr	1.49165	-------	1.4871	1.1340	Short = 10
	Zhc/Zhi	4.75791	1.9401	-------	4.1631	Long = 3
	Zhpa/Zhl	4.04230	1.3132	5.1773	-------	
Run 2	Zhpr	1.86589	-------	0.9990	1.0143	Short = 10
	Zhc/Zhi	3.73727	2.2950	-------	4.4632	Long = 3
	Zhpa/Zhl	4.60656	1.0230	4.9991	-------	
Run 3	Zhpr	1.14171	-------	1.5501	1.4162	Short = 10
	Zhc/Zhi	3.80171	1.7774	-------	5.3583	Long = 3
	Zhpa/Zhl	6.90139	0.6874	3.2829	-------	

B)

	Nm(xy)		
	Zhpr	Zhc/Zhi	Zhpa/Zhl
Zhpr	---	0.46	0.47
Zhc/Zhi	2.14	---	4.17
Zhpa/Zhl	1.18	5.76	---

Number of migrants from 'x' axis cluster into 'y' axis cluster

Table 5. A list of collapsed (identical) control region mitochondrial DNA haplotypes based on a combined data set from Ramey et al. 2005 and King et al. 2006. A total of 63 different haplotypes were found. Haplotypes are named to represent subspecies where haplotype is found. Pr= *Z. h. preblei*, C = *Z. h. campestris*, Pa = *Z. h. pallidus*, I = *Z. h. intermedius*, L = *Z. h. luteus*, and Zp = *Z. princeps*.

List of haplotype names:

1. PrC01
DCCOZhprTK86026 [60]
AbWYZhprTK86098
AbWYZhprTK86124
BCCOZhprTK86021
BCCOZhprTK86034
BCCOZhprTK86048
BCCOZhprTK86090
BCCOZhprTK86105
BCCOZhprXM871
BCCOZhprXM872
BCCOZhprXM876
BCCOZhprXM877
DCCOZhprTK86029
DCCOZhprTK86030
DCCOZhprTK86031
DCCOZhprTK86032
DCCOZhprTK86080
DCCOZhprTK86083
DCCOZhprTK86115
DCCOZhprTK86116
EbCOZhprTK86163
GCCOZhprXM874
JCCOZhprTK51406
LCCOZhprTK86109
ZHprDCCOMAY215
ZHprDCCOMAY229
ZHprDCCOMAY234
ZHprDCCOMAY268
ZHprDCCOMAY281
ZHprDCCOMAY374
ZHprDCCOMAY385
ZHprDCCOMAY408
ZHprDCCOMAY416
ZHprDCCOMAY452
ZHprDCCOMAY494
ZHprDCCOMAY497
ZHprDCCOMAY517
ZHprDCCOMAY532
ZHprDCCOMAY694

ZHprDCCOMAY714
ZHprDCCOMAY748
ZHprDCCOMAY798
ZHprDCCOMAY817
ZHprDCCOMAY822
ZHprDCCOMAY880
ZHprDCCOMAY946
ZHprDCCOMAY964
ZHprDCCOMAY9813
ZHprDCCOMAY9814
ZHprDCCOMAY940
ZHprDCCOWH98100
ZHprDCCOWH98107
ZHprDCCOWH98109
ZHprDCCOWH98110
ZHprDCCOWH98301
ZHprLCCOSP169
ZHprLCCOSP223
ZHprLCCOSP861
ZHprLCCOYG9803
CCSDZhcaK110013

2. PrC02

LCCOZhpr9A43 [35]
LCCOZhpr9B89
LCCOZhprTK86081
LCCOZhprTK86117
LCWYZhprTK86074
PCWYZhprTK86094
ZHprLCCOBG9801
ZHprLCCOBG9802
ZHprLCCOCER9801
ZHprLCCOCER9802
ZHprLCCOCER9803
ZHprLCCOCER9804
ZHprLCCOCER980
ZHprLCCOHRK981
ZHprLCCOHRK982
ZHprLCCOHRK984
ZHprLCCOMC9801
ZHprLCCOMC9803
ZHprLCCONFP9801
ZHprLCCONFP9802
ZHprLCCOPGC9801
ZHprLCCOSP125
ZHprLCCOSP170
ZHprLCCOSP243
ZHprLCCOSP336
ZHprLCCOSP367
ZHprLCCOSP375
ZHprLCCOSP674

ZHprLCCOSP746
ZHprLCCOYG9801
AbWYZhprTK86095
AbWYZhprTK86096
AbWYZhprTK86097
CCSDZhcaK109984
CCSDZhcaK109985

3. PrC03
DCCOZhprTK86120 [58]
DCCOZhprTK86121
DCCOZhprTK86122
ECCOZhprTK86093
ECCOZhprTK86106
ECCOZhprTK86107
ECCOZhprTK86118
ECCOZhprTK86166
ECCOZhprTK86167
ECCOZhprXM1166
ECCOZhprXM875
ECCOZhprXM879
ZHprDCCOMAY127
ZHprDCCOMAY254
ZHprDCCOMAY368
ZHprDCCOMAY429
ZHprDCCOMAY706
ZHprDCCOMAY785
ZHprDCCOWH9801
ZHprDCCOWH9802
ZHprDCCOWH9803
ZHprDCCOWH98102
ZHprDCCOWH98103
ZHprDCCOWH9810
ZHprDCCOWH98106
ZHprDCCOWH98108
ZHprDCCOWH98120
ZHprDCCOWH98300
ZHprDCCOWH98303
ZHprDCCOWH98304
ZHprDCCOWH98305
ZHprDCCOWH98306
ZHprDCCOWH98309
ZHprDCCOWH98311
ZHprDCCOWH98312
ZHprDCCOWH98313
ZHprECCO003
ZHprECCO004
ZHprECCO005
ZHprECCO011
ZHprECCO015
ZHprECCO016

ZHprECCO020
ZHprECCO021
ZHprECCO027
ZHprECCO080
ZHprECCO087
ZHprECCO088
ZHprECCO091
ZHprECCO092
ZHprECCO093
ZHprECCO095
ZHprECCO100
ZHprECCO102
ZHprECCO103
ZHprECCO104
CCMTZhcaK123592
CCSDZhcaK109978

4. PrC04
DCCOZhprTK86196 [39]
ECCOZhprTK86165
ECCOZhprTK86169
ECCOZhprTK8617
ECCOZhprTK8613
ECCOZhprTK86182
ECCOZhprTK86183
ECCOZhprTK86185
TCCOZhprTK86088
ZHprDCCOWH9805
ZHprDCCOWH9811
ZHprDCCOWH98104
ZHprDCCOWH98121
ZHprECCO00
ZHprECCO007
ZHprECCO008
ZHprECCO010
ZHprECCO013
ZHprECCO018
ZHprECCO019
ZHprECCO024
ZHprECCO025
ZHprECCO026
ZHprECCO079
ZHprECCO081
ZHprECCO082
ZHprECCO083
ZHprECCO084
ZHprECCO085
ZHprECCO086
ZHprECCO089
ZHprECCO090
ZHprECCO094

ZHprECCO096
ZHprECCO097
ZHprECCO098
ZHprECCO099
ZHprECCO101
CCSDZhcaK109972

5. PrZp01
AbWYZhprTK86070 [10]
AbWYZhprTK86123
DCCOZPPTK86086
DCCOZPPTK8608
TCCOZPPTK8605
ZpAbWY001
ZpAbWY004
ZpAbWY005
ZpAbWY006
ZpAbWY007

6. Pr01
AbWYZhprTK86202 [1]

7. Pr02
AbWYZhprTK86113 [1]

8. Pa01
BCNEZhpaUNL9 [10]
OCKSZhpaKU47
OCKSZhpaKU48
ZhpaBCNE030
ZhpaBCNE032
ZhpaBCNE040
ZhpaBCNE047
ZhpaKCNE021
ZhpaKCNE024
ZhpaKCNE025

9. Pa02
DCKSZhpaKU40 [4]
LCKSZhpaKU44
MAMOZhpaKU5
MAMOZhpaKU52

10. Pa03
BONEZhpaUNL7 [1]

11. Pa 04
HONEZhpaUNL42 [4]
TCNEZhpaUNL55
ZhpaBeCSD008
ZhpaBeCSD014

12. Pa05
ACNEZhpaUNL2 [18]
ACNEZhpaUNL3
ACNEZhpaUNL4
ACNEZhpaUNL5
DCNEZhpaUNL23
DGNEZhpaUNL26
HONEZhpaUNL41
LCNEZhpaUNL46
ZhpaBCNE031
ZhpaBCNE036
ZhpaBCNE039
ZhpaBCNE043
ZhpaBCNE046
ZhpaKCNE019
ZhpaKCNE026
ZhpaKCNE027
ZhpaKCNE028
ZhpaBCNE048

13. L01
NCAZZhluMSB6 [1]

14. L02
ApAZZhluMSB4 [2]
ApAZZhluMSB40951

15. L03
LACOZhluDMNH8631 [1]

16. L04
LACOZhluDMNH8632 [2]
LACOZhluDMNH8634

17. L05
BCNEZhpaUNL1 [2]
BCNEZhpaUNL12

18. PaI01
BCNEZhpaUNL16 [32]
GCNEZhpaUNL27
GCNEZhpaUNL28
HCNEZhpaUNL35
HCNEZhpaUNL36
HCNEZhpaUNL37
MCNEZhpaUNL51
TCIOZhinKU116269
ZhpaBCNE029
ZhpaBCNE033
ZhpaBCNE034
ZhpaBCNE038
ZhpaBCNE041
ZhpaBCNE042
ZhpaBeCSD004
ZhpaBeCSD005
ZhpaBeCSD006
ZhpaBeCSD007
ZhpaBeCSD009
ZhpaBeCSD010
ZhpaBeCSD011
ZhpaBeCSD012
ZhpaBeCSD013
ZhpaBeCSD015
ZhpaBeCSD016
ZhpaBeCSD017
ZhpaKCNE018
ZhpaKCNE020
ZhpaKCNE023
ZhpaBCNE049
ZhpaBeCSD002
ZhpaBeCSD003

19. L06
LACOZhluDMNH8630 [10]
OCNMMSB9
OCNMZhluMSB61684
OCNMZhluMSB61690
OCNMZhluMSB61693
OCNMZhluMSB61696
OCNMZhluMSB61712
OCNMZhluNK871
RANMZhluMSB58369
SONMZhluNK884

20. L07
BCNMZhluNK9976 [2]

VCNMZhluMSB30

21. Pa06
BeSDZhpaKU54 [1]

22. PaL01
BeCSDZhpaKU53 [26]
RANMZhluMSB58370
SCNMZhluMSB23
SCNMZhluMSB24
SCNMZhluMSB25
SCNMZhluMSB26
SCNMZhluMSB27
SCNMZhluMSB56980
SCNMZhluNK856
ZhlSCNMMSB3826
ZhlSCNMMSB3827
ZhlSCNMMSB3828
ZhlSCNMMSB382
ZhlSCNMMSB3831
ZhlSCNMMSB3832
ZhlSCNMMSB3833
ZhlSCNMMSB3834
ZhlSCNMMSB3835
ZhlSCNMMSB3836
ZhlSCNMMSB3838
ZhlSCNMMSB3840
ZhlSCNMMSB3841
ZhlSCNMMSB3842
ZhlSCNMMSB3843
ZhlSCNMMSB3844
ZhlSCNMMSB3845

23. L08
ZhlSCNMMSB3837 [2]
ZhlSCNMMSB3839

24. Pa07
BCNEZhpaUNL8 [5]
ZhpaBCNE035
ZhpaBCNE044
ZhpaBCNE045
ZhpaKCNE022

25. PaL02
ApAZZhluMSB5 [7]
ApAZZhluNK1584

LACOZhluDMNH8633
LACOZhluDMNH8635
LCKSZhpaKU45
NCAZZhluMSB7
NCAZZhluMSB8

26. I01
MCIAZhinKU108068 [1]

27. I02
HCILZhinKU127252 [1]

28. I03
WCINZhinKU112830 [1]

29. I04
DCNDZhinKU123033 [1]

30. CI01
LaSDZhcaKU112663 [2]
WCSDZhiKU115730

31. CI02
BCNDZhinKU115700 [28]
BCNDZhinKU115702
BCNDZhinKU115710
BCNDZhinKU120018
BCNDZhinKU120019
CCMTZhcaK123593
CCMTZhcaK123598
CCMTZhcaK123599
DCNDZhinKU123021
DCNDZhinKU123022
DCNDZhinKU123031
DCNDZhinKU123032
MCNDZhinDMNS7764
PCSDZhcaK101558
PCSDZhcaKU101564
WCSDZhiKU115731
WCSDZhiKU115732
WCSDZhinKU159190
WCWYZhcaTK86190
WCWYZhcaTK86191
ZhcCCSD061
ZhcCCSD066
ZhcCCSD070
ZhcPCSD079
ZhcPCSD080

ZhcPCSD081
ZhcPCSD082
ZhcPCSD083

32. I05
BCNDZhinKU120017 [1]

33. C01
CCWYZhcaKU20839 [1]

34. C02
CCWYZhcaKU20843 [2]
ZhcCCWY054

35. C03
WCWYZhcaKU42469 [1]

36. C04
ZhcCCWY034 [4]
ZhcCCWY037
ZhcCCWY053
ZhcCCWY088

37. C05
CCWYZhcaKU20844 [50]
HCSDZhcaKU83557
HCSDZhcaKU87040
HCSDZhcaKU87042
LaSDZhcaKU112660
WCWYZhcaKU42471
ZhcCCSD056
ZhcCCSD057
ZhcCCSD058
ZhcCCSD059
ZhcCCSD060
ZhcCCSD062
ZhcCCSD063
ZhcCCSD065
ZhcCCSD067
ZhcCCSD068
ZhcCCSD069
ZhcCCSD072
ZhcCCSD073
ZhcCCSD075
ZhcCCSD076
ZhcCCSD077
ZhcCCSD085
ZhcCCSD086
ZhcCCWY028

ZhcCCWY030
ZhcCCWY031
ZhcCCWY032
ZhcCCWY033
ZhcCCWY035
ZhcCCWY036
ZhcCCWY038
ZhcCCWY039
ZhcCCWY040
ZhcCCWY041
ZhcCCWY042
ZhcCCWY043
ZhcCCWY044
ZhcCCWY045
ZhcCCWY046
ZhcCCWY047
ZhcCCWY048
ZhcCCWY049
ZhcCCWY050
ZhcCCWY051
ZhcCCWY052
ZhcCCWY055
ZhcCCWY087
ZhcCCWY089
ZhcPCSD084

38. I06
DCSDZhinKU147018 [8]
DCSDZhinKU153196
ECIAZhinKU116263
ECIAZhinKU11626
LCSDZhinKU153203
ZhiMCMNMSB41532
ZhiMCMNMSB80783
ZhiMCMNMSB80784

39. I07
DCSDZhinKU153201 [1]

40. C06
ZhcCCSD071 [3]
ZhcCCSD074
ZhcCCSD078

41. C07
PCSDZhcaKU101552 [1]

42. C08
LaSDZhcaKU109970 [1]

43. I08
ECIAZhinKU116264 [4]
UCSDZhinKU153229
WCIAZhinKU104062
ZhiMCMNMSB80770

44. I09
BrCSDZhinKU140722 [1]

45. I10
LCSDZhinKU153205 [2]
MCSDZhinKU153215

46. I11
ZhiMCMNMSB41533 [2]
ZhiMCMNMSB80767

47. I12
ZhiBrCSD003 [5]
ZhiBrCSD010
ZhiBrCSD017
ZhiBrCSD018
ZhiBrCSD032

48. I13
ZhiMCMNMSB80780 [2]
ZhiMCMNMSB80786

49. I14
ZhiMCMNMSB41518 [8]
ZhiMCMNMSB80766
ZhiMCMNMSB80768
ZhiMCMNMSB80771
ZhiMCMNMSB80773
ZhiMCMNMSB80774
ZhiMCMNMSB80779
ZhiMCMNMSB80782

50. I15
BrCSDZhinKU147020 [18]
BrCSDZhinKU153176
BrCSDZhinKU153177
BrCSDZhinKU153180
BrCSDZhinKU153181
ZhiBrCSD005
ZhiBrCSD006
ZhiBrCSD007
ZhiBrCSD009
ZhiBrCSD011

ZhiBrCSD014
ZhiBrCSD016
ZhiBrCSD023
ZhiBrCSD026
ZhiBrCSD027
ZhiBrCSD028
ZhiBrCSD029
ZhiBrCSD030

51. I16
ZhiMCMNMSB80785 [1]

52. I17
BVIAZhinKU116266 [18]
BrCSDZhinKU140721
MCSDZhinKU153209
MCSDZhinKU153212
MOSDZhinKU153221
WCSDZhinKU15319
ZhiBrCSD004
ZhiBrCSD008
ZhiBrCSD012
ZhiBrCSD013
ZhiBrCSD015
ZhiBrCSD019
ZhiBrCSD024
ZhiBrCSD031
ZhiMCMNMSB80769
ZhiMCMNMSB80772
ZhiMCMNMSB80778
ZhiMCMNMSB80781

53.C09
CCMTZhcaK123595 [1]

54. Zp01
PaWYZPIdTK86039 [2]
PaWYZPIdTK86041

55. Zp02
TCWYZPUtTK86075 [3]
TCWYZPUtTK86155
TCWYZPUtTK86175

56. Zp03
TCWYZPUtTK86135 [1]

57. PaZp01

DCKSZhpaKU30814 [2]
PaWYZPIdTK86040

58. Zp05
FCWYZPIdTK86028 [3]
FCWYZPIdTK86037
FCWYZPIdTK86112

59. Zp06
CCCOZPPTK103545 [1]

60. Zp07
LACOZPPTK103593 [1]

61. Zp08
ZpAbWY002 [2]
ZpAbWY003

62. Zp09
LACOZPPTK103589 [1]

63. Zp10
LCWYZPPDMNH9316 [1]

Table 6. A summary of likelihoods scores for each STRUCTURE run for King et al. data, average likelihood scores for each K for all runs, and a visual representation of the absolute values of these scores.

Runs	K								
	1	2	3	4	5	6	7	8	9
1	-25,162.00	-22,257.80	-20,596.30	-20,067.20	-19,544.60	-20,006.30	-19,721.60	-20,104.20	-77,538.20
2	-25,165.80	-22,257.10	-20,593.60	-19,903.10	-19,378.00	-19,247.30	-19,745.60	-77,544.40	-80,319.90
3	-25,168.50	-22,257.70	-20,590.40	-20,067.00	-19,377.00	-19,994.60	-20,092.90	-19,976.70	-19,629.00
4	-25,157.90	-22,258.40	-20,589.90	-19,904.90	-19,715.10	-20,061.60	-19,708.60	-19,173.80	-19,113.90
5	-25,162.30	-22,266.00	-20,595.00	-20,067.50	-19,918.20	-19,243.90			
6	-25,156.70	-22,257.10	-20,593.00	-19,902.30	-19,542.30	-19,020.50			
7	-25,160.50	-22,263.30	-20,591.60	-20,069.80	-19,936.20	-19,025.60			
8	-25,155.90	-22,258.70	-20,591.50	-20,066.80	-19,717.40	-20,006.30			
9	-25,160.60	-22,259.00	-20,593.30	-20,071.40	-19,939.80	-19,020.50			
10	-25,157.50	-22,254.20	-20,597.30	-20,069.30	-19,931.20	-20,004.40			
Aver. -Ln	-25,160	-22,258	-20,593	-20.018	-19,699	-19.563	-19,817		

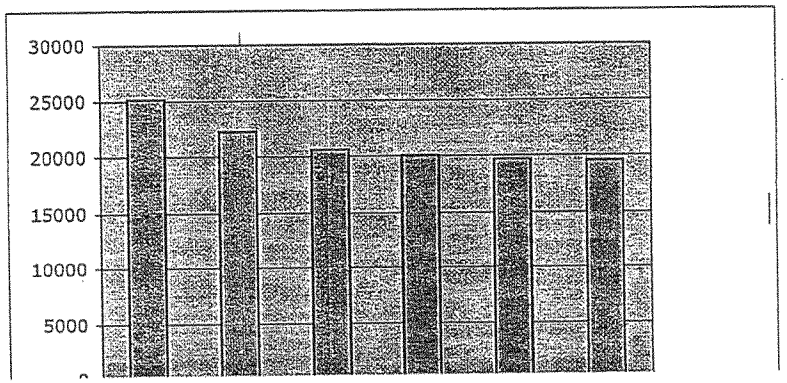


Table 7. Inferred ancestry of all individuals based on the run with the best likelihood score at K = 3 from the King et al. 2006 microsatellite data. Probabilities in bold text indicate cluster with highest assignment. Individuals in bold red text indicate individuals with mixed ancestry (no probability > 0.80) and individuals that belong to one subspecies but have highest probability ancestry assigned to a cluster with predominately individuals from a different subspecies are indicated in green. Individuals 1-94 are *Z. h. preblei*, species for all other samples are given in label. Abbreviations for sample sites are as in Table 1.

Inferred ancestry of individuals:

Label	(%Miss) :	Inferred clusters		
		1	2	3
1 LCC01_CER-9	(0) :	0.002	**0.997**	0.001
2 LCC01_CER-9	(0) :	0.002	**0.997**	0.001
3 LCC01_CER-9	(0) :	0.003	**0.995**	0.001
4 LCC01_CER-9	(0) :	0.002	**0.997**	0.001
5 LCC01_CER-9	(0) :	0.002	**0.996**	0.001
6 LCC01_CER-9	(9) :	0.002	**0.996**	0.001
7 LCC01_HRK-9	(0) :	0.003	**0.996**	0.001
8 LCC01_HRK-9	(0) :	0.002	**0.997**	0.001
9 LCC01_HRK-9	(0) :	0.003	**0.995**	0.002
10 LCC01_HRK-9	(0) :	0.002	**0.997**	0.001
11 LCC01_MC-98	(0) :	0.002	**0.997**	0.001
12 LCC01_MC-98	(0) :	0.002	**0.997**	0.001
13 LCC01_NFP-9	(0) :	0.002	**0.997**	0.001
14 LCC01_NFP-9	(0) :	0.003	**0.996**	0.001
15 LCC02_BG-98	(0) :	0.005	**0.993**	0.001
16 LCC02_BG-98	(0) :	0.017	**0.982**	0.001
17 LCC02_PGC-9	(0) :	0.002	**0.997**	0.001
18 LCC02_SP-12	(0) :	0.003	**0.995**	0.001
19 LCC02_SP-16	(0) :	0.004	**0.995**	0.001
20 LCC02_SP-17	(0) :	0.003	**0.996**	0.001
21 LCC02_SP-22	(0) :	0.003	**0.996**	0.001
22 LCC02_SP-24	(0) :	0.002	**0.996**	0.001
23 LCC02_SP-33	(0) :	0.003	**0.996**	0.001
24 LCC02_SP-36	(0) :	0.006	**0.993**	0.002
25 LCC02_SP-37	(0) :	0.012	**0.986**	0.002
26 LCC02_SP-67	(0) :	0.003	**0.995**	0.001
27 LCC02_SP-74	(0) :	0.002	**0.996**	0.001
28 LCC02_SP-86	(0) :	0.006	**0.993**	0.001
29 LCC02_YG-98	(0) :	0.003	**0.996**	0.001
30 LCC02_YG-98	(0) :	0.005	**0.994**	0.001
31 DCC01_MAY-1	(0) :	0.002	**0.997**	0.001
32 DCC01_MAY-1	(9) :	0.002	**0.997**	0.001
33 DCC01_MAY-2	(0) :	0.002	**0.997**	0.001

34 DCC01_MAY-2	(0)	: 0.004	**0.995** 0.001
35 DCC01_MAY-2	(0)	: 0.004	**0.995** 0.001
36 DCC01_MAY-2	(0)	: 0.002	**0.996** 0.002
37 DCC01_MAY-2	(0)	: 0.002	**0.997** 0.001
38 DCC01_MAY-2	(0)	: 0.002	**0.997** 0.001
39 DCC01_MAY-3	(0)	: 0.002	**0.996** 0.002
40 DCC01_MAY-3	(0)	: 0.002	**0.995** 0.003
41 DCC01_MAY-3	(0)	: 0.003	**0.996** 0.001
42 DCC01_MAY-4	(0)	: 0.002	**0.997** 0.001
43 DCC01_MAY-4	(0)	: 0.003	**0.996** 0.001
44 DCC01_MAY-4	(0)	: 0.002	**0.997** 0.001
45 DCC01_MAY-4	(0)	: 0.003	**0.996** 0.001
46 DCC01_MAY-4	(0)	: 0.005	**0.994** 0.001
47 DCC01_MAY-4	(0)	: 0.003	**0.996** 0.001
48 DCC01_MAY-5	(0)	: 0.008	**0.987** 0.005
49 DCC01_MAY-5	(0)	: 0.002	**0.997** 0.001
50 DCC01_MAY-6	(0)	: 0.002	**0.997** 0.001
51 DCC01_MAY-7	(0)	: 0.002	**0.996** 0.001
52 DCC01_MAY-7	(0)	: 0.002	**0.998** 0.001
53 DCC01_MAY-7	(0)	: 0.002	**0.996** 0.001
54 DCC01_MAY-7	(4)	: 0.002	**0.996** 0.001
55 DCC01_MAY-7	(0)	: 0.002	**0.997** 0.001
56 DCC01_MAY-8	(0)	: 0.002	**0.997** 0.001
57 DCC01_MAY-8	(0)	: 0.002	**0.997** 0.001
58 DCC01_MAY-8	(0)	: 0.002	**0.997** 0.001
59 DCC01_MAY-9	(4)	: 0.003	**0.995** 0.001
60 DCC01_MAY-9	(0)	: 0.002	**0.997** 0.001
61 DCC01_MAY-9	(0)	: 0.003	**0.996** 0.001
62 DCC01_MAY-9	(0)	: 0.002	**0.997** 0.001
63 DCC01_MAY-9	(0)	: 0.007	**0.990** 0.003
64 DCC01_MAY-9	(4)	: 0.006	**0.988** 0.006
65 DCC02_WH-98	(0)	: 0.002	**0.997** 0.001
66 DCC02_WH-98	(0)	: 0.005	**0.994** 0.002
67 DCC02_WH-98	(0)	: 0.002	**0.997** 0.001
68 DCC02_WH-98	(0)	: 0.002	**0.997** 0.001
69 DCC02_WH-98	(0)	: 0.002	**0.997** 0.001
70 DCC02_WH-98	(0)	: 0.002	**0.983** 0.015
71 DCC02_WH-98	(0)	: 0.002	**0.997** 0.001
72 DCC02_WH-98	(0)	: 0.001	**0.997** 0.001
73 DCC02_WH-98	(0)	: 0.001	**0.998** 0.001
74 DCC02_WH-98	(0)	: 0.002	**0.997** 0.001
75 DCC02_WH-98	(0)	: 0.002	**0.997** 0.001
76 DCC02_WH-98	(9)	: 0.002	**0.996** 0.001
77 DCC02_WH-98	(4)	: 0.003	**0.930** 0.067
78 DCC02_WH-98	(0)	: 0.002	**0.996** 0.002
79 DCC02_WH-98	(0)	: 0.002	**0.997** 0.001

80 DCC02_WH-98	(0)	: 0.002 **0.996** 0.001
81 DCC02_WH-98	(4)	: 0.002 **0.997** 0.001
82 DCC02_WH-98	(0)	: 0.012 **0.986** 0.002
83 DCC02_WH-98	(0)	: 0.001 **0.997** 0.001
84 DCC02_WH-98	(0)	: 0.002 **0.997** 0.001
85 DCC02_WH-98	(0)	: 0.002 **0.997** 0.001
86 DCC02_WH-98	(9)	: 0.002 **0.997** 0.001
87 DCC02_WH-98	(0)	: 0.002 **0.997** 0.001
88 DCC02_WH-98	(0)	: 0.002 **0.996** 0.001
89 DCC02_WH-98	(0)	: 0.039 **0.956** 0.005
90 DCC02_WH-98	(0)	: 0.002 **0.997** 0.001
91 DCC02_WH-98	(4)	: 0.002 **0.996** 0.001
92 DCC02_WH-98	(4)	: 0.002 **0.997** 0.001
93 DCC02_WH-98	(4)	: 0.002 **0.997** 0.001
94 DCC02_WH-98	(4)	: 0.002 **0.997** 0.001
95 ECC01_Zhp-0	(0)	: 0.002 **0.996** 0.001
96 ECC01_Zhp-0	(0)	: 0.002 **0.997** 0.001
97 ECC01_Zhp-0	(0)	: 0.004 **0.995** 0.002
98 ECC01_Zhp-0	(0)	: 0.003 **0.995** 0.001
99 ECC01_Zhp-0	(0)	: 0.002 **0.997** 0.002
100 ECC01_Zhp-0	(0)	: 0.010 **0.988** 0.002
101 ECC01_Zhp-0	(0)	: 0.002 **0.996** 0.002
102 ECC01_Zhp-0	(0)	: 0.002 **0.997** 0.001
103 ECC01_Zhp-0	(0)	: 0.003 **0.996** 0.001
104 ECC01_Zhp-0	(0)	: 0.002 **0.997** 0.001
105 ECC01_Zhp-0	(0)	: 0.010 **0.987** 0.003
106 ECC01_Zhp-0	(0)	: 0.002 **0.997** 0.001
107 ECC01_Zhp-0	(0)	: 0.002 **0.997** 0.001
108 ECC01_Zhp-0	(0)	: 0.002 **0.997** 0.001
109 ECC01_Zhp-0	(0)	: 0.002 **0.996** 0.002
110 ECC01_Zhp-0	(0)	: 0.003 **0.995** 0.002
111 ECC01_Zhp-0	(0)	: 0.002 **0.996** 0.001
112 ECC01_Zhp-0	(0)	: 0.004 **0.995** 0.002
113 ECC01_Zhp-0	(0)	: 0.006 **0.993** 0.002
114 ECC01_Zhp-0	(0)	: 0.003 **0.995** 0.002
115 ECC01_Zhp-0	(0)	: 0.003 **0.996** 0.001
116 ECC01_Zhp-0	(0)	: 0.002 **0.997** 0.001
117 ECC02_Zhp-0	(0)	: 0.003 **0.995** 0.002
118 ECC02_Zhp-0	(0)	: 0.002 **0.997** 0.001
119 ECC02_Zhp-0	(0)	: 0.006 **0.992** 0.002
120 ECC02_Zhp-0	(0)	: 0.003 **0.996** 0.001
121 ECC02_Zhp-0	(0)	: 0.003 **0.996** 0.002
122 ECC02_Zhp-0	(0)	: 0.004 **0.993** 0.003
123 ECC02_Zhp-0	(0)	: 0.003 **0.996** 0.001
124 ECC02_Zhp-0	(0)	: 0.002 **0.997** 0.001
125 ECC02_Zhp-0	(0)	: 0.002 **0.997** 0.001

126 ECC02_Zhp-0 (0) : 0.002 **0.997** 0.001
127 ECC02_Zhp-0 (0) : 0.004 **0.994** 0.001
128 ECC02_Zhp-0 (0) : 0.002 **0.997** 0.001
129 ECC02_Zhp-0 (0) : 0.003 **0.996** 0.001
130 ECC02_Zhp-0 (0) : 0.002 **0.997** 0.001
131 ECC02_Zhp-0 (0) : 0.002 **0.997** 0.001
132 ECC02_Zhp-0 (0) : 0.003 **0.995** 0.001
133 ECC02_Zhp-0 (0) : 0.004 **0.994** 0.002
134 ECC02_Zhp-0 (0) : 0.002 **0.997** 0.001
135 ECC02_Zhp-0 (0) : 0.002 **0.997** 0.001
136 ECC02_Zhp-0 (0) : 0.002 **0.996** 0.002
137 ECC02_Zhp-0 (0) : 0.002 **0.997** 0.001
138 ECC02_Zhp-1 (0) : 0.002 **0.997** 0.001
139 ECC02_Zhp-1 (0) : 0.002 **0.996** 0.001
140 ECC02_Zhp-1 (0) : 0.014 **0.983** 0.003
141 ECC02_Zhp-1 (0) : 0.072 **0.927** 0.002
142 ECC02_Zhp-1 (0) : 0.019 **0.979** 0.003

143 CCWY_Zhc-02 (0) : **0.996** 0.003 0.001
144 CCWY_Zhc-03 (0) : **0.977** 0.022 0.001
145 CCWY_Zhc-03 (0) : **0.988** 0.011 0.002
146 **CCWY_Zhc-03** (4) : **0.546** **0.452** 0.002
147 CCWY_Zhc-03 (0) : **0.990** 0.008 0.001
148 CCWY_Zhc-03 (0) : **0.993** 0.006 0.001
149 CCWY_Zhc-03 (0) : **0.995** 0.003 0.002
150 CCWY_Zhc-03 (0) : **0.986** 0.004 0.010
151 CCWY_Zhc-03 (0) : **0.979** 0.019 0.002
152 CCWY_Zhc-03 (0) : **0.980** 0.019 0.001
153 CCWY_Zhc-03 (0) : **0.979** 0.017 0.004
154 CCWY_Zhc-04 (0) : **0.990** 0.007 0.004
155 **CCWY_Zhc-04** (0) : **0.696** **0.303** 0.002
156 CCWY_Zhc-04 (0) : **0.977** 0.021 0.002
157 CCWY_Zhc-04 (0) : **0.980** 0.018 0.001
158 **CCWY_Zhc-04** (0) : **0.680** **0.318** 0.002
159 CCWY_Zhc-04 (0) : **0.895** 0.103 0.002
160 CCWY_Zhc-04 (0) : **0.992** 0.006 0.001
161 CCWY_Zhc-04 (0) : **0.996** 0.002 0.001
162 CCWY_Zhc-04 (0) : **0.921** 0.077 0.002
163 CCWY_Zhc-04 (0) : **0.881** 0.118 0.001
164 CCWY_Zhc-05 (0) : **0.852** 0.146 0.001
165 CCWY_Zhc-05 (0) : **0.985** 0.012 0.002
166 CCWY_Zhc-05 (0) : **0.994** 0.005 0.002
167 CCWY_Zhc-05 (0) : **0.983** 0.016 0.001
168 CCWY_Zhc-05 (0) : **0.977** 0.021 0.002
169 CCWY_Zhc-05 (0) : **0.995** 0.003 0.002
170 CCWY_Zhc-08 (0) : **0.985** 0.012 0.003

171 CCWY_Zhc-08 (0) : **0.987** 0.011 0.002
172 **CCWY_Zhc-08** (0) : **0.692 0.306** 0.001
173 CCSD_Zhc-05 (0) : **0.993** 0.005 0.002
174 CCSD_Zhc-05 (0) : **0.995** 0.003 0.001
175 CCSD_Zhc-05 (0) : **0.972** 0.026 0.001
176 CCSD_Zhc-05 (0) : **0.992** 0.007 0.002
177 CCSD_Zhc-06 (0) : **0.954** 0.043 0.004
178 CCSD_Zhc-06 (14) : **0.953** 0.045 0.002
179 CCSD_Zhc-06 (4) : **0.874** 0.122 0.004
180 CCSD_Zhc-06 (0) : **0.936** 0.063 0.002
181 CCSD_Zhc-06 (0) : **0.994** 0.005 0.001
182 CCSD_Zhc-06 (0) : **0.995** 0.003 0.001
183 CCSD_Zhc-06 (0) : **0.977** 0.019 0.004
184 CCSD_Zhc-06 (0) : **0.994** 0.004 0.002
185 CCSD_Zhc-06 (0) : **0.980** 0.019 0.001
186 CCSD_Zhc-06 (0) : **0.996** 0.002 0.002
187 CCSD_Zhc-07 (0) : **0.994** 0.004 0.001
188 CCSD_Zhc-07 (0) : **0.995** 0.003 0.002
189 CCSD_Zhc-07 (0) : **0.990** 0.008 0.002
190 CCSD_Zhc-07 (0) : **0.986** 0.012 0.002
191 CCSD_Zhc-07 (0) : **0.993** 0.006 0.002
192 CCSD_Zhc-07 (0) : **0.990** 0.009 0.001
193 CCSD_Zhc-07 (0) : **0.988** 0.008 0.004
194 CCSD_Zhc-07 (0) : **0.993** 0.006 0.001
195 CCSD_Zhc-07 (0) : **0.996** 0.003 0.001
196 CCSD_Zhc-07 (4) : **0.991** 0.007 0.002
197 CCSD_Zhc-08 (0) : **0.995** 0.003 0.001
198 CCSD_Zhc-08 (0) : **0.994** 0.004 0.001
199 CCSD_Zhc-08 (0) : **0.904** 0.093 0.003
200 CCSD_Zhc-08 (0) : **0.992** 0.006 0.002
201 CCSD_Zhc-08 (0) : **0.990** 0.008 0.002
202 CCSD_Zhc-08 (0) : **0.995** 0.004 0.001
203 CCSD_Zhc-08 (0) : **0.991** 0.007 0.002

204 BRCSD_Zhi-0 (0) : **0.991** 0.005 0.004
205 BRCSD_Zhi-0 (4) : **0.991** 0.006 0.002
206 BRCSD_Zhi-0 (0) : **0.995** 0.003 0.002
207 BRCSD_Zhi-0 (0) : **0.997** 0.001 0.002
208 BRCSD_Zhi-0 (0) : **0.995** 0.003 0.002
209 BRCSD_Zhi-0 (0) : **0.995** 0.002 0.003
210 BRCSD_Zhi-0 (0) : **0.996** 0.002 0.002
211 BRCSD_Zhi-0 (0) : **0.996** 0.001 0.002
212 BRCSD_Zhi-0 (0) : **0.995** 0.003 0.002
213 BRCSD_Zhi-0 (0) : **0.996** 0.002 0.002
214 BRCSD_Zhi-0 (0) : **0.928** 0.053 0.019
215 BRCSD_Zhi-0 (0) : **0.995** 0.003 0.003

216 BRCSD_Zhi-0	(0)	:	**0.991** 0.006 0.002
217 BRCSD_Zhi-0	(0)	:	**0.997** 0.002 0.002
218 BRCSD_Zhi-0	(0)	:	**0.993** 0.004 0.003
219 BRCSD_Zhi-0	(0)	:	**0.996** 0.002 0.002
220 BRCSD_Zhi-0	(0)	:	**0.996** 0.002 0.002
221 BRCSD_Zhi-0	(0)	:	**0.997** 0.001 0.001
222 BRCSD_Zhi-0	(0)	:	**0.996** 0.002 0.003
223 BRCSD_Zhi-0	(0)	:	**0.996** 0.002 0.002
224 BRCSD_Zhi-0	(0)	:	**0.988** 0.010 0.002
225 BRCSD_Zhi-0	(0)	:	**0.992** 0.003 0.006
226 BRCSD_Zhi-0	(0)	:	**0.993** 0.002 0.005
227 BRCSD_Zhi-0	(0)	:	**0.995** 0.003 0.002
228 BRCSD_Zhi-0	(0)	:	**0.990** 0.008 0.002
229 BRCSD_Zhi-0	(0)	:	**0.997** 0.002 0.001
230 BRCSD_Zhi-0	(0)	:	**0.997** 0.002 0.002
231 BRCSD_Zhi-0	(0)	:	**0.976** 0.003 0.021
232 MCMN_MSB-41	(0)	:	**0.993** 0.004 0.003
233 MCMN_MSB-41	(0)	:	**0.755** 0.005 **0.240**
234 MCMN_MSB-41	(0)	:	**0.961** 0.003 0.036
235 **MCMN_MSB-80**	(0)	:	**0.725** 0.003 **0.272**
236 MCMN_MSB-80	(0)	:	**0.994** 0.004 0.002
237 MCMN_MSB-80	(0)	:	**0.990** 0.003 0.007
238 MCMN_MSB-80	(0)	:	**0.979** 0.014 0.007
239 MCMN_MSB-80	(0)	:	**0.988** 0.009 0.003
240 MCMN_MSB-80	(0)	:	**0.977** 0.003 0.020
241 MCMN_MSB-80	(0)	:	**0.991** 0.004 0.004
242 MCMN_MSB-80	(0)	:	**0.976** 0.011 0.013
243 MCMN_MSB-80	(0)	:	**0.974** 0.002 0.024
244 MCMN_MSB-80	(0)	:	**0.919** 0.003 0.077
245 MCMN_MSB-80	(0)	:	**0.953** 0.004 0.043
246 MCMN_MSB-80	(0)	:	**0.981** 0.014 0.005
247 MCMN_MSB-80	(0)	:	**0.993** 0.004 0.003
248 MCMN_MSB-80	(0)	:	**0.990** 0.002 0.008
249 MCMN_MSB-80	(0)	:	**0.910** 0.004 0.086
250 MCMN_MSB-80	(0)	:	**0.991** 0.005 0.003
251 MCMN_MSB-80	(0)	:	**0.987** 0.010 0.003
252 **MCMN_MSB-80**	(0)	:	**0.736** 0.003 **0.262**
253 BCSD_Zhpa-0	(0)	:	0.003 0.001 **0.995**
254 BCSD_Zhpa-0	(0)	:	0.013 0.006 **0.981**
255 BCSD_Zhpa-0	(0)	:	0.002 0.031 **0.967**
256 BCSD_Zhpa-0	(0)	:	0.002 0.002 **0.995**
257 BCSD_Zhpa-0	(0)	:	0.014 0.002 **0.984**
258 BCSD_Zhpa-0	(0)	:	0.007 0.002 **0.990**
259 BCSD_Zhpa-0	(0)	:	0.002 0.002 **0.996**
260 BCSD_Zhpa-0	(0)	:	0.006 0.007 **0.987**

261 BCSD_Zhpa-0	(0)	:	0.005 0.002	**0.993**
262 BCSD_Zhpa-0	(0)	:	0.003 0.002	**0.995**
263 BCSD_Zhpa-0	(0)	:	0.003 0.005	**0.993**
264 BCSD_Zhpa-0	(0)	:	0.002 0.003	**0.995**
265 BCSD_Zhpa-0	(0)	:	0.009 0.014	**0.976**
266 BCSD_Zhpa-0	(0)	:	0.002 0.003	**0.995**
267 BCSD_Zhpa-0	(0)	:	0.002 0.007	**0.991**
268 BCSD_Zhpa-0	(0)	:	0.005 0.001	**0.994**
269 KBCNE_Zhpa-	(0)	:	0.005 0.001	**0.994**
270 KBCNE_Zhpa-	(0)	:	0.008 0.002	**0.990**
271 KBCNE_Zhpa-	(0)	:	0.008 0.002	**0.990**
272 KBCNE_Zhpa-	(0)	:	0.003 0.001	**0.996**
273 KBCNE_Zhpa-	(4)	:	0.002 0.001	**0.997**
274 KBCNE_Zhpa-	(0)	:	0.006 0.005	**0.990**
275 KBCNE_Zhpa-	(4)	:	0.003 0.004	**0.992**
276 KBCNE_Zhpa-	(0)	:	0.002 0.001	**0.997**
277 KBCNE_Zhpa-	(0)	:	0.019 0.032	**0.950**
278 KBCNE_Zhpa-	(9)	:	0.009 0.003	**0.988**
279 KBCNE_Zhpa-	(0)	:	0.005 0.003	**0.992**
280 KBCNE_Zhpa-	(0)	:	0.003 0.005	**0.992**
281 KBCNE_Zhpa-	(0)	:	0.021 0.009	**0.970**
282 KBCNE_Zhpa-	(0)	:	0.003 0.005	**0.992**
283 KBCNE_Zhpa-	(0)	:	0.007 0.007	**0.986**
284 KBCNE_Zhpa-	(0)	:	0.008 0.002	**0.991**
285 KBCNE_Zhpa-	(0)	:	0.003 0.003	**0.994**
286 KBCNE_Zhpa-	(0)	:	0.013 0.083	**0.904**
287 KBCNE_Zhpa-	(0)	:	0.004 0.007	**0.990**
288 KBCNE_Zhpa-	(0)	:	0.004 0.002	**0.995**
289 KBCNE_Zhpa-	(0)	:	0.004 0.004	**0.991**
290 KBCNE_Zhpa-	(0)	:	0.004 0.002	**0.995**
291 KBCNE_Zhpa-	(0)	:	0.003 0.002	**0.994**
292 KBCNE_Zhpa-	(0)	:	0.008 0.007	**0.986**
293 KBCNE_Zhpa-	(0)	:	0.018 0.004	**0.978**
294 KBCNE_Zhpa-	(0)	:	0.009 0.004	**0.987**
295 KBCNE_Zhpa-	(0)	:	0.008 0.024	**0.969**
296 KBCNE_Zhpa-	(0)	:	0.006 0.024	**0.970**
297 KBCNE_Zhpa-	(0)	:	0.005 0.004	**0.991**
298 KBCNE_Zhpa-	(0)	:	0.002 0.002	**0.997**
299 KBCNE_Zhpa-	(0)	:	0.019 0.009	**0.972**
300 KBCNE_Zhpa-	(0)	:	0.027 0.002	**0.971**
301 SCNM_MSB-38	(0)	:	0.001 0.001	**0.998**
302 SCNM_MSB-38	(0)	:	0.001 0.001	**0.998**
303 SCNM_MSB-38	(0)	:	0.001 0.001	**0.998**
304 SCNM_MSB-38	(0)	:	0.001 0.002	**0.997**
305 SCNM_MSB-38	(0)	:	0.002 0.002	**0.996**

306 SCNM_MSB-38	(0)	:	0.002 0.002	**0.996**
307 SCNM_MSB-38	(0)	:	0.001 0.001	**0.997**
308 SCNM_MSB-38	(0)	:	0.001 0.001	**0.997**
309 SCNM_MSB-38	(0)	:	0.002 0.002	**0.997**
310 SCNM_MSB-38	(0)	:	0.001 0.001	**0.998**
311 SCNM_MSB-38	(0)	:	0.002 0.003	**0.995**
312 SCNM_MSB-38	(0)	:	0.001 0.002	**0.997**
313 SCNM_MSB-38	(0)	:	0.001 0.001	**0.998**
314 SCNM_MSB-38	(0)	:	0.001 0.001	**0.998**
315 SCNM_MSB-38	(0)	:	0.001 0.001	**0.998**
316 SCNM_MSB-38	(0)	:	0.002 0.002	**0.997**
317 SCNM_MSB-38	(0)	:	0.001 0.001	**0.998**
318 SCNM_MSB-38	(0)	:	0.001 0.001	**0.998**
319 SCNM_MSB-38	(0)	:	0.001 0.001	**0.998**
320 SCNM_MSB-38	(0)	:	0.001 0.001	**0.998**

Table 8. Summary of results for MIGRATE analysis of King et al. microsatellite data between three hypothesized populations based on four separate runs. Because of slight insistencies in the first three runs a forth was attempted in which length of the run was doubled A) Theta is equal to the estimated effective population size and Mxy is equal to the relative importance of migration from cluster on 'x' axis into cluster on 'y' axis relative to mutation rate in introducing new variants into the population. B) Nm estimates based on the results of the Migrate run 4.

A)

Runs	Clusters	Θ	Mxy(m/μ)			Chains
			Zhpr	Zhc/Zhi	Zhpa/Zhl	
Run 1	Zhpr	1.27503	---	4.5161	1.9411	Short = 10
	Zhc/Zhi	1.27120	7.5431	---	3.5490	Long = 3
	Zhpa/Zhl	1.39109	2.6982	3.1101	---	
Run 2	Zhpr	1.19892	---	4.3438	1.9371	Short = 10
	Zhc/Zhi	1.31505	5.9209	---	3.2387	Long = 3
	Zhpa/Zhl	1.47370	3.1404	3.2982	---	
Run 3	Zhpr	1.20181	---	4.9202	1.8748	Short = 10
	Zhc/Zhi	1.31586	21.2357	---	7.3766	Long = 3
	Zhpa/Zhl	1.41384	2.9735	3.0185	---	
Run 4	Zhpr	1.39925	---	3.4569	1.7511	Short = 20
	Zhc/Zhi	1.39302	5.9200	---	3.1183	Long = 6
	Zhpa/Zhl	2.98891	2.1854	2.2228	---	

B)

	Nm(xy)		
	Zhpr	Zhc/Zhi	Zhpa/Zhl
Zhpr	---	1.21	2.45
Zhc/Zhi	2.06	---	1.09
Zhpa/Zhl	1.63	3.32	---

Number of migrants from 'x' axis cluster into 'y' axis cluster

Table 9. A summary of results for the nested clade analysis as per the Templeton (2004) inference key. Clades are labeled as in Figures 7-9. Geographical distribution of important clades (in bold) are shown in Figures 10-13.

Clades	NCA inferences
Clade 1-3	Allopatric fragmentation but the sampling scheme may be inadequate because only 6 samples were taken from Kansas and it is unclear if *Z. hudsonius* exists here.
Clade 1-5	Contiguous Range Expansion
Clade 1-9	Contiguous Range Expansion
Clade 1-10	Inconclusive
Clade 1-14	Restricted Gene Flow w/IBD
Clade 1-19	Restricted Gene Flow w/IBD
Clade 2-1	Restricted Gene Flow w/IBD
Clade 2-2	Restricted Gene Flow w/IBD
Clade 2-4	Restricted Gene Flow w/IBD
Clade 2-5	Insufficient Genetic Resolution to discriminate between range expansion/colonization and restricted dispersal / gene flow
Clade 2-9	Inconclusive
Clade 3-1	Contiguous range expansion but like with clade 1-3 this depends on adequate sampling in Eastern Colorado and Kansas
Clade 3-2	Contiguous Range Expansion
Clade 3-4	Restricted Gene Flow w/IBD
Clade 4-2	When you compare all nested clades 3-2, 3-3, and 3-4 you get restricted gene flow / dispersal with some long distance dispersal but it depends on the sampling design in the area between 3-2+3-4 and 3-3. If you just compare 3-2 and 3-4 ignoring 3-3 samples then you get Restricted Gene Flow with IBD.
Clade 5-1	Possible fragmentation but may need better sampling between clades 4-1 and 4-2.
Clade 5-3	Inadequate Geographical Sampling

Exhibit E

Science: Conjectures and Refutations

Karl R. Popper · (excerpt, 1953; pub. 1957)

WHEN I RECEIVED the list of participants in this course and realized that I had been asked to speak to philosophical colleagues I thought, after some hesitation and consolation, that you would probably prefer me to speak about those problems which interest me most, and about those developments with which I am most intimately acquainted. I therefore decided to do what I have never done before: to give you a report on my own work in the philosophy of science, since the autumn 1919 when I first begin to grapple with the problem, "When should a theory be ranked as scientific?" or "Is there a criterion for the scientific character or status of a theory?"

The problem which troubled me at the time was neither, "When is a theory true?" nor "When is a theory acceptable?" My problem was different. I wished to distinguish between science and pseudo-science; knowing very well that science often errs, and that pseudoscience may happen to stumble on the truth.

I knew, of course, the most widely accepted answer to my problem: that science is distinguished from pseudo-science—or from "metaphysics"—by its empirical method, which is essentially inductive, proceeding from observation or experiment. But this did not satisfy me. On the contrary, I often formulated my problem as one of distinguishing between a genuinely empirical method and a non-empirical or even pseudo-empirical method—that is to say, a method which, although it appeals to observation and experiment, nevertheless does not come up to scientific standards. The latter method may be exemplified by astrology, with its stupendous mass of empirical evidence based on observation—on horoscopes and on biographies.

But as it was not the example of astrology which led me to my problem, I should perhaps briefly describe the atmosphere in which my problem arose and the examples by which it was stimulated. After the collapse of the Austrian empire there had been a revolution in Austria: the air was full of revolutionary slogans and ideas, and new and often wild theories. Among the theories which interested me Einstein's theory of relativity was no doubt by far the most important. The three others were Marx's theory of history, Freud's psycho-analysis, and Alfred Adler's so-called "individual psychology."

There was a lot of popular nonsense talked about these theories, and especially about relativity (as still happens even today), but I was fortunate in those who introduced me to the study of this theory. We all—the small circle of students to which I belonged—were thrilled with the result of Eddington's eclipse observations which in 1919 brought the first important confirmation of Einstein's theory of gravitation. It was a great experience for us, and one which had a lasting influence on my intellectual development.

The three other theories I have mentioned were also widely discussed among students at the time. I myself happened to come into personal contact with Alfred Adler, and even to cooperate with him in his social work among the children and young people in the working-class districts of Vienna where he had established social guidance clinics.

It was the summer of 1919 that I began to feel more and more dissatisfied with these three theories—the Marxist theory of history, psycho-analysis, and individual psychology; and I began to feel dubious about their claims to scientific status. My problem perhaps first took the simple form, "What is wrong with Marxism, psycho-analysis, and individual psychology? Why are they so different from physical theories, from Newton's theory, and especially from the theory of relativity?"

To make this contrast clear I should explain that few of us at the time would have said that we believed in the truth of Einstein's theory of gravitation. This shows that it was not my doubting the truth of those three other theories which bothered me, but something else. Yet neither was it that I nearly felt mathematical physics to be more exact than sociological or psychological type of theory. Thus what worried me was neither the problem of truth, at that stage at least, nor the problem of exactness or measurability. It was rather that I felt that these other three theories, though posing as science, had in fact more in common with primitive myths than with science; that they resembled astrology rather than astronomy.

I found that those of my friends who were admirers of Marx, Freud, and Adler, were impressed by a number of points common to these theories, and especially by their apparent explanatory power. These theories appear to be able to explain practically everything that happened within the fields to which they referred. The study of any of them seemed to have the effect of an intellectual conversion or revelation, open your eyes to a new truth hidden from those not yet initiated. Once your eyes were thus opened you saw confirmed instances everywhere:

the world was full of verifications of the theory. Whatever happened always confirmed it. Thus its truth appeared manifest; and unbelievers were clearly people who did not want to see the manifest truth; who refuse to see it, either because it was against their class interest, or because of their repressions which were still "un-analyzed" and crying aloud for treatment.

The most characteristic element in this situation seemed to me the incessant stream of confirmations, of observations which "verified" the theories in question; and this point was constantly emphasize by their adherents. A Marxist could not open a newspaper without finding on every page confirming evidence for his interpretation of history; not only in the news, but also in its presentation—which revealed the class bias of the paper—and especially of course what the paper did not say. The Freudian analysts emphasized that their theories were constantly verified by their "clinical observations." As for Adler, I was much impressed by a personal experience. Once, in 1919, I reported to him a case which to me did not seem particularly Adlerian, but which he found no difficulty in analyzing in terms of his theory of inferiority feelings, Although he had not even seen the child. Slightly shocked, I asked him how he could be so sure. "Because of my thousandfold experience," he replied; whereupon I could not help saying: "And with this new case, I suppose, your experience has become thousand-and-one-fold."

What I had in mind was that his previous observations may not have been much sounder than this new one; that each in its turn had been interpreted in the light of "previous experience," and at the same time counted as additional confirmation. What, I asked myself, did it confirm? No more than that a case could be interpreted in the light of a theory. But this meant very little, I reflected, since every conceivable case could be interpreted in the light Adler's theory, or equally of Freud's. I may illustrate this by two very different examples of human behavior: that of a man who pushes a child into the water with the intention of drowning it; and that of a man who sacrifices his life in an attempt to save the child. Each of these two cases can be explained with equal ease in Freudian and Adlerian terms. According to Freud the first man suffered from repression (say, of some component of his Oedipus complex), while the second man had achieved sublimation. According to Adler the first man suffered from feelings of inferiority (producing perhaps the need to prove to himself that he dared to commit some crime), and so did the second man (whose need was to prove to himself that he dared to rescue the child). I could not think of any human behavior which could not be interpreted in terms of either theory. It was precisely this fact—that they always fitted, that they were always confirmed—which in the eyes of their admirers constituted the strongest argument in favor of these theories. It began to dawn on me that this apparent strength was in fact their weakness.

With Einstein's theory the situation was strikingly different. Take one typical instance—Einstein's prediction, just then confirmed by the finding of Eddington's expedition. Einstein's gravitational theory had led to the result that light must be attracted by heavy bodies (such as the sun), precisely as material bodies were attracted. As a consequence it could be calculated that light from a distant fixed star whose apparent position was close to the sun would reach the earth from such a direction that the star would seem to be slightly shifted away from the sun; or, in other words, that stars close to the sun would look as if they had moved a little away from the sun, and from one another. This is a thing which cannot normally be observed since such stars are rendered invisible in daytime by the sun's overwhelming brightness; but during an eclipse it is possible to take photographs of them. If the same constellation is photographed at night one can measure the distance on the two photographs, and check the predicted effect.

Now the impressive thing about this case is the risk involved in a prediction of this kind. If observation shows that the predicted effect is definitely absent, then the theory is simply refuted. The theory is incompatible with certain possible results of observation—in fact with results which everybody before Einstein would have expected.[1] This is quite different from the situation I have previously described, when it turned out that the theories in question were compatible with the most divergent human behavior, so that it was practically impossible to describe any human behavior that might not be claimed to be a verification of these theories.

These considerations led me in the winter of 1919-20 to conclusions which I may now reformulate as follows.

1. It is easy to obtain confirmations, or verifications, for nearly every theory—if we look for confirmations.
2. Confirmations should count only if they are the result of risky predictions; that is to say, if, unenlightened by the theory in question, we should have expected an event which was incompatible with the theory—an event which would have refuted the theory.
3. Every "good" scientific theory is a prohibition: it forbids certain things to happen. The more a theory forbids, the better it is.

4. A theory which is not refutable by any conceivable event is non-scientific. Irrefutability is not a virtue of a theory (as people often think) but a vice.
5. Every genuine test of a theory is an attempt to falsify it, or to refute it. Testability is falsifiability; but there are degrees of testability: some theories are more testable, more exposed to refutation, than others; they take, as it were, greater risks.
6. Confirming evidence should not count except when it is the result of a genuine test of the theory; and this means that it can be presented as a serious but unsuccessful attempt to falsify the theory. (I now speak in such cases of "corroborating evidence.")
7. Some genuinely testable theories, when found to be false, are still upheld by their admirers—for example by introducing ad hoc some auxiliary assumption, or by reinterpreting the theory ad hoc in such a way that it escapes refutation. Such a procedure is always possible, but it rescues the theory from refutation only at the price of destroying, or at least lowering, its scientific status. (I later described such a rescuing operation as a "conventionalist twist" or a "conventionalist stratagem.")

One can sum up all this by saying that the criterion of the scientific status of a theory is its falsifiability, or refutability, or testability.

II

I may perhaps exemplify this with the help of the various theories so far mentioned. Einstein's theory of gravitation clearly satisfied the criterion of falsifiability. Even if our measuring instruments at the time did not allow us to pronounce on the results of the tests with complete assurance, there was clearly a possibility of refuting the theory.

Astrology did not pass the test. Astrologers were greatly impressed, and misled, by what they believed to be confirming evidence—so much so that they were quite unimpressed by any unfavorable evidence. Moreover, by making their interpretations and prophesies sufficiently vague they were able to explain away anything that might have been a refutation of the theory had the theory and the prophesies been more precise. In order to escape falsification they destroyed the testability of their theory. It is a typical soothsayer's trick to predict things so vaguely that the predictions can hardly fail: that they become irrefutable.

The Marxist theory of history, in spite of the serious efforts of some of its founders and followers, ultimately adopted this soothsaying practice. In some of its earlier formulations (for example in Marx's analysis of the character of the "coming social revolution") their predictions were testable, and in fact falsified.[2] Yet instead of accepting the refutations the followers of Marx re-interpreted both the theory and the evidence in order to make them agree. In this way they rescued the theory from refutation; but they did so at the price of adopting a device which made it irrefutable. They thus gave a "conventionalist twist" to the theory; and by this stratagem they destroyed its much advertised claim to scientific status.

The two psycho-analytic theories were in a different class. They were simply non-testable, irrefutable. There was no conceivable human behavior which could contradict them. This does not mean that Freud and Adler were not seeing certain things correctly; I personally do not doubt that much of what they say is of considerable importance, and may well play its part one day in a psychological science which is testable. But it does mean that those "clinical observations" which analysts naïvely believe confirm their theory cannot do this any more than the daily confirmations which astrologers find in their practice.[3] And as for Freud's epic of the Ego, the Super-ego, and the Id, no substantially stronger claim to scientific status can be made for it than for Homer's collected stories from Olympus. These theories describe some facts, but in the manner of myths. They contain most interesting psychological suggestions, but not in a testable form.

At the same time I realized that such myths may be developed, and become testable; that historically speaking all—or very nearly all—scientific theories originate from myths, and that a myth may contain important anticipations of scientific theories. Examples are Empedocles' theory of evolution by trial and error, or Parmenides' myth of the unchanging block universe in which nothing ever happens and which, if we add another dimension, becomes Einstein's block universe (in which, too, nothing ever happens, since everything is, four-dimensionally speaking, determined and laid down from the beginning). I thus felt that if a theory is found to be non-scientific, or "metaphysical" (as we might say), it is not thereby found to be unimportant, or insignificant, or "meaningless," or "nonsensical."[4] But it cannot claim to be backed by empirical evidence in the scientific sense —although it may easily be, in some genetic sense, the "result of observation."

(There were a great many other theories of this pre-scientific or pseudo-scientific character, some of them, unfortunately, as influential as the Marxist interpretation of history; for example, the racialist interpretation of history—another of those impressive and all-explanatory theories which act upon weak minds like revelations.)

Thus the problem which I tried to solve by proposing the criterion of falsifiability was neither a problem of meaningfulness or significance, nor a problem of truth or acceptability. It was the problem of drawing a line (as well as this can be done) between the statements, or systems of statements, of the empirical sciences, and all other statements—whether they are of a religious or of a metaphysical character, or simply pseudo-scientific. Years later—it must have been in 1928 or 1929—I called this first problem of mine the "problem of demarcation." The criterion of falsifiability is a solution to this problem of demarcation, for it says that statements or systems of statements, in order to be ranked as scientific, must be capable of conflicting with possible, or conceivable, observations.

---

[1] This is a slight oversimplification, for about half of the Einstein effect may be derived from the classical theory, provided we assume a ballistic theory of light.

[2] See, for example, my Open Society and Its Enemies, ch. 15, section iii, and notes 13-14.

[3] "Clinical observations," like all other observations, are interpretations in the light of theories; and for this reason alone they are apt to seem to support those theories in the light of which they were interpreted. But real support can be obtained only from observations undertaken as tests (by "attempted refutations"); and for this purpose criteria of refutation have to be laid down beforehand; it must be agreed which observable situations, if actually observed, mean that the theory is refuted. But what kind of clinical responses would refute to the satisfaction of the analyst not merely a particular analytic diagnosis but psycho-analysis itself? And have such criteria ever been discussed or agreed upon by analysts? Is there not, on the contrary, a whole family of analytic concepts, such as "ambivalence" (I do not suggest that there is no such thing as ambivalence), which would make it difficult, if not impossible, to agree upon such criteria? Moreover, how much headway has been made in investigating the question of the extent to which the (conscious or unconscious) expectations and theories held by the analyst influence the "clinical responses" of the patient? To say nothing about the conscious attempts to influence the patient by proposing interpretations to him, etc.) Years ago I introduced the term "Oedipus effect" to describe the influence of a theory or expectation or prediction upon the event which it predicts or describes: it will be remembered that the causal chain leading to Oedipus' parricide was started by the oracle's prediction of this event. This is a characteristic and recurrent theme of such myths, but one which seems to have failed to attract the interest of the analysts, perhaps not accidentally. (The problem of confirmatory dreams suggested by the analyst is discussed by Freud, for example in *Gesammelte Schriften*, III, 1925, where he says on p. 315: "If anybody asserts that most of the dreams which can be utilized in an analysis ... owe their origin to [the analyst's] suggestion, then no objection can be made from the point of view of analytic theory. Yet there is nothing in this fact," he surprisingly adds, "which would detract from the reliability of our results.")

[4] The case of astrology, nowadays a typical pseudo-science, may illustrate this point. It was attacked, by Aristotelians and other rationalists, down to Newton's day, for the wrong reason—for its now an accepted assertion that the planets had an "influence" upon terrestrial ("sublunar") events. In fact Newton's theory of gravity, and especially the lunar theory of the tides, was historically speaking an offspring of astrological lore. Newton, it seems, was most reluctant to adopt a theory which came from the same stable as for example the theory that "influenza" epidemics are due to an astral "influence." And Galileo, no doubt for the same reason, actually rejected the lunar theory of the tides; and his misgivings about Kepler may easily be explained by his misgivings about astrology.

Exhibit F

16 October 1964, Volume 146, Number 3642

SCIENCE

Strong Inference

Certain systematic methods of scientific thinking may produce much more rapid progress than others.

John R. Platt

Scientists these days tend to keep up a polite fiction that all science is equal. Except for the work of the misguided opponent whose arguments we happen to be refuting at the time, we speak as though every scientist's field and methods of study are as good as every other scientist's, and perhaps a little better. This keeps us all cordial when it comes to recommending each other for government grants.

But I think anyone who looks at the matter closely will agree that some fields of science are moving forward very much faster than others, perhaps by an order of magnitude, if numbers could be put on such estimates. The discoveries leap from the headlines—and they are real advances in complex and difficult subjects, like molecular biology and high-energy physics. As Alvin Weinberg says (*1*), "Hardly a month goes by without a stunning success in molecular biology being reported in the Proceedings of the National Academy of Sciences."

Why should there be such rapid advances in some fields and not in others? I think the usual explanations that we tend to think of—such as the tractability of the subject, or the quality or education of the men drawn into it, or the size of research contracts—are important but inadequate. I have begun to believe that the primary factor in scientific advance is an intellectual one. These rapidly moving fields are fields where a particular method of doing scientific research is systematically used and taught, an accumulative method of inductive inference that is so effective that I think it should be given the name of "strong inference." I believe it is important to examine this method, its use and history and rationale, and to see whether other groups and individuals might learn to adopt it profitably in their own scientific and intellectual work.

In its separate elements, strong inference is just the simple and old-fashioned method of inductive inference that goes back to Francis Bacon. The steps are familiar to every college student and are practiced, off and on, by every scientist. The difference comes in their systematic application. Strong inference consists of applying the following steps to every problem in science, formally and explicitly and regularly:

1) Devising alternative hypotheses;

2) Devising a crucial experiment (or several of them), with alternative possible outcomes, each of which will, as nearly as possible, exclude one or more of the hypotheses;

3) Carrying out the experiment so as to get a clean result;

1′) Recycling the procedure, making subhypotheses or sequential hypotheses to refine the possibilities that remain; and so on.

It is like climbing a tree. At the first fork, we choose—or, in this case, "nature" or the experimental outcome chooses—to go to the right branch or the left; at the next fork, to go left or right; and so on. There are similar branch points in a "conditional computer program," where the next move depends on the result of the last calculation. And there is a "conditional inductive tree" or "logical tree" of this kind written out in detail in many first-year chemistry books, in the table of steps for qualitative analysis of an unknown sample, where the student is led through a real problem of consecutive inference: Add reagent A; if you get a red precipitate, it is subgroup alpha and you filter and add reagent B; if not, you add the other reagent, B′; and so on.

On any new problem, of course, inductive inference is not as simple and certain as deduction, because it involves reaching out into the unknown. Steps 1 and 2 require intellectual inventions, which must be cleverly chosen so that hypothesis, experiment, outcome, and exclusion will be related in a rigorous syllogism; and the question of how to generate such inventions is one which has been extensively discussed elsewhere (*2*, *3*). What the formal schema reminds us to do is to try to make these inventions, to take the next step, to proceed to the next fork, without dawdling or getting tied up in irrelevancies.

It is clear why this makes for rapid and powerful progress. For exploring the unknown, there is no faster method; this is the minimum sequence of steps. Any conclusion that is not an exclusion is insecure and must be rechecked. Any delay in recycling to the next set of hypotheses is only a delay. Strong inference, and the logical tree it generates, are to inductive reasoning what the syllogism is to deductive reasoning, in that it offers a regular method for reaching firm inductive conclusions one after the other as rapidly as possible.

"But what is so novel about this?" someone will say. This is *the* method of science and always has been; why give it a special name? The reason is that many of us have almost forgotten

The author is professor of biophysics and physics at the University of Chicago, Chicago, Ill. This is the text of an address given before the Division of Physical Chemistry of the American Chemical Society in September 1963, under the title "The New Baconians."

it. Science is now an everyday business. Equipment, calculations, lectures become ends in themselves. How many of us write down our alternatives and crucial experiments every day, focusing on the *exclusion* of a hypothesis? We may write our scientific papers so that it looks as if we had steps 1, 2, and 3 in mind all along. But in between, we do busywork. We become "method-oriented" rather than "problem-oriented." We say we prefer to "feel our way" toward generalizations. We fail to teach our students how to sharpen up their inductive inferences. And we do not realize the added power that the regular and explicit use of alternative hypotheses and sharp exclusions could give us at every step of our research.

The difference between the average scientist's informal methods and the methods of the strong-inference users is somewhat like the difference between a gasoline engine that fires occasionally and one that fires in steady sequence. If our motorboat engines were as erratic as our deliberate intellectual efforts, most of us would not get home for supper.

Molecular Biology

The new molecular biology is a field where I think this systematic method of inference has become widespread and effective. It is a complex field; yet a succession of crucial experiments over the past decade has given us a surprisingly detailed understanding of hereditary mechanisms and the control of enzyme formation and protein synthesis.

The logical structure shows in every experiment. In 1953 James Watson and Francis Crick proposed that the DNA molecule—the "hereditary substance" in a cell—is a long two-stranded helical molecule (*4*). This suggested a number of alternatives for crucial test. Do the two strands of the helix stay together when a cell divides, or do they separate? Matthew Meselson and Franklin Stahl used an ingenious isotope-density-labeling technique which showed that they separate (*5*). Does the DNA helix always have two strands, or can it have three, as atomic models suggest? Alexander Rich showed it can have either, depending on the ionic concentration (*6*). These are the kinds of experiments John Dalton would have liked, where the combining entities are not atoms but long macromolecular strands.

Or take a different sort of question: Is the "genetic map"—showing the statistical relationship of different genetic characteristics in recombination experiments—a one-dimensional map like the DNA molecule (that is, a linear map), as T. H. Morgan proposed in 1911, or does it have two-dimensional loops or branches? Seymour Benzer showed that his hundreds of fine microgenetic experiments on bacteria would fit only the mathematical matrix for the one-dimensional case (*7*).

But of course, selected crucial experiments of this kind can be found in every field. The real difference in molecular biology is that formal inductive inference is so systematically practiced and taught. On any given morning at the Laboratory of Molecular Biology in Cambridge, England, the blackboards of Francis Crick or Sidney Brenner will commonly be found covered with logical trees. On the top line will be the hot new result just up from the laboratory or just in by letter or rumor. On the next line will be two or three alternative explanations, or a little list of "What he did wrong." Underneath will be a series of suggested experiments or controls that can reduce the number of possibilities. And so on. The tree grows during the day as one man or another comes in and argues about why one of the experiments wouldn't work, or how it should be changed.

The strong-inference attitude is evident just in the style and language in which the papers are written. For example, in analyzing theories of antibody formation, Joshua Lederberg (*8*) gives a list of nine propositions "subject to denial," discussing which ones would be "most vulnerable to experimental test."

The papers of the French leaders François Jacob and Jacques Monod are also celebrated for their high "logical density," with paragraph after paragraph of linked "inductive syllogisms." But the style is widespread. Start with the first paper in the *Journal of Molecular Biology* for 1964 (*9*), and you immediately find: "Our conclusions . . . might be invalid if . . . (i) . . . (ii) . . . or (iii). . . . We shall describe experiments which eliminate these alternatives." The average physicist or chemist or scientist in any field accustomed to less closely reasoned articles and less sharply stated inferences will find it a salutary experience to dip into that journal almost at random.

Resistance to Analytical Methodology

This analytical approach to biology has sometimes become almost a crusade, because it arouses so much resistance in many scientists who have grown up in a more relaxed and diffuse tradition. At the 1958 Conference on Biophysics, at Boulder, there was a dramatic confrontation between the two points of view. Leo Szilard said: "The problems of how enzymes are induced, of how proteins are synthesized, of how antibodies are formed, are closer to solution than is generally believed. If you do stupid experiments, and finish one a year, it can take 50 years. But if you stop doing experiments for a little while and *think* how proteins can possibly be synthesized, there are only about 5 different ways, not 50! And it will take only a few experiments to distinguish these."

One of the young men added: "It is essentially the old question: How *small* and *elegant* an experiment can you perform?"

These comments upset a number of those present. An electron microscopist said, "Gentlemen, this is off the track. This is philosophy of science."

Szilard retorted, "I was not quarreling with third-rate scientists: I was quarreling with first-rate scientists."

A physical chemist hurriedly asked, "Are we going to take the official photograph before lunch or after lunch?"

But this did not deflect the dispute. A distinguished cell biologist rose and said, "No two cells give the same properties. Biology is the science of heterogeneous systems." And he added privately, "You know there are *scientists*; and there are people in science who are just working with these oversimplified model systems—DNA chains and in vitro systems—who are not doing science at all. We need their auxiliary work: they build apparatus, they make minor studies, but they are not scientists."

To which Cy Levinthal replied: "Well, there are two kinds of biologists, those who are looking to see

if there is one thing that can be understood, and those who keep saying it is very complicated and that nothing can be understood. . . . You must study the *simplest* system you think has the properties you are interested in."

As they were leaving the meeting, one man could be heard muttering, "What does Szilard expect me to do—shoot myself?"

Any criticism or challenge to consider changing our methods strikes of course at all our ego-defenses. But in this case the analytical method offers the possibility of such great increases in effectiveness that it is unfortunate that it cannot be regarded more often as a challenge to learning rather than as a challenge to combat. Many of the recent triumphs in molecular biology have in fact been achieved on just such "oversimplified model systems," very much along the analytical lines laid down in the 1958 discussion. They have not fallen to the kind of men who justify themselves by saying, "No two cells are alike," regardless of how true that may ultimately be. The triumphs are in fact triumphs of a new way of thinking.

High-Energy Physics

This analytical thinking is rare, but it is by no means restricted to the new biology. High-energy physics is another field where the logic of exclusions is obvious, even in the newspaper accounts. For example, in the famous discovery of C. N. Yang and T. D. Lee, the question that was asked was: Do the fundamental particles conserve mirror-symmetry or "parity" in certain reactions, or do they not? The crucial experiments were suggested; within a few months they were done, and conservation of parity was found to be excluded. Richard Garwin, Leon Lederman, and Marcel Weinrich did one of the crucial experiments. It was thought of one evening at suppertime; by midnight they had rearranged the apparatus for it; and by 4 a.m. they had picked up the predicted pulses showing the nonconservation of parity (*10*). The phenomena had just been waiting, so to speak, for the explicit formulation of the alternative hypotheses.

The theorists in this field take pride in trying to predict new properties or new particles explicitly enough so that if they are not found the theories will fall. As the biologist W. A. H. Rushton has said (*11*), "A theory which cannot be mortally endangered cannot be alive." Murray Gell-Mann and Yuval Ne'eman recently used the particle grouping which they call "The Eightfold Way" to predict a missing particle, the Omega-Minus, which was then looked for and found (*12*). But one alternative branch of the theory would predict a particle with one-third the usual electronic charge, and it was not found in the experiments, so this branch must be rejected.

The logical tree is so much a part of high-energy physics that some stages of it are commonly built, in fact, into the electronic coincidence circuits that detect the particles and trigger the bubble-chamber photographs. Each kind of particle should give a different kind of pattern in the electronic counters, and the circuits can be set to exclude or include whatever types of events are desired. If the distinguishing criteria are sequential, they may even run through a complete logical tree in a microsecond or so. This electronic preliminary analysis, like human preliminary analysis of alternative outcomes, speeds up progress by sharpening the criteria. It eliminates hundreds of thousands of the irrelevant pictures that formerly had to be scanned, and when it is carried to its limit, a few output pulses, hours apart, may be enough to signal the existence of the antiproton or the fall of a theory.

I think the emphasis on strong inference in the two fields I have mentioned has been partly the result of personal leadership, such as that of the classical geneticists in molecular biology, or of Szilard with his "Midwest Chowder and Bacteria Society" at Chicago in 1948–50, or of Max Delbrück with his summer courses in phage genetics at Cold Spring Harbor. But it is also partly due to the nature of the fields themselves. Biology, with its vast informational detail and complexity, is a "high-information" field, where years and decades can easily be wasted on the usual type of "low-information" observations or experiments if one does not think carefully in advance about what the most important and conclusive experiments would be. And in high-energy physics, both the "information flux" of particles from the new accelerators and the million-dollar costs of operation have forced a similar analytical approach. It pays to have a top-notch group debate every experiment ahead of time; and the habit spreads throughout the field.

Induction and Multiple Hypotheses

Historically, I think, there have been two main contributions to the development of a satisfactory strong-inference method. The first is that of Francis Bacon (*13*). He wanted a "surer method" of "finding out nature" than either the logic-chopping or all-inclusive theories of the time or the laudable but crude attempts to make inductions "by simple enumeration." He did not merely urge experiments, as some suppose; he showed the fruitfulness of interconnecting theory and experiment so that the one checked the other. Of the many inductive procedures he suggested, the most important, I think, was the conditional inductive tree, which proceeded from alternative hypotheses (possible "causes," as he calls them), through crucial experiments ("Instances of the Fingerpost"), to exclusion of some alternatives and adoption of what is left ("establishing axioms"). His Instances of the Fingerpost are explicitly at the forks in the logical tree, the term being borrowed "from the fingerposts which are set up where roads part, to indicate the several directions."

Many of his crucial experiments proposed in Book II of *The New Organon* are still fascinating. For example, in order to decide whether the weight of a body is due to its "inherent nature," as some had said, or is due to the attraction of the earth, which would decrease with distance, he proposes comparing the rate of a pendulum clock and a spring clock and then lifting them from the earth to the top of a tall steeple. He concludes that if the pendulum clock on the steeple "goes more slowly than it did on account of the diminished virtue of its weights . . . we may take the attraction of the mass of the earth as the cause of weight."

Here was a method that could separate off the empty theories!

Bacon said the inductive method could be learned by anybody, just like

learning to "draw a straighter line or more perfect circle . . . with the help of a ruler or a pair of compasses." "My way of discovering sciences goes far to level men's wit and leaves but little to individual excellence, because it performs everything by the surest rules and demonstrations." Even occasional mistakes would not be fatal. "Truth will sooner come out from error than from confusion."

It is easy to see why young minds leaped to try it.

Nevertheless there is a difficulty with this method. As Bacon emphasizes, it is necessary to make "exclusions." He says, "The induction which is to be available for the discovery and demonstration of sciences and arts, must analyze nature by proper rejections and exclusions; and then, after a sufficient number of negatives, come to a conclusion on the affirmative instances." "[To man] it is granted only to proceed at first by negatives, and at last to end in affirmatives after exclusion has been exhausted."

Or, as the philosopher Karl Popper says today, there is no such thing as proof in science—because some later alternative explanation may be as good or better—so that science advances only by disproofs. There is no point in making hypotheses that are not falsifiable, because such hypotheses do not say anything; "it must be possible for an empirical scientific system to be refuted by experience" (*14*).

The difficulty is that disproof is a hard doctrine. If you have a hypothesis and I have another hypothesis, evidently one of them must be eliminated. The scientist seems to have no choice but to be either soft-headed or disputatious. Perhaps this is why so many tend to resist the strong analytical approach—and why some great scientists are so disputatious.

Fortunately, it seems to me, this difficulty can be removed by the use of a second great intellectual invention, the "method of multiple hypotheses," which is what was needed to round out the Baconian scheme. This is a method that was put forward by T. C. Chamberlin (*15*), a geologist at Chicago at the turn of the century, who is best known for his contribution to the Chamberlin-Moulton hypothesis of the origin of the solar system.

Chamberlin says our trouble is that when we make a single hypothesis, we become attached to it.

"The moment one has offered an original explanation for a phenomenon which seems satisfactory, that moment affection for his intellectual child springs into existence, and as the explanation grows into a definite theory his parental affections cluster about his offspring and it grows more and more dear to him. . . . There springs up also unwittingly a pressing of the theory to make it fit the facts and a pressing of the facts to make them fit the theory. . . .

"To avoid this grave danger, the method of multiple working hypotheses is urged. It differs from the simple working hypothesis in that it distributes the effort and divides the affections. . . . Each hypothesis suggests its own criteria, its own means of proof, its own method of developing the truth, and if a group of hypotheses encompass the subject on all sides, the total outcome of means and of methods is full and rich."

Chamberlin thinks the method "leads to certain distinctive habits of mind" and is of prime value in education. "When faithfully followed for a sufficient time, it develops a mode of thought of its own kind which may be designated the habit of complex thought. . . ."

This charming paper deserves to be reprinted in some more accessible journal today, where it could be required reading for every graduate student—and for every professor.

It seems to me that Chamberlin has hit on the explanation—and the cure—for many of our problems in the sciences. The conflict and exclusion of alternatives that is necessary to sharp inductive inference has been all too often a conflict between men, each with his single Ruling Theory. But whenever each man begins to have multiple working hypotheses, it becomes purely a conflict between ideas. It becomes much easier then for each of us to aim every day at conclusive disproofs—at *strong* inference—without either reluctance or combativeness. In fact, when there are multiple hypotheses which are not anyone's "personal property" and when there are crucial experiments to test them, the daily life in the laboratory takes on an interest and excitement it never had, and the students can hardly wait to get to work to see how the detective story will come out. It seems to me that this is the reason for the development of those "distinctive habits of mind" and the "complex thought" that Chamberlin described, the reason for the sharpness, the excitement, the zeal, the teamwork—yes, even international teamwork—in molecular biology and high-energy physics today. What else could be so effective?

When multiple hypotheses become coupled to strong inference, the scientific search becomes an emotional powerhouse as well as an intellectual one.

Unfortunately, I think, there are other areas of science today that are sick by comparison, because they have forgotten the necessity for alternative hypotheses and disproof. Each man has only one branch—or none—on the logical tree, and it twists at random without ever coming to the need for a crucial decision at any point. We can see from the external symptoms that there is something scientifically wrong. The Frozen Method. The Eternal Surveyor. The Never Finished. The Great Man With a Single Hypothesis. The Little Club of Dependents. The Vendetta. The All-Encompassing Theory Which Can Never Be Falsified.

Some cynics tell a story, which may be apocryphal, about the theoretical chemist who explained to his class,

"And thus we see that the C-Cl bond is longer in the first compound than in the second because the percent of ionic character is smaller."

A voice from the back of the room said, "But Professor X, according to the Table, the C-Cl bond is shorter in the first compound."

"Oh, is it?" said the professor. "Well, that's still easy to understand, because the double-bond character is higher in that compound."

To the extent that this kind of story is accurate, a "theory" of this sort is not a theory at all, because it does not exclude anything. It predicts everything, and therefore does not predict anything. It becomes simply a verbal formula which the graduate student repeats and believes because the professor has said it so often. This is not science, but faith; not theory, but theology. Whether it is hand-waving or number-waving or equation-waving, a theory is not a theory unless it can be disproved. That is, unless it can be falsified by some possible experimental outcome.

In chemistry, the resonance theorists will of course suppose that I am criticizing *them*, while the molecular-orbital theorists will suppose I am

criticizing *them*. But their actions—our actions, for I include myself among them—speak for themselves. A failure to agree for 30 years is public advertisement of a failure to disprove.

My purpose here, however, is not to call names but rather to say that we are all sinners, and that in every field and in every laboratory we need to try to formulate multiple alternative hypotheses sharp enough to be capable of disproof.

Systematic Application

I think the work methods of a number of scientists have been testimony to the power of strong inference. Is success not due in many cases to systematic use of Bacon's "surest rules and demonstrations" as much as to rare and unattainable intellectual power? Faraday's famous diary (*16*), or Fermi's notebooks (*3, 17*), show how these men believed in the effectiveness of daily steps in applying formal inductive methods to one problem after another.

Within 8 weeks after the discovery of x-rays, Roentgen had identified 17 of their major properties. Every student should read his first paper (*18*). Each demonstration in it is a little jewel of inductive inference. How else could the proofs have gone so fast, except by a method of maximum effectiveness?

Organic chemistry has been the spiritual home of strong inference from the beginning. Do the bonds alternate in benzene or are they equivalent? If the first, there should be five disubstituted derivatives; if the second, three. And three it is (*19*). This is a *strong*-inference test—not a matter of measurement, of whether there are grams or milligrams of the products, but a matter of logical alternatives. How else could the tetrahedral carbon atom or the hexagonal symmetry of benzene have been inferred 50 years before the inferences could be confirmed by x-ray and infrared measurement?

We realize that it was out of this kind of atmosphere that Pasteur came to the field of biology. Can anyone doubt that he brought with him a completely different method of reasoning? Every 2 or 3 years he moved to one biological problem after another, from optical activity to the fermentation of beet sugar, to the "diseases" of wine and beer, to the disease of silkworms, to the problem of "spontaneous generation," to the anthrax disease of sheep, to rabies. In each of these fields there were experts in Europe who knew a hundred times as much as Pasteur, yet each time he solved problems in a few months that they had not been able to solve. Obviously it was not encyclopedic knowledge that produced his success, and obviously it was not simply luck, when it was repeated over and over again; it can only have been the systematic power of a special method of exploration. Are bacteria falling in? Make the necks of the flasks S-shaped. Are bacteria sucked in by the partial vacuum? Put in a cotton plug. Week after week his crucial experiments build up the logical tree of exclusions. The drama of strong inference in molecular biology today is only a repetition of Pasteur's story.

The grand scientific syntheses, like those of Newton and Maxwell, are rare and individual achievements that stand outside any rule or method. Nevertheless it is interesting to note that several of the great synthesizers have also shown the strong-inference habit of thought in their other work, as Newton did in the inductive proofs of his *Opticks* and Maxwell did in his experimental proof that three and only three colors are needed in color vision.

A Yardstick of Effectiveness

I think the evident effectiveness of the systematic use of strong inference suddenly gives us a yardstick for thinking about the effectiveness of scientific methods in general. Surveys, taxonomy, design of equipment, systematic measurements and tables, theoretical computations—all have their proper and honored place, provided they are parts of a chain of precise induction of how nature works. Unfortunately, all too often they become ends in themselves, mere time-serving from the point of view of real scientific advance, a hypertrophied methodology that justifies itself as a lore of respectability.

We praise the "lifetime of study," but in dozens of cases, in every field, what was needed was not a lifetime but rather a few short months or weeks of analytical inductive inference. In any new area we should try, like Roentgen, to see how fast we can pass from the general survey to analytical inferences. We should try, like Pasteur, to see whether we can reach strong inferences that encyclopedism could not discern.

We speak piously of taking measurements and making small studies that will "add another brick to the temple of science." Most such bricks just lie around the brickyard (*20*). Tables of constants have their place and value, but the study of one spectrum after another, if not frequently re-evaluated, may become a substitute for thinking, a sad waste of intelligence in a research laboratory, and a mistraining whose crippling effects may last a lifetime.

To paraphrase an old saying, Beware of the man of one method or one instrument, either experimental or theoretical. He tends to become method-oriented rather than problem-oriented. The method-oriented man is shackled; the problem-oriented man is at least reaching freely toward what is most important. Strong inference redirects a man to problem-orientation, but it requires him to be willing repeatedly to put aside his last methods and teach himself new ones.

On the other hand, I think that anyone who asks the question about scientific effectiveness will also conclude that much of the mathematicizing in physics and chemistry today is irrelevant if not misleading.

The great value of mathematical formulation is that when an experiment agrees with a calculation to five decimal places, a great many alternative hypotheses are pretty well excluded (though the Bohr theory and the Schrödinger theory both predict exactly the same Rydberg constant!). But when the fit is only to two decimal places, or one, it may be a trap for the unwary; it may be no better than any rule-of-thumb extrapolation, and some other kind of qualitative exclusion might be more rigorous for testing the assumptions and more important to scientific understanding than the quantitative fit.

I know that this is like saying that the emperor has no clothes. Today we preach that science is not science unless it is quantitative. We substitute correlations for causal studies, and physical equations for organic reasoning. Measurements and equations are supposed to sharpen thinking, but, in my observation, they more often tend

to make the thinking noncausal and fuzzy. They tend to become the object of scientific manipulation instead of auxiliary tests of crucial inferences.

Many—perhaps most—of the great issues of science are qualitative, not quantitative, even in physics and chemistry. Equations and measurements are useful when and only when they are related to proof; but proof or disproof comes first and is in fact strongest when it is absolutely convincing without any quantitative measurement.

Or to say it another way, you can catch phenomena in a logical box or in a mathematical box. The logical box is coarse but strong. The mathematical box is fine-grained but flimsy. The mathematical box is a beautiful way of wrapping up a problem, but it will not hold the phenomena unless they have been caught in a logical box to begin with.

What I am saying is that, in numerous areas that we call science, we have come to like our habitual ways, and our studies that can be continued indefinitely. We measure, we define, we compute, we analyze, but we do not exclude. And this is not the way to use our minds most effectively or to make the fastest progress in solving scientific questions.

Of course it is easy—and all too common—for one scientist to call the others unscientific. My point is not that my particular conclusions here are necessarily correct, but that we have long needed some absolute standard of possible scientific effectiveness by which to measure how well we are succeeding in various areas—a standard that many could agree on and one that would be undistorted by the scientific pressures and fashions of the times and the vested interests and busywork that they develop. It is not public evaluation I am interested in so much as a private measure by which to compare one's own scientific performance with what it might be. I believe that strong inference provides this kind of standard of what the maximum possible scientific effectiveness could be—as well as a recipe for reaching it.

Aids to Strong Inference

How can we learn the method and teach it? It is not difficult. The most important thing is to keep in mind that this kind of thinking is not a lucky knack but a system that *can* be taught and learned. The molecular biologists today are living proof of it. The second thing is to be explicit and formal and regular about it, to devote a half hour or an hour to analytical thinking every day, writing out the logical tree and the alternatives and crucial experiments explicitly in a permanent notebook. I have discussed elsewhere (*3*) the value of Fermi's notebook method, the effect it had on his colleagues and students, and the testimony that it "can be adopted by anyone with profit."

It is true that it takes great courtesy to teach the method, especially to one's peers—or their students. The strong-inference point of view is so resolutely critical of methods of work and values in science that any attempt to compare specific cases is likely to sound both smug and destructive. Mainly one should try to teach it by example and by exhorting to self-analysis and self-improvement only in general terms, as I am doing here.

But I will mention one severe but useful private test—a touchstone of strong inference—that removes the necessity for third-person criticism, because it is a test that anyone can learn to carry with him for use as needed. It is our old friend the Baconian "exclusion," but I call it "The Question." Obviously it should be applied as much to one's own thinking as to others'. It consists of asking in your own mind, on hearing any scientific explanation or theory put forward, "But sir, what experiment could *dis*prove your hypothesis?"; or, on hearing a scientific experiment described, "But sir, what hypothesis does your experiment *dis*prove?"

This goes straight to the heart of the matter. It forces everyone to refocus on the central question of whether there is or is not a testable scientific step forward.

If such a question were asked aloud, many a supposedly great scientist would sputter and turn livid and would want to throw the questioner out, as a hostile witness! Such a man is less than he appears, for he is obviously not accustomed to think in terms of alternative hypotheses and crucial experiments for himself; and one might also wonder about the state of science in the field he is in. But who knows?—the question might educate him, and his field too!

On the other hand, I think that throughout most of molecular biology and nuclear physics the response to The Question would be to outline immediately not one but several tests to disprove the hypothesis—and it would turn out that the speaker already had two or three graduate students working on them!

I almost think that government agencies could make use of this kind of touchstone. It is not true that all science is equal, or that we cannot justly compare the effectiveness of scientists by any method other than a mutual-recommendation system. The man to watch, the man to put your money on, is not the man who wants to make "a survey" or a "more detailed study" but the man with the notebook, the man with the alternative hypotheses and the crucial experiments, the man who knows how to answer your Question of disproof and is already working on it.

There are some really hard problems, some high-information problems, ahead of us in several fields, problems of photosynthesis, of cellular organization, of the molceular structure and organization of the nervous system, not to mention some of our social and international problems. It seems to me that the method of most rapid progress in such complex areas, the most effective way of using our brains, is going to be to set down explicitly at each step just what the question is, and what all the alternatives are, and then to set up crucial experiments to try to disprove some. Problems of this complexity, if they can be solved at all, can be solved only by men generating and excluding possibilities with maximum effectiveness, to obtain a high degree of information per unit time—men willing to work a little bit at thinking.

When whole groups of us begin to concentrate like that, I believe we may see the molecular-biology phenomenon repeated over and over again, with order-of-magnitude increases in the rate of scientific understanding in almost every field.

References and Notes

1. A. M. Weinberg, *Minerva* **1963**, 159 (winter 1963); *Phys. Today* **17**, 42 (1964).
2. G. Polya, *Mathematics and Plausible Reasoning* (Princeton Univ. Press, Princeton, N.J., 1954), vol. 1, *Induction and Analogy in Mathematics*; vol. 2, *Patterns of Plausible Inference*.
3. J. R. Platt, *The Excitement of Science* (Houghton Mifflin, Boston, 1962); see especially chapters 7 and 8.
4. J. D. Watson and F. H. C. Crick, *Nature* **171**, 737 (1953).
5. M. Meselson and F. Stahl, *Proc. Natl. Acad. Sci. U.S.* **44**, 671 (1958).
6. A. Rich, in *Biophysical Science: A Study*

Program, J. L. Oncley *et al.*, Eds. (Wiley, New York, 1959), p. 191.
7. S. Benzer, *Proc. Natl. Acad. Sci. U.S.* **45**, 1607 (1959).
8. J. Lederberg, *Science* **129**, 1649 (1959).
9. P. F. Davison, D. Freifelder, B. W. Holloway, *J. Mol. Biol.* **8**, 1 (1964).
10. R. L. Garwin, L. M. Lederman, M. Weinrich, *Phys. Rev.* **105**, 1415 (1957).
11. W. A. H. Rushton, personal communication.
12. See G. F. Chew, M. Gell-Mann, A. H. Rosenfeld, *Sci. Am.* **210**, 74 (Feb. 1964); *ibid.* **210**, 60 (Apr. 1964); *ibid.* **210**, 54 (June 1964).
13. F. Bacon, *The New Organon and Related Writings* (Liberal Arts Press, New York, 1960), especially pp. 98, 112, 151, 156, 196.
14. K. R. Popper, *The Logic of Scientific Discovery* (Basic Books, New York, 1959), p. 41. A modified view is given by T. S. Kuhn, *The Structure of Scientific Revolutions* (Univ. of Chicago Press, Chicago, 1962), p. 146; it does not, I believe, invalidate any of these conclusions.
15. T. C. Chamberlin, *J. Geol.* **5**, 837 (1897). I am indebted to Professors Preston Cloud and Bryce Crawford, Jr., of the University of Minnesota for correspondence on this article and a classroom reprint of it.
16. M. Faraday, *Faraday's Diary 1820–62* (Bell, London, 1932–36).
17. H. L. Anderson and S. K. Allison, *Rev. Mod. Phys.* **27**, 273 (1955).
18. E. C. Watson [*Am. J. Phys.* **13**, 281 (1945)] gives an English translation of both of Roentgen's first papers on x-rays.
19. See G. W. Wheland, *Advanced Organic Chemistry* (Wiley, New York, 1949), chapter 4, for numerous such examples.
20. B. K. Forscher, *Science* **142**, 339 (1963).

Glacier Geophysics

Dynamic response of glaciers to changing climate may shed light on processes in the earth's interior.

Barclay Kamb

In ordinary experience ice is a strong, rigid substance, and to casual observation glaciers appear to be solidly permanent features of the landscapes where they occur. The finding that these great masses of ice are actually in motion, flowing like fluids, has long attracted attention and has provoked much scientific controversy (*1*). Only within the past 15 years or so have we arrived at an understanding of how and why glaciers flow, through concepts of solid-state physics and of the new materials sciences and through development of new and better means of making physical measurements on glaciers.

Ten percent of the earth's land area is at present covered by ice; during the recent ice ages the ice-covered area was almost three times as large. On at least four occasions ice invaded most of Canada, the northern part of the United States, northern Europe, and many mountain regions of the world. There is no general agreement yet on the cause of this phenomenon (*2*). In the search for an explanation, glacier geophysics aims to provide a quantitative connection between climatic change and glacier fluctuation, so that glaciers can be interpreted confidently as long-term monitors of climate. To provide such a connection requires an intimate understanding of the dynamics of glacier motion. It has only recently been realized, for example, that there are inherent instabilities in glacier response, so that a small climatic change may produce a large glacial advance or retreat.

The flow of glaciers also serves as a reminder that, on a time scale of millions of years, "solid" rocks themselves flow like fluids, in the complex and little understood processes by which the earth builds great mountain ranges and other structures of continental dimensions, the processes of tectonophysics. In this similarity, glacier geophysics and tectonophysics have an important meeting ground. Glaciers constitute great outdoor laboratories in which concepts and theories derived from indoor laboratory experimentation can be tested on time scales and distance scales more nearly appropriate to the phenomena of solid-earth deformation, and yet still accessible to human observation and measurement. Flow in glaciers produces striking internal structures (see cover) that are analogs of structures in certain metamorphic rocks from which great deformations of the earth's crust have been inferred. By studying how and why these structures originate in glaciers we can hope to get a better understanding of deep and fundamental earth processes that will probably forever be hidden from direct investigation.

This article sketches recent ideas and measurements bearing on the glacier flow process, its expression in dynamic response of glaciers, and the possibility of using these concepts to increase our understanding of solid deformation processes in the earth generally.

The author is professor of geology and geophysics at the California Institute of Technology, Pasadena.

Glacier Flow

Flow velocities of most valley glaciers are in the range of 0.1 to 2 meters per day, and are in general an increasing function of glacier size and valley slope. In ice falls, like those seen in the cover photograph, velocities of up to about 6 meters per day occur commonly. Exceptional motions of as much as 30 meters per day have been reported for the great glaciers flowing from the ice sheet into fjords on the west coast of Greenland, such as Jakobshavn.

These velocities are actual downslope ice motions under gravity. The apparent advancing or retreating movement of the glacier snout (terminus) represents, of course, a balance between ice wastage (ablation) and forward motion, and is usually much slower than the ice motion itself. A striking exception is the movement of certain glaciers, such as the famous Black Rapids Glacier of Alaska, which at times advance suddenly and catastrophically at speeds of up to 50 or even 100 meters per day (*3*).

Although velocity measurements are made by long-established methods of precise surveying, only recently have the motions of individual glaciers been measured in sufficient detail to define completely the velocity field at the surface of the ice. Data for a portion of Saskatchewan Glacier (Canada), one of the most completely studied so far (*4*), are shown in Fig. 1. Figure 1*b*

Exhibit G

Testimony of
Rob Roy Ramey II, Ph.D.
Committee on Natural Resources
United States House of Representatives
July 31, 2007

My qualifications

As a field biologist and conservation geneticist, I have 27 years of experience in conservation, research and management of threatened and endangered wildlife. I have worked with: peregrine falcons; California condors; goshawks; rainforest birds; desert, Sierra Nevada, and Rocky Mountain bighorn sheep, argali sheep of Asia, meadow jumping mice, and African elephants. I have studied parasites and pathogens including: Psoroptic scabies mites; respiratory bacteria, and HIV. I earned a Ph.D. from Cornell University in Ecology and Evolutionary Biology; a master's degree from Yale University in Wildlife Ecology; and a bachelor's degree in Biology and Natural History from the University of California Santa Cruz. My postdoctoral experience included research at University of Colorado, Boulder and as a visiting scientist at the Center for Reproduction of Endangered Species at the San Diego Zoo. I was Curator of Vertebrate Zoology at the Denver Museum of Nature & Science and served as a consulting Science Advisor to the Office of the Assistant Secretary of the Interior in Washington, D.C. I am member of the Caprinae Specialist Group at the International Union for the Conservation of Nature (IUCN). I presently consult on endangered species scientific issues and conduct scientific research with Wildlife Science International, Inc.

Introduction

This hearing is focused on questionable actions of the current administration relative to science. However, to avoid science falling prey to partisan politics, there is a need to focus briefly on the larger question of what distinguishes science from non-science. The fundamental distinction between science and non-science is the criterion of falsifiability. In other words, all hypotheses must be testable. When clear-cut criteria are laid out in advance of data collection and all information considered (the scientific method), then there is less room for bias through the selective interpretation of the information. For the Endangered Species Act (ESA), which relies on scientific information, that means that data must be publicly available, conclusions open to question, and all information considered - including contrary information. If ESA decisions are not made in such an open and transparent way, then the moral authority of the ESA is compromised and valuable resources are diverted away from conservation.

I write today because there does appear to be a "Crisis in Confidence" with some of the "science" used in Endangered Species Act decisions. This is an issue that crosses administrations and sides of the aisle. The examples below show that there is a "crisis" occurring, for reasons other than what you may have been led to believe. There can also be serious consequences for those who dare to ask questions about information used in some ESA decisions.

Case 1: The Preble's Mouse Jumping Mouse

In the case of the Preble's mouse (listed as an endangered subspecies), the record will ultimately show that special interest groups, individuals, and academics with vested financial interests, and some U.S. Fish and Wildlife Service (USFWS) staff, have managed to maintain an invalid subspecies as an ESA-listing by obfuscation, intimidation, and ignoring contrary evidence. I have five years of experience on this issue because I was the scientist who led the work that questioned the validity of the Preble's mouse subspecies and its presumed rarity, and concluded that it was not a valid subspecies.

Obfuscation

The USFWS erroneously reported twice in the Federal Register that the Preble's delisting petitions relied primarily on the results of our study. That is contrary to the fact that our research was only mentioned on half a page of the 106-page delisting petitions. The delisting petitions provided abundant information that these mice are more common and widespread than previously thought. Yet the USFWS has still failed to address these data over three years later.

The USFWS Denver office organized two sets of peer reviews of our research prior to publication. However, they had failed to rigorously review the weak evidence that was used previously in *support* of the listing.

After our original research refuting the validity of the Preble's mouse as a subspecies was published in 2005, the FWS at Region 6 went looking for another study that would support the listing. Shortly thereafter, a report came out by a USGS biologist that concluded that Preble's was a valid subspecies and made a wholesale portrayal of our work as inaccurate. This USGS report was leaked to the press by a pro-listing environmental group amidst much media fanfare. Most of the press did not bother to read any of the original papers, or our responses. The key difference between these studies was how the problem was approached. We set criteria in advance of data collection and measured to those thresholds, whereas the USGS study relied on post-hoc interpretations and used a level of divergence so low that almost any population could be listed as endangered under the ESA, effectively removing such decisions from the realm of science.

In March of 2006, the staff at Region 6 sought to rush through approval of a peer review panel composed largely of agency biologists and scheduled for a time when I could not attend. After their efforts failed, another peer review panel was organized. The lead author of the USGS study, as well as environmental groups, influenced the structure and composition of the panel. A double standard was applied to evaluating panelist's conflicts of interest and to evaluating the evidence itself. Instead of reviewing <u>all</u> of the available science, the panel arbitrarily created its own burden of proof, which it then unilaterally

applied only to our study. Rather than focus on the real issue of appropriate thresholds that can be used to define subspecies, they diverted attention by focusing criticism on results from a handful of specimens in our study. The panel failed to acknowledge that reanalysis of our data without these specimens, did not alter the overall results or conclusions of our study. Ultimately, if this panel's recommendations are followed and applied to other cases, it would mean that many inadequately defined subspecies would not be potentially falsifiable (i.e. could never be questioned). This effectively puts ESA-listed subspecies evaluations outside the realm of scientific investigation.

We respectfully disagreed with the conclusions of the USGS study and prepared a response paper. That paper was accepted for publication in February 2007, however, the lead author of the USGS study managed to delay publication of our paper for months.

Intimidation

Over the course of two years I was harassed and intimidated by USFWS Denver staff, most notably, the leader of the Preble's Recovery Team who cursed me in harassing telephone messages, wrote fallacious slander about me to my supervisors, and threatened to withhold research funding for the project. A Preble's mouse consultant, representing a coalition of environmental groups, USFWS staff, and academics, all of who have financial stake in the Preble's listing or others like it, put pressure on my employer.

Ignoring contrary evidence

Most contrary information to the Preble's listing is absent from the USFWS Preble's Meadow Jumping Mouse Home Page. The USFWS gives dismissive treatment to contrary information in Federal Register notices, or does not provide it to peer reviewers. This speaks volumes about the selective use of information by this agency. For example:

- The USFWS has not acknowledged that this supposed subspecies was originally based on measurements of only three specimens, nor have they acknowledged that the original scientist who described this subspecies in 1953 went on record in 2004 rejecting the validity of the subspecies.

- The USFWS has not acknowledged that an earlier (1997) genetics study that was used in support of the listing was never published and the data were never made publicly available, despite repeated requests. In short, that study was never subjected to a rigorous review.

- The USFWS has not acknowledged that the 1995 distribution study that was used in support of the listing was based on minimal effort and never published.

- The USFWS kept over a decade of Preble's trapping data in their files but never analyzed them. Independent analysis of those data showed that the supposedly rare Preble's mouse subspecies was far more common and widespread than previously thought.

- Contrary information missing from the USFWS website includes:

1) A 1981 dissertation that examined 9,000 specimens of jumping mice and concluded that there were *no subspecies* of meadow jumping mice.

2) A series of five papers in the journal *Animal Conservation* that followed our original study, including a 2006 response paper by my coauthors and myself. The only paper from this series that appeared on the website was the paper which supports the continued ESA listing.

3) A 1986 experimental study that showed that another species of rodent, the meadow vole, out-competes the meadow jumping mouse. In other words, when meadow vole numbers are high, meadow jumping mouse numbers are low and they are hard to catch.

4) An independent quantitative analysis of both the raw genetic data from our 2005 paper and the data from the USGS study. That quantitative analysis used thresholds from the literature and found no support for Preble's as a subspecies, let alone as an Evolutionary Significant Unit (ESU) or Distinct Vertebrate Population Segment (DPS).

5) Our August 2006 response to the Preble's review panel report that we provided to the USFWS.

6) Our response paper to the USGS study that we provided to the USFWS.

7) A 2003 study published in *Conservation Biology* that revealed that the Preble's subspecies ESA listing actually encouraged landowners to take steps that were counterproductive to conservation.

Case Two: The Coastal California Gnatcatcher

Two peer reviews of the coastal California gnatcatcher taxonomy were conducted by the USFWS (listed as an endangered subspecies). One internal peer review by federal agency biologists omitted substantial contrary information that was in the public record. The omitted contrary information included six technical reports reanalyzing the original data used to describe the subspecies, one peer-reviewed paper on gnatcatcher taxonomy, and a deposition by the scientist who described it as a new subspecies. In that deposition, the scientist recanted the reliability of key measurements, admitted to substituting estimates for missing data, and told of destroying original copies of his data *before* he finished his dissertation and published the results. Despite these revelations, the scientists who conducted the internal agency peer review then made a Powerpoint presentation to senior decision makers at the Department of Interior in Washington, D.C. That presentation

made no mention of the omitted contrary information and thus the subspecies listing of the coastal California gnatcatcher was maintained.

Case Three: Critical Habitat of Desert Bighorn Sheep in the Peninsular Ranges of California

The recovery plan for desert bighorn sheep in the Peninsular Ranges of southern California (listed as an endangered DPS) specifically called for a quantitative habitat analysis. Consequently, an extensive database of 21,055 bighorn sheep observations was compiled. However, Critical Habitat was subjectively defined by the USFWS and based upon the opinions of Recovery Team members rather than on a quantitative analysis of the observation data.

Several colleagues and I published a scientific paper on the determination of Critical Habitat for this population. We had to obtain the bighorn observation data under a Freedom of Information Act request because the local USFWS office would not release the data when requested. When we obtained the data, we found that it had been stripped of many attributes. When I asked for these additional data, I was told by the USFWS to go to the individual researchers. When I went to the individual researchers I was told: *"The USFWS data was deliberately provided in a format that would not facilitate a detailed analysis by those unfamiliar with the manner in which it was collected."*

In our subsequent analyses, we found that over 60 percent of designated Critical Habitat in the northern Santa Rosa Mountains had a near zero probability of bighorn sheep use. Critical Habitat for this DPS has been vacated in part and remanded for new rulemaking by the Court. In this case, both our analysis and the Court did not agree with the USFWS staff's so-called "science".

Conclusion

Congress and the Department of Interior could ask: "Why don't we ask the right questions in the first place *before* questionable subspecies and populations are added to the Endangered Species list?"

Obfuscation, intimidation, and ignoring of contrary evidence have contributed to the continued ESA-listing of the Preble's mouse subspecies. As shown with the second and third examples, the Preble's case is not an isolated incident; it is symptomatic of deeper problems within agencies charged with administration of the ESA. While there are many competent and dedicated staff within these agencies, there are neither adequate safeguards nor oversight to prevent other staff from cherry-picking, engaging in subjective interpretations, or completely ignoring contrary information altogether. There are scant few with the expertise or the time needed to detect such occurrences.

There are productive steps that could be taken to ensure that ESA decisions are based upon science rather than opinion and politics, while ensuring that priority for conservation effort goes to truly endangered species. I have suggested a number of these in previous Congressional testimony and publications.

Briefly, these include:

1) Take steps to ensure that all information, including contrary information, is considered in peer reviews, listing/delisting decisions and biological opinions. Consistent questions and standards in these peer reviews would serve conservation. Rather than internal agency peer reviews, require external/independent reviewers.

2) Require that data used in peer reviews, listing/delisting decisions, and biological opinions be publicly available.

3) Establish legally-definable minimum thresholds for the uniqueness of taxa that can be listed. Set the bar at a quantifiable and biologically meaningful level of distinctiveness.

4) Establish quantitative thresholds for "significance" used in DPS listings. This could be quantified in terms of percent range and/or census numbers.

5) Establish a quantitative approach for designating Critical Habitat.

6) Require compliance with priority rankings in order to allocate listing and recovery effort.

7) Take steps to eliminate financial and other conflicts of interest in Recovery Teams and peer reviews.

8) Evaluate hypothetical threats using a well-defined problem analysis approach.

In conclusion, I urge this Committee to pursue this reasonable and science-based path to protecting endangered species.

Thank you for the opportunity to write to you about these issues.

The Gazette
gazette.com

Exhibit I

Our View - Friday

September 6, 2007 - 10:47PM

Both sides now
More to Julie MacDonald case than meets the eye

There are at least two sides to every story. But until recently we had never heard the other side of Julie Mac-Donald's story, freeing her enemies to paint as unflattering a portrait of the former Interior Department official as possible. But getting a more complete and balanced story is important, since the case could continue to impact many Endangered Species Act decisions — including the proposed de-listing of the Preble's meadow jumping mouse.

MacDonald resigned as deputy assistant secretary for fish, wildlife and parks in May, not long after an inspector general report seemed to confirm that she misused her position to browbeat subordinates into altering agency science to advance political aims. As well, the IG confirmed that she provided internal agency documents to outside parties. No laws were broken, according to the IG, but her actions were questionable and created the appearance that politics unduly influenced ESA-related decisions.

Because the report was leaked to the media minus any rebuttal (which Macdonald says was never solicited by the IG), this simplistic, one-sided version of events became accepted "truth" among those who have their own political reasons for discrediting MacDonald and the Bush administration. And the repercussions didn't end with MacDonald's resignation.

The IG's report, along with politicized congressional probes of "politicized" science inside federal agencies, have been seized upon by administration foes and knee-jerk ESA defenders to raise doubts about the the integrity of every ESA-related decision in which MacDonald took part. The media now routinely report these allegations as fact. H. Dale Hall, the director of the U.S. Fish and Wildlife Service, has ordered reviews of eight or nine major ESA decisions, including the Preble's mouse, in response to the furor. The IG's report is serving as a basis for new legal challenges of agency decisions.

But it appears to us, now that we've read the IG's report and acquired a copy of MacDonald's response — which has never before been reported on, as far as we know — that she's been railroaded. So many partisans and interest groups have a vested interest in her continued vilification, however, and in the claim of politicized science inside the administration, that the record may be impossible to set straight.

Advertisement

PrinterStitial® Ads by Format Dynamics.

FormatDynamics

Print Powered By FormatDynamics

But let us at least try, by taking a closer look at a few points of contention. The IG's report insinuated that MacDonald altered range estimates for a protected bird, the southwest willow flycatcher, because a critical habitat designation might impact her " ranch" in California. But MacDonald's property — which is not a sprawling "ranch," but 80 acres of row crops — is nearly 300 miles from flycatcher habitat. She also notes that her alteration of the bird's range was approved by the agency's supposed experts, including Hall. If Hall believed the science was unsound, why didn't he object?

The IG suggested that MacDonald took an inordinate interest in ESA rulings involving California out of self-interest, because she's from there. MacDonald counters that she was responsible for signing off on all ESA decisions as part of her job. "Most ESA listing and critical habitat designations are in California," she points out. And these are often among the most contentious, high-profile cases.

The IG claimed MacDonald attempted to alter river flow requirements for the Kootenai sturgeon. She says all she was trying to do was bring clarity to "confusing and internally inconsistent language in the rule," in the interest of preventing a flawed ruling and future legal problems. She includes e-mail exchanges that seem to support her explanation.

The IG report suggested MacDonald made unreasonable demands for supporting documents from staff. She says she was authorized to make such requests and that they were "not unusual." The IG seemed to confirm allegations that she rejected the scientific findings of agency biologists. MacDonald said the law requires that the best available science be used, but she found that "FWS did not always consider all the data and often ' cherry picked' for sources and reviewers which supported their position." That's a serious allegation. We wonder why the IG isn't looking into the selective or self-serving use of science by agency insiders.

Much has been made, by the IG and others, of the fact that MacDonald is not a biologist. "She has no formal educational background in natural sciences, such as biology, " noted the IG. But this criticism " implies that the absence of a degree in biology makes one unfit to review and comment on FWS rulemakings," wrote McDonald. " That perspective, if applied to virtually any supervisory position, would result in many executives being found unfit." All she attempted to do — and was encouraged to do by her boss — was ensure that statements contained in rules were supported by data. "It requires no specialized knowledge," according to MacDonald — just a willingness to question what gets passed up the chain of command.

Much of the rest of the allegations boil down to matters of personal style. Some underlings and field personnel apparently felt intimidated by

Advertisement

FormatDynamics

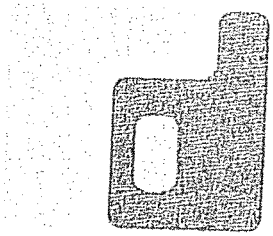
Print Powered By FormatDynamics

MacDonald, or chafed at her persistent questions. They evidently were used to rubber stamps from political appointees. Some feelings and egos were bruised in the process. And these people served as the primary sources for the IG's obviously half-baked, one-sided report.

None of this made it into the IG report — which MacDonald says she never even saw before it was leaked to the media. None of this has been factored into the simplistic story line and morality play presented by the rest of the media. Readers wanting to read the IG's report and MacDonald's rebuttal can find links in today's opinion section at Gazette.com. Fair and open-minded folks may come away — as we did — doubting the widely-accepted version of events.

We're reminded of the question former Secretary of Labor Raymond Donovan asked, after he was similarly dragged through the mud. Where does Julie MacDonald go to get her reputation back?

Advertisement



Print Powered By FormatDynamics

The Gazette
gazette.com

Our View - Sunday

THE GAZETTE

November 4, 2007 - 12:50AM

Seeing double
Mouse saga takes another bizarre turn

It was fitting that the announcement came down the morning after Halloween, given the trick-or-treat quality of the Preble's meadow jumping mouse saga. Now you see an endangered species, now you don't. Now you see an endangered species, now . . . well, now you see an endangered species in Colorado, but not one in Wyoming, according to the decision handed down by the U.S. Fish and Wildlife Service on Thursday.

A decision that should have brought more clarity and closure to the situation will sow more confusion, more controversy and more suspicions that politics is unduly influencing endangered species decisions.

As we fully expected, the agency ruled that the mouse is a valid subspecies, siding with those who would use today's advanced DNA diagnostic tools to slice and dice whole species into a potentially infinite number of subgroups. This will likely create a regulatory and administrative nightmare that takes the Endangered Species Act well beyond its original intent. The law is hard enough to manage and adequately fund when focused just on species; but it will become impossible to administer and fund if someone at the policymaking level doesn't impose some reasonable thresholds — draws a clear line on the strands of DNA, that is — on what differentiates one species or subspecies from another. This sort of reductio ad absurdum regulating couldn't have been anticipated when the law was written; it must be reviewed and updated in the light of these new developments.

Equally troubling is how agency insiders at the regional level rigged this outcome, by working to discredit and vilify the researcher who first challenged the mouse's status as a subspecies, Dr. Rob Roy Ramey — and how they went out and found a hired gun, federal scientist Dr. Tim King, to try and shoot down Ramey's work. Sen. Wayne Allard has asked that the regional office's actions be investigated. We fear that request has fallen into a black hole. But the intrigues that led to this decision would make Machiavelli blush.

The second puzzling precedent was the agency's decision to de-list the mouse in Wyoming but keep it listed in Colorado — which seems to create a subspecies of a subspecies, which might now be called the Colorado Preble's meadow jumping mouse. Mice in Wyoming don't need federal protection, they argue, because the North Platte River basin is under less development pressure than Colorado's South Platte basin. But if these creatures are plentiful enough in

Advertisement

PrinterStitial® Ads by Format Dynamics.

FormatDynamics

Print Powered By Format Dynamics

Wyoming to rate de-listing, and if Wyoming's jumping mice are identical to Colorado's jumping mice, they are not threatened at all and the de-listing should apply to both states. This strange regulatory double standard raises more questions than it resolves.

"I have a difficult time comprehending how a mouse could nest along Colorado's northern border and wake up one morning listed as an 'endangered species,' cross over into Wyoming to forage for food, and no longer be listed," said Allard, who called the decision a cave-in to "environmental extremists who plan on using this decision to extort private property owners and prevent the responsible growth of Colorado's economy."

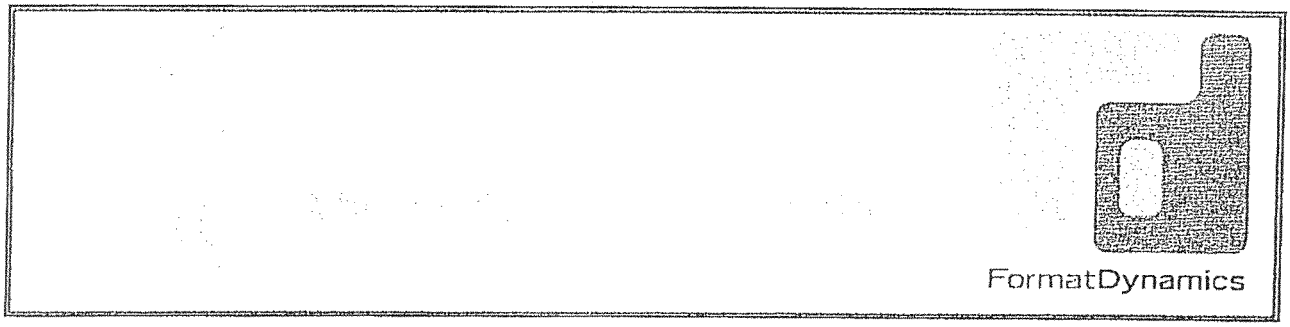
Agency officials will vehemently deny politics played any part in this decision, but it's worth noting that the governor and attorney general of Wyoming have been very (and admirably) vocal in arguing for a de-listing (Thursday's announcement came as a result of a court-ordered deadline, after Wyoming filed suit to force a decision). Colorado's governor and Legislature, in contrast, haven't been aggressive enough in fending off the regulatory juggernaut or questioning the science behind the listing.

"Wyoming kicked and screamed and fought until it was dropped from the plan" says Bob Hoff, a local Realtor who has been active in the fight against the listing.

The "logic" of tying listing decisions to development trends means the mouse will permanently remain listed in Colorado, unless 1) additional data on mouse populations eventually prove that there are more of them in the state than the service estimates, or 2) the dispute between Ramey and King about how to interpret DNA evidence swings in Ramey's direction. The regulators have thrown in their lot with King & Co., because more species mean bigger budgets, more biologists and more power. Congress must end the confusion by establishing clear, common-sense standards for what delineates one subspecies from another, at least as far as the law is concerned.

Agency officials admitted during post-announcement questioning that they really don't know how many mice there are. As well, they lack any firm numerical benchmarks by which to judge whether the regulatory actions that come with a listing are achieving recovery. "It is now nine years and $150 million taxpayer dollars later and this latest report still shows and admits that the service doesn't know much about the Preble's," says Hoff. "When it was listed in 1998, USFW thought there might be 500 or 600 Preble's in Colorado and Wyoming. When USFW did a Draft Recovery Plan in late 2001, it said that about 20,000 Preble's would be sufficient in the two states to avoid extinction. When they did a Critical Habitat Plan in 2003, you find, by closely examining the plan, that they thought there might be 32,000 Preble's in the two states. Continuing surveys since then have brought the estimated population up to approximately 60,000."
"We have recovery now," says Hoff. But the agency won't admit it.

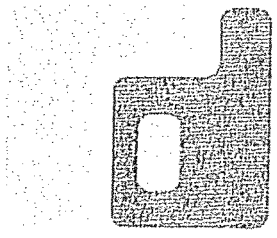
Advertisement



Print Powered By FormatDynamics

The decision shows that the Endangered Species Act is "carried out through selective citation of information, ignoring contrary information and discrediting of anyone who challenges FWS conclusions," Ramey told us. "It underscores the fact that there are no standards or thresholds with which to define 'unique' or 'endangered' under the Endangered Species Act. Decisions are based on a subjective approach that is open to bias and politics. This is precisely why the ESA has a poor record of recovering anything."

Advertisement



PrinterStitial® Ads by Format Dynamics.

FormatDynamics

Print Powered By FormatDynamics

Our View - Sunday

Colorado Springs Gazette
August 5, 2007 - 1:14AM
Fur should fly
Lamborn, Allard on the mouse's case

Democrats didn't get the big bang they were hoping for from a House Natural Resources Committee hearing Tuesday — the latest in a series aimed at nailing Bush administration officials for allegedly politicizing endangered species decisions.

The inquisitors were hoping for a smoking gun linking Vice President Dick Cheney to the die-off of salmon in Oregon's Klamath River, stemming from a water dispute in 2001, which was the focus of a recent expose (read: hit piece) by The Washington Post. But no damning revelations were produced by the dogs and ponies paraded before the committee. That's why the event didn't get much press.
But one matter of local and regional interest was raised.

Rep. Doug Lamborn placed into the record a six-page letter from Sen. Wayne Allard to Interior Secretary Dirk Kempthorne, claiming that the regional office of the U.S. Fish and Wildlife Service is conspiring to keep the Preble's meadow jumping mouse from being de-listed, through a selective interpretation of science and an effort to discredit Rob Roy Ramey, the conservation biologist whose DNA and morphological analysis refuted the rodent's status as a subspecies. (We wrote about Allard's allegations in detail Wednesday). Lamborn pressed USFWS Director H. Dale Hall to investigate these allegations, as Hall tries to make good on his pledge to safeguard the integrity of science at the agency.

This followed by less than a week a request by Lamborn for a full briefing from Kempthorne on the situation inside USFWS, where a decision on de-listing the mouse has repeatedly been delayed. We're glad someone is holding the agency's feet to the fire, given the costs and property-rights impacts this fiasco has had on people living in mouse habitat.

The credibility of endangered species science, and the suspicion that agency insiders might be finessing outcomes to suit their personal or ideological agendas, dates back to at least 2001, when federal biologists were caught planting samples of lynx fur in Washington state, suggesting the presence of animals that weren't actually there. Sounds were made at the time about shoring up agency science. But Ramey's work, along with these latest revelations, raise new doubts and suspicions.

Lamborn also had testimony from Ramey placed into the record, which echoed many of Allard's allegations and cited other cases in which endangered species decisions were

based on dubious science. We detailed some of Ramey's allegations Wednesday.

If the committee and the agency are serious about ferreting out slanted science, Lamborn said, the public also needs to know whether agency insiders, outside academics and environmental groups are unduly influencing decisions. "What we are talking about today involves transparency," Lamborn said. "My colleagues on the other side of the aisle are questioning decisions that were made (by the Bush administration) and that is fine, but if they are serious about transparency, and not just using this hearing as an opportunity to score political points, then they will join me in calling for all information regarding this process to see the light of day."

Hall gave Lamborn assurances that there would be a comprehensive review of the Preble's mouse process, including Allard's and Ramey's allegations. We'll be watching closely to see that there is.

We appreciate the initiative Allard and Lamborn are showing on this always contentious issue. Daring to question the Endangered Species Act, or the sometimes shoddy science underpinning it, is guaranteed to make one a target for those who misuse the law to boost their regulatory power and anti-development agendas — as Ramey found out.

Those who live in or near mouse habitat — those who have incurred costs and had property rights impacted as a result of this listing — are entitled to a thorough and independent investigation of the Allard and Ramey allegations. Something's rotten inside USFWS, it seems. And if Kempthorne, Hall and agency personnel really want to clear away the strong whiff of scandal, some fur should fly inside the agency.

Rocky Mountain News
Carroll: The Preble's travesty
Thursday, August 2 at 12:31 AM

The director of the U.S. Fish and Wildlife Service, Dale Hall, is concerned about the scientific integrity of the process for listing endangered species. He's right to be concerned, too, but he'll miss the real scandal if he doesn't broaden his focus.

Hall has ordered a review of eight decisions influenced by a former high-level Interior official who resigned in May after an internal probe concluded she'd pressured scientists.

Fine. Review her work. But at the end of the day, don't try to tell us that defenseless agency scientists are cowering before political intimidation. Because some of them appear quite adept at hard ball themselves.

The history of the Preble's jumping mouse, whose habitat includes the Front Range, is a case in point.

Incredibly, one of the decisions Hall has ordered reviewed is the 2005 proposal to "delist" Preble's as a threatened subspecies.

Why doesn't he review the entire history of how the agency has handled the Preble's listing? It qualifies as a travesty.

When federal officials declared the mouse threatened in 1998, they relied upon a shoddy, cursory analysis of the mouse's range and population.

The lawsuit demanding the listing actually claimed only a few hundred Preble's mice survived — an implausible judgment since refuted by a growing body of evidence.

Meanwhile, the agency took as gospel the definition of Preble's as a separate subspecies that emerged in the early 1950s after the study of three specimens by a scientist who has since disavowed his conclusion. And it discounted contrary analysis.

The agency did have a genetic analysis on its side; trouble is, its data have not been released for independent review.

The capper, however, was the treatment of Rob Roy Ramey, former chairman of the Denver Museum of Nature and Science's zoology department, after his research questioned whether Preble's is a distinct subspecies at all. Ramey announced his findings in December 2003, and he's been a marked man ever since.

A year ago, I documented the pressure put on Ramey and his employer based upon correspondence and e-mails I reviewed. It was clear that attempts to gag Ramey included angry complaints from a Fish and Wildlife official to museum leaders that apparently included a threat to suspend funding related to Preble's research.

This same official, Ramey says, denounced him in inflammatory phone messages.

Fish and Wildlife took public action, too. Seemingly determined to neutralize Ramey's research with a contrary study, it tapped a federal scientist whose previous work defining subspecies practically guaranteed he'd "refute" Ramey — which in due course he did. (Incredibly, there are no generally accepted criteria defining subspecies in such cases, and the agency resists pleas by scientists such as Ramey to establish them.)

Sen. Wayne Allard's office has since obtained copies of other correspondence shining a light on Fish and Wildlife's behind-the-scenes efforts to undermine Ramey's work, which Allard laid out in a letter sent this week to Interior Secretary Dirk Kempthorne.

Meanwhile, Fish and Wildlife has yet to seriously tackle the main argument made in the 2003 petitions requesting that Preble's be taken off the threatened list — not Ramey's work, but the fact that officials grossly underestimated the prevalence of the mouse. If Hall is interested in polishing the agency's image, he might begin by demanding an end to such foot-dragging.

Vincent Carroll is editor of the editorial pages. Reach him at carrollv@RockyMountainNews.com.

Colorado Springs Gazette
Our View - Wednesday

August 1, 2007 - 1:10AM

We smell a rat
Fish and Wildlife accused of gaming the science

Sen. Wayne Allard leveled serious allegations of scientific and regulatory malpractice against the U.S. Fish and Wildlife Service Monday, claiming in a letter to Interior Secretary Dirk Kempthorne that the agency waged a behind-the-scenes campaign to keep the Preble's meadow jumping mouse on the endangered species list by ignoring or discrediting science that contradicted its regulatory wishes.

Further, the letter says agency officials conspired with outside academics and environmental groups to discredit Dr. Rob Ramey, the biologist who first challenged the mouse's status as a subspecies, based on DNA and morphological analysis. Ramey's findings embarrassed the agency, stirred controversy and led to a still-pending petition to de-list the mouse.

Ramey echoes some of the allegations in written testimony submitted to the House Natural Resources Committee for a hearing held Tuesday, claiming that "obfuscation, intimidation and ignoring contrary evidence have contributed to the continued ESA-listing of the Preble's mouse subspecies."

These are serious enough allegations, coming from credible enough sources, that Kempthorne should ask for a thorough and independent investigation. The Resources Committee should also investigate, as long as it is looking into allegations that the Bush administration is tailoring science to conform with political agendas.

If the allegations are true, a housecleaning is in order at the Region 6 office of USFWS. A much broader review of ESA science also is called for, not just dealing with alleged efforts by political appointees to skew the science, but the role agency insiders, agenda-driven academics and environmental groups play in listing and de-listing decisions.

Internal agency e-mails Allard's office has reviewed "paint what appears to be a troubling picture of a coordinated effort on behalf of U.S. Fish and Wildlife Service staff to retain the listed status of the Preble's regardless of what the best available science tells us," Allard wrote. He says agency staff collaborated with other government personnel, academics and outside groups to skew public perceptions in favor of keeping the mouse listed, by attempting to discredit Ramey and by ignoring science that doesn't support de-listing.

"It appears that FWS staff set their minds on rebutting Ramey whatever the cost," Allard wrote, because they felt "threatened and angered" by Ramey's work. The agency secretly went out and found a hired gun, also on the federal payroll, who could be counted on to contradict Ramey's conclusions. They put pressure on Ramey's employer, the Denver Museum of Nature and Science, which ultimately led to Ramey's exit from the job (under circumstances first written about by Vince Carroll at the Rocky Mountain News.) Agency personnel worked with outside allies and a government contractor to game a scientific review of the conflicting reports in their favor, according to Allard.

"With the help of interested parties in academia, and perhaps environmental groups, FWS employed and funded an agency ally, USGS researcher Dr. Tim King, to protect Preble's listed status," Allard

writes. "After this, FWS staff influenced what was to be an 'independent review' of the genetic and taxonomic issues related to Prebles."

This culminated, as readers may recall, in the creation of a supposedly independent panel, assembled by a contractor, the Sustainable Ecosystems Institute, tasked with sorting out the seemingly contradictory findings of King and Ramey. SEI came down on the side of King, after a two-day hearing in Fort Collins that Ramey likened to an inquisition rather than a scientific hearing. Allard suggests the process may have been rigged in King's favor.

"Upon review of numerous correspondences I am concerned that outside influences may have been exerted in the Preble's panel." Allard points to the incomplete posting of papers and documents on the agency's Preble's mouse page as further evidence that bureaucrats are attempting to spin public perceptions and present one-sided science. The agency also consistently ignores studies or data that indicate the mouse is more abundant than claimed.

It's a long and potentially damning list of allegations — which can be read in its entirety by using the "Opinion" link at Gazette.com. The senator showed courage in bringing them forward, given the vilification that's heaped on those who dare challenge the validity of ESA science or the credibility of USFWS.

In his testimony (which also is posted at Gazette.com), Ramey echoes some of Allard's allegations — including the attempts to smear and discredit him by one USFWS staff member. He cites other cases of slipshod science being used in listing or critical habitat decisions. Among other things, he says the agency for a decade ignored trapping data that show the mouse is more plentiful than claimed.

"In the case of the Preble's mouse," Ramey wrote, "the record will ultimately show that special interest groups, individuals and academicians with vested financial interests, and some U.S. Fish and Wildlife staff, have managed to maintain an invalid . . . ESA listing by obfuscation, intimidation and ignoring contrary evidence." Ramey, who worked for a time as a scientific trouble-shooter under contract to the Department of Interior, said the "Preble's case is not an isolated incident," but is "symptomatic of deeper problems within agencies charged with administration of the ESA."

These allegations deserve the prompt and aggressive attention of Kempthorne and USFWS Director H. Dale Hall, who has pledged to restore the integrity of science at the agency. Hall and the department's inspector general have been focusing on the alleged meddling in ESA science by former Interior Department official Julie MacDonald. But agenda-driven science by agency insiders, colluding with outside parties, deserves equal attention.

Only a comprehensive, outside review of ESA related decision-making — including the allegations by Allard and Ramey — will lift this cloud from the law and the agency.

Our View - Sunday

July 29, 2007 - 12:08AM
A twisted tail
Preble's mouse case takes another turn

Former Interior Department official Julie MacDonald may in fact be the wicked witch of the West, who bullied and badgered underlings into tailoring endangered species science to suit the Bush administration's political agenda. Or, as we suspect, she was a hard-charging political appointee who became the target of a witch hunt because she ruffled the feathers of career bureaucrats and challenged agency scientists to justify their conclusions and decisions.

But the decision to de-list or not de-list the Preble's meadow jumping mouse should be made strictly on the science — of which there is now plenty, thanks to agency outsiders who dared to question the listing. Using the political firestorm swirling around MacDonald as an excuse for further delay also amounts to "politicizing" the process.

Fish and Wildlife Service Director H. Dale Hall last week announced that at least eight ESA decisions in which Mac-Donald was involved will be "re-examined," in light of the allegations. Hall called the MacDonald affair a "blemish" on the "scientific integrity" of the agency he intended to expunge.

But there's been no decision to re-examine, at least in the Preble's case, despite a mountain of new (if sometimes contradictory) scientific data. Let's first have a decision, Director Hall, so those of us in mouse habitat aren't living in regulatory limbo. Then you and others can fret over MacDonald's undue influence, if she had any.

We, too, welcome an objective and comprehensive review of "scientific integrity" at the agency — if it goes well beyond the partisan focus on MacDonald. We hope Hall also will take a closer look at the flimsy scientific basis for the mouse's original listing, for instance.

We hope he'll also investigate why agency officials, when confronted with DNA and morphological analysis by Dr. Rob Roy Ramey which questioned the listing, went out shopping for a second opinion (from a researcher on the federal payroll) they knew would refute Ramey's work. As well, we hope Hall takes a second look at whether this creature is as rare as claimed, since some have also questioned this "scientific" finding.

A broader review would show that Hall is really interested in upholding scientific integrity at USFWS, rather than just playing to witch-hunters on Capitol Hill and in environmental groups — who wrote the book on politicized science.

In past discussions with MacDonald's one-time boss, former Assistant Secretary of Interior Craig Manson, he told us that he directed her to bring more rigor to the agency's scientific work. That's important, Manson said, given the impact endangered species regulations can have on property owners and the economy. And while Manson doesn't necessarily endorse all MacDonald's actions, or her personal style, he believes her intentions were good — and that senior officials need to do more than rubber stamp the work of staff, some of whom may have their own agendas.

Ramey, whose research first called into question the mouse's status as a subspecies (and who

knows MacDonald, through his former work as a DOI consultant), believes a double standard is applied to scientific evidence inside the agency. "No one is asking how good the evidence is in listing decisions or biological opinions," he says. "Rather, the service seems focused on destroying anyone who questions its authority."

Ramey likens the heat he has taken for challenging the Preble's listing and offering critiques of how ESA is administered "to the Inquisition of the Middle Ages, or of McCarthyism in the 1950s, when dogma and the authority of certain officials could not be questioned without serious consequences."

The evidence used in many listing decisions is a house of cards, Ramey told us. "In the case of the Preble's mouse, we checked just one corner of this house of cards and the whole house shook. Subjective and unsubstantiated opinion often pass as science at the service because there are no standards or thresholds to measure evidence against," he said.

Perhaps MacDonald became a target because she, too, dared to question agency bureaucrats and biologists.

State Games a chance to shine

Let the games begin! No, not the Olympics. They're still a year off. We're talking about the 2007 State Games of America, which begin here in Colorado Springs on Thursday, when Olympic skater Michelle Kwan lights the torch at the opening ceremonies.

The events, which will attract 10,000 athletes of all ages and skill levels from 46 states, "will be the largest multi-sport, Olympic-style event in Colorado history," says Mike Moran, communications director for The Colorado Springs Sports Corporation. Along with cementing this city's national and global reputation as a center of sports, the games could provide a $11 million economic boost.

"We have something for everyone starting Thursday, with our gala opening ceremony at the Colorado Springs World Arena and figure skating great Michelle Kwan lighting the torch to open the Games," Moran tells us. And making it a success is a city-wide endeavor, involving 2,000 local volunteers and 90 businesses, corporations or foundations that have signed up as sponsors (including The Gazette). Tickets and more information are available at www.stategames.org.

The games are scheduled to return to the Springs in 2009, and next year we'll play host to the first-ever State Games of the West, which are expected to attract 8,000 athletes from Colorado and other Western states.

We know we speak for everyone in the Springs when we welcome athletes, spectators and dignitaries to the games. Play hard and have a wonderful time.

Rocky Mountain News

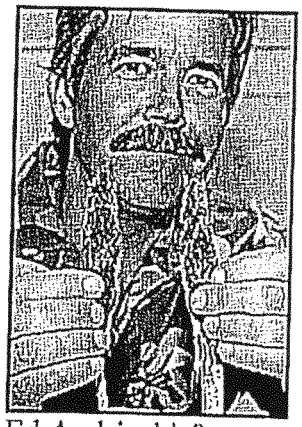
To print this page, select **File** then **Print** from your browser

URL: http://www.rockymountainnews.com/drmn/opinion_columnists/article/0,2777,DRMN_23972_4595830,00.html

On Point: The mouse that roared

April 5, 2006

Rob Roy Ramey's distinguished career at the Denver Museum of Nature & Science began its death spiral not long after he released research questioning whether a Front Range mouse is a unique subspecies and therefore worthy of federal protection. Coincidence? Ramey, the former chairman of the museum's zoology department, doesn't think so, and he has documents to back him up.



Ed Andrieski &copy AP © 2005

Rob Roy Ramey holds a meadow jumping mouse, left, from Mercer County, N.D., and a Preble's meadow jumping mouse, right, from El Paso County, Colo.

Ramey's conclusions, first announced in December 2003 and refined until their publication last year in *Animal Conservation*, a journal of the Zoological Society of London, startled and upset many of those involved in endangered species protection and environmental activism. Among those most irate, evidently, was an official in the U.S. Fish & Wildlife Service, whose complaints became so vocal by September 2004, according to Ramey, that museum executives met with him to smooth his feathers. But he remained unappeased - or so it seems based upon a letter the museum's chief curator and another top official sent to him on Oct. 26, 2004.

"Labeling Dr. Ramey as 'pro-development' is unfortunate and inaccurate," they advised. Moreover, they suggested, "to suspend all funding for continued understanding of this animal," as the federal official apparently threatened to do, "seems counter to the principles of the USFWS" - as of course it was.

But Fish & Wildlife was hardly alone in its displeasure with Ramey's research on the Preble's meadow jumping mouse. Environmentalists repeatedly tried to impugn Ramey's credibility (*Westword's* David Holthouse did a fine job of recounting some of these attacks in an article published in January of last year), while professionals in the research and consulting communities weighed in, too.

For example, Ramey shared with me the copy of an e-mail he received during a particularly tense period in 2004 from Carron Meaney, a private consultant and "research associate" at the museum, warning Ramey "there are a lot of people who question your approach and have concerns about working with the museum in the future. I love the DMNS, and am very concerned to watch the alienation your behavior has wrought between the museum and the biology community."

Not the most subtle warning, was it?

Ramey says that despite the brave front the museum presented to Fish & Wildlife, in fact his superiors fretted repeatedly about outside pressure. At one point, he says, a museum official asked if there was anything in his past that could "embarrass" the institution. Another suggested Ramey was being "co-opted" by developers. And his relationship with the museum continued to deteriorate into the early months of last year, until he saw the writing on the wall and officially resigned.

But make no mistake: Ramey insists his departure, here publicly detailed for the first time, was a direct consequence of the inflamed reaction to his research. And that reaction, let's also be clear, had less to do with science than with the *political* agenda of environmentalists and their allies in federal and state agencies who are determined to use the Endangered Species Act to influence land-use decisions up and down the Front Range.

Not surprisingly, the Fish & Wildlife Service and Denver Museum of Nature & Science dispute Ramey's version of events. "We had nothing to do with what they did with Mr. Ramey," Fish & Wildlife's Sharon Rose told me. Moreover, "it was never expressed by us that there were any problems with his work."

Museum officials were even more emphatic, while lavishing praise on Ramey himself.

"We strongly support Rob's research," said CEO George Sparks. "He's a great scientist."

"We don't make personnel decisions based upon what someone (on the outside) wants," Sparks also insisted. "Ethically it wouldn't be right."

Richard Stucky, curator of paleoecology and evolution, went further. There "was no direct feedback to me at the museum" suggesting Ramey had become a problem, he said.

There are several obvious objections to such wholesale denials, even if museum officials had legitimate reasons for disenchantment with Ramey that they feel they can't disclose. To begin with, Sparks didn't join the museum until November 2004 and was in no position to assess the reaction to Ramey's research during many critical months. Stucky, meanwhile, is one of two museum officials who signed the 2004 letter protesting the description of Ramey as "pro-development." If that term doesn't qualify as "direct feedback," perhaps it's no surprise Stucky can't remember other examples, either.

For that matter, Stucky was copied with Meaney's e-mail in which she mentioned "colleagues at CU-Boulder, DU, UNC, USFWS, and the coalition of environmental organizations here in the Front Range" as being upset with Ramey. And yet Stucky failed to object when Sparks assured me, "I don't think there was any unhappiness with the research."

Right. No unhappiness whatever - outside of CU-Boulder, DU, UNC, USFWS, and that coalition of environmental groups.

Thursday: More on why the Preble's posse went after Ramey.

Vincent Carroll writes On Point several times a week (carrollv@RockyMountainNews.com).

MORE CARROLL COLUMNS »

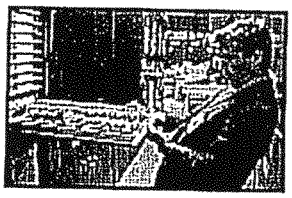
Copyright 2006, Rocky Mountain News. All Rights Reserved.

Rocky Mountain News

To print this page, select **File** then **Print** from your browser

URL: http://www.rockymountainnews.com/drmn/opinion_columnists/article/0,2777,DRMN_23972_4598923,00.html

On Point: Species act abused



Rob Roy Ramey

April 6, 2006

Last in a series

Concern for a common rodent, misleadingly called the Preble's meadow jumping mouse, is costing local governments and private landowners in Colorado tens of millions of dollars. But lots of people have a stake in preserving this ludicrous state of affairs. They have so much invested, in fact, that when the chairman of the Zoology Department at the Denver Museum of Nature & Science released research questioning the validity of the mouse's "threatened" status under the Endangered Species Act, the resulting clamor may have cost him his job.

I recounted Rob Roy Ramey's clash with the Preble's posse in a column Wednesday that can be read at www.rockymountain news.com/drmn/opinion/. But the story doesn't end with Ramey's flight last year from the museum. Because of his findings that Preble's was indistinguishable from meadow jumping mice that range as far north as Montana, the U.S. Fish & Wildlife Service had little choice but to reconsider whether Preble's was really threatened at all.

Not so fast, replied the Preble's posse. It enlisted Tim King of the U.S. Geological Survey, a scientist famously able to discern distinct "subspecies" where others have failed to see them, to resuscitate the Preble's fiction. And King dutifully delivered. His research, released in January, claims to discover significant genetic differences between Preble's and other jumping mice, giving the Fish & Wildlife Service the excuse it needed to freeze the Preble's delisting process.

How could Ramey's and King's studies reach such starkly different conclusions? Because the Endangered Species Act gives biologists astonishing freedom to define - essentially, to invent - separate populations that require federal protection.

Most Americans don't realize that the Endangered Species Act protects not only species, but also subspecies and "distinct population segments" - and that those last two terms open the door to manipulation and abuse.

"Federal biologists and environmental groups use these terms to put inappropriate groups in the ESA list," explains Matthew Cronin, research associate professor for the School of Natural Resources at the University of Alaska at Fairbanks, in an article in *Range* magazine. "By my count, 70 percent of the ESA's listings of mammals in the United States are subspecies or populations."

"There is so much variation among populations of most species," Cronin notes, "that some combination of characters will distinguish each population from others and consequently there is no clear limit to the number of subspecies that can be recognized.

"Widespread species thus can be divided into any number of different sets of 'subspecies' simply by selecting different characteristics on which to base them."

He might as well be describing the meadow jumping mouse.

Naturally King was able to conclude Preble's is a different subspecies. There are scientists who could probably locate a separate subspecies, or at least a "distinct population segment," of some creature in a randomly chosen backyard.

Cronin would revise the Endangered Species Act so that it protected only full species. Ramey wouldn't go so far, but he too believes "we need to establish consistent, legally definable thresholds of genetic uniqueness to qualify for listings," and has even made this case in testimony before Congress. That's what really inflames the Preble's posse: that legitimate scientists would dare to expose the scandal of how the Endangered Species Act is being abused to pursue personal and activist agendas.

No one seems to understand this more clearly than Colorado Attorney General John Suthers and his counterpart from

Wyoming, Patrick Crank. Last month they wrote a six-page letter to then Interior Secretary Gale Norton and Fish & Wildlife Director Dale Hall imploring them to help prevent a "serious mistake by the Fish & Wildlife Service Region 6 in Denver" concerning the Preble's mouse. Suthers and Crank argued, correctly, that Preble's should have been delisted even without Ramey's research given how many of the mice have been found in recent years, some in places no one even suspected they lived.

"The Preble's mouse is not threatened," they write, "it just was not well trapped in 1998" when it was first declared a threatened subspecies.

The letter goes on to recount the series of curious decisions by Region 6 that have thwarted every attempt to delist Preble's, adding that "we are growing more concerned about the impartiality of Region 6 on the Preble's mouse petitions as time wears on."

As they should be. By Fish & Wildlife's own estimate, the cost of the Preble's listing to public and private landowners is as much as $18 million a year, and yet still the agency dithers rather than retract a mistake. But thanks to Rob Roy Ramey, at least the dithering is a great deal more awkward for the federal bureaucrats than it ever was before.

Vincent Carroll, editor of the editorial pages, writes On Point several times a week. Reach him at carrollv@RockyMountainNews.com.

MORE CARROLL COLUMNS »

Copyright 2006, Rocky Mountain News. All Rights Reserved.

On Point: Trouble with Preble's

Anne Ruggles ©

The Preble's meadow jumping mouse

Vincent Carroll
email | bio
January 26, 2006

"Science is like a captured spy; torture it long enough and it will say anything."

- William Ruckelshaus, first director of the Environmental Protection Agency

Science seems to be of two minds regarding the Preble's meadow jumping mouse, a little critter whose very name provokes heartburn in Front Range developers.

First, science concluded that Preble's was a separate subspecies, an opinion that survived several decades.

Then science announced that Preble's was a fraud, indistinguishable from a mouse whose range runs into Montana and South Dakota.

Now, as if right on cue to halt Preble's removal from the government's "threatened" list, science has sprinted up to breathlessly proclaim Preble's a unique subspecies after all. This latest study relies upon "an enormous amount of data," one supporter enthused, which was "very objectively" analyzed.

Perhaps. But however serious the latest study, so was the research that debunked Preble's in 2003. That work was reviewed, revised, refined and published just last year in *Animal Conservation*, a journal of the Zoological Society of London. So hang tight: It remains to be seen which group of scientists tortured the spy.

In one important sense, it doesn't even matter who did. Preble's isn't "threatened" even if it *is* unique. When the U.S. Fish & Wildlife Service declared Preble's in danger in 1998, it had scant knowledge of the mouse's true range or numbers - and discoveries in the past eight years have not been kind to the agency's assumptions. The mouse is far more numerous, and its range far more extensive, than the government thought.

Most of Preble's-occupied sites are so remote, moreover, that subdivisions will never come knocking on their doors.

Cross Country / *By Stephen Moore*

Of Mice and Men

Denver

Here in Colorado, the hottest political issue of the day may not be the war in Iraq or the out-of-control federal budget, but rather the plight of a tiny mouse. Back in 1998, a frisky eight-inch rodent known as the Preble's meadow jumping mouse gained protective status under the 1973 Endangered Species Act (ESA). What has Coloradans hot under the collar is that some 31,000 acres of local government and privately owned land in the state and stretching into Wyoming—an area larger than the District of Columbia—was essentially quarantined from all development so as not to disrupt the mouse's natural habitat. Even the Fish and Wildlife Service concedes that the cost to these land owners could reach $183 million.

What we have here is arguably the most contentious dispute over the economic impact of the ESA since the famous early-'90s clash between the timber industry and the environmentalist lobby over the "endangered" listing of the spotted owl in the Northwest. That dispute eventually forced the closure of nearly 200 mills and the loss of thousands of jobs. Last week the war over the fate of the Preble's mouse escalated when a coalition of enraged homeowners, developers and farmers petitioned the Department of the Interior to have the mouse immediately delisted as "endangered" because of reliance on faulty data.

The property-rights coalition would seem to have a fairly persuasive case based on the latest research on the mouse. It turns out that not only is the mouse not endangered, it isn't even a unique species.

The man who is almost singlehandedly responsible for exposing the truth about the Preble's mouse is Rob Roy Ramey, a biologist and lifelong conservationist, who used to serve as a curator at the Denver Museum of Nature and Science. Mr. Ramey's research—published last year in the peer-reviewed journal Animal Conservation—concluded that the Preble's mouse "is not a valid subspecies based on physical features and genetics." The scientist who conducted the original research classifying Preble's as unique now agrees with Mr. Ramey's assessment. Even scientists who defend extending the mouse's "endangered" status admit that it is 99.5% genetically similar to other strains of mice.

Nor is the mouse on the road to extinction. "The more people look for these mice, the more they find. Every time scientists do a new count, we find more of the Preble's mouse," Mr. Ramey says. It's now been found inhabiting twice as many distinct areas as once thought. These *are* mice, after all, and the one thing rodents are proficient at is breeding. The full species of the meadow jumping mouse, far from being rare, can be found over half the land area of North America.

"The federal government has effectively shut off tens of millions of dollars of economic development," complains coalition spokesman Kent Holsinger, "based on saving a species that we now know doesn't even exist." But green groups and Department of Interior bureaucrats, who regard the ESA as a sacred pact—the modern-day equivalent of Noah's Ark, as former Interior Secretary Bruce Babbitt called it—pledge to fight any change in status.

Not surprisingly, Mr. Ramey has been accused of being "dishonest," a "whore for industry" and a "shill for the Bush administration." Under intense political pressure from environmental activists, he was removed from his curator's job at the museum. "I've been nearly stampeded by a herd of agitated elephants in Africa and suspended from some of the highest cliffs in North America, but nothing prepared me for the viciousness of the attacks from the environmentalist lobby," he tells me.

> *Property rights vs. 'endangered' species.*

Meanwhile, the Preble's mouse continues to impose huge costs on local communities. One water district in Colorado was recently required to build two tunnels for the mice under a man-made pond to spare the critters the inconvenience of having to scurry around it. Regulators asked local officials if it would be feasible to grow grass in the tunnels for the mice, which was only slightly less absurd than padding the mouse thoroughfares with red carpet. The extra cost to the water project to make it mouse-friendly? More than $1 million. The Fish and Wildlife Service also has the authority to assess penalties on property owners if they even inadvertently spoil mouse habitat. Owners can even be fined if their cats do what cats do: chase and apprehend mice.

A comprehensive 2003 survey found that more than one in four land owners impacted by the Preble's mouse regulation "admitted to actively degrading habitat following the species listing in 1998." This is often precisely what happens in these situations: Because most of 1,500 or so species that have been listed as threatened since 1972 are anything but, people have no respect for the designation and attempt to force the species away from their land. They call it "shoot 'em, shovel 'em and shut up." For truly endangered species, the ESA is a disaster.

Many individuals have been so strong-armed by federal bureaucrats that t[illegible] have come to believe—with good reasc that the original and widely supported intent of the ESA has been subverted into a back-door means to slam the brakes on economic development. "It's a cost-free way for the government and the greens to impose land-use control on property owners," says R.J. Smith, an ESA expert at the Competitive Enterprise Institute.

Therein lies the crux of the problem. The law tries to achieve the societal policy goal of saving species from extinction by imposing all of the costs on a hapless few. House Resources Committee Chairman Richard Pombo has sensibly proposed reforms that allow landowners to get fair compensation from the government if their land is depressed in value due to a wetlands or endangered species designation. That seems equitable: If society wants to preserve habitat for the common good, then the cost should be borne by all taxpayers, not individual landowners, who would no longer regard endangered species as an economic plague.

If anything good can come out of the Preble's mouse fiasco in Colorado, it will be that it has awakened Congress to the reality that the ESA isn't just failing property owners but the very irreplaceable species it was designed to protect.

Mr. Moore is a member of The Wall Street Journal's editorial board.

Exhibit J

Animal Conservation. Print ISSN 1367-9430

Response to Vignieri *et al.* (2006): Should hypothesis testing or selective *post hoc* interpretation of results guide the allocation of conservation effort?

R. R. Ramey II[1], J. D. Wehausen[2], H.-P. Liu[3], C. W. Epps[4] & L. M. Carpenter[5]

1 Department of Conservation Biology, Denver Zoological Foundation, Denver, CO, USA
2 University of California, White Mountain Research Station, Bishop, CA, USA
3 Department of Biological Sciences, University of Denver, Denver, CO, USA
4 Department of ESPM, University of California, Berkeley, CA, USA
5 Department of Zoology, Denver Museum of Nature & Science, Denver, CO, USA

Keywords: *Zapus*; subspecies; taxonomy; conservation; Endangered Species Act.

Correspondence
Rob Roy Ramey II, Department of Conservation Biology, Denver Zoological Foundation, 2300 Steele St., Denver, CO 80205, USA.
Tel/Fax: 303 258 9535. Email: ramey@spot.colorado.edu

Received 15 May 2006; accepted 6 June 2006

doi:10.1111/j.1469-1795.2006.00056.x

In their response to Ramey *et al.* (2005), Vignieri *et al.* (2006, this issue; hereafter VEA) claim that they are concerned about erroneous application and interpretation of morphometric, genetic and ecological data. We share this concern, which is why we used a consistent hypothesis-testing approach to test the taxonomic validity of Preble's meadow jumping mouse *Zapus hudsonius preblei* as a subspecies and its uniqueness as a distinct population segment. We used critical tests that were set in advance of data collection to avoid subjective *post hoc* interpretation of results. We also used multiple lines of evidence for our tests of uniqueness to avoid erroneous conclusions (Ramey *et al.*, 2005). We do not agree with VEA that four lines of corroborating evidence can be considered to be 'narrow in scope'.

Contrary to their stated goals, VEA did not accurately portray our goals, methods, results or conclusions. They selectively cited information and relied on speculation and *post hoc* interpretation of results to support their claims that *Z. h. preblei* is a distinct subspecies and an 'evolutionary distinct mouse'. We contend that the approach used by VEA was less than objective and, if widely applied, could result in the misallocation of conservation effort to many non-distinct local populations.

Morphometric analyses

At the center of this debate is the separation of *Z. h. preblei* as a subspecies by Krutzsch (1954) based on measurements of only three skulls and comparisons of only four skins – sample sizes that no modern taxonomist would accept. In their attempt to defend this taxonomy, VEA try to discredit all of our morphometric analyses, while ignoring the work of Jones (1981) that found no morphological support for any subspecies of *Zapus hudsonius*. VEA state that our analyses suffered from intercorrelated data because 26 of 36 correlations among the nine skull measurements were significant at $P<0.001$. Yet, these were the same measurements used by Krutzsch (1954), whose conclusions they attempt to defend. Traditional frequentist statistical tests that emphasize *P*-values have come under strong criticism (Cherry, 1998; Johnson, 1999; Anderson, Burnham & Thompson, 2000). Indeed, the *P*-values that VEA cite for correlations in our data reflect the large cumulative sample size we used, rather than statistically important levels of correlation among variables used in multivariate analyses of shape variation.

Krutzsch's sample sizes precluded meaningful statistical tests, and he used none; yet, VEA concluded that his finding of a smaller interorbital breadth in *Z. h. preblei* was a 'definitive finding'. VEA claim that interobital breadth was the only one of the morphometric variables we measured that Krutzsch (1954) found to distinguish *Z. h. preblei* and that our finding of a difference for that character confirmed Krutzsch's (1954) conclusion. What Krutzsch (1954) actually stated was that *Z. h. preblei* was smaller than *Zapus hudsonius campestris* in most of the nine skull dimensions measured, a hypothesis that our data clearly refuted. Such univariate tests that VEA appear to espouse were replaced decades ago in morphometric analyses by multivariate analyses of shape variation (Reyment, Blackith & Campbell, 1984), which was the approach we used.

VEA criticize us for ignoring unquantified characters that Krutzsch (1954) included as the basis of his taxonomy, describing these as 'shape differences noted by a trained morpho-taxonomist'. They fail to realize that this 'trained morpho-taxonomist' (Krutzsch) does not accept his

 © 2006 The Authors. Journal compilation © 2006 The Zoological Society of London

taxonomy and has publicly stated that our research 'clearly invalidates *Z. h. preblei* and demonstrates its relationship to *Z. h. campestris*'. He went on to state: 'Perhaps most significant is the model you provide to unequivocally establish the uniqueness of an organism and its relationships before declaring it in danger of extinction. Such an analytical approach would prevent implementation of a process to support an agenda or a point of view. I can think of other listed endangered species that could have benefited for a prior, detailed, scientific appraisal' (P. Krutzsch in email to R. R. Ramey, entered into the U.S. Congressional Record on 28 April 2004).

Ecological analysis

Contrary to VEA's claims, we did not deny that *Z. h. preblei* seems to be currently isolated. What we questioned was *how long* this isolation has existed. Nor did we 'present nothing' that could be interpreted as a test of ecological exchangeability. We cited the original morphological research of Krutzsch (1954) and Jones (1981) as well as the literature reviews of Whitaker (1972, 1999), Clark & Stromberg (1987) and Cryan (2004) in support of our claim that no adaptive differences have been described between *Z. h. preblei* and other subspecies. Although it is possible that some critical adaptive difference had been 'missed' in the 106 years of study, starting with Preble (1899), none seem to have been noticed.

VEA make the assertion that 'the potential for ecological differentiation among these populations (putative subspecies of *Z. hudsonius*) is high'. However, the evidence and rationale they provide is speculative. VEA base their claims on Küchler's (1964) potential natural vegetation (PNV) classifications. PNV classifications are based on hypothetical 'climax' vegetation that could potentially occupy a site without disturbance or climatic change (Zerbe, 1998). PNV classifications are not mutually exclusive categories. For example, each of the PNV classifications cited in VEA has overlap in plant species. PNV classifications are qualitative, generalized descriptions of vegetation communities that do not take into account the mosaic nature of natural landscapes, including successional stages, nor do they accurately characterize moist riparian habitat occupied by *Z. hudsonius* in the Great Plains. VEA ignore the fact that *Z. hudsonius* is a generalist species in its food habits (eating seeds, insects, fruit and fungi) and habitat preferences (Quimby, 1951; Jones, 1981), making adaptation to specific forage species less likely. VEA's assertion that the potential for ecological differentiation is high is therefore questionable. Most importantly, speculation is an inappropriate basis for definitions of subspecies or lower levels of population distinction (Ball & Avise, 1992; Crandall *et al.*, 2000; Cronin, 2006); yet VEA declared *Z. h. preblei* a 'habitat-specific subspecies group'.

Molecular genetic analyses

VEA have made a case on the small value of the unscaled migration rates (m) derived from our analyses of mtDNA variation, mistakenly suggesting that these rates reflect the number of migrants per generation. In fact, the scaled migration rates (N_em) reflect a theoretical number of migrants per generation of 0.09–0.87 among putative subspecies. Although this value is low and suggests the possibility of continuing divergence because of genetic drift, we consider the relative ranking of gene flow rates between putative subspecies as more informative. This analysis suggests that *Z. h. preblei* and *Z. h. campestris* have recently experienced gene flow at higher levels than any other comparison, except *Z. h. campestris* and *Zapus hudsonius intermedius*.

VEA inaccurately report that reciprocal monophyly was the sole criterion we used for accepting divergence among subspecies. VEA seek to explain away the shared haplotypes among subspecies by labeling them as 'contaminant' haplotypes rather than acknowledging that shared variation is a common biological phenomenon. They attribute this 'contamination' to incomplete lineage sorting. Their table 1 shows that 22.6% of *Z. h. campestris* mtDNA sequences were *Z. h. preblei* haplotypes. This is hardly incomplete lineage sorting. Even if the mtDNA results for these seven samples are excluded from analyses it does not change the results to a degree that would lead to the alteration of our conclusions (MDIV: range of M 0–0.32; AMOVA 0.52 between *Z. h. preblei* and *Z. h. campestris; Z. h. preblei* is paraphyletic with low bootstrap support).

VEA seek to invoke selective *post hoc* interpretations to explain away our microsatellite results. They equate statistical significance (in F_{ST}) with biological significance and selectively cite other mammal subspecies comparisons in support of their claim of 'strong differentiation' of *Z. h. preblei*. VEA incorrectly report that '95% of the northern population of *Z. h. preblei*' was assigned. What we did find was that 94% of the southern population could be assigned (table 6, Ramey *et al.*, 2005), but we did not use any cut-off value for confidence of assignment (q). Therefore, some of these assignments were only slightly better than coin flips. VEA contradict themselves in stating that we 'employed too few loci' while also concluding that our microsatellite results add 'further strong support of differentiation' of *Z. h. preblei*.

Z. h. preblei and the US-Endangered Species Act (ESA)

VEA suggest a double standard in evaluating evidence used in ESA listings. They state that Ramey *et al.* (2005) 'should most certainly not be presented as an adequate basis for the making of taxonomic decisions regarding a (US-ESA) listed taxon'. Yet they ignore the fact that *Z. h. preblei* was US-ESA listed based on far fewer data – Krutzsch's (1954) study of just a few specimens and an unpublished qualitative mtDNA study for which the data were never made public (Riggs, Dempcy & Orrego, 1997).

VEA raise some important questions with regard to subspecies and populations relative to the ESA. How should conservation effort be allocated relative to (1) hypothesized adaptive uniqueness, (2) geographic isolation of recent

origin and (3) populations showing minor differentiation at a few neutral loci that may be due to recent anthropogenic population bottlenecks?

We agree with VEA that it is impossible to predict future patterns of speciation. However, the US-ESA is not a biodiversity law that mandates the protection of all potential pathways to speciation (e.g. weakly differentiated populations or hypothetical evolutionary trajectories). VEA's suggestion that the ESA should protect all potential speciation pathways is impractical, logically inconsistent and not a view supported by the courts. It is impractical because there is great uncertainty in predicting potential speciation pathways. It is logically inconsistent because the evolutionary potential for some species can only be realized through the extinction of other species (e.g. in cases where one species is limited by another), leading to conflicting listing and recovery goals. Lastly, VEA's approach is in conflict with a recent US Ninth Circuit Court ruling that while 'the USFWS can draw conclusions based on less than conclusive evidence, . . . it cannot base its conclusions on no evidence' (National Association of Homebuilders vs. Norton, No. CIV-00-0903-PHX, 2001). In other words, US-ESA decisions cannot be based on speculation or hypothetical scenarios alone.

In listing *Z. h. preblei* as 'threatened', the US Fish & Wildlife Service (USFWS) concluded that there was a loss of populations over a significant portion of its range (USFWS, 1998). Post-listing surveys have shown this conclusion to be erroneous. Historically (pre-1980), the range of *Z. h. preblei* was thought to be restricted to 14 eighth-order hydrologic units along the eastern edge of the Rocky Mountains in Colorado and Wyoming (State of Wyoming, 2003; data from Wyoming Natural Diversity Database and Colorado Natural Heritage Program), of which nine were thought to be occupied at the time of listing based on minimal survey efforts (USFWS, 1998). This rodent is now known to occur in all historically occupied hydrologic units in both Colorado and Wyoming. In addition, it has been captured in three hydrologic units north and east of its presumed historic range: the Upper Laramie Hydrologic Unit in Wyoming as well as the Kiowa and Chico Hydrologic Units in Colorado (State of Wyoming, 2003; see tables 4 and 5). Although development and habitat alteration have certainly caused some local extirpations, the number of occupied locations within these hydrologic units has increased over fourfold with greater survey effort, to over 126. Consequently, it appears that data on taxonomic uniqueness and geographical distribution used for ESA listing were both questionable. Yet, VEA propose to maintain the status quo of *Z. h. preblei* under the ESA. This raises fundamental questions regarding the allocation of conservation effort.

The US Government Accountability Office recently reported that the time and costs that are required to recover US-ESA listed species, subspecies and distinct vertebrate populations are largely unknown (US Government Accountability Office, 2006). With the costs and duration of most US-ESA listings unknown, it would seem that prioritization in the allocation of conservation effort would become imperative. However, this has not been the case. Although a prioritization scheme was established in the 1982 amendments to the US-ESA, it was based on taxonomic uniqueness, and it has subsequently been found that there is no correlation between priority rank and conservation expenditure (Restani & Marzluff, 2001, 2002). In other words, expenditures on local populations of otherwise common species (like *Z. h. preblei*) often exceed the expenditures for full species that are at greater risk of extinction. For example, in a ranking of US-ESA expenditures in 2004, *Z. h. preblei* ranked 125 out of 1260 listed taxa (USFWS, 2006). That put spending for *Z. h. preblei* well above that for blue whales – an endangered species (rank 391) and only slightly behind the California condor – an endangered monotypic genus (ranked 119).

In the case of *Z. h. preblei*, the only verifiable figures on the cost for the 23 632 ha critical habitat designation were conservatively estimated by the USFWS at $79 to $183 million from 2005 to 2015 (USFWS, 2003). Virtually, all of these funds will be spent on consultations rather than more permanent protection, such as land purchases or conservation easements. The development of long-term regional habitat conservation plans accounts for less than 4% of the expenditures. The estimate does not include costs incurred between the time of the listing and the designation of Critical Habitat from 1998 to 2003. It is conceivable that the total allocation of conservation effort for this population could exceed half a billion dollars within the next 20 years.

The United States may be unique in its ability to allocate such resources to non-distinct but presumably threatened populations of common species. However, it is clear that this conservation approach comes at the expense of many full species that are far more endangered. With many full species endangered worldwide, and limited resources to save them, many nations may not find the US-ESA model to be a desirable or sustainable approach to conservation.

References

Anderson, D.R., Burnham, K.P. & Thompson, W.L. (2000). Null hypothesis testing: problems, prevalence, and an alternative. *J. Wildl. Mgmt.* **64**, 912–923.

Ball, M.R. & Avise, J.C. (1992). Mitochondrial DNA phylogeographic differentiation among avian populations and the evolutionary significance of subspecies. *Auk* **109**, 626–636.

Cherry, S. (1998). Statistical tests in publications of The Wildlife Society. *Wildl. Soc. Bull.* **26**, 947–953.

Clark, T.W. & Stromberg, M.R. (1987). *Mammals in Wyoming*. Lawrence: University of Kansas Museum.

Crandall, K.A., Bininda-Emonds, O.R.P., Mace, G.M. & Wayne, R.K. (2000). Considering evolutionary processes in conservation biology. *Trends Ecol. Evol.* **15**, 290–295.

Cronin, M.A. (2006). Commentary: a proposal to eliminate redundant terminology for intra-species groups. *Wildl. Soc. Bull.* **34**, 237–241.

Cryan, P.M. (2004). *Synthesis of existing information on meadow jumping mice (Zapus hudsonius) in the northern Great Plains*. An information review compiled for the U.S. Fish and Wildlife Service, Region 6, Denver, CO. USGS Fort Collins Science Center, Fort Collins, CO.

Johnson, D.H. (1999). The insignificance of statistical significance testing. *J. Wildl. Mgmt.* **63**, 763–772.

Jones, G.S. (1981). *The systematics and biology of the genus Zapus (Mammalia, Rodentia, Zapodidae)*. Unpublished PhD dissertation. Indiana State University, Terre Haute.

Krutzsch, P.H. (1954). North American jumping mice (genus *Zapus*). *Univ. Kansas Publ. Mus. Nat. Hist.* **4**, 349–472.

Küchler, A.W. (1964). *Potential natural vegetation of the conterminous United States: a map and manual*. American Geographical Society Special Publication, Vol. 36. Princeton, NJ: Princeton Polychrome Press.

Preble, E.A. (1899). Revision of the jumping mice of the genus *Zapus*. *North Am. Fauna* **15**, 1–41.

Quimby, D.C. (1951). The life history and ecology of the jumping mouse, *Zapus hudsonius*. *Ecol. Monogr.* **21**, 61–95.

Ramey, R.R., Liu, H.P., Epps, C.W., Carpenter, L. & Wehausen, J.D. (2005). Genetic relatedness of the Preble's meadow jumping mouse (*Zapus hudsonius preblei*) to nearby subspecies of *Z. hudsonius* as inferred from variation in cranial morphology, mitochondrial DNA, and microsatellite DNA: implications for taxonomy and conservation. *Anim. Conserv.* **8**, 329–346.

Restani, M. & Marzluff, J.M. (2001). Avian conservation under the Endangered Species Act: expenditures versus recovery priorities. *Conserv. Biol.* **15**, 1292–1299.

Restani, M. & Marzluff, J.M. (2002). Funding extinction? Biological needs and political realities in the allocation of resources to endangered species recovery. *Bioscience* **52**, 169–177.

Reyment, R.A., Blackith, R.E. & Campbell, N.A. (1984). *Multivariate morphometrics*. Orlando: Academic Press.

Riggs, L.A., Dempcy, J.M. & Orrego, C. (1997). *Evaluating distinctness and evolutionary significance of Preble's meadow jumping mouse: phylogeography of mitochondrial DNA non-coding region variation*. Final report submitted to Colorado Division of Wildlife (appendices).

State of Wyoming (2003). *Petition to remove Preble's meadow jumping mouse (Zapus hudsonius preblei) from the list of endangered and threatened wildlife*. Cheyenne, WY: State of Wyoming, Office of the Governor.

US Fish & Wildlife Service (USFWS) (1998). Final rule to list the Preble's meadow jumping mouse as a threatened species. *The Federal Register* **53**, 26517–26530.

US Fish & Wildlife Service (USFWS) (2003). *Final environmental assessment designation of critical habitat for Preble's meadow jumping mouse*. Appendix 10.5. Addendum to economic analysis of critical habitat designation for the Preble's meadow jumping mouse. Ecological Services Field Office, Cheyene, Wyoming.

US Fish & Wildlife Service (USFWS) (2006). *Federal and state endangered and threatened species expenditures: fiscal year 2004*. Washington, DC: US Fish & Wildlife Service Endangered Species Program.

US Government Accountability Office (2006). *Endangered species: time and costs required to recover species are largely unknown*. GAO-06-463R. Washington, DC: US Government Accountability Office.

Vignieri, S.N., Hallerman, E.M., Bergstrom, B.J., Hafner, D.J., Martin, A.P., Devers, P., Grobler, P. & Hitt, N. (2006). Mistaken view of taxonomic validity undermines conservation of an evolutionarily distinct mouse: a response to Ramey *et al.* (2005). *Anim. Conserv.* **9**, 237–243.

Whitaker, J.O. (1972). *Zapus hudsonius*. *Mamm. Species* **11**, 1–7.

Whitaker, J.O. (1999). Meadow jumping mouse (*Zapus hudsonius*). In *The Smithsonian book of North American mammals*. Wilson, D.E. & Ruff, S. (Eds). Washington, DC: Smithsonian Institution Press.

Zerbe, S. (1998). Potential natural vegetation: validity and applicability in landscape planning and nature conservation. *Appl. Veg. Sci.* **1**, 165–172.

Molecular Ecology (2007)

doi: 10.1111/j.1365-294X.2007.03397.x

Exhibit K

COMMENTARY

How King *et al.* (2006) define an 'evolutionary distinction' of a mouse subspecies: a response

ROB ROY RAMEY II,* JOHN D. WEHAUSEN,† HSIU-PING LIU,‡ CLINTON W. EPPS§ and LANCE M. CARPENTER¶

*Wildlife Science International, Inc., PO Box 386, Nederland, Colorado 80466, USA, †University of California, White Mountain Research Station, 3000 East Line Street, Bishop, CA 93514, USA, ‡University of Denver, Department of Biological Sciences, 2190 E. Iliff, Denver, CO 80208, USA, §University of California, Department of ESPM, 137 Mulford Hall, Berkeley, CA 94720-3114, USA, ¶Department of Zoology, Denver Museum of Nature & Science, 2001 Colorado Blvd, Denver, CO 80205, USA

Present address: Colorado Division of Wildlife, 6060 Broadway, Denver, CO 80216, USA

King *et al.* (2006; hereafter KEA) produced a second independent set of genetic data on the Preble's meadow jumping mouse (formerly *Zapus hudsonius preblei*) and their analyses thereof led to the opposite conclusions of Ramey *et al.* (2005) (hereafter REA) regarding the uniqueness of that subspecies. KEA argued that their different conclusions result from sampling design, tissues used (museum specimens vs. fresh ear punches), amount of molecular genetic data used [longer and more mitochondrial DNA (mtDNA) sequences, more microsatellite loci], analytical methods used and criteria for defining subspecies. We respectfully disagree with their interpretation of differences between the studies and their wholesale portrayal of our work as inaccurate. We find the difference between conclusions is largely a function of basic conceptual and philosophical differences. While the KEA sampling represented a notable effort, it fell short in both sampling design and strength of inference that they attempted to present so forcefully.

Morphometric considerations

KEA chose not to use several key sources of contrary information that did not support their conclusions. These included a range wide study of over 9000 specimens of *Zapus* (Jones 1981), which did not find support for recognizing any subspecies of *Zapus hudsonius*. Also not cited was a literature review conducted by one of the coauthors of KEA (Cryan 2004), which could not find any published literature or reports suggesting adaptive or ecological differences among putative subspecies; a literature that spans 107 years of study. KEA also ignored the fact that we retested the original quantitative basis of Krutzsch's (1954) description of *Z. h. preblei* and found no support for his results (that were based on measurements of just three skulls). That alone would be sufficient basis to reject the taxonomic separation of *Z. h. preblei*. Rather than acknowledging that finding, KEA dismissed the use of morphology in general because it might not reflect genetic differences, an argument they attempted to support by selective use of references. However, by that same argument, these mice should never have been listed under the United States Endangered Species Act (US-ESA) — a listing that KEA now strive to defend. Of some significance to this issue is the fact that Krutzsch, who originally described *Z. h. preblei*, no longer accepts it as a valid subspecies (Ramey *et al.* 2006).

Correspondence: Rob Roy Ramey II, Fax: +1 303 2589535; E-mail: ramey@spot.colourado.edu

Conceptual basis and thresholds for uniqueness

We based our analyses on a definition of subspecies provided by Ball & Avise (1992) to avoid the long history of taxonomic subspecies decisions having no definitional basis (Cronin 2006). Ball & Avise (1992) proposed that subspecies should represent major subdivisions in the gene-pool diversity of species. By that definition, subspecies are similar to evolutionary significant units (ESUs) as discussed by Moritz (1994a) in requiring deeper historical phylogenetic separation, an important criterion of Crandall *et al.* (2000), for recognizing distinct populations. Although KEA claimed to use the same conceptual basis, they actually employed a far lower threshold for subspecies than REA that appears to be equivalent to what Moritz (1994b) defined as management units. Their null hypothesis was that subspecies of *Zapus husdonius* comprise a single homogeneous unit. The evidence that KEA considered adequate to reject that hypothesis was *'significant phylogeographical separation of mtDNA alleles between subspecies combined with congruent phylogeographical structure for nuclear loci'*. The issue is: what constitutes a significant difference?

© 2007 The Authors
Journal compilation © 2007 Blackwell Publishing Ltd

KEA failed to acknowledge the ways in which the molecular results of REA were similar to their results. These include: (i) shallow levels of evolutionary divergence found among putative subspecies for mtDNA and microsatellites; (ii) support in mtDNA analyses for a *Z. h. pallidus/luteus* clade and a *Z. h. preblei/campestris/ intermedius* clade; (iii) not even near reciprocal monophyly among putative subspecies; and (iv) few unique microsatellite alleles in *Z. h. preblei* despite a larger sample size for this putative subspecies. These similarities are important because of their bearing on how different conceptual approaches to subspecies affected differences in conclusions.

Statistical significance versus biological significance

In their null-hypothesis test of genetic homogeneity, KEA equated statistical significance with biological significance, an analytical approach that deviates from REA. It is well known that with larger sample sizes it is possible to find statistical significance in almost any comparison, especially when intervening geographical variation is ignored. As pointed out by Hedrick (2001): *'the statistical power for determining differentiation between groups is closely related to the number of independent alleles, so that even for a few highly variable microsatellite loci, there can be extremely high statistical power. When there is such high statistical power, extremely small molecular genetic differences between groups become statistically significant.'*

Although KEA found a high level of statistical significance in their comparisons (using 27 microsatellites), the degree of differentiation among *Zapus hudsonius preblei*, *Z. h. campestris* and *Z. h. intermedius* were the lowest of any of the pairwise comparisons for mtDNA and microsatellites. The low degree of differentiation is illustrated by the fact that only four unique alleles were reported in *Z. h. preblei* (out of 279 in total), the lowest number of unique alleles for any subspecies sampled.

KEA reported high levels of correct assignment to subspecies using the program STRUCTURE. These authors attribute this to *Z. h. preblei* having 'considerable evolutionary differentiation' from other putative subspecies and to shortcomings of REA. However, KEA failed to acknowledge that this high level of correct assignment could also be an artifact of sampling design and number of loci surveyed (Rosenberg *et al.* 2005). KEA's findings raise a valid critique of all such studies — use of assignment tests such as STRUCTURE may not be an appropriate tool for evaluating taxonomic separation because of the sensitivity of these tests to the number of loci employed. Future efforts employing these types of analyses may need to establish threshold assignment probabilities for a set number of loci with a given amount of variation per locus to allow comparability between studies.

Sampling distribution

KEA sampled seven populations of *Zapus hudsonius preblei* but only one or two populations from each of the other putative subspecies. In contrast, REA sampled many populations, but few individuals per population, across the range of each putative subspecies. An ideal study design with unlimited resources would incorporate both approaches, as well as sampling across the entire species; however, this is not often practical because of logistical and funding constraints. Given the choice, which strategy provides the most objective test of subspecies uniqueness?

KEA claim their sampling strategy allows more appropriate statistical testing, but they do not acknowledge that their approach created artificial gaps in the distribution of genetic variation, leading to an 'isolation-by-sampling design' effect among all five of the subspecies. This sampling strategy predisposes the results to an exaggeration of genetic distances among putative subspecies. This assertion is supported by the fact that genetic distances from KEA were strongly correlated with geographical distances for the *Z. h. preblei/campestris/intermedius* lineage ($R^2 = 0.82$), including a sample from southeastern Wyoming that is intermediate between *Z. h. preblei* and *Z. h. campestris* (see KEA Fig. 3).

Sources of material

KEA criticized REA for using museum specimens, claiming a wide variety of problems associated with such tissue, based mostly on literature for ancient DNA samples, not museum specimens collected within the past 45 years. In truth, Ramey *et al.* (2005) used a mixture of museum specimens and frozen tissue. However, all of the *Zapus hudsonius preblei* specimens used by KEA are subject to the same issues they raise regarding museum specimens because these ear punches were obtained without bleaching the ear punch between samples or wearing laboratory gloves to reduce cross contamination (R. Taylor, personal communication entered into the record on 7/6/06; Riggs *et al.* 1997).

If only newly trapped individuals are used, as advocated by KEA, investigations will be limited in the extent to which current patterns of variation might be parsed relative to historical natural processes vs. recent anthropogenic effects (e.g. bottlenecks).

mtDNA Sequences

KEA assert that the shared mtDNA haplotypes found by REA between *Zapus hudsonius preblei* and *Z. h. campestris* are the result of contamination of museum specimens, and they present a reanalysis of these specimens in support of their assertion. While this may be a point well taken, KEA did not consider any alternative explanations (e.g. nuclear paralogs, heteroplasmy, different amplification primers

© 2007 The Authors
Journal compilation © 2007 Blackwell Publishing Ltd

and conditions, and template quality). Additionally, the use by KEA of different primers, annealing temperatures (48 rather than 60 degrees) and buffers suggests conditions that could lead to different amplification success and results.

Despite efforts to portray our work as inaccurate, KEA changed several key methods and details of results at the proof stage *after* we pointed out those errors. First, KEA originally claimed that we amplified with primers L15926 and H16498, then changed this in the proofs to L15320 and ZAP5PLr (which they claimed did not work for disputed museum specimens). We actually used L15320 and ZAP5PLr, and we then used nested primers L15398 and H16498 for nested polymerase chain reaction (PCR) for a subset of weakly amplifying specimens (Ramey *et al.* 2005). Second, Table 1 of Appendix B in KEA clearly mixed up REA's mtDNA haplotypes and sample numbers but was changed by KEA in the proofs.

Even if the mtDNA sequences in question are excluded from analysis, it does not alter the basic conclusions of REA. That is because our critical tests did not rely on any sharing of haplotypes among putative subspecies. Instead, our subspecies test relied on: (i) morphological analyses to test the original quantitative basis of *Z. h. preblei*; (ii) mtDNA reciprocal monophyly, AMOVA; and (iii) the proportion and frequency of unique microsatellite alleles and pairwise F_{ST} values. With the samples in question excluded, AMOVA results just exceed our threshold but *Z. h. preblei* is still not even close to being reciprocally monophyletic. Similar results are obtained if all mtDNA sequences obtained by REA using nested PCR are excluded from analyses and KEA's data are substituted for REA's *Z. h. campestris* sequences.

Are KEA's mtDNA sequences 'diagnostic'?

KEA claim their mtDNA results are 'diagnostic' in support of *Zapus hudsonius preblei* as a subspecies and as it being on 'its own independent evolutionary trajectory'. Although they present additional mtDNA sequence data, *Z. h. preblei* remains paraphyletic with low bootstrap support.

Standardization between studies

KEA misrepresented that they had not obtained necessary samples from the Denver Museum of Nature and Science. The submission of a proposal along with a request is a standard requirement for destructive sampling of any museum specimens. KEAs request was fulfilled *after* King provided a proposal for the use of specimens.

AMOVA

We concur with KEA that the AMOVA criterion that we proposed for mtDNA may not be an ideal measure with which to test the uniqueness of subspecies or distinct populations. As found by KEA, if there are slight differences among mtDNA haplotypes, but those haplotypes are fixed or nearly fixed in populations, this will have a substantial effect on the value of Φ_{ST}. That could lead to the erroneous designation of a weakly differentiated population as a subspecies or distinct population segment (DPS).

Conclusions

As noted in REA, thresholds for identifying subspecies and DPS's below the level of subspecies have been lacking. It is legitimate to debate thresholds, but the need for them is obvious — there are many endangered taxa and not enough resources to conserve them. If conservation effort is allocated to nondistinct or weakly differentiated populations, other more unique taxa (e.g. full species) will lose out. Hypothesis testing relative to these thresholds can provide objective assessments of degree of uniqueness and a basis for prioritizing the allocation of conservation effort.

The publicly available record shows that KEA changed their interpretation of results at least twice, from subspecies being 'weakly differentiated' to 'evolutionarily distinct'; and the number of potential DPS's of *Zapus hudsonius preblei* changed from two, to three, to none. This subjectivity is symptomatic of an approach that lacks clearly defined thresholds, and epitomizes the problem that REA attempted to address. We believe that few if any subspecies or DPSs proposed for US-ESA listing or delisting will be falsifiable under the general approach and low level of genetic differentiation that KEA used to accept subspecies. General application of that approach may move the allocation of conservation effort outside the realm of scientific inquiry.

Keywords: conservation, Endangered Species Act, subspecies, taxonomy, Zapus

Received 5 January 2007; revision accepted 20 April 2007

References

Ball MR, Avise JC (1992) Mitochondrial DNA phylogeographic differentiation among avian populations and the evolutionary significance of subspecies. *Auk*, **109**, 626–636.

Crandall. KA, Bininda-Emonds ORP, Mace GM, Wayne RK (2000) Considering evolutionary processes in conservation biology. *Trends in Ecology and Evolution*, **15**, 290–295.

Cronin MA (2006) A proposal to eliminate redundant terminology for intra-species groups. *Wildlife Society Bulletin*, **34**, 237–241.

Cryan PM (2004) Synthesis of existing information on meadow jumping mice (*Zapus hudsonius*) in the northern Great Plains. An information review compiled for the U.S. Fish and Wildlife Service, Region 6, Denver, Colorado. USGS Fort Collins Science Center, Fort Collins, CO

Hedrick PW (2001) Conservation genetics: where are we now? *Trends in Ecology and Evolution*, **16**, 629–636.

© 2007 The Authors
Journal compilation © 2007 Blackwell Publishing Ltd

Jones GS (1981) The systematics and biology of the genus *Zapus* (Mammalia, Rodentia, Zapodidae) unpublished PhD Dissertation. Indiana State University, Terre Haute.

King TL, Switzer JF, Morrison CL *et al.* (2006) Comprehensive genetic analyses reveal evolutionarily distinction of a mouse (*Zapus hudsonius preblei*) proposed for delisting from the U.S. Endangered Species Act. *Molecular Ecology*, 15, 4331–4359.

Krutzsch PH (1954) North American jumping mice (genus *Zapus*). *University of Kansas Publication Museum of Natural History*, 4, 349–472.

Moritz C (1994a) Defining evolutionarily significant units for conservation. *Trends in Ecology and Evolution*, 9, 373–375.

Moritz C (1994b) Applications of mitochondrial DNA analysis in conservation: a critical review. *Molecular Ecology*, 3, 401–411.

Ramey RRII, Liu H-P, Epps CW, Carpenter LM, Wehausen JD (2005) Genetic relatedness of the Prebleís meadow jumping (*Zapus hudsonius preblei*) to nearby subspecies of *Z. hudsonius* as inferred from variation in cranial morphology, mitochondrial DNA and microsatellite DNA: implications for taxonomy and conservation. *Animal Conservation*, 8, 329–346.

Ramey RR, Wehausen JD, Liu HP, Epps CW, Carpenter L (2006) Response to Vignieri et al. (2006): Should hypothesis testing or selective post hoc interpretation of results guide the allocation of conservation effort? *Animal Conservation*, 9, 244–247.

Riggs LA, Dempcy JM, Orrego C (1997) *Evaluating Distinctiveness and Evolutionary Significance of Prebleís Meadow Jumping Mouse: Phylogeography of Mitochondrial DNA Non-Coding Region Variation*. Report to the Colorado Division of Wildlife, Denver, CO.

Rosenberg NA, Mahajan S, Ramachandran S, Zhao C, Pritchard JK, Feldman MW (2005) Clines, clusters, and the effect of study design on the inference of human population structure. *PLOS Genetics*, 1, 660–671.

Rob R. Ramey is a conservation geneticist and field biologist with 26 years of research experience on the taxonomy and population structure of endangered wildlife in North America, Central America, Africa, and Asia. He consults on endangered species conservation issues in the U.S.A. and abroad. For the past 33 years John D. Wehausen has researched numerous aspects of the population biology and nutritional ecology of bighorn sheep in high mountain and deserts ranges of California. As a Research Scientist at the University of California, San Diego, he is also engaged in morphometric and genetic research concerning taxonomic questions of North American wild sheep. Hsiu-Ping Liu is a Research Assistant Professor at the University of Denver where she conducts genetics research on endangered invertebrates. Clinton W. Epps is a postdoctoral researcher at the University of California, Berkeley, where he is studying habitat fragmentation and connectivity of large mammal populations in the United States and Africa. Lance M. Carpenter is a biologist with the Colorado Division of Wildlife working on the conservation of non-game species.

© 2007 The Authors
Journal compilation © 2007 Blackwell Publishing Ltd

Exhibit L

Philip H Krutzsch, 3/31/04 2:39 PM -0700, Ms

X-Authentication-Warning: ptah.u.arizona.edu: krutzsch owned process doing -bs
Date: Wed, 31 Mar 2004 14:39:41 -0700 (MST)
From: Philip H Krutzsch <krutzsch@U.Arizona.EDU>
To: Rob Roy Ramey <Ramey@spot.colorado.edu>
Subject: Ms
X-Spam-Checker-Version: SpamAssassin 2.63 (2004-01-11) on ruff
X-Spam-Status: No, hits=-4.9 required=5.0 tests=BAYE_00 autolearn=ham
version=2.63
X-Spam-Level:
Status:

Dear Rob:I received the Ms and have read it with great intrest! The tools of today's systamatists are indeed at the cutting edge! The study clearly invalidates Z.h.preblei and demonstrates its relationship to Z.h.campestris as well as seperating it from geographicly adjacent Zapodid taxa.
You should be very satisfied with the indepth and reproducable analyses you present.
Perhaps most significant is the modal you provide to unequivocally establish the uniqueness of an organism and its relationships before declareing it in danger of extinction!!! Such an analytical approach would prevent implentation of a process to support an agenda or a point of view. I can think of other listed endangered species that could have benefited for a prior, detailed, scientific appraisal! Thank you for letting me see your eloquent study. Phil

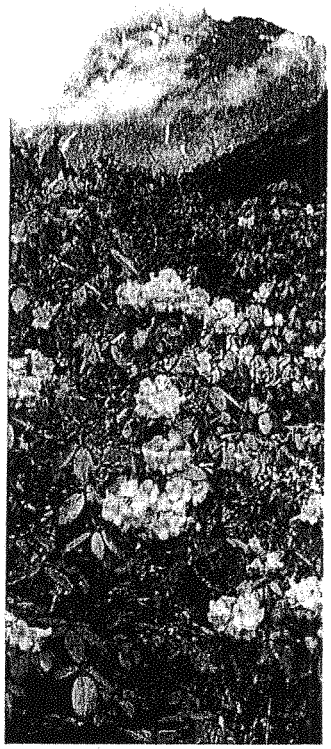
Philip H. Krutzsch
Department of Cell Biology and Anatomy
The University of Arizona
Tucson, AZ 85724

krutzsch@u.arizona.edu
Phone: 520/887-2791
Fax: 520/626-2097

LETTERS

World's Largest Flora Completed

CHINA IS HOME TO MORE THAN 31,000 species of vascular plants, more than any country except Brazil and Colombia. More than half of Chinese vascular plant species are found nowhere else, including many, such as *Ginkgo* and *Metasequoia*, which were once widespread around the Northern Hemisphere, but now survive only in China. Numerous noted botanical explorers and collectors from Europe, America, and China contributed valuable material to the herbaria of leading botanical institutions and greatly enriched the gardens of the world through their discoveries. The completion by Chinese botanists of the *Flora Republicae Popularis Sinicae* (FRPS), which outlines the characteristics of the country's huge flora, is an event of great significance; no flora of comparable size has ever been completed.



Rhododendrons in the Kama Valley, or Valley of the Flowers, east of Mount Everest in Tibet Autonomous Region, China.

CREDIT: GALEN ROWELL/CORBIS

This publication of this work was formally begun in 1958, but it was initiated in the 1930s by Hu Xiansu (better known as H. H. Hu) (*1*). Work on the flora virtually ceased during the chaotic "Proletarian Cultural Revolution" (1966–76). After 1978, Chinese botanists resumed and greatly accelerated their efforts, with major financial support from the National Natural Science Foundation of China, the Chinese Academy of Sciences, and the Ministry of Science and Technology. Finally, after 45 years of extraordinary effort by 312 Chinese botanists representing four generations, the Flora has been completed. It consists of 126 books, which constitute 80 volumes; it includes 31,141 species, 3407 genera, and 300 families of vascular plants. The final part was published in October 2004. The Flora includes all native and naturalized plant species, as well as China's economically important cultivated plants, such as crops, and plants that are grown in plantations.

Exhibit M

More than 20,000 species are illustrated in plates of line drawings. FRPS [illegible] ed into a database and will be made accessible through the Internet by the Institute of Botany, Chinese Academy of Sciences, Beijing.

Although FRPS provides an important step forward for the knowledge of Chinese plants, it is based on a relatively short period of study by the nation's botanists. Modern taxonomic research by Chinese botanists was not begun until 1916 (*1*), with earlier studies carried out mainly by European and American scientists. As a result, much of the important reference material is held by European and American institutions and was not always easily accessible to Chinese botanists, particularly during the "Cultural Revolution." The material that Chinese botanists have had available for study is mainly based on that assembled within China, most of it since 1949. Consequently, FRPS has certain deficiencies.

Because of these problems, an international collaborative project, the Flora of China project, was organized to produce a collaborative, revised English edition of FRPS. This project involves many Chinese and non-Chinese taxonomists from throughout the world and is supported by various funding agencies in China and the United States, including the National Natural Science Foundation of China, the Chinese Academy of Sciences, and the U.S. National Science Foundation, as well as the C.V. Starr, Kadoorie, and Stanley Smith foundations. Ten volumes of text and ten volumes of accompanying illustrations have been published to date (*2*). The project will ultimately result in the publication of 25 volumes of text and 25 volumes of illustrations and is expected to be completed by 2010.

By completing FRPS, Chinese botanists have made a great contribution to the understanding of the world's plants and have laid a more secure foundation for their conservation and sustainable use. Given the rapid development of China's economy and the consequent pressures on natural resources, this information is of vital importance. It is also hoped that the Flora may also present a useful model for botanists from other nations that are in the process of developing knowledge about their plant resources and encountering pressures similar to those felt in China.

QIN-ER YANG,[1] GUANGHUA ZHU,[2] DEYUAN HONG,[1] ZHENGYI WU,[3] PETER H. RAVEN[2]

[1]Laboratory of Systematic & Evolutionary Botany, Institute of Botany, Chinese Academy of Sciences, Beijing 100093, China. [2]Missouri Botanical Garden, St. Louis, MO 63166, USA. [3]Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650204, China.

References and Notes

1. W. J. Haas, *Arnoldia* **48**, 9 (1988).
2. The volumes are available online at http://flora.huh.harvard.edu/china/ and www.mobot.org/MOBOT/Research/asiaprojects.shtml.
3. We thank Xingguo Han, director of the Institute of Botany, Chinese Academy of Sciences, for his helpful discussion.

What Constitutes a Proper Description?

IN THEIR REPORT "THE HIGHLAND MANGABEY *Lophocebus kipunji*: a new species of African monkey" (20 May, p. 1161), T. Jones *et al.* attempt to describe a distinctive, new species of mangabey from Tanzania. The description of the new mangabey is based on two photographs, one of an adult male designated as the holotype, and one of unknown sex designated as a paratype. No voucher material was obtained, and the authors state, "The number of individuals in each of the two populations of this species is undoubtedly very small; no live individual should be collected at this time to serve as the holotype." Contrary to the statements in the published description, the photographs do not function as name-bearing types (*1*). Thus, *Lophocebus kipunji* Ehart, Butynski, Jones, and Davenport is not an available name and has no formal standing in zoology.

The photographs are not valid substitutes for a type specimen. The function of a type specimen in nomenclature is to provide an objective basis for the application of a species-group name. Jones and colleagues are encouraged to acquire a specimen, or part(s) thereof, and prepare a new description of this, as yet, undescribed species.

ROBERT M. TIMM,[1] ROB ROY RAMEY II,[2] AND THE NOMENCLATURE COMMITTEE OF THE AMERICAN SOCIETY OF MAMMALOGISTS

[1]Department of Ecology and Evolutionary Biology and Biodiversity Research Center, University of Kansas, Lawrence, KS 66045, USA. [2]Department of Zoology, Denver Museum of Nature and Science, 2001 Colorado Boulevard, Denver, CO 80205, USA.

Reference

1. International Commission on Zoological Nomenclature (ICZN), *International Code of Zoological Nomenclature* (ICZN, London, ed. 4, 1999). "Article 16.4. Species-group names: fixation of name-bearing types to be explicit. Every new specific and subspecific name published after 1999,... must be accompanied in the original publication[:] 16.4.1. by the explicit fixation of a holotype, or syntypes for the nominal taxon,..., and, 16.4.2 where the holotypes or syntypes are extant specimens, by a statement of intent that they will be (or are) deposited in a collection and a statement indicating the name and location of that collection. (see Recommendation 16C)."

THE DISCOVERY OF A NEW SPECIES OF MONKEY is very important and heartening to preservationists everywhere ("The highland mangabey *Lophocebus kipunji*: a new species of African monkey," T. Jones *et al.*, Reports, 20 May, p. 1161). Unfortunately, as a taxonomic description, the Report leaves much to be desired and seems destined to sow confusion in future synonymies.

There are no hard and fast rules for the protocol of a species description, but certain features should be adhered to. It is usual to start with a brief taxonomic hierarchy, placing the new taxon in the set of animals; thus, Class, Order, Family, Genus, Species name (i.e., the proposed Linnaean binomial), "Sp. Nov.," "New Sp.," or some designation clearly marking the name as new.

"Ehardt *et al.*" give the citation for the new species as "Ehardt, Butynski, Jones and Davenport," that is, four of the seven authors of the paper. The purpose of the citation is to identify the paper, not to assign credit, and all of the authors should be cited.

This paper has not properly designated a type specimen. There is no provision under the *International Code of Zoological Nomenclature* (*1*) for designating a photograph as a type. The authors were understandably reluctant to collect a specimen of this rare species, but the proper course of action would have been to announce the discovery of the new species, publishing all of the excellent descriptive material and their quite convincing case for calling it new, without, however, naming it.

They have published a nomen nudum, a name that, because it is not backed by a type specimen, has no standing under the Code and that other taxonomic workers are free to ignore. Moreover, they rendered their name (*kipunji*) unavailable under the rules, meaning that not only is their entirely appropriate name, *Lophocebus kipunji*, not established, but that nobody can ever establish it.

STUART O. LANDRY

Professor Emeritus of Biology, State University of New York, Binghamton, NY 13902–6000, USA.

Reference

1. International Commission on Zoological Nomenclature (ICZN), *International Code of Zoological Nomenclature* (ICZN, London, ed. 4, 1999).

Response

LANDRY AND TIMM *ET AL.* SHOW A LAUDABLE concern about descriptions of new animal species that either are not, or appear not to be, compliant with the currently applicable *International Code of Zoological Nomenclature* (*1*). The aims of the Code, and of the Commission responsible for its periodic revision and implementation (ICZN), have always been to minimize chaos in animal nomenclature, hence ICZN's Mission Statement: "achieving stability and sense in the scientific naming of animals."

The destabilizing effect of publishing non–Code-compliant descriptions of new animal taxa is as old as the Code itself. Perhaps the most recent example is the invalid description of the fossil duck *Vegavis iaai* (*2*). Because no description for the generic name was provided, the binomial proposed for an extremely important

Published by AAAS

fossil is invalid, or "unavailable" in the Code's terminology.

By contrast, Jones *et al.* undertook a series of consultations with the ICZN Secretariat and with several eminent taxonomists to ensure that their description of the highland mangabey *Lophocebus kipunji* was Code-compliant. Although under Article 16.4.2, it is stated that authors of new taxa must publish a statement of intent that extant types will be deposited in a collection, Article 73.1.4 provides an opportunity for the description of new taxa without the necessity of providing dead type specimens: "Designation of an illustration of a single specimen as a holotype is to be treated as designation of the specimen illustrated; the fact that the specimen no longer exists or cannot be traced does not of itself invalidate the designation." The Article, as formulated, thereby permits the description of threatened animals or those for whom the collection of specimens is otherwise impractical, impossible, or unethical. This situation has been dealt with in detail by Wakeham-Dawson *et al.* (*3*).

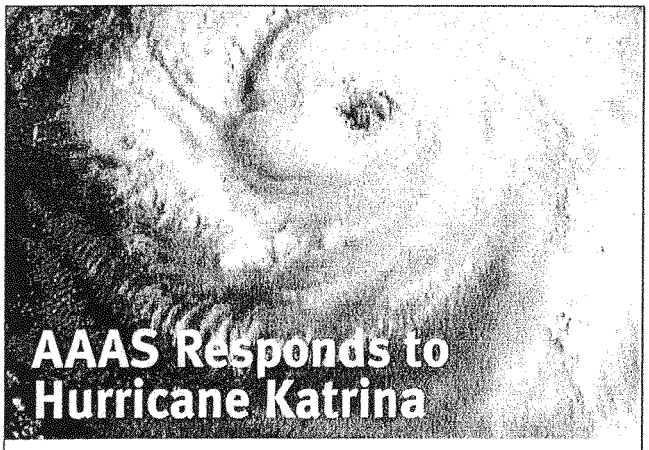
This photo, of an adult male highland mangabey *Lophocebus kipunji*, was designated by Jones *et al.* as the holotype.

CREDIT: T. R. B. DAVENPORT

The description of *L. kipunji* is also Code-compliant in all other respects, and the objections raised by Landry are unsupported. Although often the case, it is not required, nor always appropriate, that authorship of a publication describing a new taxon and its discovery be the same as the authorship of the name assigned under the Code. For *L. kipunji*, the authorship of the name (Ehardt, Butynski, Jones, and Davenport) specifically designates the authority assigning the name of the new species.

The allowance under the Code for designation of surviving specimens as holotypes needs to be more widely recognized, given contemporary concerns for the conservation of threatened species. There is no doubt that many newly described taxa will be threatened (*L. kipunji* will be designated as "critically endangered" in the IUCN Red List). Dead animal specimens should not be understood to be essential to the process of establishing new taxa. In such cases, supplementation with evidence such as sonograms and oscillograms of species-specific vocalizations, and molecular information (now readily derived from noninvasive samples, e.g., hair, urine, and feces) may contribute to validation. It should also be more widely recognized that establishing the taxonomic rank of new taxa and ensuring the availability of names are critical to the conservation listings (regional, national, and international) that assist in prioritizing, initiating, and supporting conservation efforts. Even the perception of the necessity for physical specimens under the Code could hamper and delay the very processes that determine whether newly discovered taxa survive.

The well-intentioned reactions of Landry and Timm *et al.* show that the current Code is open to different interpretations on the subject of type specimens (compare Articles 16.4.2 and 73.1.4 at www.iczn.org/iczn/index.jsp). The permissiveness of the Code in allowing illustrations of type specimens to make new



AAAS and its journal *Science* share the deep sense of loss caused by Hurricane Katrina.

Donate and Find Resources

Visit our online brokering system if you are a scientist, engineer, or teacher in need of resources, or if you have resources to share.

www.aaas.org/katrina

Find *Science* Content

Read freely accessible *Science* articles related to hurricanes, coastal disasters, and disaster policy.

www.sciencemag.org/sciext/katrina

Science
AAAS

AAAS Travels

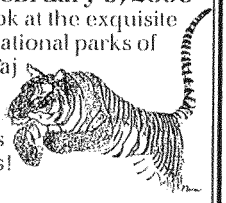
We invite you to travel with AAAS in the coming year. You will discover excellent itineraries and leaders, and congenial groups of like-minded travelers who share a love of learning and discovery.

Oaxaca December 27, 2005–January 2, 2006

Explore the rich cultural heritage from Mexico City to Oaxaca. Visit fascinating archaeological sites. $2,495 + air

India Wildlife Safari
January 21–February 5, 2006

A magnificent look at the exquisite antiquities and national parks of India, from the Taj Mahal, Agra Fort & Khajuraho Temples to tigers and Sarus cranes! $3,595 + air.



Alaska Aurora Borealis
March 2–8, 2006

Discover Alaska in winter including 20,320-ft Mt. McKinley. See ice sculptures in Fairbanks and the Aurora Borealis with lectures at the Geophysical Institute. $2,495 + air.

Turkey Eclipse Yacht Adventure
March 21–April 1, 2006

Explore Greek and Roman sites from Dalaman to Antalya. See the Total Solar Eclipse March 29. $4,995 + air.

China Feathered Dinosaur
March 18–April 5, 2006

Explore highlights of Beijing, Xian and cruise the Yangtze River, plus the world's finest fossil sites of feathered dinosaurs, the species at the transition from reptile to bird. $5,990 + air.

Aegean Odyssey
May 24–June 7, 2006

Our classic adventure to explore the history of Western Civilization in Athens, Delphi, Delos, Santorini, & Knossos. $3,695 plus 2-for-1 air + tax from JFK.

Call for trip brochures & the Expedition Calendar

(800) 252-4910

AAAS Travels

17050 Montebello Road
Cupertino, California 95014

Email: AAASinfo@betchartexpeditions.com

names available, despite the subsequent loss or return to the wild of those types, is open to potential abuse. An obvious modest step forward would be to introduce a registration system for animal names. This would (i) alert zoologists to the appearance of newly described taxa and (ii) ensure that the names are Code-compliant and available.

Our comments support the preparation of a new edition of the Code—one that will prevent potential misinterpretations and perhaps encompass an open-access registration system. Such an effort, embracing the principle of bioinformatics, should unite all biologists involved in biodiversity conservation, systematics, evolutionary ecology, molecular biology, and related disciplines.

ANDREW POLASZEK,[1] PETER GRUBB,[2] COLIN GROVES,[3] CAROLYN L. EHARDT,[4] THOMAS M. BUTYNSKI[5]

[1]Executive Secretary, International Commission on Zoological Nomenclature, c/o Natural History Museum, London SW7 5BD UK. [2]35 Downhills Park Road, London N17 6PE, UK. [3]School of Archaeology and Anthropology, Building 14, Australian National University, Canberra 0200, Australia. [4]Department of Anthropology, University of Georgia, Athens, GA 30602–1619, USA. [5]Conservation International, Eastern Africa Regional Program, Post Office Box 68200, City Square 00200, Nairobi, Kenya.

References

1. International Commission on Zoological Nomenclature (ICZN), *International Code of Zoological Nomenclature* (ICZN, London, ed. 4, 1999), pp. 1–306.
2. J. A. Clarke, C. P. Tambussi, J. I. Noriega, G. M. Erickson, R. A. Ketcham, *Nature* **433**, 305 (2005).
3. A. Wakeham-Dawson, S. Morris, P. Tubbs, M. L. Dalebout, C. S. Baker, *Bull. Zool. Nomenclat.* **59** (no. 4), 282 (2002).

Quantifying Publication Impact

THE RANDOM SAMPLES ITEM "IMPACT FACTOR" (19 Aug., p. 1181) noted the proposal by Jorge Hirsch of the University of California, San Diego (*1*) that the total scientific output of a researcher can be judged by h, the largest number such that the researcher has at least h papers with h citations. Although this is indeed an indication of cumulative scientific impact, another measure, call it c, would also be interesting: the total number of papers from that researcher cited more than once by other research groups in the most recent calendar year. This alternative parameter c would be a much better measure of current research impact.

DOUGLASS F. TABER

Department of Chemistry and Biochemistry, University of Delaware, Newark, DE 19716, USA.
E-mail: taberdf@udel.edu

Reference

1. J. E. Hirsch, preprint available at www.arxiv.org/abs/physics/0508025.

Letters to the Editor

Letters (~300 words) discuss material published in *Science* in the previous 6 months or issues of general interest. They can be submitted through the Web (www.submit2science.org) or by regular mail (1200 New York Ave., NW, Washington, DC 20005, USA). Letters are not acknowledged upon receipt, nor are authors generally consulted before publication. Whether published in full or in part, letters are subject to editing for clarity and space.

CORRECTIONS AND CLARIFICATIONS

News Focus: "A 'Robin Hood' declares war on lucrative U.S. patents" by E. Kintisch (26 Aug., p. 1319). The story incorrectly identified the name and scope of the organization Patients not Patents. The group focuses on drug patents.

Special Section on the Great Sumatra-Andaman Earthquake: Viewpoint: "A flying start, then a slow slip" by R. Bilham (20 May, p. 1126). The mention of the Richter scale in the second sentence of the second paragraph on page 1126 was incorrect. All magnitudes cited should be moment magnitude M_w, a scale that is defined by total energy release. The second sentence of the first paragraph on page 1126 incorrectly gave the energy equivalents of the earthquake. A magnitude of $M_w = 9.15$ corresponds to 3.35×10^{18} J, or 0.8 gigatons of TNT. This is equivalent to 11 days of total U.S. energy consumption, assuming a 2005 rate of 101×10^{15} British thermal units ($\approx 10^{20}$ J) (see www.eia.doe.gov/oiaf/aeo/index.html). However, although estimates of M_w range up to 9.3, the most reliable seismic energy release estimate is 1.1×10^{18} J, corresponding to ≈0.25 gigatons of TNT.

Reports: "Supramolecular assembly of amelogenin nanospheres into birefringent microribbons" by C. Du *et al.* (4 Mar., p. 1450). In this Report on amelogenin nanosphere assemblies and their tendency to form microribbon structures, the authors included a diffraction pattern that was attributed to these microribbons (Fig. 1F and Table S2). Elia Beniash (Forsyth Institute, Boston) subsequently informed the authors that the diffraction pattern and the d-spacings reported are analogous to those of cellulose fibers, and analysis of one of the microribbons by Beniash confirmed the presence of cellulose. The authors therefore conclude that the diffraction reported in Fig. 1F belongs to a cellulose contaminant fiber and not to an amelogenin microribbon. The authors have carried out new crystallization and characterization experiments of amelogenin birefringent microribbons that were free of contamination. The dimensions of the microribbons appear to be smaller than those indicated in the Report, with a wider distribution in length and width. The shape is not regular, although a ribbon-like morphology (similar to that of cellulose) is always preserved. These amelogenin microribbons, although birefringent, show either no or a very weak x-ray diffraction pattern. This suggests the presence of a preferential orientation in the nanosphere assembly, without any regular periodicity. The authors apologize for these errors and any inconvenience they may have caused.

Published by AAAS

Exhibit N

35 Wiens, D. (1978) Mimicry in plants. *Evol. Biol.* 11, 365–403
36 Givnish, T.J. (1989) Ecology and evolution of carnivorous plants. In *Plant–Animal Interactions* (Abramson, W.G., ed.), pp. 243–290, McGraw-Hill
37 Zamora, R. (1999) Conditional outcomes of interactions: the pollinator-prey conflict of an insectivorous plant. *Ecology* 80, 786–795
38 Gloßner, F. (1992) Ultraviolet patterns in the traps and flowers of some carnivorous plants. *Bot. Jahr. Syst.* 113, 577–587
39 Moran, J.A. *et al.* (1999) Aspects of pitcher morphology and spectral characteristics of six Bornean *Nepenthes* pitcher plant species: implications for prey capture. *Ann. Bot.* 83, 521–528
40 Moran, J.A. (1996) Pitcher dimorphism, prey composition and the mechanisms of prey attraction in the pitcher plant *Nepenthes rafflesiana* in Borneo. *J. Ecol.* 84, 515–525
41 Thum, M. (1986) Segregation of habitat and prey in two sympatric carnivorous plant species, *Drosera rotundifolia* and *Drosera intermedia*. *Oecologia* 70, 601–605
42 Karlsson, P.S. *et al.* (1987) Trapping efficiency of three carnivorous *Pinguicula* species. *Oecologia* 73, 518–521
43 Schwaegerle, K.E. (1983) Population growth of the pitcher plant, *Sarracenia purpurea* L., at Cranberry Bog, Licking County, Ohio. *Ohio J. Sci.* 83, 19–22
44 Brewer, J.S. (2001) A demographic analysis of fire-stimulated seedling establishment of *Sarracenia alata* (Sarraceniaceae). *Am. J. Bot.* 88, 1250–1257
45 Caswell, H. (2000) *Matrix Population Models: Construction, Analysis and Interpretation*, Sinauer
46 Fisher, R.A. (1930) *The Genetical Theory of Natural Selection*, Clarendon
47 Ellison, A.M. (2001) Interspecific and intraspecific variation in seed size and germination requirements of *Sarracenia* (Sarraceniaceae). *Am. J. Bot.* 88, 429–437
48 Godt, M.J.W. and Hamrick, J.L. (1996) Genetic structure of two endangered pitcher plants, *Sarracenia jonesii* and *Sarracenia oreophila* (Sarraceniaceae). *Am. J. Bot.* 83, 1016–1023
49 Godt, M.J.W. and Hamrick, J.L. (1998) Allozyme diversity in the endangered pitcher plant *Sarracenia rubra* ssp. *alabamensis* (Sarraceniaceae) and its close relative *S. rubra* ssp. *rubra*. *Am. J. Bot.* 85, 802–810
50 Menges, E. (1990) Population viability analysis for an endangered plant. *Conserv. Biol.* 4, 41–62
51 Chiang, C. *et al.* (2000) Effects of 4 years of nitrogen and phosphorus additions on Everglades plant communities. *Aquat. Bot.* 68, 61–78
52 Doak, D.F. and Morris, W. (1999) Detecting population-level consequences of ongoing environmental change without long-term monitoring. *Ecology* 80, 1537–1551
53 Brewer, J.S. (1999) Short-term effects of fire and competition on growth and plasticity of the yellow pitcher plant, *Sarracenia alata* (Sarraceniaceae). *Am. J. Bot.* 87, 1537–1551.
54 Barker, N.G. and Williamson, G.B. (1988) Effects of a winter fire on *Sarracenia alata* and *S. psittacina*. *Am. J. Bot.* 75, 286–305
55 Schulze, E-D. *et al.* (1991) The utilization of nitrogen from insect capture by different growth forms of *Drosera* from southwest Australia. *Oecologia* 87, 240–246
56 Schulze, W. and Schulze, E-D. (1990) Insect capture and growth of the insectivorous *Drosera rotundifolia* L. *Oecologia* 82, 427–429
57 Adamec, L. (1995) Ecophysiological study of the aquatic carnivorous plant *Aldrovanda vesiculosa* L. *Acta Bot. Gallica* 142, 681–684
58 Lledo, M.D. *et al.* (1998) Systematics of Plumbaginaceae based upon analysis of *rbcL* sequence data. *Syst. Bot.* 23, 21–29

Conservation genetics: where are we now?

Philip W. Hedrick

Genetic studies in endangered species have become widespread in the past decade, and with new information from various genome projects, new applications and insights are forthcoming. Generally, neutral variants are used for conservation applications, and when combined with highly variable loci and/or many more markers, these approaches should become much more informative. Conservation genetics is also concerned with detrimental and adaptive variation, which are more difficult to identify and characterize; however, the ability to predict the extent of such variation might become more successful and applied in future conservation efforts. Neutral variants might be used to identify adaptive variants, but the overlay of different mutational processes and selective regimes suggests that extreme caution should be used in making such identifications.

The recent extinction of many species, and continuing threats to many more has made conservation biology crucial in the 21st century. Although ecological, political, economic, and other forces might be of primary concern for avoiding the extinction of most endangered species, for long-term persistence, genetics and related considerations has also been a focus of conservation effort. In particular, the application of new molecular techniques[1] has made examination of genetics in endangered species feasible and genetic analysis has become widely used in conservation research.

Philip W. Hedrick
Dept of Biology, Arizona State University, Tempe, AZ 85287, USA.
e-mail: philip.hedrick@asu.edu

Here, as an organizational theme for examining the status and possible future of conservation genetics, I consider the three major types of genetic variation – NEUTRAL, DETRIMENTAL and ADAPTIVE (see Glossary) – and discuss how each is used in the conservation of endangered species. Of course, whether a particular variant is neutral, detrimental or adaptive depends on the environment, population size, genetic background and so on. For example, a particular allele that is adaptive, such as providing resistance to an infectious disease in one environment, could be detrimental when the pathogen is absent because of a pleiotropic cost associated with that allele. Alternatively, genetic variants that are neutral in one situation could be adaptive in another.

I also consider one of the great promises of neutral molecular variation, that of the use of the extent and pattern of neutral variation to predict the amount and significance of detrimental and adaptive variation. Although it has not proven compelling in some cases, with more informative loci and more genes, we might be able to utilize such observed associations better in the future.

http://tree.trends.com 0169-5347/01/$ – see front matter © 2001 Elsevier Science Ltd. All rights reserved. PII: S0169-5347(01)02282-0

Box 1. Potentially misleading interpretations of molecular genetic data

It is often presumed that statistical significance between groups for neutral molecular markers indicates the presence of biologically important differences, or that populations have been separated long enough for biologically important differences to accumulate. Traditional molecular markers often provided inadequate statistical power to estimate the differences between groups in endangered species because they displayed too little variation. However, with the discovery of highly variable loci, such as microsatellites, or large numbers of independent markers, such as single nucleotide polymorphisms, the statistical power to differentiate between groups is now often very high.

Let us briefly examine the association of statistical significance based on molecular markers and biological meaningfulness of comparisons of groups[a,b]. There can either be both no significant statistical and no meaningful biological difference between groups, or there can be a significant statistical difference between groups reflecting a meaningful biological difference (Table I). In both cases, statistics based on molecular markers result in an appropriate evaluation of the real biological situation.

Table I. Classification of the four general states of association of statistical significance of molecular markers and the biological importance of the difference between groups, with some possible examples

Statistical significance	Biological importance	Possible examples
No	No	Recent divergence of groups
Yes	Yes	Long-term separation of groups
No	Yes	High gene flow but strong selective differences
Yes	No	High statistical power for molecular markers

However, problems result when statistical significance does not reflect biological meaningfulness, a conflict that can occur in two basic forms: there might be no statistical significance when there are actual biologically meaningful differences between groups, or there can be statistical significance between groups when there is no meaningful biological difference. In the first instance, there might be no significant difference based on molecular genetic markers but other, adaptively important loci, might be highly differentiated between populations. For example, in Scots pine *Pinus sylvestris* from Finland, several different molecular markers show little differentiation between northern and southern populations[c]. However, many important adaptive quantitative traits show high levels of genetic differentiation between these populations in common experimental environments. In this case, the molecular data appear to be adequately reflecting the high level of gene flow in Scots pine. However, the selective forces between populations are so strong that they overcome the effects of gene flow and result in large adaptive genetic differences between populations. In this case, the error is not a typical 'false negative', because the result is correct for the neutral nuclear markers. The error results from not directly assaying, or being able to assay, the genes involved in adaptation.

The final classification could become a major concern in both evolutionary and conservation biology as large numbers of highly variable markers become available in many species. In general, statistical power for determining differentiation between groups is closely related to the number of independent alleles (S. Kalinowski, pers. commun.), so that, even for a few highly variable microsatellite loci, there can be extremely high statistical power. When there is such high statistical power, extremely small molecular genetic differences between groups become statistically significant. This is not a typical 'false positive' because the differences detected are real, but so small that they do not reflect biologically meaningful differences.

To determine a biologically meaningful difference, we need to define some measure or effect related to the likelihood of the accumulation of significant biological differences. One potential way to examine the relationship between biological and statistical significance is to evaluate the statistical power to detect a known biological effect. For example, the statistical power to detect a one-generation genetic bottleneck of different sizes can be compared to the ancestral population for different numbers of loci[a]. In this case, assuming that each of ten loci had five alleles and the sample size is 40, the statistical power to detect a bottleneck of size 32 is >0.8. It is unlikely that a bottleneck of that size for one generation would have any biological effects, but there would be high statistical power to detect it with highly polymorphic loci. In general, we need to quantify the extent of the evolutionary effect that we are able to detect with highly variable molecular markers and evaluate whether this effect is likely to have important biological consequences.

References

a Hedrick, P.W. (1999) Perspective: highly variable loci and their interpretation in evolution and conservation. *Evolution* 53, 313–318

b Waples, R.S. (1998) Separating the wheat from the chaff: patterns of genetic differentiation in high gene flow species. *J. Hered.* 89, 438–450

c Karhu, A. *et al.* (1996) Do molecular markers reflect patterns of differentiation in adaptive traits of conifers? *Theor. Appl. Genet.* 93, 215–221

Neutral variation

The extent and pattern of molecular variation within a population is generally consistent with neutral variation; that is, a balance predicted by a reduction in variation from genetic drift and an increase in variation from mutation. When the population is small, even if selection is acting on the variation at a given gene, genetic drift could have a greater effect than would selection on allele frequencies. In general, neutrality of genetic variants can be assumed when the selection coefficient s (either the selective disadvantage of a detrimental allele or the advantage of an adaptive allele) is $<1/(2N_e)$, where N_e is the effective population size[2]. Therefore, because there are generally low effective population sizes in endangered species, genetic variants are more likely to be effectively neutral in endangered than in common species (the connection between single gene variation and quantitative genetic traits is discussed in Ref. 3).

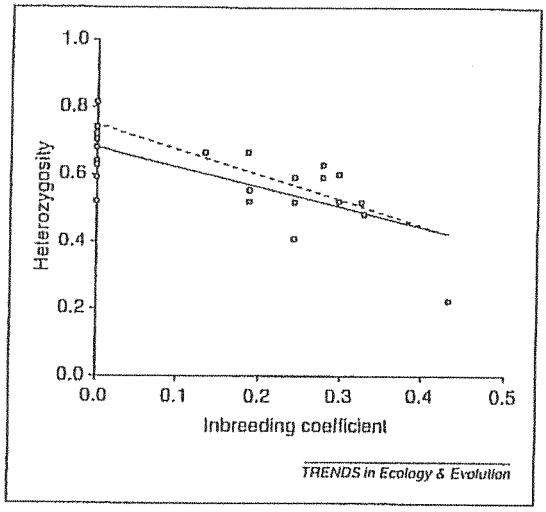


Fig. 1. The relationship between heterozygosity from 29 microsatellite loci and the inbreeding coefficient from pedigree information for 29 individual gray wolves *Canis lupus*. The solid line represents the observed regression; the broken line represents the expected relationship assuming that the heterozygosity of noninbred individuals is 0.75. $R^2 = 0.521$. Reproduced, with permission, from Ref. 20.

Most recent conservation genetics research has focused on the use of neutral molecular markers. These applications have been primarily used to identify species, evolutionarily significant units (ESUs), management units (MUs) and the origin of individuals[4–7]. Highly variable genetic markers, such as microsatellite loci, have allowed the quantification of patterns that are not apparent when using genetic markers with less variation. The use of large numbers of single-nucleotide polymorphisms (SNPs) might also provide high genetic resolution. However, the application of highly variable loci (or extremely large numbers of markers) must be used with some caution[8] because statistically significant differences might not reflect biologically important differences (Box 1), or might give a different signal compared with those from other markers[9].

Molecular genetic markers hold great promise for several other conservation applications, including approaches to measuring fundamental parameters important in conservation, such as effective population size[10,11], past bottlenecks[12,13], and sex-specific gene flow[14] or founder contributions[15]. Molecular markers can also be used to infer the historical and geographical relationships between groups[16,17]. Although the power to infer such relationships is substantial, data from ancient specimens can now provide additional insight into the relationship of contemporary groups[18].

In addition to identifying individuals within a particular group, highly variable loci or large numbers of markers might allow identification of the relationships between individuals or their individual levels of inbreeding[19]. For example, to examine the predictive value of microsatellite variants, the heterozygosity at 29 microsatellite loci in a captive population of gray wolves *Canis lupus* was compared with that from the known pedigree (Fig. 1)[20]. The predicted level of heterozygosity from the pedigree data and the observed heterozygosity from the microsatellites were almost identical.

Detrimental variation

Perhaps the most important early contribution of genetics to conservation was the recognition of the importance of INBREEDING DEPRESSION, which is thought to be due to increasing the homozygosity of detrimental alleles[21]. In addition to the expected decline in fitness with inbreeding, the mean population fitness can also decline over time, because detrimental mutations with a small selective disadvantage in a small population will become fixed, much as if they were neutral[22]. It is useful to distinguish between these effects[23] and to define the GENETIC LOAD as the reduction in mean population fitness from detrimental variation compared with that of a population without lowered fitness. Inbreeding depression and genetic load have been of major concern when dealing with endangered species[24] and inbreeding avoidance has become a priority in captive breeding programs.

Levels of inbreeding depression and genetic load

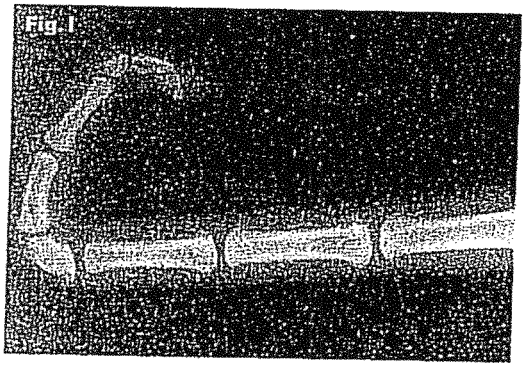
In a large population at equilibrium, substantial standing detrimental genetic variation is expected, as is a large reduction of fitness if inbreeding was to occur (Table 1). There is also little genetic load: because of the efficacy of selection in large populations, most of the detrimental variants are recessive and occur at a low frequency. If the population declines in size, purging of detrimental variation should take place, especially for alleles of large detrimental effect, thereby reducing inbreeding depression. Some detrimental variants might become fixed, particularly those of smaller effect, thus causing an increase in genetic load[22]. If the population remains small for an extended period, more detrimental variation could be purged, further reducing inbreeding depression, but more detrimental variants could be fixed, resulting in a higher genetic load. Such a population might show no lowered fitness upon inbreeding but, because of the fixation of detrimental loci, all individuals in the population might have a low fitness and the population might have a high genetic load.

Table 1. Scenarios that produce generally different predicted levels of inbreeding depression and genetic load

Scenario	Inbreeding depression	Genetic load
Large population at equilibrium	High	Low
Recently small population	Intermediate (purged some detrimentals of large effect)	Intermediate (fixed some detrimentals of small effect)
Long-term small population	Low (purged detrimentals of medium and large effect)	High (fixed many detrimentals of small effect)

Box 2. Gene flow and genetic restoration

Populations of some endangered species have become so small that they have lost genetic variation and appear to possess a high frequency of deleterious genetic variants, even to the level of fixation. To avoid extinction from this genetic deterioration, some populations might benefit from the introduction of individuals from related populations or even subspecies for genetic restoration, that is, elimination of deleterious variants and recovery to normal levels of genetic variation. An extreme example is the last remaining population of the Florida panther *Puma concolor coryi*, which has a suite of traits that suggests genetic drift has fixed (or nearly fixed) the population for previously rare and potentially deleterious traits. These traits, which are found in high frequency only in the Florida panther and are unusual in other puma subspecies, include high frequencies of cryptochordism (unilateral undescended testicles), kinked tail for the last five vertebra (Fig. I; reproduced, with permission, from Ref. a), cowlick on the back, and the poorest semen quality recorded in any felid[a]. In addition, a large survey of microsatellite loci has shown that Florida panthers have much lower molecular variation than do other North American populations of pumas[b].



The potential positive and negative genetic effects of introducing individuals from genetically diverse but geographically isolated populations into apparently inbred populations were theoretically evaluated before the introduction of Texas cougars *P. c. stanleyana* (recently, it has been proposed that all the North American subspecies be synonymized[b]) into Florida[c]. Assuming 20% gene flow from outside in the first generation and 2.5% every generation thereafter, the fitness generally quickly improves. One concern about this approach is that any locally adapted alleles could be swamped by outside gene flow. However, fitness from advantageous alleles is only slightly reduced with this level of gene flow.

A program to release Texas cougar females from the closest natural population from Texas was initiated in 1995 to restore genetically fitness in the Florida population. Five of the eight introduced Texas females produced offspring with resident Florida panther males and several F1 and F2 offspring have been produced[d]. At this point ~20% of the overall ancestry is from the introduced Texas cougars. Of the animals with Texas ancestry, none have a kinked tail and only one has a cowlick, a dramatic change from earlier proportions (Table I)[e]. Although cryptochordism and semen quality have not been evaluated in many individuals with Texas ancestry, the frequency of detrimental traits that have accumulated in the Florida panther already appears to have been greatly reduced by the introduction of Texas cougars.

Table I. The proportion of Florida panthers with a kinked tail and the distinctive cowlick before and after the introduction of Texas cougars[a]

	Before 1990	F1 and F2
Proportion with kinked tail	0.88 (48)	0.00 (15)
Proportion with cowlick	0.93 (46)	0.14 (14)

[a]Sample size is in parentheses; F1 and F2 animals.

References

a Roelke, M.E. *et al.* (1993) The consequences of demographic reduction and genetic depletion in the endangered Florida panther. *Curr. Biol.* 3, 340–350

b Culver, M. *et al.* (2000) Genomic ancestry of the American puma (*Puma concolor*). *J. Hered.* 91, 186–197

c Hedrick, P.W. (1995) Gene flow and genetic restoration: the Florida panther as a case study. *Conserv. Biol.* 9, 996–1007

d Land, E.D. and Lacy, R.C. (2000) Introgression level achieved through Florida panther genetic restoration. *Endangered Species Update* 17, 100–105

e Land, D. *et al.* (2000). *Florida Panther Genetic Restoration and Management: Annual Performance Report 1999–2000.* Florida Fish and Wildlife Conservation Commission

Two caveats should be mentioned. First, during this process, some populations (or even species) might become extinct and the ones going extinct could be those with a higher genetic load. Consequently, the remaining populations might not have as high a genetic load as would be expected from the standing amount of detrimental genetic variation. Second, genetic load might be documented as a low estimate of fitness compared with other populations, or by comparing the fitness of progeny from between- and within-population crosses. However, carrying out such breeding experiments might not be possible, or the groups might differ in other characteristics[25].

In *Drosophila melanogaster* approximately half the effect of inbreeding depression is thought to be from nearly recessive lethals and half from detrimentals of small effect but that have higher dominance[22,26]. However, *D. melanogaster* generally has an extremely large effective population size and the genetic architecture of their detrimental genetic variation probably reflects the first scenario in Table 1. Alternatively, for many endangered species, genetic

drift has been important, either because of a current small population size or a history of bottlenecks: the second scenario of Table 1 might therefore be more appropriate. As a result, endangered species might have a different genetic architecture with fewer variants of large detrimental effect, lower inbreeding depression, and perhaps higher genetic load, than do species with histories of larger population sizes.

If the genetic load increases in a population, the population might decrease in size, such that detrimental mutants of larger effect become effectively neutral and, subsequently, are more likely to become fixed. This feedback process has been named 'mutation meltdown' and, in theory, could result in the extinction of small populations[27,28]. However, it is not clear how significant mutation meltdown actually is because the extinction probability owing to other causes might be high in such small populations[29] and other factors could also counter its effects[30].

The magnitude and specific detrimental effects of alleles on fitness are highly variable because they might greatly depend on how these genotypes interact with the environment. Recent natural experiments are broadly consistent in reporting greater inbreeding depression in more stressful environments[24]. Inbreeding depression in captive populations or laboratory environments is generally thought to be underestimated compared with that in a natural environment. For example, the effect of inbreeding on male reproductive success in wild mice under laboratory conditions was minor, whereas in semi-natural conditions, inbred males had only ~20% of the success of outbred males[31]. In a comprehensive examination in *Drosophila*, the extent of inbreeding depression was greatly increased under stressful laboratory conditions[32].

Specific deleterious variants

Populations of some endangered species possess an extremely high frequency of deleterious genetic variants[33–35] and this genetic deterioration can result in extinction. GENETIC RESTORATION of populations can take place via the introduction of individuals from related populations or subspecies[30] (Box 2). Gene flow from outside populations has appeared to restore the fitness of several populations that were suffering from a high frequency (or fixation) of detrimental alleles[33–35].

Little progress has been made on the genetic characterization of genes with detrimental effects segregating in endangered species. However, the effort in the human and other genome projects to identify genes causing inherited disorders should provide information about homologous genes in endangered species. In addition, the ability to map genes affecting fitness-related traits portends imminent knowledge of the detailed architecture of genes affecting inbreeding depression, namely, the number and location of the genes, the distribution of their effects and their dominance, and the interaction (epistasis) of different genes.

There are documented examples of inherited disorders in captive populations of endangered species[37,38], which would have important negative consequences for animals reintroduced to the wild. Although these recessive alleles are only present at a low frequency, many individuals in such a population could be heterozygous for them. Reducing the frequency of these alleles could be complicated and must be undertaken carefully to avoid jeopardizing the remaining genetic variation in these species[39]. For example, eliminating the recessive allele for chondrodystrophy, a form of dwarfism, from the California condor *Gymnogyps californianus* would require over half the population to be prevented from breeding[38]. Conversely, it appears that hereditary blindness in a captive population of gray wolves could be reduced without greatly influencing the genetic variation in the rest of the genome[40]. Disease alleles that have such severe negative effects are often easy to distinguish, but other detrimental alleles, which are present in every population, might not be as easy to identify[39]. Selection will also act to reduce the frequency of detrimental alleles, although if they are recessive the effect will be rather slow. If the population size is small, some detrimental alleles will be effectively neutral and purifying selection will not be effective. However, in a small population the expected frequency of recessive detrimental alleles of large effect is much lower than in an infinite population[41].

Adaptive variation

The extent and pattern of adaptive (advantageous) variation is crucial to the long-term survival of endangered species. In particular, if there is no adaptive variation in a population that is facing a new environmental challenge, such as a new disease or competition from an introduced species, there is no potential for adaptive response except from new mutations or gene flow from other taxa. However, determining the extent and pattern of adaptive variation in the present or presumed future environments is rather difficult. Experimental tests of fitness and adaptiveness in a variety of environments could be carried out, but this is difficult, even in a model organism such as *D. melanogaster*, and practically impossible in an endangered species.

The extensive molecular data available today might provide new ways to determine whether adaptive selection has operated in the past on a given gene and, therefore, whether it might operate in the future. For example, rather than being able to measure the impact of selection in a single or a few generations by determining differential viability or reproduction, the cumulative effect over many generations could be observed by analyzing DNA variation. Two such approaches are the higher rate of nonsynonymous (amino acid changing) to synonymous substitutions and the pattern of sequence variation within and between species[42,43].

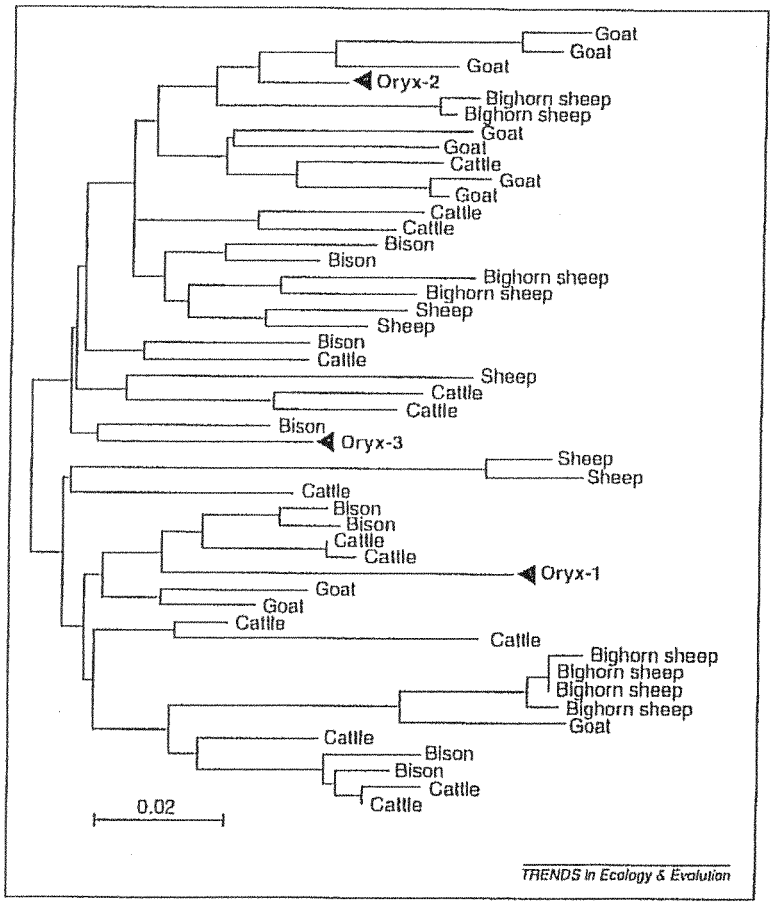


Fig. 2. A neighbor-joining tree with the three Arabian oryx *Oryx leucoryx* class II major histocompatibility alleles, indicated by arrows, and related sequences from other ruminants. Reproduced, with permission, from Ref. 50.

Using such techniques, several genes appear to show adaptive variation[43,44] but these approaches do not usually allow identification of specific selected alleles.

In general, there are few obvious molecular ways to differentiate alleles that are adaptive, neutral or detrimental (except for detrimental variants that have stop codons or those for which there is detailed structural information on the molecule[45] or alleles that have quantifiable adaptive characteristics[46]). However, favorable alleles that have become fixed by a selective sweep are expected to show low variation at, and high linkage disequilibrium with, tightly linked markers[47]. This pattern has proved useful in estimating the age of adaptive polymorphic alleles at the G6PD locus in humans[48] and could be used to identify alleles at other loci that have recently increased in frequency because of their adaptive importance.

For genes in the major histocompatibility complex (MHC) involved in pathogen resistance[49], it might be possible to make an educated guess as to the importance of particular MHC alleles. For example, the three class II MHC alleles found at a locus in the Arabian oryx *Oryx leucoryx* are extremely divergent[50], averaging 15 amino-acid differences (Fig. 2), with nearly all of the nucleotide differences resulting in amino acid differences at the functionally important antigen-binding site. When these sequences are placed in a phylogenetic tree with sequences from related species, they are widely distributed and the allelic lineages are maintained even across species, unlike the prediction for neutral loci. These findings suggest that long-term balancing selection has occurred and imply that the different variants have contributed to pathogen resistance.

The best general approach for maintaining genetic variation in a captive population is to minimize mean kinship[51,52]. A breeding scheme to select for any specific putatively adaptive allele might result in a faster loss of variation at most other loci in the genome. Taken to the extreme, if the contribution of one individual with a rare allele is greatly increased, then the pedigree will overly reflect the ancestral contributions of that individual. Selecting for a rare adaptive allele in captive pedigrees appears to be worse than the mean kinship approach, but the magnitude of the effect depends on the depth and complexity of the pedigree and the population size[53].

Neutral variation as an indicator of detrimental and adaptive variation

The extent and pattern of neutral genetic variation has been used as a guide to the amount and pattern of detrimental and adaptive variation. If this association were always positive and strong, it could be a good predictor of how much detrimental variation might be present that could lower fitness and how much adaptive variation might be present to deal with future challenges. With greater numbers of more variable loci, estimates of the extent and pattern of neutral genetic variation will presumably become more accurate. However, different evolutionary scenarios might be responsible for different amounts of neutral variation (Table 2) and these scenarios might in turn result in different amounts or patterns of detrimental[23] or adaptive variation. Positive associations might occur when the population size has been large or small for a long time or where stochastic effects dominate the extent of genetic variation. For example, there is a high correlation of (neutral) microsatellite and (adaptive) MHC loci variation in Gila topminnow *Poeciliopsis o. occidentalis*[54] and desert bighorn sheep *Ovis canadensis*[55,56] populations, two species in which stochastic factors appear to be important in the present spatial pattern of variation.

However, there might be situations in which such an inference based on a positive association is unfounded. For example, some time after a bottleneck, there might be a negative association between neutral and detrimental variation, because the amount of detrimental variation could recover faster as a result of a higher mutation rate. This might not be the case for all neutral variation because some genes, such as microsatellite loci, have mutation rates that are as high as those for detrimental variation. A negative association could also occur when separately bottlenecked populations become

Table 2. The amount of observed neutral variation and general predictions of the amounts of detrimental and adaptive variation under different evolutionary scenarios

Scenario		Observed Neutral variation	Predicted Detrimental variation	Adaptive variation
Equilibrium	Large population	High	High	High
	Small population	Low	Low	Low to medium (retention more than neutrality)
Bottleneck	Shortly after	Low (loss of alleles more than heterozygosity)	Low (loss of lethals more than detrimentals)	Low to medium (retention more than neutrality)
	Some time after	Low to high (depending upon mutation rate of variants)	High (assuming high mutation rate)	Low to medium (retention more than neutrality, function of mutation rate)
	Mixture of separately bottle-necked populations	High (owing to fixing of different alleles in different populations)	Low (owing to purging of variants within each population)	Low to medium (retention more than neutrality)
Metapopulation	With extinction, recolonization, and low gene flow	Low (owing to low effective population size)	Low (owing to purging)	Low to medium (depending upon selection model)
	No extinction and more gene flow	High (as if one large population)	High (as if one large population)	High (as if one large population)

mixed, resulting in high neutral variation but low detrimental variation. Finally, in a metapopulation, there might be a positive association between neutral and detrimental variation, but depending on the level of extinction, recolonization, and gene flow, the levels of variation might be either extremely low or high.

In some specific cases, it appears that there might be little genetic variation for some markers and substantial variation for others. For example, the cheetah *Acinonyx jubatus* was documented to have low genetic variation for allozymes and other genetic markers[57], and great concern was raised about its long-term survival prospects. However, further studies showed that cheetahs have substantial genetic variation for microsatellites[58], and captive cheetahs appear to exhibit inbreeding depression for juvenile survival[59], indicating variation for detrimental alleles. These differences have been explained with scenarios that include a bottleneck[58] or a metapopulation with a small effective population size[60] and different mutation rates for different markers.

Overall, the positive correlation between neutral and adaptive variation might not be particularly high. However, high neutral variation might indicate the potential for significant adaptive variation. Low neutral variation could indicate low adaptive variation, but the present population might either be well adapted or poorly adapted to its environment. Furthermore, gene flow between populations could result in low differentiation for neutral markers between populations, but there might still be strong adaptive differences in the populations. The extent and pattern of neutral variation is the result of nonselective forces and can potentially be used to identify the past importance of finite size, bottlenecks and population structure. However, adaptive variants might differ in mutation rate from neutral markers and selection might result in rather different patterns of variation within and between populations. In other words, neutral variants might be used as a guide to understanding nonselective effects, but the overlay of different mutational processes and selective regimes suggests that great caution should be used in making such predictions.

Prospects

Genetics will probably play an even more important role in conservation in the future than it does now. The utilization of information from the human and other genome projects will provide substantially more background understanding and the appropriate use of these data should be of great benefit to conservation. In particular, techniques to screen and analyze large amounts of data, whether it is variation at marker loci or DNA sequence data, will be used to determine specific groups and individuals. However, to complement the high statistical power from these data, an evolutionary perspective is required to evaluate their biological importance. In addition, these data will allow an understanding of past evolutionary events in endangered species, whether they are nonselective, such as bottlenecks or gene

Glossary

Adaptive variation: genetic variation that produces an advantage in fitness. A population might be fixed for an adaptive variant or polymorphic because the variant is newly arisen or is maintained by balancing selection.

Detrimental variation: variation that has a negative effect on fitness, often brought into the population by mutation and sometimes increased by genetic drift.

Genetic load: the reduction in mean fitness resulting from detrimental variation for a population compared to a population without lowered fitness.

Genetic restoration: the elimination or reduction of deleterious variants and recovery to normal levels of genetic variation for a population in poor genetic health by the introduction of individuals from another genetically healthy population.

Inbreeding depression: the decline in progeny fitness that occurs as the result of inbreeding.

Neutral variation: variation that has a small selective coefficient(s) relative to the population size, such that $s<1/(2N_e)$, where s is either the selective disadvantage of a detrimental allele or the selective advantage of an adaptive allele and N_e is the effective population size.

flow, or selective, such as detrimental or adaptive mutations. Examination of the pattern of variation at tightly linked loci might allow the determination of whether alleles have recently undergone selection to increase their frequency. This could be particularly important because the present-day small population size of some endangered species might make variants effectively neutral, but, if a population recovers to larger numbers these variants could become detrimental or adaptive.

References

1 Summucks, P. (2000) Efficient genetic markers for population biology. *Trends Ecol. Evol.* 15, 199–203
2 Kimura, M. (1983) *The Neutral Theory of Molecular Evolution*, Cambridge University Press
3 Lynch, M. (1996, A quantitative-genetic perspective on conservation issues. In *Conservation Genetics* (Avise, J. and Hamrick, J., eds), pp. 471–501, Chapman and Hall
4 Cournuet, J-M. *et al.* (1999) New methods employing multilocus genotypes to select or exclude populations as origins of individuals. *Genetics* 153, 1989–2000
5 Crandall, K.A. *et al.* (2000) Considering evolutionary processes in conservation biology. *Trends Ecol. Evol.* 15, 290–295
6 Davies, N. *et al.* (1999) Determining the source of individuals: multilocus genotyping in nonequilibrium population genetics. *Trends Ecol. Evol.* 14, 17–21
7 Moritz, C. (1999) Conservation units and translocation: strategies for conserving evolutionary processes. *Hereditas* 130, 217–228
8 Hedrick, P.W. (1999) Perspective: highly variable loci and their interpretation in evolution and conservation. *Evolution* 53, 313–318
9 Balloux, F. *et al.* (2000) Microsatellites can be misleading: an empirical and simulation study. *Evolution* 54, 1414–1422
10 Kuhner, M.K. *et al.* (1998) Maximum likelihood estimation of population growth rates based on the coalescent. *Genetics* 149, 429–434
11 Schwartz, M.K. *et al.* (1998) Review of DNA-based census and effective population size estimators. *Anim. Conserv.* 1, 293–299
12 Lulkart, G. and Cornett, J-M. (1998) Empirical evaluation of a test for identifying recently bottlenecked populations from allele frequency data. *Conserv. Biol.* 12, 228–237
13 Reich, D.E. *et al.* (2001) Linkage disequilibrium in the human genome. *Nature* 411, 199–204
14 Latta, R.G. and Mitton, J.B. (1997) A comparison of population differentiation across four classes of gene markers in limber pine (*Pinus flexilis* James). *Genetics* 146, 1153–1163
15 Carvajal-Carmona, L.G. *et al.* (2000) Strong Amerind/white sex bias and a possible Sephardic contribution among the founders of a population in Northwest Colombia. *Am. J. Hum. Genet.* 67, 1287–1295
16 Avise, J. (2000) *Phylogeography: The History and Formation of Species*, Harvard University Press
17 Cruzan, M.B. and Templeton, A. (2000) Paleoecology and coalescence: phylogeographic analysis of hypotheses from the fossil record. *Trends Ecol. Evol.* 15, 492–497
18 Leonard, J.A. *et al.* (2000) Population genetics of Ice age brown bears. *Proc. Natl. Acad. Sci. U. S. A.* 97, 1651–1654
19 Lynch, M. and Ritland, K. (1999) Estimation of pairwise relatedness with molecular markers. *Genetics* 152, 1753–1766
20 Ellegren, H. (1999) Inbreeding and relatedness in Scandinavian grey wolves *Canis lupus*. *Hereditas* 130, 239–244
21 Charlesworth, D. and Charlesworth, B. (1999) The genetic basis of inbreeding depression. *Genet. Res.* 74, 329–340
22 Wang, J. *et al.* (1999) Dynamics of inbreeding depression due to deleterious mutations in small populations: mutation parameters and inbreeding rate. *Genet. Res.* 74, 165–178
23 Kirkpatrick, M. and Jarne, P. (2000) The effects of a bottleneck on inbreeding depression and the genetic load. *Am. Nat.* 155, 154–167
24 Hedrick, P.W. and Kalinowski, S.T. (2000) Inbreeding depression in conservation biology. *Annu. Rev. Ecol. Syst.* 31, 139–162
25 Wang, J. (2000) Effects of population structure and selection strategies on the purging of inbreeding depression due to deleterious mutations. *Genet. Res.* 76, 75–86
26 Lynch, M. *et al.* (1999) Perspective: spontaneous deleterious mutation. *Evolution* 53, 645–663
27 Higgins, K. and Lynch, M. (2001) Metapopulation extinction caused by mutation accumulation. *Proc. Nat. Acad. Sci. U. S. A.* 98, 2928–2933
28 Lynch, M. *et al.* (1995) Mutation meltdowns in sexual populations. *Evolution* 49, 1067–1088
29 Lande, R. (1995) Mutation and conservation. *Conserv. Biol.* 9, 782–791
30 Whitlock, M.C. (2000) Fixation of new alleles and the extinction of small populations: drift load, beneficial alleles, and sexual selection. *Evolution* 54, 1855–1861
31 Meagher, S. *et al.* (2000) Male-male competition magnifies inbreeding depression in wild house mice. *Proc. Natl. Acad. Sci. U. S. A.* 97, 3324–3329
32 Bijlsma, R. *et al.* (1999) Environmental dependence of inbreeding depression and purging in *Drosophila melanogaster*. *J. Evol. Biol.* 12, 1125–1137
33 Land, D. *et al.* (2000) *Florida Panther Genetic Restoration and Management: Annual Performance Report 1999–2000*. Florida Fish and Wildlife Conservation Commission
34 Madsen, T. *et al.* (1999) Restoration of an inbred adder population. *Nature* 402, 34–35
35 Westemeier, R.L. *et al.* (1998) Tracking the long-term decline and recovery of an isolated population. *Science* 282, 1695–1698
36 Hedrick, P.W. (1995) Gene flow and genetic restoration: the Florida panther as a case study. *Conserv. Biol.* 9, 995–1007
37 Laikre, L. (1999) Hereditary defects and conservation genetic management of captive populations. *Zoo Biol.* 18, 91–99
38 Ralls, K. *et al.* (2000) Genetic management of chondrodystrophy in California condors. *Anim. Conserv.* 3, 145–153
39 Laikre, L. *et al.* (1993) Hereditary blindness in a captive wolf (*Canis lupus*) population: frequency reduction of deleterious alleles in relation to gene conservation. *Conserv. Biol.* 7, 592–601
40 Lacy, R.C. (2000) Should we select genetic alleles in our conservation breeding programs? *Zoo Biol.* 19, 279–282
41 Wright, S. (1937) The distribution of gene frequencies in populations. *Proc. Natl. Acad. Sci. U. S. A.* 23, 307–320
42 Richman, A. (2000) Evolution of balanced genetic polymorphism. *Mol. Ecol.* 9, 1953–1963
43 Yang, Z. and Bielawski, J.P. (2000) Statistical methods for detecting molecular adaptation. *Trends Ecol. Evol.* 15, 496–503
44 Hughes, A.L. (1999) *Adaptive Evolution of Genes and Genomes*, Oxford University Press
45 Gao, X. *et al.* (2001) Effect of a single amino acid substitution in the MHC class I molecule on the rate of progression to AIDS. *New Engl. J. Med.* 344, 1668–1675
46 Yokoyama, S. *et al.* (1999) Adaptive evolution of color vision of the Comoran coelacanth (*Latimeria chalumnae*). *Proc. Natl. Acad. Sci. U. S. A.* 96, 6279–6284
47 Hudson, R.R. *et al.* (1997) DNA variation at the *Sod* locus of *Drosophila melanogaster*: an unfolding story of natural selection. *Proc. Natl. Acad. Sci. U. S. A.* 94, 7725–7729
48 Tishkoff, S.A. (2001) Microsatellite/RFLP haplotype analysis of G6PD A– and Med deficiency mutation: implications for the origins of malarial resistance in humans. *Science* 293, 455–462
49 Hedrick, P.W. and Kim, T.J. (2000). Genetics of complex polymorphisms: parasites and maintenance of the major histocompatibility complex variation. In *Evolutionary Genetics: From Molecules to Morphology* (Singh, R.S. and Krimbas, C.B., eds). pp. 204–234. Cambridge University Press
50 Hedrick, P.W. *et al.* (2000) Major histocompatibility complex (MHC) variation in the Arabian oryx. *Evolution* 54, 2145–2151
51 Ballou, J.D. *et al.*, eds (1995) *Population Management for Survival and Recovery*. Columbia University Press, New York
52 Caballero, A. and Toro, M.A. (2000) Interrelations between effective population size and other pedigree tools for the management of conserved populations. *Genet. Res.* 75, 331–343
53 Miller, P.S. (1995) Selective breeding programs for rare alleles: examples from the Przewalski's horse and California condor pedigrees. *Conserv. Biol.* 9, 1262–1273
54 Hedrick, P.W. *et al.* (2001) Genetic variation in the endangered Gila and Yaqui topminnows: microsatellite and MHC variation. *Mol. Ecol.* 10, 1399–1412
55 Gutierrez-Espeleta, G.A. *et al.* (2000) Genetic variation and population structure in desert bighorn sheep: implications for conservation. *Conserv. Genet.* 1, 3–15
56 Gutierrez-Espeleta, G.A. *et al.* (2001) Is the decline of desert bighorn sheep from infectious disease the result of low MHC variation? *Heredity* 86, 439–450
57 O'Brien, S.J. *et al.* (1985) Genetic basis for species vulnerability in the cheetah. *Science* 227, 1428–1434
58 Menotti-Raymond, M. and O'Brien, S. J. (1995) Evolutionary conservation of ten microsatellite loci in four species of Felidae. *J. Hered.* 86, 319–322
59 Wielebnowski, N. (1996) Reassessing the relationship between juvenile mortality and genetic monomorphism in captive cheetahs. *Zoo Biol.* 15, 353–369
60 Hedrick, P.W. (1996) Bottleneck(s) or metapopulation in cheetahs. *Conserv. Biol.* 10, 897–899

Exhibit O

TESTIMONY OF STEVEN P. QUARLES

before the

Subcommittee on Fish, Wildlife and Water

Environment and Public Works Committee

United States Senate

May 9, 2001

Issues Concerning the Listing of Distinct Population Segments of Vertebrates under the Endangered Species Act of 1973

My name is Steven P. Quarles. I am counsel to, and appearing on behalf of, the American Forest & Paper Association (AF&PA) and the QuadState County Government Coalition. AF&PA is the national trade association representing the forest products industry. It has more than 130 member companies that grow, harvest, and process wood and wood fiber and manufacture a wide variety of products including pulp, paper, paperboard and wood products. AF&PA has more than 60 association members that represent all facets of the forest products industry and represent more than 10,000 companies. AF&PA's member companies, as a condition of membership, must also commit to conduct their business in accordance with the principles and objectives of the Sustainable Forestry Initiative (SFI)SM program. QuadState County Government Coalition is a coalition of six counties in the four States that

share portions of Mojave and Colorado Deserts — Mojave County in Arizona; Imperial, Kern and San Bernardino Counties in California; Lincoln County in Nevada; and Washington County in Utah. The Coalition's principal concern is the effect of the listing of the Mojave population of the desert tortoise on its member counties' economic and revenue bases, their public works, and the businesses and properties of their constituents.

The topic to which the Subcommittee has devoted this hearing is timely and significant. Individual determinations or "listings" of species to be endangered species or threatened species ("listed species") under the Endangered Species Act ("ESA") have frequently been contentious because the stakes are high; imposed with the listings is the full panoply of the ESA's controls over human behavior to benefit the listed species. However, for most of the ESA's life span of nearly three decades, the general process (and the underlying science and law) of listing species as endangered species or threatened species has not garnered the same degree of controversy, or at least attention, as many of the other processes and decisions under that Act.

Controversy over and attention to the species' listing process are now at hand, however — triggered by recent actions of both the Clinton and Bush Administrations. On November 17, 2000, FWS Director Jamie Clark announced that the agency lacks sufficient funds to conduct any species' listings, including responding to any listing petitions, in fiscal year 2001 beyond those mandated by court order. This Clinton listings moratorium was followed by a legislative proposal in President Bush's budget to waive for fiscal year 2002 the ESA's statutory deadline for species listings (and designations of critical habitat) and to limit use of the available funding to implementing already issued court

orders and those listings (and designations) the Secretary of the Interior in her discretion determines to be important. Both the Clinton moratorium and the Bush budget language, if enacted, should spawn litigation and enlist the judiciary in the intensifying species' listing controversy.

My topic will focus on one issue: the overbroad definition of "species" eligible for listing under the ESA. In particular, I will emphasize the authority to list distinct population segments of vertebrates, efforts by Congress to determine the use of that authority, and how that authority has been abused by the ESA-implementing agencies (the U.S. Fish and Wildlife Service ("FWS") and the National Marine Fisheries Service ("NMFS") (collectively, the "Services)). The Services' abuse of the distinct population segment concept has resulted in the expansion of their species' listing authority well beyond the expectations of Congress and, in particular, this Committee, and the infiltration of the concept into other ESA decisionmaking processes for which it was not authorized or intended. I conclude with suggestions on ways Congress, in this time of funding scarcity, could limit the applicability of the distinct population segment concept for listing vertebrate species — a concept that provides the least amount of protection for genetic heritage, a principal purpose of the ESA.

Background

The ESA only permits the Services to list an endangered species or threatened species if it is a "species" unit as defined by ESA § 3(16), and only if that "species" unit meets the definitions of "endangered species" or "threatened species" in ESA § 3(6) and 3(20) which requires a degree of risk over a "significant portion of [the] range" occupied by the species unit. 16 U.S.C. §§ 1532(6) and (20), 1533(a)(1)

(FWS shall "determine whether any species is an endangered species or threatened species"), (a)(2), (a)(3), (b)(1), (b)(3)(A), and (b)(6)(A) (final "determination as to whether a species is an endangered species or a threatened species").

The ESA defines the crucial term "species" in an unusual way. An ESA-listable "species" unit includes not only a true biological species (those individuals that can interbreed and produce viable offspring), but also a recognized "subspecies of fish or wildlife or plants," and — in the case of a vertebrate species — "any distinct population segment of any species of vertebrate fish or wildlife which interbreeds when mature" ("DPS"). 16 U.S.C. § 1532(16).

The ESA listing of any "species" unit has adverse consequences for private and public land uses within the range of that species. ESA § 9 and regulations prohibit the "take" of almost all listed wildlife species — the "take" prohibition bars any economic land use which would inadvertently harm even one member of that wildlife species. *See* 16 U.S.C. § 1538(a)(1); 50 C.F.R. §§ 17.3 and 222.102; *Babbitt v. Sweet Home Chapter of Communities for a Great Oregon*, 515 U.S. 687 (1995). Further, federally-assisted actions cannot be approved until an ESA § 7 consultation procedure has been completed, and must be disapproved if the action is likely to jeopardize the existence of the listed species unit. *See* 16 U.S.C. § 1536.

Each of the three branches of government has treated the authority to list distinct population segments of vertebrates in a strikingly different manner. If the analogy were to ships, two of the branches — Congress and the Executive — have passed in the night sailing in opposite directions; the third — the Judiciary — has never left port. Since the ESA's

enactment, Congress has made several efforts to reduce, met by countervailing efforts of the Services to expand, the scope of listings below the subspecies level. The federal courts have rarely been asked to pass judgment on these opposing efforts.

Congressional Efforts to Constrain Listings of Distinct Populations

Distinct population segments did not enjoy protection under the ESA's predecessors. The Endangered Species Preservation Act of 1966 referred only to "species"; "subspecies" were added in the Endangered Species Conservation Act of 1969. The ESA's first attempt to provide protection to species units below subspecies was quite liberal. As enacted in 1973, the ESA defined "species" to "include[] any subspecies of fish or wildlife or plants and any other group of fish or wildlife of the same species or smaller taxa in common spatial arrangement that interbreed when mature."

Contrary to popular belief, many of the populations of charismatic megafauna that have been designated as endangered or threatened species were listed under this early, quite generous "common spatial arrangement" standard-less standard. These include species, such as the grizzly bear and bald eagle, that may have been rare in the lower 48 States but are common in Canada and Alaska. The listing documents made no attempt to demonstrate any degree of distinctness between the lower 48 species unit and the northern species unit; instead, they readily admitted that the two species units were not reproductively segregated and had no genetic or other biological differences. Moreover, the FWS made no effort to determine whether these "species" were suffering declines in Canada

(or, for that matter, in the rest of the U.S., *i.e.*, Alaska). Indeed, as one author noted, the FWS engaged in the ludicrous fiction of "defin[ing] the grizzlies' entire range as the lower forty-eight states even though it was obviously well aware that grizzlies existed in Canada and Alaska." (Daniel Rohlf, "There's Something Fishy Going On Here: A Critique of the National Marine Fisheries Service's Definition of Species Under the Endangered Species Act", 24, Envtl. L. 617, 653 (1994).)

By 1978, Congress had had second thoughts about this loose "common spatial arrangement" authority for species' listings. To restrict sub-subspecies listings, Congress amended the ESA by replacing the original definition of species with the present definition and its DPS language. Criticism continued, however; none more withering than that of the General Accounting Office ("GAO"). In 1979 testimony before this Committee and a report, the GAO raised concern over any loose definition of a "distinct population" in what has become the well-known squirrels-in-the-park analogy. The agency stated that the definition:

permitted FWS to list populations of species, regardless of their size, location, or total numbers. Thus, squirrels in a specific city park could be listed as endangered, even though an abundance of squirrels lived in other parks in the same city and elsewhere.... Such listings could increase the number of potential conflicts between endangered and threatened species and Federal, State, and private projects and programs.... However, the purpose of the Endangered Species Act is to conserve endangered and threatened species and their critical habitats, not preserve every individual animal and plant.

52, 58 (GAO Rep. CED 79 65, 1979). Although the particular quote referred to the 1973 "species" definition, two lawyers and a scientist from NMFS pointed out in a law review article that subsequent GAO statements indicated the same concern was relevant to the revised 1978 "species" definition. (Karl Gleaves, Michael Kuruc, Patricia Montanio, "The Meaning of 'Species' Under the Endangered Species Act," 13 Pub. Land L. Rev. 25, 31, n.30 (1992).)

This Committee took note of the GAO's criticism (even if it viewed the problem as FWS's interpretation of the statutory definition) and the GAO's suggestion that the authority to list DPSs be deleted from the ESA. However, after FWS and others emphasized the importance of the listing flexibility afforded by the DPS portion of the "species" definition, this Committee decided to retain that definition in the 1979 ESA amendments and issue a stern admonition to the Services to use the DPS listing authority "sparingly":

[T]he General Accounting Office recommended that the subcommittee consider an amendment to the definition of species currently contained in the act which would prevent the FWS from listing geographically limited populations of vertebrates as threatened or endangered. It is the GAO's contention...that FWS has interpreted the term "species" to include any population of the animal, regardless of its size, location, or total numbers. According to the GAO, this could result in the listing of squirrels in a specific city park, even if there is an abundance of squirrels in other parks in the same city, or elsewhere in the country.... *[T]he committee is aware of the great potential for abuse of this authority and expects FWS to use the ability to list populations sparingly and only when the biological evidence indicates that such action is warranted.*

S. Rep. No. 151, 96th Cong., 1st Sess. (1979) at 6-7

(emphasis added).

The Services' Unrestrained Use of the Distinct Population Segment Listing Authority

Contrary to the "sparingly" stricture of this Committee, the Services have been more *unsparing* in their use of the DPS listing authority. They produced two documents purportedly defining and confining their DPS authority — NMFS's "Policy on Applying the Definition of Species Under the Endangered Species Act to Pacific Salmon," 56 Fed. Reg. 58612 (Nov. 20, 1991), which established the DPS concept of "evolutionarily significant unit" ("ESU"), and the Services' joint "Policy Regarding the Recognition of Distinct Vertebrate Population Segments Under the Endangered Species Act", 61 Fed. Reg. 4722 (Feb. 7, 1996). Both documents quoted, and paid lip service to, this Committee's "sparingly" admonition. However, the Services' actual performance in listing DPSs is clear evidence that their DPS policies do nothing to limit and, arguably, substantially expand the authority to list DPSs.

According to a draft manuscript prepared by the Pierce Atwood law firm for an upcoming edition of the American Bar Association's *Natural Resources and Environment* magazine, since the existing "species" definition was added to the ESA in 1978, 59 DPSs have been listed by the Services, with the precise number dependant upon interpretation of decisions that, initially, did not always clearly identify the listings as DPSs. I suspect that there could be vigorous debate over whether 59 DPS listings in 22 years represents sparing use of the DPS listing authority. But once the trend — a rapid increase in DPS listings in the last several years — is examined, the nays should have it. The

pace of DPS listings was relatively constant for the first 17 years (7 in 1978-1985; 6 in 1986-1990 (including NMFS' first listing of a West Coast salmonid DPS); and 8 in 1991-1995). From 1996 through 2000, the Services have listed 38 DPSs, more than quadrupling the number of listings of the previous five years. This trend can be expected to continue; about 35 DPSs are currently involved in rulemaking processes.

By contrast, despite the existence of the Services' two policies which ostensibly were to bring rigor to the DPS listing process, the Pierce Atwood authors could find only 13 instances over the past 22 years when either of the Services concluded that a particular population did not qualify as a DPS (excluding instances where DPS status was denied for de-listing purposes and where a DPS existed but was not listed for conservation reasons). The Services have concluded that species units have qualified as DPSs more than 80% of the time. These statistics suggest that the Services rarely determine that a species unit — identified by petitioners or by themselves — does not qualify as a DPS, notwithstanding Congress' admonition to use the DPS listing authority "sparingly."

Reasons for the Liberal Use of the Distinct Population Segment Listing Authority

How did this frequent use of the DPS listing authority happen in the face of the Congressional caution? I can think of at least three reasons.

1. No scientific agreement on the DPS concept. In general, common and consistent scientific understanding and usage of *any* units below the species level is absent. As one

scientist put it: "The discussion of what makes a subunit within a species, be it a subspecies, race, ecotype, variety, or stock is such a durable source of dispute among systematic biologists that scientific consensus may never be achieved." (Robert Taylor, "Biological Uncertainty in the Endangered Species Act," 7 Natural Resources and the Environment 6 (1993).)

This scientific uncertainty becomes more severe at the DPS level. In the preamble to their 1996 joint DPS policy, the Services stated: "Available scientific information provides little specific enlightenment in interpreting the phrase 'distinct population segment.' This term is not commonly used in scientific discourse, although 'population' is an important term in a variety of contexts." 61 Fed. Reg. 4722. With so little common scientific understanding of, or agreement on, the term DPS, it is too easy for the Services to use "the best scientific and commercial data available", as required by ESA § 4(b) for listing decisions, to reach whatever conclusion they may wish. 16 U.S.C. § 1533(b)(1)(A). A committee of the National Research Council, in a 1995 report commissioned by members of Congress, while supporting a population listing concept of its own devising ("evolutionary unit"), admitted that even the one criterion everyone — Congress, the 1991 NMFS policy, and the (later) 1996 joint Services' policy — could agree on for a listable population — "distinctness" — is as much a matter of judgment as science:

The most difficult questions generally arise at taxonomic levels below the subspecies level. Because evolutionary units at such levels are not discrete but exist along a continuum, it is a *policy judgment as well as a scientific judgment* to determine the significance of an evolutionary unit . . . . [S]cience alone does not lead to a conclusion that any

objectively definable degree of distinction is more significant than another.

National Research Council, *Science and the Endangered Species Act*, 56 (1995) (emphasis added).

In other words, DPS is in the eye of the beholder.

2. The DPS policies of the Services are riddled with discretion. Certainly, an important purpose of the Services' two DPS policies was to provide a measure of scientific rigor to the DPS decisionmaking in the listing process. The fact is they only pretend to do so. As one critic wrote about the more thoroughly analyzed and justified 1991 NMFS policy:

Much like the Wizard of Oz employed smoke and mirrors to lend him an air of might and wisdom, NMFS invokes science in an effort to portray its definitions of distinct populations eligible for listing as beyond question. When one looks behind the curtain, however, it becomes apparent that NMF's ESU policy merely trades the discretion inherent in historic approaches to listing populations for a more technically complex but equally discretionary scheme. The tremendous discretion inherent in NMFS' ESU policy stems from two related sources: scientific uncertainty and extremely imprecise definitions of the two ESU criteria: reproductive isolation and whether a population represents an important component in the "evolutionary legacy" of a species. (Rohlf at 644.)

When the Pierce Atwood authors reviewed the broader, less fully explained 1996 joint DPS policy, they found so much discretion that they concluded: "We are, in other words, back to the listing of squirrels in the city park."

With this amount of discretion, the eyesight of the beholder can be quite poor, and yet suffice.

3. Neither Congress nor the courts have provided helpful guidance. The Service's discretion in designating DPSs is unconstrained by any useful legislative or judicial direction. Congress has chosen not to define DPS, and the "legislative history provides some discussion of the concept but provides no specific guidance. It is probably safe to conclude not only that the meaning of 'distinct population' is ambiguous, but also that Congress has not directly addressed or resolved this precise question." (Gleaves at 37-38.) The preamble to the 1991 NMFS policy concurs that "NMFS does not believe that the intent of Congress is clear as to the meaning of 'distinct population.' The ESA allows vertebrate populations that are 'distinct' to be considered 'species,' but does not explain how distinctness should be measured." 56 Fed. Reg. 58613. Few courts have addressed, and none has provided a significant decision on, the meaning and application of the DPS concept.

Examples of Misuse or Abuse of The Distinct Population Segment Concept in Listing Decisions

The list of methods by which the Services expand their DPS listing authority by misuse or abuse of that authority could be lengthy. I will mention only three:

1. Designation of DPSs by political boundaries. In the early days, the Services listed species populations by international borders (*e.g.*, grizzly), by State borders (*e.g.*, bald eagles), and even by parishes (American alligator). Today, the Services typically eschew State and local

boundaries for DPS listing purposes, but still adhere in their joint 1996 policy to the notion that national boundaries are perfectly permissible means of delineating DPSs. As I have noted, when the national border of the lower 48 States is used, the Services typically make no attempt to determine any reproductive isolation of, or other distinctness for, the U.S. portion of the multinational population, nor do they make any effort to learn the status of the portion of the population across the border (which is often abundant) or the other country's management regime for that population portion. All too often, this is little more than species jingoism — a fervor to claim citizenship for as many species as we can. Moreover, since the Services ignore whether the species with the lower 48 DPSs are abundant in Alaska, they appear to side with the Seward's Folly crowd that preferred the State to remain in foreign ownership or, at a minimum, they wish to restore a biological form of territorial status to our 49th State.

Unfortunately, Congress is complicit here. In 1973 and again in 1979, House and Senate reports expressed their intent to allow the Services to list domestic populations of species whose principal ranges are in another country, asserting (in the Senate report) that "the U.S. population of an animal should not necessarily be permitted to become extinct simply because the animal is more abundant elsewhere in the world." H. R. Rep. No. 412, 93d Cong., 1st Sess. 10 (1973); S. Rep. No. 151, 96th Cong., 1st Sess. 7 (1979); quoted in Rohlf at 628-629.

Many scientists emphatically disagree. Unless the U.S. population is both reproductively isolated and biologically significant, both the National Research Council in its 1995 report and NMFS in its 1991 DPS policy found that there were no "sound scientific reasons" to delineate

populations by political boundaries. NRC Report at 58; 56 Fed. Reg. 58613.

2. Designation of DPSs that are not reproductively isolated. One would have thought that an easy call for a criterion (but admittedly not always an easy matter of scientific proof) to determine a "distinct population segment" would be that the population must be reproductively isolated. After all, the plain ESA language connotes reproductive isolation. A population is "distinct" if it is separate from other members of the same biological species. A population develops "distinct" characteristics if it has a separate gene pool. The ESA phrase "population...which interbreeds when mature" suggests a population which interbreeds among itself but not with other populations. The NMFS lawyers and scientist in their law review article concur that this is the plain meaning of "distinct population":

A common dictionary definition of "distinct" is "separate" or "apart from." In addition, as a biological term, "population" includes the idea of reproductive isolation or separation. (Gleaves at 46.)

However, in their continual search for discretion, the Services have all but discarded the necessity to find reproductive isolation. For example, the 1991 NMFS policy states that the first criterion for delineating a DPS is that the Pacific salmon stock "must be *substantially* reproductively isolated from other conspecific population units" and declares that the "first criterion, reproductive isolation, *does not have to be absolute*, but it must be strong enough to permit evolutionarily important differences to accrue in different population units." 56 Fed. Reg. 58618 (emphasis added). The Services' joint 1996 policy weakens the reproductive

isolation factor further. The policy does demand that the DPS be "*markedly* separated" but this can be as a result of "physical, physiological, ecological, or behavioral factors." And, once again, "the standard *adopted does not require absolute separation* of a DPS from other members of its species. . . . The standard adopted is believed to allow entities recognized under the Act to be identified *without requiring an unreasonably rigid test for distinctness*." 61 Fed. Reg. 4725, 4724 (emphasis added).

As one observer put it: "FWS has likewise waffled on the importance of genetic make-up in distinguishing between population segments. Predictably, the agency cited the presence or absence of genetic distinctiveness in instances in which it found reproductive isolation to be important, and downplayed genetics in cases where it had made listing decisions despite a lack of such isolation." (Rohlf at 657.)

3. Designation of DPSs by disregarding the ESA's definition of "species" and relying instead on the ESA's definitions of "threatened species" and "endangered species." In at least one listing of a population, the FWS abandoned any attempt to find any "distinct" quality to the population segment. It could not. It admitted the three-State population of Marbled Murrelets it wished to list was not reproductively isolated or particularly biologically distinct from the Canadian population. Therefore, it looked away from the statutory definition of species and DPSs, and, instead, discovered helpful language in the ESA's definitions of "endangered species" and "threatened species." 16 U.S.C. § 1532(6) and (20). FWS adopted an entirely different and certainly clever approach to defining DPSs by suggesting that the "significant portion of its range" language in both the "endangered species" and "threatened species" definitions could be made to serve as a means to delineate

distinct populations without the need to demonstrate reproductive isolation or genetic or other differences. If this approach is followed in future listings, any population segment of any relative size could qualify for listing and the statutory requirement that the population be "distinct" will be sapped of all meaning.

The Services Have Inappropriately "Re-Listed" Smaller Species Units, Often As the Distinct Population Segments, In Decisionmaking Under Other Sections of the ESA

I would also like to bring to the Committee's attention our view that the Services are inappropriately redefining species units after their listing into smaller species units in decisionmaking under sections of the ESA other than the ESA § 4 listing section.

The ESA is quite clear that ESA § 7 compliance, ESA § 4(f) recovery plans, and species de-listings decisions are to be made with reference to the same "species" unit that has been listed. *See* 16 U.S.C. §§ 1533(f), 1536(a)(2) and (b). Yet, in the case of the red-cockaded woodpecker ("RCW") and in several other instances where the Services have listed a biological species, they are assessing ESA § 7 compliance not with reference to jeopardy to the listed biological species as a whole, but jeopardy to smaller subgroupings, such as an individual population of RCWs. This approach makes it far more likely that a localized action will be found to jeopardize a localized population and to violate ESA § 7. We believe that this approach is unlawful under the ESA, is contentious, and should be discontinued.

There has also been unwarranted "population creep" into ESA § 4(f) recovery planning and species' de-listing actions. In several instances, where the FWS or NMFS has listed a

larger "species" unit (*e.g.*, the Mojave population of desert tortoises or grizzly bears in the lower 48 States), the agency has subdivided that "species" unit for de-listing purposes into smaller populations. These new multiple populations were, of course, designated without the formal rulemaking required for the listing of the original all-encompassing population (grizzly) or DPS (desert tortoise). The affected public was deprived of all the procedural protections of Administrative Procedure Act rulemaking which the ESA promised in its § 4 listing provisions. 16 U.S.C. § 1532(a)(1). Moreover, this division of the listed species, subspecies, or populations into smaller populations, often renamed with such titles as "recovery units," likely extends the time for de-listing. For example, if five recovery units/mini-populations are designated in the recovery plan and four out of the five meet recovery plan standards for recovery, the FWS or NMFS likely could conclude that the entire listed population should be de-listed. But, under the Services' practice, the agency would de-list only the four recovery units/mini-populations and leave ESA burdens in place for the remaining recovery unit/mini-population. And, that listing could last for a very long time because, in most instances where this practice has been followed, at least one of the recovery units/mini-populations has little chance of ever recovering.

The desert tortoise rendition of this practice is particularly interesting for another reason. After listing the Mojave population of the desert tortoise in 1990, the FWS divided that DPS into 6 smaller DPSs termed "evolutionarily significant units" in the 1994 recovery plan. FWS, *Desert Tortoise (Mojave Population) Recovery Plan*, June 1994, p. 19. NMFS formally adopted use of ESUs in its 1991 DPS policy for Pacific salmon. But, FWS has never adopted, or invited public comment on application of, such a policy for the terrestrial species within its jurisdiction. Instead, it applied

another agency's standard for certain anadromous fish to the tortoise. This isn't "population creep," its more of a "population scramble."

Congress Should Consider Amending The ESA So That It Protects Only An Imperiled Biological Species, Or To Create A Presumption Against Listings Below The True Species Level, And To Limit The Use Of Species Elsewhere In The ESA To The Form Of Species Actually Listed.

If the local government and land use interests regulated by the ESA had been fully engaged when the ESA's broad definition of "species" was adopted in 1973 and then narrowed somewhat in 1978, they likely would have preferred that the ESA simply define a "species" as a recognized biological species. That definition would eliminate the trivialization of the ESA that occurs when the Services list and create ESA compliance burdens for a subspecies or distinct population which is locally rare, even though the biological species as a whole is not imperiled. It also both would address the concerns raised by the Clinton listing moratorium and Bush budget's legislative proposal by conserving the Services' resources and would conform with the original legislative intent to: (1) protect the "genetic heritage" associated with a species' unique gene pool, while (2) realizing that "it is beyond our capability to acquire all the habitat" needed by locally-rare populations "without at the same time dismantling our own civilization." H.R. Rep. No. 93-412, at 4-5 (1973).

Indeed, in 1978, the House of Representatives voted to limit the ESA to a biological species. The full House adopted Representative Duncan's amendment defining "species" consistent with its biological usage (a group of "physically similar organisms capable of interbreeding but generally

incapable of producing fertile offspring through breeding with organisms outside this group"). 124 Cong. Rec. 38154-56 (Oct. 14, 1978). Rep. Duncan stated:

The legislation before us today is entitled, and I think this is important, "the Endangered Species Act." It is not entitled, and I think this is important,..."An Endangered Subspecies Act," or the "Endangered Variation of a Species Act." The amendment preserves, again, the integrity of the legislation while sufficiently tightening up the definitions so that we do not afford protection of this legislation, to the detriment of man, to every individual creature on the face of the Earth that might differ in one degree or another from one of its brothers.

124 Cong. Rec. 38154.[1]

However, given the history of ESA listings of subspecies and distinct population segments of vertebrate species, we suspect that removal entirely of the listing authority for either subspecies or DPSs would be a difficult undertaking. We also, as suggested by the focus of my testimony, find far more troublesome the sub-subspecies listing authority than the subspecies listing authority.

I would therefore suggest, as a possible alternative, that the ESA could be amended to: (1) require the Services to list a species or subspecies if it is biologically threatened or endangered; but (2) grant the Services the discretion to list or not list a DPS. Indeed, it may be appropriate for Congress to state a presumption, or default position, that DPSs should not be listed under the ESA, and to require the Services to provide judicially-reviewable reasoning if they decide to list such variations-of-a-species. This Committee suggested such a presumption when its report on the 1979

ESA amendments stated that:

the committee is aware of the great potential for abuse of this authority and expects FWS to use the ability to list populations sparingly and only when the biological evidence indicates that such action is warranted.

S. Rep. No. 151, 96th Cong., 1st Sess. (1979) at 6-7.

However, the ESA as written arguably requires the listing of any "species" unit (be it a biological species, a subspecies, or a distinct population segment of a vertebrate species) if that species unit is biologically endangered or threatened over a significant portion of its range. *See* 16 U.S.C. §§ 1532(6), (16) and (20), 1533(a) and (b)(1). Congress could remove these nagging questions on the Services' discretion to not list a DPS by amending the ESA to clearly make listing at the population level discretionary and to provide in the form of a statutory command its previous Committee report admonition that such listing authority be exercised only "sparingly."

Finally, I would urge the Committee to put an end to the inappropriate "population creep." The Services should be directed to determine ESA § 7 compliance, prepare ESA § 4(f) recovery plans, and conduct ESA § 4 de-listings on the basis of the same "species" units that have been listed under ESA § 4.

[1] Though the House passed Rep. Duncan's amendment, he was not named to the Conference

Committee. The conferees reinserted the "species" definition that had been reported by the House Committee.

Exhibit P

Response to the report: Evaluation of Scientific Information Regarding Preble's Meadow Jumping Mouse (prepared by the Sustainable Ecosystems Institute).

Rob Roy Ramey II*, John D. Wehausen[§], Hsiu-Ping Liu†, Clinton W. Epps[#], and Lance M. Carpenter**

* *Department of Conservation Biology, Denver Zoological Foundation, Steele St., Denver, Colorado 80205, USA*
§ University of California, White Mountain Research Station, 3000 East Line Street, Bishop, CA 93514, USA
†University of Denver, Department of Biological Sciences, 2190 E. Iliff, Denver, CO 80208, USA
 # University of California, Department of ESPM, 137 Mulford Hall, Berkeley, CA 94720-3114
** *Department of Zoology, Denver Museum of Nature & Science, 2001 Colorado Blvd., Denver, CO 80205, USA*

10 August 2006

We respectfully disagree with the Preble's review panel (report by Arbogast et al. 2006) on a number of points and find their conclusions biased and inadequately founded in science. The panel opined that the lines of evidence in Ramey et al. (2005) are insufficient to overturn *Z. h. preblei* as a subspecies and that additional data need to be gathered to "clarify" the issue. Yet no thresholds were advanced by the panel that could be used to objectively test this subspecies with additional data. It is our contention that the stance taken by the panel amounts to support for an approach that precludes falsification of virtually any ESA-listed subspecies or DPS.

Instead of reviewing all of the available science, the panel arbitrarily created its own burden of proof, which it then unilaterally applied only to Ramey et al. (2005). Rather than critically analyze the underlying data upon which *Z. h. preblei* became a subspecies and was ESA-listed (e.g. Krutzsch 1954; Riggs et al. 1997), the panel only examined the most recent papers and correspondence while ignoring much contrary information. If all available scientific information are considered, the U.S. Fish and Wildlife Service currently has sufficient scientific information to conclude that *Z. h. preblei* does not qualify as a valid subspecies or DPS.

Cranial Morphometry

We do not agree with the panel's claim that *"two of the lines of evidence presented by REA (their analyses of cranial morphometrics and ecological exchangeability) are based on insufficient data to support their suggestions for taxonomic change."* The description of *Z. h. preblei* by Krutzsch (1954) was based on far less data and small sample sizes that precluded statistical tests. As we noted in our response to Vigneiri et al. (Ramey at al.

2006): ***"At the center of this debate is the separation of Z. h. preblei as a subspecies by Krutzsch (1954) based on measurements of only 3 skulls and comparisons of only 4 skins. ... Krutzch's sample sizes precluded meaningful statistical tests, and he used none."*** The panel's opinion that our morphometric analyses are inadequate shows bias in ignoring the weak inference and small sample size used originally to describe this subspecies in 1954.

Based on their statements, the panel is willing to accept poor discriminant analysis assignments that are in many cases differ little from flips of a coin in discrimination ability (posterior probabilities of >0.50) in support of this putative subspecies. Similarly, the panel is willing to accept principal components analysis plots (an exploratory tool lacking inferential statistical capabilities that is not designed for hypothesis testing) as a means to define subspecies. The panel is unwilling to accept the fact that the only quantitative basis for Krutzsch's original description of *Z. h. preblei* is not supported by univariate statistical analysis using much larger sample sizes (n=40 per subspecies). Additionally, the panel does not acknowledge the fact that the scientist who originally described *Z. h. preblei*, Krutzsch, no longer supports this subspecies description (Ramey et al. 2006). This should settle the issue but has been ignored by the USFWS and the panel (see Ramey et al. 2006).

We do not agree with the panel that a *"thorough analysis of the original characters and specimens used by Krutzsch (1954) to describe Z. h. preblei is required"*. Why should those three original specimens provide different results? If *Z. h. preblei* is a good subspecies, then any adequate sampling of its population should show statistically meaningful differences from other subspecies. We performed such an analysis and obtained clear results to the contrary. Relative to characters we did not measure, the following points are pertinent.

1) Krutzsch did not actually measure the auditory bullae or the inflation of the frontal region; those characters were only described qualitatively, thus conclusions had no objective basis. The panel's opinion that these could somehow be "coded for systematic analysis", ignores the fact that Krutzsch never measured them in the first place. Furthermore, the panel did not provide specifics as to how this could be done, nor thresholds that could be applied to such data to test (and potentially falsify) the hypothesis of uniqueness for *Z. h. preblei.*
2) Although Krutzsch's (1954) methods indicated that he measured incisive foramina width and length, no values for those measurements were reported.
3) We measured and analyzed the same nine measurements for which Krutzsch (1954) reported actual measurement data (mean, ranges, and sample sizes in Table 5, Krutzsch 1954), and we did not measure those qualitative skull characteristics (discussed above) for which he did not report measurements.
4) After examining pelage in several hundred museum specimens with the pelage descriptions in hand (sides duller, less black-tipped hair on dorsal stripe), we concluded that these were subjective qualitative assessments that would not be repeatable. (The attached photograph gives some idea of the variation within, and overlap among, putative subspecies.) In fact, the species, *Z. princeps* and *Z. hudsonius*, cannot be reliably

distinguished on pelage alone, which was why Conner and Shenk (2003) developed their cranial morphometric test to distinguish them and noted the following : *"Furthermore, the taxa* [the species: *Z. princeps* and *Z. hudsonius*] *are ecologically and physically similar and no reliable technique exists to distinguish live specimens in the field."* In other words, *those species* are not reliably distinguishable using pelage color or pattern. Based on our examination of several hundred museum skins, *the subspecies* are even less so.

The best available science shows that morphologically, *Z. h. preblei* is not reliably distinguishable from *Z. h. campestris* and *Z. h. intermedius*.

Genetic Analyses

In their discussion of differences in mtDNA results of the two studies, the panel stated: "In this section we discuss how the mtDNA sampling, quantity and quality issues outlined above may have lead REA (Ramey et al. 2005) and KEA (King et al. 2006) to come to different conclusions regarding the taxonomic status of *Z. h. preblei.*" However, the panel failed to notice that our critical tests did not rely on any sharing of haplotypes among putative subspecies; instead, our subspecies test relied on: morphological analyses to test the original quantitative basis of *Z. h. preblei*, mtDNA reciprocal monophyly and Analysis of Molecular Variance, and the proportion and frequency of unique microsatellite alleles and pairwise F_{ST}. Our tests for distinct populations used the approach of Crandall et al. (2000) and did not rely on sharing of mtDNA haplotypes among putative subspecies.

The panel presents a neighbor-joining tree but fails to tell the reader that this is one of a number of *potential* graphical representations of evolutionary relationships among mtDNA haplotypes. Many other trees that are equally parsimonius can be found (as discussed in Ramey et al. 2005). The reliability of the branches of the single tree presented by the panel was not indicated by any bootstrap support values. As both Ramey at al. (2005) and KEA both showed, the bootstrap support for all but the two major mtDNA lineages representing *Z. h pallidus/luteus* and *Z. h. preblei/intermedius/campestris* are not reliable. Therefore, none of the phylogenetic analysis to date support *Z. h. preblei* as being reciprocally monophyletic or even close to being so. This result was confirmed by Crandall and Marshall (2006) in their analysis of KEA's data.

The panel and KEA equate *statistical significance* with *biological significance* in their evaluation of microsatellite data. This is the same pitfall that basic statistic textbooks urge students to avoid: *statistical significance* should not be blindly equated with *practical significance*. In other words, while a difference among populations might be statistically significant, the actual difference in means can be small in magnitude and with substantial overlap in range of values. KEA's data shows this pattern with extensive shared microsatellite alleles with other subspecies, especially *Z. h. campestris* and *Z. h. intermedius*. Statistical significance can also be an artifact of sampling design. As several authors have shown that when a large number of individuals are sampled for a

large number of microsatellite loci, and modern statistical tests are applied (as in KEA), it is very likely that statistically significant differences will be found among populations even with high levels of interbreeding and there will be a high level of correct assignment of individuals to populations (Cornuet et al. 1999; Pritchard et al. 2000; Cegelski et al. 2003; Baudouin et al. 2004; Waples and Gaggiotti 2006). Application of such an approach to defining subspecies and DPSs, means that population-level differences could qualify as ESA-listable units. This would lead to an unlimited number of potential ESA-listable units. It also means that these designations (and subsequent ESA listings) could be acceptable even if they were an artifact of sampling design. This was clearly pointed out in Crandall and Marshall (2006), and by Crandall and Ramey to the panel. Please refer to our discussion of statistical significance vs. biological significance in our comments on KEA submitted to the USFWS and the panel: *Is the Preble's meadow jumping mouse an evolutionarily distinct subspecies? Comments on the report by King et al. (2006)* and to the technical report by Crandall and Marshall (2006).

Additional problems with equating statistical significance in genetic results with biological significance were pointed out to the panel but were ignored. These included: 1) the fact that very recent human induced genetic bottlenecks and isolation can increase apparent genetic divergence among populations (such as *Z. h. preblei* north and south of Denver as discussed in Ramey et al. 2005 but not acknowledged by KEA, Vigneiri et al. (2006), or the panel); 2) a low level of female dispersal relative to males can result in higher levels of mtDNA divergence than nuclear markers (trapping data show that females disperse less often and over shorter distances); 3) a faster mutation rate in small mammals (due to small body size and short generation time) can result in higher levels of genetic divergence over the same period of time than in larger mammals. If genetic results are accepted without taking these factors into account, then ESA listings could be based on statistical significance that is of little or no biological significance.

The Scientific Basis for Uniqueness under the ESA

We do not agree with the panel's claim that ecological exchangeability is inadequate. The panel mistakenly reports that we used tests of ecological exchangeability upon which to base subspecies synonymy. In fact we used this as one of the tests to determine whether *Z. h. preblei* could be considered a DPS (Ramey et al. 2005). By their own logic, the panel could not find our evidence to be inadequate because *we did not use it to specifically test this subspecies in the first place.* However, as we have found in our review of the literature, in 106 years of study no one has noticed any adaptations that would preclude ecological exchangeability among the putative subspecies *Z. h. preblei* and nearby subspecies (Please see Ramey et al. 2005 and 2006 for a more extensive treatment). Relative to what the U.S. Fish and Wildlife Service is supposed to consider in making its findings on the listing of *Z. h. preblei*, the question is: "What do we know?", not: "What do we not know?" If the panel's approach is extended to other cases, it would mean that speculation about as yet undescribed or hypothetical uniqueness is adequate justification to create or maintain an ESA-listed subspecies. This amounts to proposing that science not be the basis of such decisions.

The panel noted that *"However, we also note that Z. h. preblei appears to be at a stage in its evolution in which clearly determining taxonomic rank will not be easy to do [our emphasis], and that large groups of scientists are unlikely to reach a unanimous consensus concerning its status."* It would appear from this statement that this (and other) subspecies could be listed indefinitely if different conclusions can be reached by different authors depending upon how the results are "interpreted", while more data are continually called for. The panel's statement underscores the central conceptual issue that we addressed in Ramey et al. (2005): consistent thresholds for defining conservation units below the level of species have been lacking. Until such thresholds are established, both subspecies and Distinct Vertebrate Population Segments (DPS's) will remain subjectively defined. We have consistently argued that unless reasonable thresholds are set in advance and consistently applied, these classifications will continue to be based on opinion rather than scientific hypothesis testing (please refer to Ramey et al. 2005, 2006a, and the conclusions of 2006b).

The panel goes on to recommend an extensive research program to *"further clarify this issue"*; however, the panel failed to provide any critical tests that could be used to potentially falsify *Z. h. preblei* as a taxon. We do not agree with the panel's subjective approach and contend that it will *clarify nothing* because no thresholds or critical tests are proposed. Furthermore, we find that the absence of these (and their poorly defined *burden of proof*) makes this subspecies not falsifiable and therefore, makes the whole inquiry into its taxonomic status a moot point.

The panel presented *Z. h. preblei* as a *"valid, formally recognized subspecies"*; however, it is important to recognize that there was no standard for describing subspecies in 1954, nor is there currently. Scientists and amateurs alike have been free to use whatever approach they wanted. The International Code of Zoological Nomenclature (or *The Code*) only sets forth procedural guidelines for taxonomic description and not thresholds of uniqueness, data quality, or evolutionary basis. For example, *The Code* requires a type specimen (a body or parts thereof, Timm et al. 2005) as well as publication of the taxonomic revision and its distribution to at least four libraries. *The Code* does not require that the publication of taxonomic revision be peer-reviewed. The Preble's subspecies description in Krutzsch (1954) is a half page in length. The repeated citation of it in books on mammal taxonomy is simply a restatement (or summary) of the original description (see Appendix 2). Under *The Code*, *Z. h. preblei* and *Z. h. intermedius* are officially synonymous with *Z. h. campestris* with the publication of Ramey et al. (2005).

While additional geographic and taxonomic sampling could be expanded so that *"evolutionary and biogeographic history, as well as the taxonomic status of Z. h. preblei could be evaluated more critically within this broader framework"*, we have consistently argued that this putative subspecies can be (and was) synonymized based on available scientific information. Such a genus-wide study across the entire *Zapus* genus has already been conducted by Jones (1981). That study involved morphological, ecological, and evolutionary information (including the fossil record), and examined specimens from

123 collections, totaling almost 9,900 individuals. Jones concluded that: *"There is no evidence of any population of Zapus hudsonius being sufficiently isolated or distinct to warrant subspecific status"* (pages V and 303 from Jones 1981). We find it discouraging that despite the fact that a copy of Jones (1981) was personally handed to the panel, the panel chose to make brief mention of it (to criticize it) and otherwise ignore the results and conclusions of this critical study. If the USFWS had looked more closely at this study before the listing of *Z. h. preblei* in 1998, this subspecies would never have been listed.

As we have pointed out in Ramey et al. (2006) and to the panel, while additional work could be done on the Kansas Museum of Natural History specimens in question (there are multiple explanations for differences), these mtDNA sequences can simply be excluded from analyses and the same basic result is obtained. In fact, as we presented to the panel, if all nested PCR results are excluded from analysis and KEA's mtDNA data from *Z. h. campestris* are substituted for ours, the same basic results are obtained.

The panel did not address the substantive issues raised by Ramey et al. (2006) about Vigneiri et al.'s (2006) use of *post hoc* interpretation of results, speculative approach to on ecological uniqueness, and misrepresentation of facts. Our paper, titled: *Response to Vignieri et al. (2006): Should hypothesis testing or selective post-hoc interpretation of results guide the allocation of conservation effort?* (The panel cited this as if it were an unpublished report when it was peer reviewed, accepted for publication, and "in press" before the panel convened.) For the reasons detailed in Ramey et al. (2005 and 2006) we disagree with the approach of VEA, KEA, and the panel, all of which have relied on post-hoc interpretation of results in support of their conclusions.

Conclusion

In the field of conservation biology, there is a nearly universal tendency to err on the side of protection, even when the data in support of it are questionable. The panel's conclusions suggest that they accept any geographic isolation (no matter how recent) and genetic divergence (no matter how minor) to be sufficient to defend a subspecies (no matter how weak the evidence was to describe the subspecies in the first place). As such, we find that the panel uncritically equates statistical significance with biological significance. We do not think that this serves conservation or the public's best interest when applied to local populations of very common and widespread species, like meadow jumping mice which range over *half of North America* (see map in Ramey et al. 2005).

In our experience, it is a common occurrence that ESA-related peer reviews are not held to any quantitative threshold of uniqueness that could be consistently used to test the validity a subspecies or DPS. Without the application of thresholds, peer reviews essentially become *de facto* opinion surveys, with the inherent value-laden perspectives of any opinion survey. If reviewers do not fully give consideration to all the relevant evidence, it will compromise the completeness, and therefore the outcome, of any review. If this panel's recommendation is followed and applied to other cases, it would mean that

many inadequately defined subspecies would not be potentially falsifiable. This effectively puts ESA-listed subspecies evaluations outside the realm of scientific investigation. Probably the greatest twentieth century contribution to epistemology was Popper's (1958 or whatever citation you prefer) criterion that falsifiability separates science from non-science. It is our opinion that the panel abrogated their responsibility in presenting a biased interpretation of the available information that failed to recognize the basic implications of their conclusions relative to falsifiability and the application of science to the ESA. Our analysis of their conclusions finds that they amount to advocating that listings under the ESA do not need a scientific basis.

Literature Cited

Arbogast, B. S., J. P. Dumbacher, and S. J. Steppan (2006) Evaluation of scientific information regarding Preble's meadow jumping mouse. Sustainable Ecosystems Institute, Portland, OR.

Baudouin, L., S. Pity, and J. M. Cornuet (2004) Analytical Bayesian approach for assigning individuals to populations. *Journal of Heredity* 95(3):217–224.

Cegelski, C. C., L. P. Waits, and N. J . Andeson (2003) Assessing population structure and gene flow in Montana wolverines (*Gulo gulo*) using assignment-based approaches. *Molecular Ecology* 12: 2907–2918 .

Conner, M. M., and T. M. Shenk (2003) Distinguishing *Zapus hudsonius preblei* from *Zapus princeps princeps* by using repeated cranial measurements. *Mammalogy* 84(4):1456-1463.

Cornuet, J-M. S. Piry, G. Luikart, A. Estoup and M. Solignac (1999) New Methods Employing Multilocus Genotypes to Select or Exclude Populations as Origins of Individuals. *Genetics* 153:1989–2000.

Crandall, K. A., O. R. P. Bininda-Emonds, G. M. Mace, and R. K. Wayne (2000) Considering evolutionary processes in conservation biology. *Trends in Ecology and Evolution* 15:290-295.

Crandall, K. A. and J. C. Marshall (2005) An assessment of the threatened subspecific status of the Preble's meadow jumping mouse (*Zapus hudsonius preblei*) based on current molecular datasets. Report prepared by Genoma LLC - for the state of Wyoming, Office of the Attorney General.

Jones, G. S. (1981) The systematics and biology of the genus *Zapus* (Mammalia, Rodentia, Zapodidae) unpublished Ph.D. dissertation. Indiana State University, Terre Haute.

Krutzsch, P. H. (1954) North American jumping mice (genus *Zapus*). *University of*

Kansas Publications, Museum of Natural History 4: 349-472.

Pritchard, J. K., M. Stephens and P. Donnelly (2000) Inference of Population Structure Using Multilocus Genotype Data. *Genetics* 155: 945–959.

Ramey, R.R., Liu, H. P. Epps, C. W. Carpenter, L. and Wehausen, J. D. (2005) Genetic relatedness of the Preble's meadow jumping mouse (*Zapus hudsonius preblei*) to nearby subspecies of *Z. hudsonius* as inferred from variation in cranial morphology, mitochondrial DNA, and microsatellite DNA: implications for taxonomy and conservation. *Animal Conservation.* 8:329-346.

Ramey, R. R., J. D. Wehausen, H. P. Liu, C. W. Epps, and L. Carpenter. (2006a) Response to Vignieri et a. (2006): Should hypothesis testing or selective post hoc interpretation of results guide the allocation of conservation effort? *Animal Conservation.* Published on-line July 2006.

Ramey, R .R., J. D. Wehausen, H. P. Liu, C. W. Epps, and L. Carpenter (2006b) Is the Preble's meadow jumping mouse and evolutionarily distinct subspecies? Comments on the report by King et al. (2006). Comments submitted to the U.S. Fish and Wildlife Service on 18 May 2006.

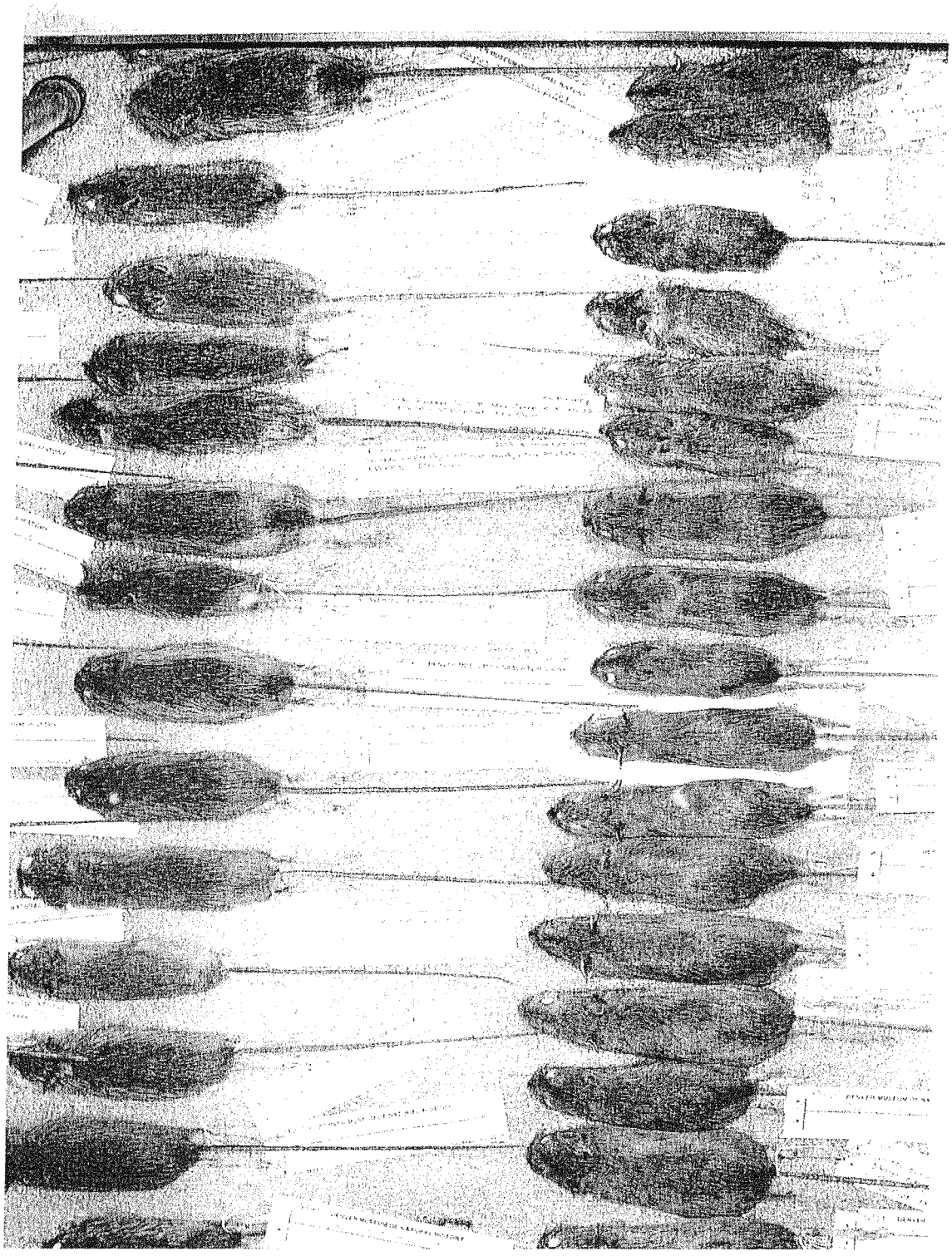
Riggs, L. A., J. M. Dempcy, and C. Orrego (1997). Evaluating distinctness and evolutionary significance of Preble's meadow jumping mouse: Phylogeography of mitochondrial DNA non-coding region variation. Final Report submitted to Colorado Division of Wildlife. 13 pp. + appendices.

Timm, R. M., R. R. Ramey, and the Nomenclature Committee of the American Society of Mammalogists. (2005) What constitutes a proper description? *Science* 309:2163-2164.

Vignieri , S. N., E. M. Hallerman, B. J. Bergstrom, D. J. Hafner, A. P. Martin, P. Devers, P. Grobler, and, N. Hitt, (2006) Mistaken view of taxonomic validity undermines conservation of an evolutionarily distinct mouse: a response to Ramey *et al. Animal Conservation*. Published on-line May 2006.

Waples, R. S., and O. Gaggiotti (2006) What is a population? An empirical evaluation of some genetic methods for identifying the number of gene pools and their degree of connectivity. *Molecular Ecology* 15:1419–1439.

Figure 1. A tray of *Z. h. preblei* specimens at the Denver Museum of Nature & Science, with two *Z. h. intermedius* specimens placed among them. (Hint: the *Z. h. intermedius* specimens are the ones on the yellow loan tags.) This photo gives some idea of the variation in size and pelage found within and among subspecies of *Z. hudsonius*.



Appendix 1. Relevant results and conclusions from Jones 1981 to the taxonomic validity of *Z. h. preblei*.

Jones 1981 examined thousands of *Zapus* specimens and traveled all over its' range in North America in the most extensive study of morphology and ecology of *Zapus* to date. Below are quotations from Jones (1981) *on Z. hudsonius* subspecific taxonomy, text in **bold** is provided for emphasis.

Abstract:
"There are two dental phenotypes of *Z. hudsonius*, one in northwestern (e.g. Alaska, British Columbia) and the other in eastern North America. This suggests an isolated population in the unglaciated portion of Alaska, in a manner similar to that theorized in the Southeast. **There is no evidence of any population of *Zapus hudsonius* being sufficiently isolated or distinct to warrant subspecific status.**" (From Page V of Jones 1981)

Background and conceptual approach:

"Both Preble (1899) and Krutzsch (1954) based their classifications on museum skins and skulls. The latter author relied heavily upon size and pelage coloration. Problems with this classification have become apparent. Utilizing Krutzsch's (1954) characters, Davis and Ernst (1971) were unable to determine whether a Minnesota population was *Z. hudsonius* or *Z. princeps*. A large number of specimens of *Z. hudsonius* collected in Tompkins Couthy, New York, by John O. Whitaker, Jr., exhibits much of the color and size variation attributed to this species throughout its range, thus challenging recognized subspecific division.

These questions, the challenge of the new systematics to consider all neontological and palentological evidence, and the need to consider the relationships of the various populations in light of the biological species concept (Whitaker, 1970) prompted this author to examine the classification of the genus *Zapus*. The present study was conducted in an attempt to develop a classification which would more acceptably reflect the relationships in the genus *Zapus*.

In pursuing this goal, specimens and other biological materials were collected during two trips through western North America, specimens in numerous museums were studied, and biological information was gathered from the literature. The biological materials and information were gathered with the intent that they might reveal relationships among the various forms and might expose primary isolating mechanisms (i.e., barriers to dispersal) or secondary isolating mechanisms (i.e., barriers to reproduction).

Specimens from 123 collections (museums, university and college collections, and personal collections), totaling almost 9,900 individuals, were studied." (From pages 2-4 of Jones 1981)

Adaptations:
"Barry (1976, 1977) described the morphology of the small and large intestines and caecum of *Z. hudsonius* and concluded that this species had evolved structurally as an omnivore." (From page 258 of Jones 1981)

"That *Zapus* is an omnivore is substantiated by the foods it consumes. Whitaker's (1963) data indicate that it is an opportunist, taking advantage of readily available foods, with a preference for seeds (i.e., when seeds are in abundance, *Z. hudsonius* is primarily a granivore)." (From page 258 of Jones 1981)

Intraspecific systematics of *Z. hudsonius*:
"Discussion of INTRASPECIFIC SYSTEMATICS: A number of subspecies of *Zapus hudsonius* have been named (Appendix G). Krutzsch (1954) recognized eleven but the present study recognizes none. No named subspecies if geographically restricted by a barrier, with the possible exception of *Z. h. preblei*. Whether or not islands such as Martha's vineyard or Prince Edward Island harbor undescribed subspecies is as yet unstudied. Only recently has a relatively large collection from Prince Edward Island been made (HHT).

"Krutzsch (1954) named *Z. hudsonius preblei* on the basis of 4 adults and 7 non-adults, stating that it averaged smaller than adjacent *Z. h. campestris* (his classification) in most cranial measurements, including least interorbital constriction, smaller auditory bullae, and narrower incisive foramina. Table 41 includes these measurements, some additional ones, and comparative measurements from other portions of the species' range (extracted from tables presented elsewhere in this paper). It is evident from the table that *Zapus hudsonius* in Colorado is generally the same size as specimens from North Dakota, Pennsylvania, New England, and British Columbia. In fact, four measurements are larger in the Colorado sample than in specimens from North Dakota which are equal to Krutzsch's *Z. h. intermedius* which he described as being smaller than *Z. h. campestris* -- length of incisive foramina, width of auditory bullae, length of upper tooth row, and breadth of M3-M3. As stated above, however, the Colorado populations appear to be isolated along with those in south-eastern Wyoming. This arid land which may isolate these populations from those in northwestern Wyoming (Lowers, 1974) and Nebraska (Berry, 1974). Armstrong (1972) suggested that *Zapus hudsonius* in this area is a relict of a previously occurring humid grassland or savanna association. **Although they are isolated, no characteristics indicate that these populations have evolved into a separate taxon."** (pages 288-289 of Jones 1981)

Evolutionary mechanisms:
"As pointed out in the discussion of speciation, *Zapus* was present and presumably isolated in what is now the south-eastern United States. With the recession of the glacier, *Zapus* moved northward, as did other species (Hadley, 1971). Prior to that range expansion, the Mississippi River and the glacier were presumable effective barriers; it was hypothesized earlier that isolation during the early Pleistocene may have resulted in speciation of *Zapus hudsonius*. But with the absence of the glacier there were and are no barriers to gene flow between populations east of the Great Plains. There is essentially

continuous distribution in the East (Fig. 31; Appendix E). The eastern subspecies which were recognized by Krutzsch (1954) occurred in successive northwest to southeast bands sharing long borders with adjacent subspecies. The Great Lakes and St. Lawrence River divided these bands in the East. An example of the status of these subspecies is Krutzsch's *Z. h. intermedius*. It crosses the Mississippi River, which assuredly would be a barrier if it were not for its northern terminus around which gene flow occurs. Further, the southwest border of *Z. h. intermedius*, which is continuous with the northeast border of Z. h. pallidus, extands from west central South Dakota to east central Missouri. *Zapus h. intermedius* was ambiguously distinguished: "All characters differentiating *Z. h. intermedius* from any contiguous subspecies are not present in every specimen, even in type series." (Krutzsch, 1954). This variation suggests a variable taxon which cannot be distinguished except by isolation. Briefly, specimens are generally lighter on the prairie than they are in the Northeast; this difference is the recognized phenomenon of populations evoking darker pelage on darker substrate, often correlated with increased rainfall. It was noted above that the Alaskan *Zapus* has the same pelage characteristics as the northern and northeastern populations...In conclusion, to distinguish subspecies where there are no barriers to gene flow and subsequently no distinct morphological differences would violate the premise that the subspecies is a genetic unit evolving towards the species." (From page 301 of Jones 1981)

Conclusions:
"There is no evidence that any population of *Zapus hudsonius* has been isolated long enough or under a set of circumstances to allow subspeciation." (From page 303 of Jones 1981)

Appendix 2: The full extent of Hall's (1981) authoritative treatment of *Z. h. preblei* in Mammals of North America.

Zapus hudsonius preblei Krutzsch

1954. *Zapus hudsonius preblei* Krutzsch, Univ. Kansas Pub., Mus. Nat. Hist., 7:452, April 21, type from Loveland, Larimer Co., Colorado.

MARGINAL RECORDS (Krutzsch, 1954:453).—Wyoming: Springhill, 12 mi. N Laramie Peak, 6300 ft.; Chugwater; *Cheyenne*. Colorado: type locality; *5 mi. E Boulder*; Semper; *3 mi. E Boulder*.

Exhibit Q

ONTARIO CANADA
Ontario's North
There's no better place in the world to do business.
FIND OUT MORE

Species inflation

Hail Linnaeus

Conservationists—and polar bears—should heed the lessons of economics

"NO SCIENCE in the world is more elevated, more necessary and more useful than economics." That was the view of Carl Linnaeus, a Swedish naturalist, born three centuries ago this week, who is better remembered for devising the system used to this day to classify living organisms.

Linnaeus sought to reveal what he saw as the divine order of the natural world so that it might be exploited for human benefit. He lived at a time when exploration and trade were bringing new specimens to the attention of European scientists. Those specimens, particularly the plants, were scrutinised as potential crops. At the turn of the 17th century there was no sense of how creatures were related to each other; descriptions and classifications were unsystematic. Linnaeus gave life to an organising hierarchy with kingdoms at the top and species at the bottom.

The system he created has proved both robust and flexible. It survived the rise of evolution. It also survived the discovery of whole categories of organism, such as bacteria, that the Swede never suspected existed. But, rather as John Maynard Keynes observed that "there is no subtler, no surer means of overturning the existing basis of society than to debauch the currency," so Linnaeus's system is being subtly debauched by over-eager taxonomists, trying to help conservation.

Go forth and multiply

As new areas are explored, the number of species naturally increases (see article). For example, the number of species of monkey, ape and lemur gradually increased until the mid-1960s, when it levelled off. In the mid-1980s, however, it started rising again. Today there are twice as many primate species as there were then. That is not because a new wave of primatologists has emerged, pith-helmeted, from the jungle with hitherto unknown specimens. It is because a lot of established subspecies have been reclassified as species.

Perhaps "reclassified" is not quite the right word. "Rebranded" might be closer. Taxonomists do not always get it right first time, of course, and what looked like one species may rightly later be seen as two. But a suspiciously large number of the new species have turned up in the limited group of big, showy animals known somewhat disparagingly as "charismatic megafauna"—in other words the species that the public, as opposed to the experts, care about.

One reason for this taxonomic inflation is that the idea of a species becoming extinct is easy to grasp, and thus easy to make laws about. Subspecies just do not carry as much political clout. The other is that upgrading subspecies into species simultaneously increases the number of rare species (by fragmenting populations) and augments the biodiversity of a piece of habitat and thus its claim for protection.

In the short term, this strategy helps conservationists by intensifying the perceived threat of extinction. In the long term, as every economist knows, inflation brings devaluation. Rarity is not merely determined by the number of individuals in a species, it is also about how unusual that species is. If there are only two species of elephant, African and Indian, losing one matters a lot. Subdivide the African population, as some taxonomists propose, and perceptions of scarcity may shift.

The trouble is that the idea of what defines a species is a lot more slippery than you might think. Since it is changes in DNA that cause species to evolve apart, looking at DNA should be a good way to divide the natural world. However, it depends which bit of DNA you look at. The standard technique says, for example, that polar bears are just brown bears that happen to be white. This is not good news for those relying on the Endangered Species Act. For a certain sort of Colorado rodent (with, alas, a nose for prime riverfront real estate) the question of whether it is "Preble's meadow jumping mouse" or a boring old meadow jumping mouse may be a matter of life or death: local property developers are on the death side. The Bahamas switched overnight from protecting their raccoons to setting up programmes to eradicate them when a look at the genetic evidence showed the animals were common Northern raccoons, not a separate species.

The 21st-century answer to this 18th-century riddle is that a species is what a taxonomist says it is. Evolution often fails to produce the clear divisions that human thought in general, and the law in particular, prefers to work with. It therefore behoves taxonomists to be honest. If they debase their currency, it will ultimately become valueless. Linnaeus the economist would have known that instinctively.

Copyright © 2007 The Economist Newspaper and The Economist Group. All rights reserved.

Exhibit R

Animal Conservation. Print ISSN 1367-9430

LETTER FROM THE EDITOR

Advocacy dressed up as scientific critique

K. A. Crandall
Editor
Department of Integrative Biology, Brigham Young University, USA

doi:10.1111/j.1469-1795.2006.00054.x

While we certainly appreciate the concerns of Martin (2006) about the quality of papers published in our journal as well as the quality and integrity of our peer-review process, we would like to reassure readers that his concerns about our review process are unfounded, and in doing so mention also that concerns about quality of analyses are ubiquitous, regardless of the methods used. Martin's (2006) first objection to the Ramey *et al.* (2005) publication is with the perceived advocacy *in the absence of peer review*. While scientific objectivity in conservation biology, in particular, is difficult (many of us become biologists *because* of our love of nature), Martin is absolutely correct that the peer-review system should, among other things, guard against blatant advocacy, especially in the face of conflicting data. Yet unlike Martin's letter, the Ramey *et al.* (2005) paper was passed through a standard peer-review process. Indeed, the first submission was rejected due to two reviewers who were unconvinced by both the analyses and the data. Both reviewers, however, recommended that the authors be encouraged to resubmit if their concerns could be addressed. As the editor assigned to this paper, I concurred with this assessment and rejected the paper with encouragement to resubmit. The Ramey team then collected the additional data (microsatellite data as a nuclear complement to their mtDNA sequence data) and performed additional analyses as suggested by the reviewers, and resubmitted their paper. This revised draft went out for a second round of full review (a more rigorous standard than in many journals). I sent this paper back to one of the original reviewers (the harshest – as is my own personal policy) as well as a 'fresh' reviewer (which is also a standard of mine to make sure that the paper still has good flow and consistency). These reviewers were more favourable to this revised draft, but still had concerns which were subsequently addressed by the authors in a final submission. As the editor, I then examined the final paper and found that the concerns of the reviewers had been addressed. While we obviously cannot disclose the identity of the reviewers, they were, contrary to Martin's speculation, experts in population genetics and conservation genetics. Thus, the peer-review process of this paper was of at least standard rigour.

Martin offers two lines of evidence of 'advocacy' by Ramey. The first is citing Ramey as someone who 'is a self-proclaimed advocate for changing the Endangered Species Act'. I would argue that most conservation biologists would be in this same camp. There are many scientific difficulties with the Act as it stands; however, opening up that for review opens up many doors for political input that could even further reduce the scientific input into such questions. Of course, as a journal, we do not solicit opinions about national conservation policy from authors before we make decisions on manuscripts. The decisions are based on peer review (as described above). Martin's second line of evidence is the following quote from Ramey *et al.* (2005): 'If defensible data are lacking and a protected organism is not distinguishable with a high degree of certainty from neighbouring, non-threatened relatives, considerable financial and logistical conservation effort may be misallocated at the expense of other endangered organisms.' In my view (as an alternative peer reviewer), this is not advocacy but straightforward common sense. Funds for endangered species are very limited. Why would you want to spend these precious resources on taxa that are originally based on weak data and do not hold up to scientific scrutiny. It seems as though Martin is advocating to ignore the science altogether whereas Ramey *et al.* (2005) clearly state 'If defensible data are lacking . . .'. Rather than making 'the task of maintaining biological diversity less expensive', Ramey seems simply to be arguing for those precious resources to be more carefully allocated to species of truly high need for conservation.

Finally, Martin critiques *his perception* of the 'peer review process orchestrated by the journal *Animal Conservation*' and rates it miserable. I am the one who 'orchestrated' the peer review along what I thought (and still think) are standard peer-review practices (as described above). In addition to our own peer review, the Ramey work has also been extensively reviewed by 14 peers via the US Forest Service and these reviews are available online (http://mountain-prairie.fws.gov/preble/). As one can quickly see from Martin's critique, advocacy is alive and well in the science of conservation biology and advocates fall out on both sides of the issues. In this, as in many other cases, conclusions are debatable, and *Animal Conservation*, like other scientific journals, is the correct place for those scientific uncertainties to be expounded and debated. In this same issue, Ramey *et al.* respond to the scientific critique of their article. While I disagree with the recommendation of retraction, certainly standard scientific practice would be to strongly support Martin's recommendation to acquire independent replication of the study for the strongest conclusion. It is through the preponderance of evidence that these legal

Animal Conservation 9 (2006) 250–251 © 2006 The Author. Journal compilation © 2006 The Zoological Society of London

issues are solved, not through some magical biological statistic.

References

Martin, A. (2006). Advocacy dressed up as science: response to Ramey *et al.* (2005). *Anim. Conserv.* **9**, 248–249.

Ramey, R.R. II, Liu, H.-P., Epps, C.W., Carpenter, L.M. & Wehausen, J.D. (2005). Genetic relatedness of the Preble's meadow jumping mouse (*Zapus hudsonius preblei*) to nearby subspecies of *Z. hudsonius* as inferred from variation in cranial morphology, mitochondrial DNA and microsatellite DNA: implications for taxonomy and conservation. *Anim. Conserv.* **8**, 329–346.

Exhibit S

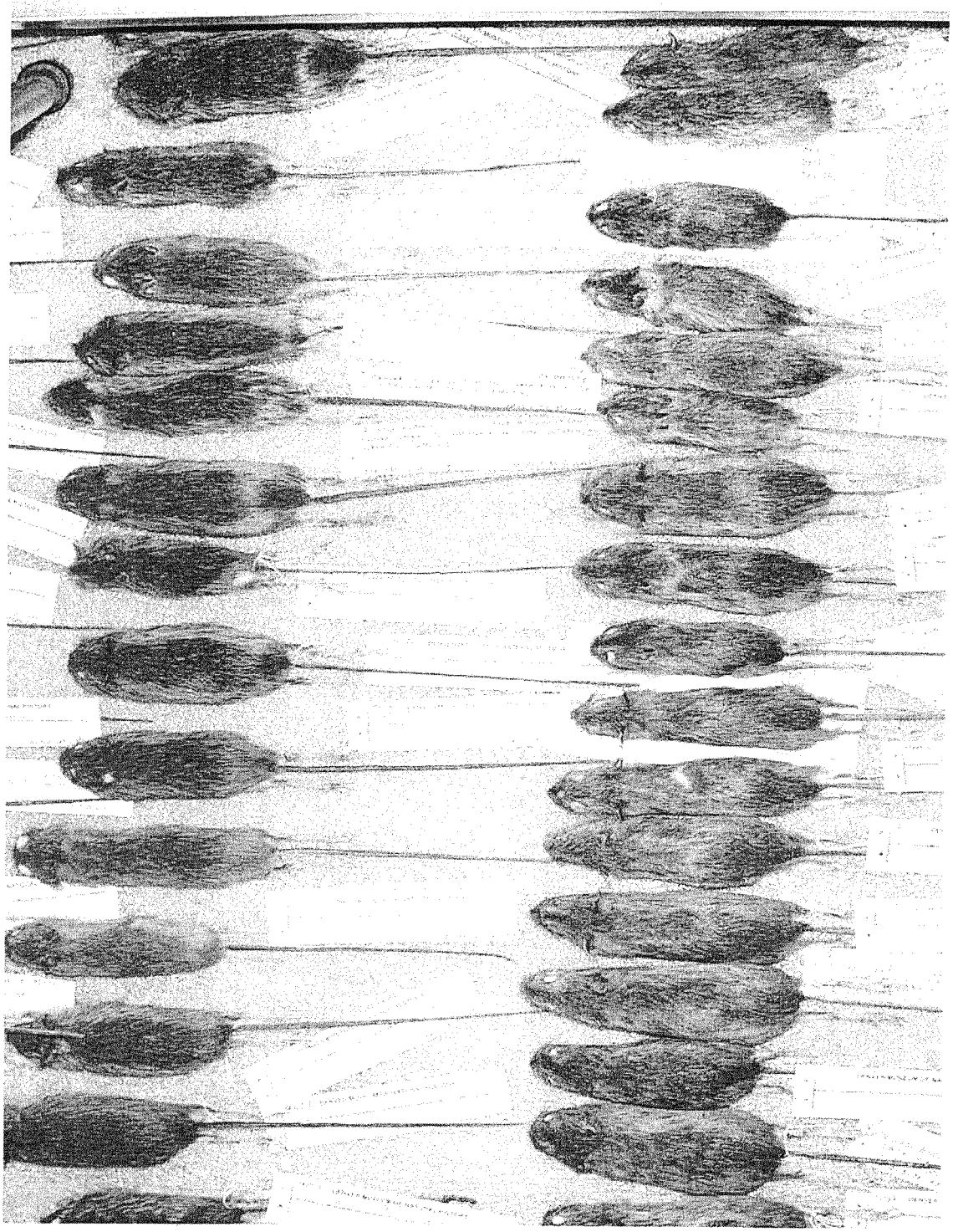


Exhibit T

DEMAND SIDE

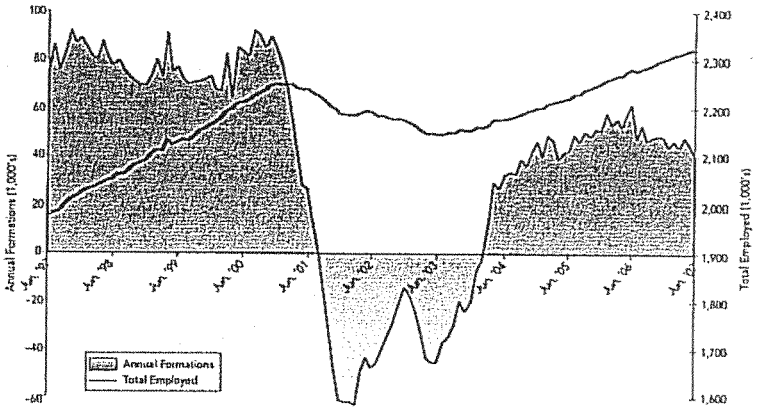
Colorado Employment 2006 vs. 2007

MARKET	Employment June '07		Employment June '06		Unemployment 6/07	6/06
Alamosa-Conejos	11,038	0.4%	11,567	0.5%	5.1%	5.8%
Canon City	19,279	0.7	19,096	0.8	4.7	5.9
Colorado Springs	303,153	11.7	300,298	11.8	4.3	4.9
Denver-Aurora-Boulder	1,608,578	62.0	1,568,422	61.6	3.8	4.8
Greeley	112,202	4.3	111,333	4.4	4.1	5.0
Durango	31,407	1.2	30,252	1.2	2.8	3.6
Edwards	33,158	1.3	31,823	1.3	3.0	4.0
Fort Collins-Loveland	167,695	6.5	164,025	6.4	3.3	4.2
Fort Morgan	13,770	0.5	14,540	0.6	3.4	4.1
Grand Junction	74,771	2.9	72,705	2.9	3.2	4.3
Montrose	20,553	0.8	20,232	0.8	3.7	4.1
Otero-Crowley	9,695	0.4	10,598	0.4	5.8	7.0
Pueblo	69,597	2.7	67,241	2.6	4.9	6.2
Silverthorne	15,675	0.6	15,163	0.6	3.0	3.7
Sterling	10,409	0.4	11,223	0.4	3.3	4.0
Colorado	2,594,827	100.0%	2,545,515	100.0%	3.8%	4.7%

Source: Colorado Department of Labor and Employment

State of Colorado Job Formation Trends

June 1998 – June 2007



Colorado continues job growth trend

Second quarter tallies show that Colorado ranked 15th in the nation in jobs formation. Our state continues to regain lost ground from just before the '02 recession — when job formation was at an all-time high — and hovers around the halfway mark in jobs formed.

Colorado also ranks inside the top 15 states for both single family and multifamily permits nationwide. Statewide, multifamily housing enjoyed a net gain of 438 units over last year, or an overall six percent growth.

State Comparison

(12-Month Annual – June 2007)

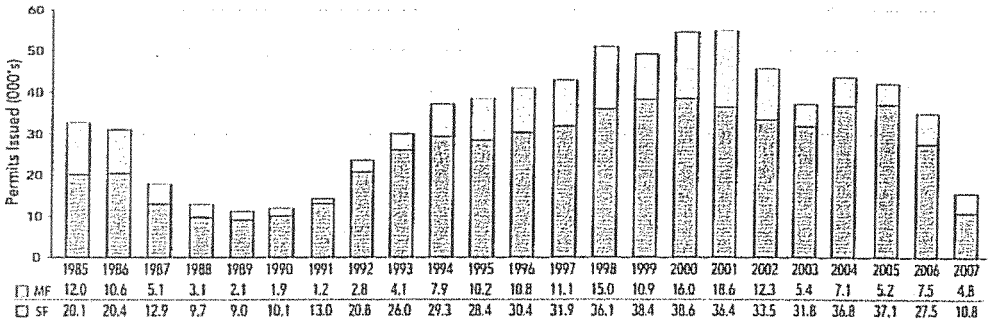
Job Formations		Job Growth	
1 Texas	213,500	1 Utah	4.5%
2 California	182,600	2 Arizona	2.9
3 Florida	112,900	3 Wyoming	2.8
4 New York	81,400	4 South Dakota	2.5
5 N. Carolina	80,500	5 Louisiana	2.5
6 Arizona	74,900	6 Kansas	2.4
7 Georgia	61,100	7 Idaho	2.4
8 Utah	55,100	8 Texas	2.1
9 Washington	51,400	9 Hawaii	2.0
10 Illinois	47,000	10 N. Carolina	2.0
11 Louisiana	46,800	11 Washington	1.8
12 Virginia	46,200	12 S. Carolina	1.7
13 Pennsylvania	46,100	13 Nevada	1.7
14 Colorado	38,668	14 North Dakota	1.7
15 Massachusetts	36,300	15 Colorado	1.7
16 Minnesota	35,200	16 Montana	1.6
17 S. Carolina	33,100	17 New Mexico	1.5
18 Kansas	32,800	18 Georgia	1.5
19 Missouri	24,500	19 Mississippi	1.4
20 Maryland	22,600	20 Florida	1.4%

Source: Metrosearch USA

SUPPLY SIDE

State of Colorado Annual Permit Trends

1995 – 2007 *(through June 2007)*



Source: SOCDS; Metrostudy

State Comparison of Permits

(12-Month Annual – June 2007)

SF Permits		MF Permits	
1 Texas	136,925	1 Texas	51,392
2 Florida	99,342	2 California	44,090
3 California	81,032	3 Florida	43,141
4 N. Carolina	75,700	4 New York	36,240
5 Georgia	68,042	5 Illinois	19,595
6 Arizona	44,364	6 Georgia	18,407
7 S. Carolina	35,111	7 Washington	16,625
8 Tennessee	33,820	8 N. Carolina	15,967
9 Virginia	32,933	9 New Jersey	12,719
10 Washington	31,742	10 Arizona	9,634
11 Illinois	30,839	11 S. Carolina	8,789
12 Pennsylvania	30,426	12 Maryland	8,386
13 Ohio	29,484	13 Colorado	7,978
14 Colorado	25,207	14 Mass.	7,571
15 Alabama	21,358	15 Missouri	7,045
16 Indiana	20,983	16 Virginia	6,972
17 Utah	20,595	17 Tennessee	6,373
18 Michigan	20,133	18 Ohio	6,214
19 Nevada	19,733	19 Alabama	5,978
20 Louisiana	19,431	20 Wisconsin	5,926

Source: Metrosearch USA

Colorado Market Comparison Annual SF vs. MF

MARKET	Annual SF	Annual +/–	Annual MF	Annual +/–
Boulder	340	-57.5%	149	-50.3%
Colorado Springs	3,288	-2.7%	359	-15.5%
Denver-Aurora	9,822	-0.6%	5,923	1,535
Fort Collins-Loveland	1,189	-9%	363	-27.8%
Grand Junction	1,387	-4.6%	92	-50%
Greeley	1,939	-0.9%	267	1
Pueblo	927	-31.6%	12	-11%
Colorado	25,207	-12.75%	7,978	438

Source: Metrosearch USA

Tom Heyden, senior market analyst with Metrostudy, compiled the data for these charts. Reach him at 720-493-2020 x5 or thayden@metrostudy.com

Exhibit U

: County Land Use Regulations

County	Land Use Regulations	Open Space Acquisitions
Adams[12]	- National Resources Conservation Overlay (NCRO) District Map includes "important wildlife areas and associated riparian areas." [3-37-02] - "A site-specific Resources Review to determine whether land is included within the NRCO District is required, prior to review of the first development application for the land." [3-37-03-02] - "Development in significant wildlife habitat is prohibited except for essential facilities." [4-10-02-04-02]	- Since 2000 Adams County Commissioners have funded 72 projects to preserve nearly 2,616 acres of open space, rivers and creeks, wildlife habitat, farmland and new park development. - The County recently purchased up to 92 acres for preservation and protection of the South Platte Wildlife Corridor.
Boulder[41]	- Development shall avoid significant natural ecosystems or environmental features, including riparian corridors. [4-800] - All land use development applications shall be required to include a wildlife impact report whenever the property is located within a Critical Wildlife Habitat or a Riparian Corridor. [7-1700] - Wildlife impact report shall include an inventory of any Species of Special County Concern, an assessment of the proposed development's impact and a recommendation regarding whether the proposal can proceed without causing material adverse impact. [7-1700, B]	- Officials in the Parks and Open Space Department have said that approximately 55 percent of the Preble's mouse habitat in Boulder County is under open space protection. - In January 2004 the Boulder County Commissioners adopted the St. Vrain Trail Master Plan. The project is intended to facilitate riparian habitat and enhancement along the St. Vrain River corridor. According to the Colorado Natural Heritage Program, the corridor contains a "fair" occurrence of Preble's meadow jumping mice.
Douglas[42]	- Rural Site Plan provides an administrative site plan process for alternative 35-acre development that considers preservation of open space,	- The Division of Open Space and Natural Resources reports nearly 45,000 acres in acquisitions of open space. Of this, approximately 11,000

[12] Adams County Development Standards and Regulations (January 10, 2005), Adams County Open Space Funded Projects: www.co.adams.co.us/services/department/open_space/funded_projects.html

[41] Boulder County Land Use Code (adopted on October 18, 1994), St. Vrain Corridor Master Plan (adopted January 2004)

[42] Douglas County Open Space Projects: www.douglas.co.us/Open%20Space/PropertyChart.htm

	rural landscapes, important wetland and riparian areas, and reduces environmental impacts. Criteria for approval consider protection and preservation of riparian areas and critical wildlife habitats. Rural site plans require permanent protection of either 50% or 67% of included land as open space by conservation easement or similar method. [Section 3-A] - Development District is designed, in part, to allow flexibility and promote layout, design, and construction of residential development that is sensitive to the natural land form and environmental conditions, such as riparian areas and wildlife habitat. [Section 15]	acres owned by Douglas County are designated for protection of natural resources and wildlife habitat among other purposes. - In 2000, Douglas County acquired North Willow Creek Ranch, a 694-acre parcel strategically located between Roxborough State Park and the Division of Wildlife's Woodhouse property. The parcel contains mixed grass prairie and riparian habitat along Little Willow Creek and provides a critical link for wildlife movement. - In July 2000 Douglas County purchased the 105-acre Snortland property adjacent to the JA Cattle Ranch which contains a portion of East Plum Creek and associated wetlands. The property provides habitat for the Preble's meadow jumping mouse. -In 2002 Douglas County purchased 475 acres on the Duncan Ranch. West Plum Creek passes through the property, providing habitat for mammals, bird and fish species and is an ideal habitat for the Preble's meadow jumping mouse.
El Paso[43]	- "It is the policy of the County that no land use be initiated which would... result in the increased destruction of wildlife habitats." [Chapter II, B] - Report shall include: 1) a narrative description of major lakes, streams, topographical features, and wildlife habitats affected by the proposal; and 2) Inspection of the proposal of such features and mitigation techniques, if necessary. [51.6]	- In 2003 the El Paso Parks, Trails and Open Space Master Plan identified Forest Lakes, Monument Branch and Black Squirrel as high priority conservation lands because of the presence of Preble's meadow jumping mice.
Jefferson[44]	-Wildlife habitat shall be preserved as required by the Board. [13.1] - Plans of the area of investigation shall assure that wildlife and vegetation factors affecting the planning, design, and construction of the subdivision are recognized, adequately interpreted and presented for use in the subdivision	- The county and various cities have acquired over 50,000 acres of open space purchased with bond and non-bond funds. In an effort to evaluate which lands should be considered for inclusion into the Master Plan, a set of values have been identified that support the Open Space mission and goals. For example, the City of

[43] El Paso County Land Development Code, El Paso Parks, Trails and Open Space Master Plan (2003)
[44] Jefferson County Land Development Code, Section 13

	development. The plans shall include the following: 1) The wildlife and vegetation habitat conditions which should be preserved or improved within the subdivision; and 2) Solutions and alternatives to preserve and/or improve the wildlife and vegetative habitat. [13.6]	Westminster plans to acquire land near Walnut Creek, in part to protect habitats for species such as the Preble's meadow jumping mouse.
Larimer[45]	- For proposed developments that will or may have an adverse impact on wildlife and wildlife habitats, an approved wildlife conservation plan is required. [8.4.5] - Criteria for adverse impact will include placement of structures in close proximity to nesting and feeding areas and species reliance on specific, unique habitat features, such as riparian areas. [8.4.6]	- In 2001 the Larimer County Commissioners adopted an Open Lands Master Plan. The plan called for the most important wildlife habitat, river corridors and wetlands to be identified and protected as open space. - With a $3.1 million contribution from GOCO, Larimer County acquired 6100 acres of the Cherokee State Wildlife Area. The project preserves an important wildlife migration corridor in northern Colorado and protects habitat for the Preble's meadow jumping mouse.

Appendix B: Municipal Land Use Regulations

City	Land Use Regulations	Open Space Acquisitions
Boulder	"The city council finds that it is necessary for the city to ensure protection for wetlands by discouraging development activities in wetlands and those activities at adjacent sites that may adversely affect wetlands. When development is permitted and the destruction of wetlands cannot be avoided, the city council finds that impacts on wetlands should be minimized and mitigation provided for unavoidable losses." [9-12-1 (c)]	The City of Boulder owns and manages more than 43,000 acres of Open Space & Mountain Parks land in and around Boulder.
Colorado Springs	"All development plans submitted for review for property wholly or partially contained within the streamside overlay zone shall be consistent with the recommendations	The Colorado Springs Department of Parks, Recreation and Cultural Services cares for approximately 1,200 acres of open space.

[45] Larimer County Land Use Code (adopted December 20, 2002), Open Space Master Plan (2001), GOCO information at www.goco.org/program/wildlife.html

	of the design manual and land suitability analysis and shall conform with the following additional review criteria: Has the project been designed to minimize impact upon wildlife habitat and the riparian ecosystem which exists on or adjacent to the site? Does the project design protect established habitat or any known populations of any threatened or endangered species or species of special concern?" [7.3.508 (B)]	
Ft. Collins	- Requires developments to avoid "natural communities or habitats" including: rivers, streams, lakes and ponds, wetlands and wet meadow, native grasslands, riparian forest, urban plains forest, riparian shrubland and foothills forest. - No fewer than 27 pages of standards related to project design, set-backs, buffer zones and other measures designed to protect wildlife, water quality, air quality, open spaces and ecological communities. Any development must also submit proof of compliance to the City with "all applicable federal wetland regulations as established in the federal Clean Water Act."[46]	As of the end of 2003, the City of Fort Collins has conserved 11,472 acres of natural areas and open space worth over $44 million.
Parker	"Development shall be undertaken in a manner that will preserve the multiple functions and quality of the Town's streams and associated riparian areas, including wetlands. More specifically, it is the intent of these regulations to designate appropriate stream buffers that will... preserve critical wildlife habitat by ensuring that new development protects and sustains significant wildlife populations." [13.10.220]	The Town of Parker currently maintains over 700 acres of open space.

[46] City of Fort Collins, Division 3.4, Environmental, Natural and Cultural Standards, at Page 70-1.

Exhibit W

19 Hands-on Restoration

William Burnham, Tom J. Cade, Alan Lieberman, J. Peter Jenny, and William R. Heinrich

The Peregrine Fund is one of the most experienced nongovernmental organizations (NGO) in hands-on restoration of endangered vertebrate species in the United States. The organization began working toward restoration of the American peregrine falcon (*Falco peregrinus anatum*) several years before the Endangered Species Act of 1973 was enacted (Cade et al. 1971; Burnham and Cade 2003; Cade 2003) and has since played an important role in the recovery of the endangered northern aplomado falcon (*Falco femoralis septentrionalis*) (Jenny 2003; Jenny et al. 2004; Montoya et al. 1997), California condor (*Gymnogyps californianus*) (Cade et al. 2004), many endemic avian Hawaiian species (Kuehler et al. 1995, 1996, 2000, 2001), and several nonnative species listed as endangered under the Endangered Species Act (ESA), in particular the Mauritius kestrel (*Falco punctatus*) and harpy eagle (*Harpia harpyja*). Most people agree abstractly with the importance of saving species from extinction. Problems arise, however, in defining what that means and how to achieve that objective. This is particularly true when legal requirements for preserving species conflict with human desires for resources.

Lessons for Species Recovery

The Peregrine Fund collaborates with state and federal agencies and other NGOs to prevent species extinction and to restore viable wild populations (Burnham 1997). Our work under the ESA serves as the basis for several lessons about what is and is not successful.

Species Restoration and the Endangered Species Act

Species cannot be successfully restored unless the reasons for the species decline are successfully mitigated. In our experience, conservation actions

under the ESA have seldom significantly ameliorated the causes of population declines. For example, the Endangered Species Act had little impact on habitat improvement for recovery of the peregrine because the insecticide DDT had been banned prior to passage of the act in 1973, and the peregrine was already protected by state and federal laws. Similarly, the California condor was protected against human persecution by the State of California and the federal Migratory Bird Treaty Act before passage of the ESA, and no actions have been taken to reduce the presence of lead, a major cause of mortality in this species (Kiff et al. 1979; Pattee et al. 1990; Cade et al. 2004). Changes in ranching and land management practices that allowed aplomado falcon restoration in Texas occurred before the falcon was listed under the ESA. And the act has yet to produce any measurable effects on the causes for declines and extinctions of endemic Hawaiian species. These examples suggest that the additional direct and indirect (habitat) protection provided under the ESA does not enhance recovery. In the case of endemic species in Hawaii, however, the act may yet have a positive effect if the right corrective actions are taken to improve habitats at biologically significant scales (removal of exotic herbivores and predators).

Most endangered species depend significantly on habitats found on private lands; some occur only on such lands (Bean and Wilcove 1997). Hawaii (225 listed species) and Texas (70 listed species) have only 16 percent and 1 percent, respectively, of federal land (Wilcove et al. 1996). The use of safe harbor agreements and nonessential experimental population status can reduce the concerns of private landowners and users of federal lands thus making it easier to work with ESA-listed species. Creating incentives for private landowners is critical in many cases to the recovery of endangered species (Brook et al. 2003).

Benefits of Species Listing

Just the threat of listing can provide benefits but also cause problems for a species. For example, peregrine falcons were taken from the wild shortly before the passage of the ESA in 1973 by individuals who knew that peregrines would be listed as endangered under the new act and thus their legal taking for any purpose would become impossible; later, these birds became the foundation for the captive population and restoration program of peregrine falcons (Burnham 2003).

Listing focuses attention on a species and can increase support for conservation actions on its behalf, as was the case with the peregrine falcon. The threat of listing has caused state wildlife departments and federal land management agencies to develop plans to address concerns and benefit species such as the greater sage-grouse (*Centrocercus urophasianus*). Even without the ESA and

listing, however, there was significant support for the conservation of the peregrine and California condor.

On the other hand, it is commonly acknowledged that the petition for listing the black-tailed prairie dog (*Cynomys ludovicianus*) resulted in large-scale poisoning of prairie dog colonies by landowners who feared intrusion on their property by the U.S. Fish and Wildlife Service (USFWS). Other examples of negative responses to listing include the Preble's meadow jumping mouse (*Zapus hudsonius preblei*) and red-cockaded woodpecker (*Picoides borealis*) (Brook et al. 2003; Pickrell 2004). Brook et al. (2003) found that listing did not enhance the prospect of survival for listed species on private property.

Once a species is listed as threatened or endangered, delisting is difficult, even when the species no longer meets listing criteria. Delisting the Arctic peregrine from threatened status, for instance, took about three and a half years from the publication of the delisting proposal to the final *Federal Register* notice. Likewise, the American peregrine falcon delisting process required four years and three months (Burnham and Cade 2003). Opposition to delisting is often motivated by a desire to continue habitat protective measures mandated by the listing of a species.

The Role of Recovery Teams

Following enactment of the Endangered Species Act, four regional recovery teams were established for the peregrine falcon. The teams were assigned the task of writing (and updating) recovery plans as well as advising the USFWS. Although there were multiple recovery teams for the peregrine, each was a manageable size and largely composed of peregrine experts. The teams advised on strategic programmatic issues as requested by the USFWS. In large part, they did the jobs requested of them, and their contributions facilitated restoration.

Another success story is the working group to recover the aplomado falcon. Although a recovery plan was written for the aplomado falcon (USFWS 1990c), a recovery team was not established. Instead, effective coordination was accomplished through regular communication among municipal, state, federal, and private cooperators, and most aplomado falcon experts are actively involved in recovery actions.

Other efforts have been less effective. For example, in California, the condor recovery team gradually evolved from a small group of experts focusing on strategic issues to a large group of stakeholders attempting to micromanage restoration actions. As a result, the effectiveness of the restoration program was diminished for a time; the situation has subsequently improved. In Hawaii, where conservation issues are nearly overwhelming and in need of quick action, recovery teams took over ten years to update and draft two recovery plan revi-

sions (ʻalala, the Hawaiian crow (*Corvus hawaiiensis*), and Hawaiian forest birds) that are only now being reviewed by the public.

Organizational structures called *working groups* were formed in many states to coordinate peregrine recovery actions (Burnham and Cade 2003). Participants were drawn from state wildlife departments, NGOs such as the Peregrine Fund, federal land agencies, and affected private landowners. Working groups are often brought together by state wildlife agencies to plan and fund recovery actions. Meetings tend to be informal, congenial gatherings, with participants frequently adjourning afterward to local bars to share a few beers.

The Players in Species Restoration Programs

For any given species restoration program, there can be many stakeholders, at several levels. Although national, and even international, cooperation is sometimes needed to implement restoration programs, successful programs generally require the input of nearby local communities, including landowners and other interested individuals. Species restoration programs require trust to succeed. Often, people do not trust governments but do trust other people. Such trust cannot be legislated; rather, it develops gradually, over time and through interactions among concerned parties.

The help of state wildlife agencies in facilitating and supporting species recovery has been key to the success of many programs, including peregrine recovery programs (Oakleaf and Craig 2003). This holds true for current efforts with the aplomado falcon in Texas, the California condor in Arizona and California, and the forest birds in Hawaii.

Successful restoration programs also require extensive participation by the private sector, along with support of local communities. In the case of the peregrine falcon, private organizations spearheaded recovery efforts, but they also collaborated closely with state wildlife and federal land management agencies where jurisdiction overlapped. Recovery programs for the aplomado falcon in Texas and the California condor in Arizona similarly involved significant participation by private organizations. In Hawaii, leaders in restoration programs within the private sector include the Zoological Society of San Diego, the Nature Conservancy, Kamehameha Schools, the Silversword Foundation, and public-private partnerships such as the Olaa-Kilauea Partnership.

The Science behind Recovery

Knowledge of an at-risk species' biology and ecology, the reasons behind its decline in population, and its primary limiting factors (e.g., winter habitat, food during breeding) are crucial (Scott, Goble, et al., this volume; Lomolino,

forthcoming). Considerable knowledge about the peregrine falcon, based on research and centuries of the species use in falconry, existed at the time of its listing. Further research documented population trends and causes of decline (Newton 2003). This information ultimately facilitated its recovery. There was also substantial additional research (often funded by federal land managing agencies) that was rarely used by the biologists actually engaged in recovery actions. Much of this work consisted of due-diligence studies that agencies believed were necessary to comply with requirements of the ESA and the National Environmental Policy Act (Act of January 1, 1970). A similar situation has developed with aplomado falcon restoration in New Mexico. Prioritizing expenditure of the limited ESA funds for information gathering is an important but often neglected task.

Programs often tout their use of "best available science" to guide species recovery, but scientists, lawyers, and other professionals are fallible and can find it difficult to separate personal opinion, bias, and agendas from pure science (Burnham and Cade 1995). Nevertheless, the need to keep science free from political alliances is critical (Brussard et al. 1994; Scott et al. 1995).

Funding and Species Recovery

Hands-on restoration programs are expensive; therefore, every effort should be made to prevent species from declining to a level requiring such action. Recovery costs increase dramatically when moving from managing a species in a functional ecosystem to conducting highly focused, hands-on species restoration (Conway 1986).

Hands-on restoration can also require long-term action. Restoration of the peregrine took about three decades, restoration of the aplomado falcon will likely require two decades, that of the California condor may extend a half century or more, and restoration of Hawaiian bird species may continue indefinitely. Obtaining sufficiently long-term funding for such projects is difficult because the private sector and the government eventually tire in their support.

Annual appropriations for threatened and endangered species conservation have never approached the limits authorized by Congress. Such funding must compete with other budgetary demands. Furthermore, increases do not necessarily mean more dollars for actual recovery actions because developing and maintaining the bureaucracy to implement the complex regulations associated with the ESA are expensive. Although public funds are critical, we believe that species recovery programs in which individuals and organizations assist financially are more likely to succeed than those that are supported only by government funding. Dollars contributed for restoration actions reflect the acceptance and commitment of the public.

Permitting and Species Recovery

ESA regulations are complex, especially in regard to permitting (Burnham and Cade 1995). Permits and the permitting process have discouraged species conservation action and hindered research and recovery action. Although the USFWS is trying to simplify the permitting process, existing regulations and other statutory protections, such as the Migratory Bird Treaty Act (Act of July 3, 1918) and the Wild Bird Conservation Act (Act of October 23, 1992), limit what can be accomplished without additional legislative changes.

Recommendations for Species Recovery

We recommend that the Endangered Species Act be restructured to emphasize incentives rather than regulations. Below, we offer specific recommendations on listing and delisting species, recovery planning and implementation, research, regulations and permitting, revisions to the Endangered Species Act, and biome conservation.

Listing and Delisting Species

The U.S. Fish and Wildlife Service has emphasized listing as a primary means of protecting imperiled species. Although we understand the importance of listing at-risk species, petitions for listing should only be accepted from established experts on the species under consideration. Delisting should also be a priority if for no other reason than to showcase proof of success. In reality, delistings are often held up for a variety of reasons. The bald eagle (*Haliaeetus leucocephalus*), for example, was originally proposed for delisting ten years ago, but action has been held up owing to concern about the adequacy of habitat protection after delisting—a misapplied application of the "precautionary principle." It would be better to transfer ESA decision making about species status, listing, and delisting to an independent panel of experts on each species, appointed by the National Research Council of the National Academy of Sciences (see Cade 1998; Greenwald et al., this volume).

Recovery Planning and Implementation

Recovery planning and implementation should be substantially restructured to clarify the roles of the various parties.

First, recovery teams should be composed of small groups of biologists (seven to nine individuals) selected on the basis of their scientific knowledge of the species in question and the threats it faces. When individuals are included

in a representative capacity, all organizations (particularly governmental organizations) will seek representation; the result will be a team that is inefficient, expensive, and difficult to manage. USFWS staff should be precluded from serving on teams, although exceptions may be made for species experts; USFWS interaction with recovery teams should be limited to facilitation of team activities and recommendations for recovery.

Second, the recovery plan should be brief, requiring only a few pages to outline the problem and make general recommendations on the recovery goals for downlisting and delisting the species. Detailed documents requiring years to write result in plans that are outdated before they are finished and thus are of limited value (Burnham and Cade 2003). Short plans also simplify revision and updating as new information becomes available.

Third, the sole task of the team should be to write the recovery plan. Recovery teams should recommend, not implement, species recovery. By allowing the recovery team to focus on planning for species recovery, they can avoid entanglement in the desires and political interests of stakeholders and thus produce a better plan.

Fourth, after the recovery plan is developed, stakeholders should meet to discuss its implementation. It is at this point that input, needs, recommendations, and involvement of stakeholders should occur. From these discussions, an implementation agreement can be developed between the USFWS, local communities, and other interested parties; recovery plans should be implemented much as habitat conservation plans are. Although complete agreement among all involved may not be possible, if stakeholders know where they stand and what is to happen, the potential for collaboration is increased. For both the California condor releases in Arizona and aplomado falcon restoration in Texas, agreements were developed for implementation of recovery actions as part of the 10(j) and safe harbor agreements.

Fifth, a working group is a useful organization to facilitate recovery if participants are limited to entities that actively contribute to the restoration effort. It functions best when led by the organizations or individuals engaged in the recovery action in cooperation with appropriate governmental agencies. Implementation should be accomplished by those best qualified in the private sector and in state and federal wildlife agencies. To the extent possible, private landowners should be included and compensated for their participation in recovery efforts.

Sixth, the role of USFWS should be limited to oversight and facilitation rather than implementation of restoration projects and programs. USFWS administrators continue the transition from ESA enforcers to recovery facilitators. Land management agencies should take the lead in recovery implementation. Finally, state governments and wildlife agencies should be given increased

responsibility for implementing recovery. In the long term, much of what the USFWS is attempting to accomplish should be transferred to qualified state agencies. Conservation of endangered species needs to become a local desire and focus.

Research and Recovery

Having the best possible information is important to guide recovery actions for species (Ruckelshaus and Darm, forthcoming), but research should not be perceived as recovery action. The primary value of research is to (1) define the reason(s) for the species' decline, (2) determine the factors limiting populations, and (3) help support and guide restoration. Recovery should include monitoring to evaluate the success or failure of restoration actions. Federal land management agencies should carefully evaluate use of ESA funding and support recovery actions first and research second.

Regulations and Permitting

Regulations related to the ESA are complex and often overlap with those of other laws and treaties (Burnham and Cade 1995); they should be reduced and simplified. A comprehensive permit (inclusive of all applicable laws) should be developed, eliminating the need for multiple permits and reports. Every effort should be made to increase the flexibility, efficiency, and effectiveness of the ESA.

Revisions to the Endangered Species Act

The ESA should be amended to provide objective definitions for "threatened" and "endangered" that incorporate specific criteria (Mace and Lande 1991) and emphasize the degree of jeopardy and urgency (Cade 1998). The "threatened" category in particular is too vague as presently defined.

The overlapping meanings and functions of "harm" and "critical habitat" need to be reexamined. The designation of critical habitat may be most useful when it is applied to special localized habitats critical to species survival, such as nest sites that limit the number of breeders (e.g., peregrine falcons) or springs that serve as the entire distribution area for a species (e.g., Bruneau Hot springsnail [*Pyrgulopsis bruneauensis*]). When it is applied to major habitat units on a wide scale encompassing millions of acres (e.g., old-growth forest for the northern spotted owl [*Strix occidentalis caurina*] or the proposed designation of major reaches of the Chihuahuan Desert in southern New Mexico for the

largely nonexistent aplomado falcon), then its use becomes questionable, even though protection of such large areas may be justified in a broader, more inclusive environmental context. Critical habitat provides little additional protection to a listed species. Moreover, designation of critical habitat lasts only as long as the species requiring it remains listed (Doremus and Pagel 2001); therefore, it is not a permanent solution to habitat protection and, in fact, can become a disincentive for species recovery. Considering the high costs involved in designating and defending critical habitat against lawsuits, the benefits appear to be problematic, even unjustified.

We recommend that the definition of "critical habitat" established by the 1978 congressional amendment be rescinded and that the definition of "take" and "harm" be suitably modified to encompass all requirements for protection of essential habitat of listed species. Where essential habitat is needed for protection on private lands, owners should be compensated through a system of purchases, leases, easements, or other economic incentives.

The act should be amended specifically to authorize safe harbor agreements. Section 10(j) should be clarified by including the "open-minded" safe harbor concept for application in a mixed land status of federal, state, tribal, and private properties.

The ESA should also include conservation and research organizations, universities, and private landowners as cooperators. Ultimately, conservation will not be accomplished by government alone. Private sector cooperation and leadership will determine the fate of many endangered species (Burnham and Cade 2003). Section 6 of the ESA should be amended to recognize this fact by authorizing funding for NGO participation.

Biome Conservation

Unless the ESA is modified or until the nation has a law focused on habitat and biome conservation, endangered species will continue to suffer from lack of private sector and landowner support. This will continue to produce conflicts over designation of "critical habitat," "take," and other punitive measures; and litigation will continue to consume dollars critically needed for recovery actions. Congress should consider passage of a new law dealing with habitat and biome conservation. A first step would be to inventory all public lands, nonprofit conservation holdings, and private land with conservation easements to determine the potential to conserve the various ecoregions and associated species. Key to this inventory would be use of gap analysis where habitats and the known and potential distribution of species are mapped (Scott et al. 1988).

Conclusion

Our experiences confirm that hands-on restoration efforts are expensive and that recovery is unlikely without a highly focused effort that has the support of the states and private sector. In those successful efforts in which we have been involved (aplomado falcon, peregrine falcon, Mauritius kestrel), the limiting biological factors were substantially reduced or eliminated over landscape scales. The ESA provided a platform for cooperation, particularly among government agencies, and added a new source of funding. Section 6 funding to states was particularly important. The section 10(j) experimental population provisions of the act have also assisted in recovery efforts both by allowing greater flexibility and by helping to build trust and support among private and state groups. Safe harbor agreements were critical to the success of aplomado recovery in Texas (Jenny 2003); we found greater support when NGO, rather than agency, personnel negotiated the agreements. Bureaucratic red tape (particularly in the wpermitting process) can be an obstacle to recovery (Burnham and Cade 2003).

We thank Frank Bond and Lloyd Kiff for review and comments and Michael Bean, Pat Burnham, and others for other assistance provided. A special thanks to Ellen Paul for researching litigation related to the Endangered Species Act.

Honorable Dirk Kempthorne
Secretary of the Interior
Department of the Interior
1849 C Street, NW
Washington, DC 20240

Exhibit X

May 6, 2007

Dear Secretary Kempthorne,

Attached is a copy of a letter I sent to Inspector General Earl Devaney responding to and correcting inaccuracies in his IG Report: File No. PI-06-0267-I. The report addresses allegations made in an anonymous letter regarding activities I undertook as Deputy Assistant Secretary of Fish and Wildlife and Parks. While much of the information included in my letter to the IG was offered to the Deputy Secretary after the report was leaked to the press, - violating my privacy rights – the offer was ignored. I, therefore, am providing you with my formal response to the allegations.

As I note in my letter to the Inspector General, at no point was I provided a formal opportunity to respond to the allegations contained in the Report. I was never formally provided an official copy of the full report, the redacted report, or the executive summary, prior to the report being leaked nor after it was leaked. In fact, I was told that Inspector General Devaney had refused to authorize my receipt of any part of the report. Eventually I was allowed to read a copy of the redacted report. I also printed out a copy of the summary report from the Center for Biological Diversity's web page. Based on the information contained in those two documents, I provided the Inspector General the attached corrections and supporting documentation. The information is intended to allow him to address the most glaring inaccuracies in a revised or corrected report. Unfortunately, even if the report is corrected, it is unlikely that the broader record will be corrected and the incorrect report will continue to circulate.

The Deputy Secretary has stated that the Interior Conduct Accountability Board provides a process for reviewing the IG report and where warranted, recommending an appropriate response. When the Accountability Board met I was not allowed to be present nor to be a part of the presentation or discussion, nor was I informed of their recommendation if there was one. The Board's existence implies that due process is available and also provides the impression that all issues were considered in an impartial manner and any action predicated on the report is valid. The reality is that I was accused and judgment was rendered, with absolutely no opportunity to defend myself or correct the record. Clearly the Board could not have come to a fair conclusion without the attached information. It is incomprehensible to me why it would be in the interest of the Interior Department or of any department to allow spurious charges against executive staff not only to remain unchallenged but also to be leaked to the press and others, apparently with the sole purpose of destroying my professional and personal reputation and providing a platform to unfairly criticize the President.

This has been a difficult experience for me. As a result of the refusal to address my responses and documentation, my professional and personal reputation and behavior have been publicly misrepresented. The inaccuracies included in the report have been widely reported, all over the world. My professional reputation and integrity is being impugned in the press as a result of the Department's cavalier response to the allegations in the report and continued leaks to the press. I have no other option than to provide you with a formal rebuttal, in order to correct the record.

Sincerely,

Julie MacDonald

Honorable Dirk Kempthorne
Secretary of the Interior
Department of the Interior
1849 C Street, NW
Washington, DC 20240

May 6, 2007

Dear Secretary Kempthorne,

Attached is a copy of a letter I sent to Inspector General Earl Devaney responding to and correcting inaccuracies in his IG Report: File No. PI-06-0267-I. The report addresses allegations made in an anonymous letter regarding activities I undertook as Deputy Assistant Secretary of Fish and Wildlife and Parks. While much of the information included in my letter to the IG was offered to the Deputy Secretary after the report was leaked to the press, - violating my privacy rights – the offer was ignored. I, therefore, am providing you with my formal response to the allegations.

As I note in my letter to the Inspector General, at no point was I provided a formal opportunity to respond to the allegations contained in the Report. I was never formally provided an official copy of the full report, the redacted report, or the executive summary, prior to the report being leaked nor after it was leaked. In fact, I was told that Inspector General Devaney had refused to authorize my receipt of any part of the report. Eventually I was allowed to read a copy of the redacted report. I also printed out a copy of the summary report from the Center for Biological Diversity's web page. Based on the information contained in those two documents, I provided the Inspector General the attached corrections and supporting documentation. The information is intended to allow him to address the most glaring inaccuracies in a revised or corrected report. Unfortunately, even if the report is corrected, it is unlikely that the broader record will be corrected and the incorrect report will continue to circulate.

The Deputy Secretary has stated that the Interior Conduct Accountability Board provides a process for reviewing the IG report and where warranted, recommending an appropriate response. When the Accountability Board met I was not allowed to be present nor to be a part of the presentation or discussion, nor was I informed of their recommendation if there was one. The Board's existence implies that due process is available and also provides the impression that all issues were considered in an impartial manner and any action predicated on the report is valid. The reality is that I was accused and judgment was rendered, with absolutely no opportunity to defend myself or correct the record. Clearly the Board could not have come to a fair conclusion without the attached information. It is incomprehensible to me why it would be in the interest of the Interior Department or of any department to allow spurious charges against executive staff not only to remain unchallenged but also to be leaked to the press and others, apparently with the sole purpose of destroying my professional and personal reputation and providing a platform to unfairly criticize the President.

This has been a difficult experience for me. As a result of the refusal to address my responses and documentation, my professional and personal reputation and behavior have been publicly misrepresented. The inaccuracies included in the report have been widely reported, all over the world. My professional reputation and integrity is being impugned in the press as a result of the Department's cavalier response to the allegations in the report and continued leaks to the press. I have no other option than to provide you with a formal rebuttal, in order to correct the record.

Sincerely,

Julie MacDonald

Earl Devaney
Inspector General
Department of the Interior
Office of the Inspector General
1849 C Street, NW
Washington, DC 20240

May 6, 2007

Dear Mr. Devaney,

I write to correct inaccurate information included in the contents of your IG Report: File No. PI-06-0267-I, initiated on the basis of an anonymous letter.

Your investigators were provided incomplete and inaccurate information during the investigative process for this report. The report was released to the press and others before I was able to identify and correct this inaccurate and incomplete information. While the contents of your report can be no better than the information provided by those interviewed, your office can take steps to ensure that the information is as complete as possible. For whatever reason, that process did not occur in my case. Accordingly, I am providing additional information, including documentation that addresses many of the specific allegations made regarding my activities as Deputy Assistant Secretary for Fish and Wildlife and Parks.

A brief synopsis of the allegations and my response to each of them follows. The referenced attachments address the allegations in detail.

- ALLEGATION: Changed the range of the southwest willow flycatcher critical habitat to avoid affecting the family ranch. FACT: As your investigators were aware, my family does not own a ranch, we have 80 acres of row crop land with an agricultural business on site. The land is nearly 300 miles north of any of the proposed or final flycatcher habitat. **Attachment 1**
- ALLEGATION: Unilaterally changed the science related to the range of the southwest willow flycatcher. FACT: The change was discussed with Director Williams and Regional Director Hall who did not object. **Attachment 1**
- ALLEGATION: Paid particular attention to all California activities because the family ranch was in California. FACT: Reviewed and was responsible for surnaming all ESA regulatory packages prior to their submittal for the Assistant Secretary's signature. **Attachment 2**
- ALLEGATION: Tried to force a change in flows for the Kootenai sturgeon, abandoned the effort when Director Hall required documentation. FACT: For 3 months requested clarification of confusing and internally inconsistent language in the rule. Did not require

any particular flow, rather required that the regulation be internally consistent and supported by the research cited. **Attachment 3**

- ALLEGATION: Placed unreasonable demands on staff by requesting 4,000 documents. FACT: Requested the citations of scientific publications supporting the rulemakings as well as public comment letters. These requests are not unusual, the Administrative Procedures Act requires that the Service provide access to such documents. **Attachment 4**
- ALLEGATION: Released non-public information in violation of departmental rules. FACT: Released information which I had the authority to release. **Attachment 6**

Had you verified the accuracy of the accusations prior to preparation and release of the report I could have provided this documentation. To date your office has denied me a full copy of the report and as a result I can provide specific evidence of inaccuracy based only on information included in news accounts and other sources. The other more personal observations of the staff interviewed are subjective in nature and generally not possible to refute factually. However **Attachment 7** addresses a number of specific statements contained in the report. I note that none of the personal observations implicates waste, fraud, or abuse.

The incomplete research and fact checking coupled with the leaks to the press and others has resulted in my reputation and behavior being widely misrepresented nationally and internationally. Your office represents a position of trust and wields tremendous power to address wrongdoing within the government. However, that power carelessly or maliciously misused has the ability to visit harm on innocent individuals. I was not allowed to read, rebut nor correct anything in the report until it was released. As a result, information which you should have known was inaccurate is now in the public domain, and practically speaking I have very little ability to undo the harm your report has done. This letter is one avenue I have taken. In the future however, I strongly recommend that in order to avoid repeating such injustice, including violating the privacy rights of the person being investigated, you address specific allegations with the accused, and confirm factual information before releasing your reports.

Yours truly,

Julie MacDonald

ATTACHMENT 1 – Southwest Willow Flycatcher

The report implies that DAS MacDonald changed a critical habitat designation to benefit her family's property holdings.

1. **Family property is over 200 miles north of flycatcher habitat**. The distance from the location of the family property to the largest city in the northernmost flycatcher habitat is roughly 300 miles. **(directions attached)**
2. **Range was changed from 25 to 18 miles, the high end of the normal range, not from 2.1 to 1.8 as stated by the Director**. The initial draft proposed to base the designation on the farthest recorded range of 25 miles. I discussed this with Director Williams because of a concern that it would be difficult to defend a range based on the exception rather than the usual range identified in the research.

> Excerpt from the proposed rule:'... *Most recorded between-year movements occurred within the same drainage from 1.6 to 29 km (1 and 18 mi), but movements as far as 40 km (25 mi) were recorded (Luff et al. 2000; Kenwood and Paxton 2001; E. Paxton, USGS, e-mail).*'

As a result, 18 miles was used because it represented the high range of where most of the movements occurred. This was after a discussion with FWS Director Steve Williams regarding the radius as well as a more thorough discussion of the fact that many many miles of critical habitat were included, based on these overlapping radii. I asked then Director Williams biologically speaking how one could develop a decision rule that properly identified habitat without also including non-habitat areas such as towns and cities (which was the case for the draft rule). Director Williams indicated that generally, overlapping ranges would not be used for species like the flycatcher, but that instead, the data would be examined to find where densest concentrations of sightings were found and those areas designated. I relayed that information to the AD for Endangered Species and also the field.

3. Regional Director Hall was consulted and did not object (**email attached**). Had RD Hall objected he could have raised those objections to Director Williams. Had Director Williams objected he would have discussed the issue with Judge Manson. At no time did I have the authority to overrule a Regional Director or the Director of the FWS. If and when resolution on an issue could not be resolved, it was elevated. Then, as Supervisor of the Director of the FWS, the Assistant Secretary was the final arbiter.

1. Distance from family property to SouthWest Willow Flycatcher northernmost critical habitat.

Dixon, CA

Drive: 299 mi (about 4 hours 43 mins)

1. Head **east** on **E a St** toward **S 7th St**	0.3 mi
2. Continue on **Dixon Ave E**	0.4 mi
3. Turn **left** at **Pedrick Rd**	2.9 mi 6 mins
4. Turn **left** to merge onto **I-80 E** toward **Sacramento**	14.6 mi 15 mins
5. Continue on **Capital City Fwy/I-80-BR E/US-50 E** (signs for **US-50/ Capital City Fwy/I-80-BR/Sacramento/S Lake Tahoe**)	5.2 mi 5 mins
6. Take the exit onto **CA-99 S** toward **Fresno**	274 mi 4 hours 11 mins
7. Take the **California Ave** exit toward **Civic Center**	0.2 mi
8. Turn **left** at **California Ave**	1.4 mi 4 mins
9. Turn **right** at **Chester Ave**	0.1 mi 1 min

Bakersfield, CA

These directions are for planning purposes only. You may find that construction projects, traffic, or other events may cause road conditions to differ from the map results.

Map data ©2007 NAVTEQ™

2. Notification of Director Hall of the use of 18 mile diameter circles to designation

Julie
MacDonald/ASFW/OS/DOI
11/05/2004 03:31 PM

To Dale Hall
cc
bcc
Subject southwest willow flycatcher

I need to talk to you about this critical habitat designation. I'd like to have a quick 60,000 foot discussion, and then can talk more specifically to your staff, but I want to run a few things by you first.

1. Extension of comment period deadline,
2. Lambreth's ruling on piping plover and the resulting effects on our designations
    a. occupied at the time of listing,
    b. PCEs must be present at the time of designation
3. Invasive species (tamarisk and russian olive)
4. The use of 18 mile diameter circles to designate,
5. Designation of unoccupied habitat.

thank you!

ATTACHMENT 2

Attachment 2

The Assistant Secretary for Fish and Wildlife and Parks has the statutory duty of supervising the Director of the Fish and Wildlife Service. Part of the supervisory duties undertaken by the Assistant Secretary's office is the review of rulemaking packages. Those rules are reviewed for legal sufficiency, scientific accuracy, policy consistency, and readability. DAS MacDonald was the primary reviewer of ESA rulemaking packages prior to the Assistant Secretary approving them for release.

1. A review by the investigators of the surnames on all the ESA regulatory packages would demonstrate that my surname is on the vast majority of the ESA regulatory packages.
2. A review of the administrative record for each of the ESA regulatory packages would demonstrate that I was meticulous in my review of all packages, not just California packages.

ATTACHMENT 3 – KOOTENAI STURGEON FLOW REQUIREMENTS

The report implies that DAS MacDonald tried to impose flow requirements that would benefit operators of a Corps of Engineers dam. The email threads below demonstrate that DAS MacDonald did not require a particular flow, but rather required that the flow required by the Service be supported by the research available. There is ample evidence attached to demonstrate that DAS MacDonald was not trying to force a particular flow, but was trying to ensure that the Service accurately conveyed the facts in the rulemaking.

Julie MacDonald/ASFW/OS/DOI

11/30/2005 11:23 AM

To Renne Lohoefener/R2/FWS/DOI@FWS

cc Michelle McBryde/ASFW/OS/DOI@DOI, Craig Manson/ASFW/OS/DOI@DOI

bcc

Subject kootnai Sturgeon

I am very concerned about this proposed rule. After reading the decision, I believe that there are significant issues that must be addressed. Michelle will be setting up a meeting with David Verhay, yourself, me, Bob Comer from the Denver Solicitor's office to discuss the issues. In addition, if you could please set up a meeting with your staff here and/or in Denver to discuss the issues raised with the rule as soon as possible I would very much appreciate it.

thank you

Renne Lohoefener/ARL/R9/FWS/DOI@FWS

12/06/2005 02:14 PM

To Julie MacDonald/ASFW/OS/DOI@DOI

cc Elizabeth Stevens/ARL/R9/FWS/DOI@FWS, Chris Nolin/ARL/R9/FWS/DOI@FWS, Rick Sayers/ARL/R9/FWS/DOI@FWS

bcc

Subject Kootenei sturgeon CH

revised proposal attached

Biology wise, it is unknown why the sturgeon doesn't spawn in the stretch of river below the dam - substrate may be wrong, water may be too fast, water may be too cold.

They try to spawn in the stretch currently critical habitat but not well.

They currently do not spawn in the proposed ch, but we think they should.

Ren

Sturgeon CH proposed 12_6_05.doc

Renne Lohoefener/ARL/R9/FWS/DOI@FWS

12/28/2005 03:31 PM

To Julie MacDonald/ASFW/OS/DOI@DOI

cc Chris Nolin/ARL/R9/FWS/DOI@FWS

bcc

Subject Kootanei sturgeon critical habitat

Julia

Revised version based on the comments you gave me over the telephone is attached. Your comments were incorporated unless noted. We will need to get this one moving upon your return as time is quickly running out.

Thanks

Ren

Sturgeon CH proposed 12_28_05.doc

Julie
MacDonald/ASFW/OS/DOI
12/29/2005 05:44 PM

To Renne Lohoefener/ARL/R9/FWS/DOI@FWS
cc
bcc
Subject Re: Kootanei sturgeon critical habitat

Renne, we need to discuss my edits. Can we get Ben Jessup or whoever he thinks necessary in the discussion? Thank you.

kootnai12.29.doc
Renne Lohoefener@FWS

Renne
Lohoefener@FWS
12/28/2005 03:31 PM

To: Julie MacDonald/ASFW/OS/DOI@DOI
cc: Chris Nolin/ARL/R9/FWS/DOI@FWS
Subject: Kootanei sturgeon critical habitat

Julia

Revised version based on the comments you gave me over the telephone is attached. Your comments were incorporated unless noted. We will need to get this one moving upon your return as time is quickly running out.

Thanks

Ren

Sturgeon CH proposed 12_28_05.doc

Dale Hall/ARL/R9/FWS/DOI@FWS
01/05/2006 05:04 PM

To Julie MacDonald/ASFW/OS/DOI@DOI
cc Elizabeth Stevens/ARL/R9/FWS/DOI@FWS, Renne Lohoefener/R2/FWS/DOI@FWS, Theresa Rabot/RO/R1/FWS/DOI@FWS
bcc
Subject Re:

Thanks Julie. Everyone please look this over to see if it depicts and clarifies, for example, the PCE of flows that meet biological information we have, while recognizing that other PCEs may not be present (nor subject to AM/Destruction consideration). Julie and I talked specifically about recognizing that cobble/rock may be burried under sediment and the depth may not be there, but still want to accurately look at flows. The considerations may vary among PCEs as existing conditions at various places under consideration. What we're trying to nail down is which PCEs occur where and which ones don't.

Dale

Julie MacDonald/ASFW/OS/DOI@DOI

Julie MacDonald/ASFW/OS/DOI@DOI
01/05/2006 03:30 PM

To Theresa Rabot/RO/R1/FWS/DOI@FWS
cc Renne Lohoefener/R2/FWS/DOI@FWS, Elizabeth Stevens/ARL/R9/FWS/DOI@FWS, Dale Hall
Subject

All, attached is a copy of the Kootenai rule with some specific edits for discussion purposes only. I took another stab at trying to articulate our point. I am not sure I have the biology right, so biologists please correct me if it's wrong.

discussion Sturgeon CH proposed 12_28_05.doc

Julie MacDonald/ASFW/OS/DOI
01/06/2006 06:58 PM

To Renne Lohoefener/ARL/R9/FWS/DOI@FWS
cc
bcc
Subject Re: Fw: K. Sturgeon PCE comparison

Thank you Renne. Following is an excerpt from the 12/29 version of the draft critical habitat document:

Paragamian et al. (2001) observed mean water column velocities between RM 141.6 and 149.4 (RKM 228 and 240.5) during spawning events and in 2002, Paragamian et al. (2002) hypothesized that spawning sturgeon may select sites further upstream with greater water velocities as depth increases due to the backwater from Kootenai Lake. Parsley and Beckman (1994) suggested, based on information from four lower Columbia River sites were white sturgeon successfully reproduce, that optimal spawning habitat may occur when mean water column velocity is 5.9 ft/s (1.8 m/s) or greater. Based on these studies it appears that white sturgeon use velocity as a cue for spawning.

I note that the peak average velocities shown in the Berenbrock work are a little less than 3fps, which is significantly below that which triggers spawning. This is consistent with the information I received on the braided reach. If indeed the sturgeon are looking for faster flows, areas farther from the backwater of the lake will certainly have more rapid flows. However, it is difficult to understand how we can find that a mean velocity which is roughly half the optimal velocity is appropriate for triggering spawning.

Perhaps I'm missing something.

Renne Lohoefener@FWS

Renne Lohoefener@FWS
01/06/2006 03:57 PM

To: Julie MacDonald/ASFW/OS/DOI@DOI
cc:
Subject: Fw: K. Sturgeon PCE comparison

----- Forwarded by Renne Lohoefener/ARL/R9/FWS/DOI on 01/06/2006 03:55 PM -----

Theresa Rabot/RO/R1/FWS/DOI
01/06/2006 03:52 PM

To Renne Lohoefener/ARL/R9/FWS/DOI@FWS, elizabeth_stevens@fws.gov, Dale Hall/ARL/R9/FWS/DOI@FWS
cc patricia_kennedy@fws.gov
Subject Fw: K. Sturgeon PCE comparison

for the 1:00

---- Forwarded by Theresa Rabot/RO/R1/FWS/DOI on 01/06/2006 12:57 PM ----

Susan

Julie MacDonald/ASFW/OS/DOI
01/20/2006 06:12 PM

To Renne Lohoefener/R2/FWS/DOI@FWS
cc
bcc
Subject kootnai comments

Renne, my rewrite. This may be wrong, but I could not make heads nor tails out of the document I got. So I tried to string together various portions of the draft into a different order and have it make sense.

kootenai comments 1-20-06.doc

Julie MacDonald/ASFW/OS/DOI
01/23/2006 09:54 AM

To Renne Lohoefener/R2/FWS/DOI@FWS
cc
bcc
Subject kootenai

Could I please get copies of the cites from the rule. Generally they can either email them, if they are not voluminous or fedex a cd containing them. And they might as well send any substantive comment letters from the first rule when they send the cites.

Thank you.

Julie
MacDonald/ASFW/OS/DOI
01/30/2006 02:43 AM

To Renne Lohoefener/ARL/R9/FWS/DOI@FWS
cc Dale Hall/ARL/R9/FWS/DOI@FWS
bcc
Subject Fw: Re: Fw: K. Sturgeon PCE comparison

I reviewed the literature send by Scott several weeks ago as cited in the rule. Parsley and Beckman (1994) which was the basis for the original requirement of 5.9f/s velocity, is a very detailed study tracking the response of white sturgeon to varying flow/depth parameters downstream from dams. The work is quite persuasive.

Unfortunately, the newest cite that the field provided (Schaffter 1997) was not included in the original cites that were forwarded in Scott's email. All I have to review is the one page reference faxed late Friday. The page forwarded includes the abstract which references finding eggs at substrate sites where flow velocities exceeded 1 m/s (or 3.26 fps (my conversion)). Apparently, the field staff now prefers the results of this study to the Parsley and Beckman study which has been part of their rule from the beginning.

Until I have a complete copy of the study, I can't make any judgement as to its validity compared to the Parsley work. Accordingly, could you please ask the staff to email me copies of the entire Schaffter work, as well as any other work upon which they now wish to rely, but which was not previously provided.

I'll review either Monday night when I get in or Tuesday morning and call you.

I must note that it would have been preferable for the staff to do this level of review of their work and findings prior to the last week before the rule was due; Ben Jessup and I had this conversation regarding the discrepancy between the 5.9fps figure in the rule and the actual peak flows of 2.6 fps with the staff quite explicitly nearly a month ago.

Thank you very much.
----Forwarded by Julie MacDonald/ASFW/OS/DOI on 01/30/2006 02:20AM ----

To: Renne Lohoefener/ARL/R9/FWS/DOI@FWS
From: Julie MacDonald/ASFW/OS/DOI
Date: 01/06/2006 06:58PM
Subject: Re: Fw: K. Sturgeon PCE comparison

Thank you Renne. Following is an excerpt from the 12/29 version of the draft critical habitat document:

Paragamian et al. (2001) observed mean water column velocities between RM 141.6 and 149.4 (RKM 228 and 240.5) during spawning events and in 2002, Paragamian et al. (2002) hypothesized that spawning sturgeon may select sites further upstream with greater water velocities as depth increases due to the backwater from Kootenai Lake. Parsley and Beckman (1994) suggested, based on information from four lower Columbia River sites were white sturgeon successfully reproduce, that optimal spawning habitat may occur when mean water column velocity is 5.9 ft/s (1.8 m/s) or greater. Based on these studies it appears that white sturgeon use velocity as a cue for spawning.

I note that the peak average velocities shown in the Berenbrock work are a little less than 3fps, which is significantly below that which triggers spawning. This is consistent with the information I received on the braided reach. If indeed the sturgeon are looking for faster flows, areas farther from the backwater of the lake will certainly have more rapid flows. However, it is difficult to understand how we can find that a

Julie MacDonald/ASFW/OS/DOI
01/31/2006 08:13 PM

To Matt J Hogan/ARL/R9/FWS/DOI@FWS
cc
bcc
Subject Fw: Kootenai Sturgeon Revised Draft Rule

Here is the string where I gave them my comments and requested we have a meeting to walk through the changes and what/why... I received absolutely no response from the FWS... not Renne, not Dale, and not Kurt. I was quite clear in my original email I had concerns about my edits and asked to get us all on the phone to discuss. Instead, you receive a rule with none of the issues resolved, about 6 hours before you have to take it to the register. I might note that this brinksmanship puts you, as Acting Assistant Secretary at risk of contempt, not me and not Dale.

---- Forwarded by Julie MacDonald/ASFW/OS/DOI on 01/31/2006 08:09 PM ----

Julie MacDonald/ASFW/OS/DOI
01/27/2006 10:55 AM

To Kurt Johnson/ARL/R9/FWS/DOI@FWS, Renne Lohoefener
cc Dale Hall, Marshall Jones/ARL/R9/FWS/DOI@FWS
Subject Re: Kootenai Sturgeon Revised Draft Rule

Where is the draft and when is the meeting going to be scheduled? This rule is due to the register next Wednesday, there was supposed to be a meeting set up yesterday and there was no meeting set up for yesterday or today. With the amount of difficulty we have had drafting this in a manner that is factually accurate, we need to see a new draft ASAP and have this meeting.

thank you.
Kurt Johnson/ARL/R9/FWS/DOI@FWS

Kurt Johnson/ARL/R9/FWS/DOI@FWS
01/25/2006 01:59 PM

To Julie MacDonald/ASFW/OS/DOI@DOI
cc
Subject Re: Kootenai Sturgeon Revised Draft Rule

Will do. Here is the draft EA that we discussed yesterday.

Kurt Johnson

Kootenai DEA 01-13-06.doc

Julie MacDonald/ASFW/OS/DOI@DOI

Julie MacDonald/ASFW/OS/DOI@DOI
01/25/2006 01:49 PM

To Kurt Johnson/ARL/R9/FWS/DOI@FWS
cc Chris Nolin/ARL/R9/FWS/DOI@FWS, John Fay/ARL/R9/FWS/DOI@FWS, Renne

Julie MacDonald/ASFW/OS/DOI
01/31/2006 01:46 PM

To Matt J Hogan/ARL/R9/FWS/DOI@FWS
cc
bcc David Bernhardt/SIO/OS/DOI@DOI; Brian Waidmann/SIO/OS/DOI@DOI
Subject kootenai sturgeon.

The rule is due today.

I sent this over the weekend, as I was worried about the rule and the basis for the designation. We are required to designate based on the best available data, and also based on the PCEs that are present.

In all the previous draft rules for this designation presented by the Service the 5.9fps number was cited as the basis for their designation and the requirement for spawning. Unfortunately, that PCE does not occur in the braided reach (which is the area the court directed them to designate). This has been an issue for over a month. The FWS staff in Region 1 presented modeling done by the USGS demonstrating that peak flows (occuring once or twice a decade) occur, but the peak velocity is 2.6-2.7 fps. Suddenly, in this new rule, they have cited new (1997) research (which was not, and has not been provided) and wish to base their new 3.3fps PCE on this data. The cite, which is referenced in the thread attached was never provided to this office. The FWS did not even give me the courtesy of a reply to the attached email, but I understand moved ahead with the rule unilaterally and presented it to you for signature today.

The new velocity number, 3.3 fps, is not achieved in the newly designated braided reach, since the maximum achieved is 2.6-2.7 fps, which is about 20% less than the minimum required. The modeling predicts the number is not reached and the model prediction is supported empirically by the fact that the sturgeon occur in that reach, but don't spawn.

The research cited refers to sturgeon in the Sacramento River, not sturgeon in the pacific nw which the earlier cite relied on for their 3.9 fps recommendation. The FWS has not demonstrated they are using the best availabe data and they are deliberately not providing the last study which they rely upon to support their new PCE. I have absolutely no basis for believing this 3.3 fps number is valid or that the PCE occurs in the reach.

Finally, I have not received a draft of the rule since my last comments were submitted on the 25th. It may be correct and it may not be, the but FWS is not providing the basis for their decision, nor are they responding to my requests.

I cannot recommend signature of this rule and I strongly recommend that we resolve these process issues with Dale as soon as possible. If my review is redundant, then I would prefer to not waste time on it.

---- Forwarded by Julie MacDonald/ASFW/OS/DOI on 01/31/2006 01:24 PM ----

Julie MacDonald/ASFW/OS/DOI
01/30/2006 02:43 AM

To Ronne Lohoefener/ARL/R9/FWS/DOI@FWS
cc Dale Hall/ARL/R9/FWS/DOI@FWS
Subject Fw: Re: Fw: K. Sturgeon PCE comparison

I reviewed the literature send by Scott several weeks ago as cited in the rule. Parsley and Beckman (1994) which was the basis for the original requirement of 5.9f/s velocity, is a very detailed study tracking the response of white sturgeon to varying flow/depth parameters downstream from dams. The work is quite persuasive.

Unfortunately, the newest cite that the field provided (Schaffter 1997) was not included in the original cites that were forwarded in Scott's email. All I have to review is the one page reference faxed late Friday. The page forwarded includes the abstract which references finding eggs at substrate sites where flow velocities exceeded 1 m/s (or 3.26 fps (my conversion)). Apparently, the field staff now prefers the results of this study to the Parsley and Beckman study which has been part of their rule from the beginning.

Until I have a complete copy of the study, I can't make any judgement as to its validity compared to the Parsley work. Accordingly, could you please ask the staff to email me copies of the entire Schaffter work, as well as any other work upon which they now wish to rely, but which was not previously provided.

I'll review either Monday night when I get in or Tuesday morning and call you.

I must note that it would have been preferable for the staff to do this level of review of their work and findings prior to the last week before the rule was due; Ben Jessup and I had this conversation regarding the discrepancy between the 5.9fps figure in the rule and the actual peak flows of 2.6 fps with the staff quite explicitly nearly a month ag
Thank you very much.
-----Forwarded by Julie MacDonald/ASFW/OS/DOI on 01/30/2006 02:20AM -----

To: Renne Lohoefener/ARLR9/FWS/DOI@FWS
From: Julie MacDonald/ASFW/OS/DOI
Date: 01/06/2006 06:58PM
Subject: Re: Fw: K. Sturgeon PCE comparison

Thank you Renne. Following is an excerpt from the 12/29 version of the draft critical habitat document:

Paragamian et al. (2001) observed mean water column velocities between RM 141.6 and 149.4 (RKM 228 and 240.5) during spawning events and in 2002, Paragamian et al. (2002) hypothesized that spawning sturgeon may select sites further upstream with greater water velocities as depth increases due to the backwater from Kootenai Lake. Parsley and Beckman (1994) suggested, based on information from four lower Columbia River sites were white sturgeon successfully reproduce, that optimal spawning habitat may occur when mean water column velocity is 5.9 ft/s (1.8 m/s) or greater. Based on these studies it appears that white sturgeon use velocity as a cue for spawning.

I note that the peak average velocities shown in the Berenbrock work are a little less than 3fps, which is significantly below that which triggers spawning. This is consistent with the information I received on the braided reach. If indeed the sturgeon are looking for faster flows, areas farther from the backwater of the lake will certainly have more rapid flows. However, it is difficult to understand how we can find that a mean velocity which is roughly half the optimal velocity is appropriate for triggering spawning.

Perhaps I'm missing something.

Renne Lohoefener@FWS

Renne Lohoefener@FWS
01/06/2006 03:57 PM

To: Julie MacDonald/ASFW/OS/DOI@DOI
cc:
Subject: Fw: K. Sturgeon PCE

Renne Lohoefener/ARL/R9/FWS/DOI@FWS	To	Julie MacDonald/ASFW/OS/DOI@DOI
	cc	Marshall Jones/ARL/R9/FWS/DOI@FWS
02/01/2006 02:36 PM	bcc	
	Subject	review of our phone conversation

History: This message has been replied to.

Thank you for taking my call and helping reach resolution. Very much appreciated. Please let me know immediately if your recollection differs from what I captured.

"successful spawning" will be changed to something like "successful production of larval sturgeon", the issue being that spawning is technically milt meeting egg

"May and June" will be changed to "May into July", the issue being some successful production does occur in early July

"intermittent depths of 1 to 16 meters" will be changed to "depths of at least 5 meters", the issue again being successful production of young fish and we don't want to require agencies to consult on lesser water depths.

"within the entire braided reach" will be changed to something like "throughout (but not uniformly occuring) in the entire braided reach" the issue being that the braided reach has a patchy distrtibution of water depths and bottom types

The draft EA will accompany the interim final rule

I believe these were the important issues and the rule will be changed to reflect consistency throughout.

I have talked to Ben and he had no issues. He has reservations about the clarity of the paragraph that reads:

"Each of the areas designated contain PCEs that provide for one or more of the life history functions of the sturgeon. In some cases the PCEs may exist as a result of ongoing federal actions. However, the Service does not foresee that continued operations of Libby Dam in manner consistent with past management would result in destruction or adverse modification of critical habitat. These conditions are part of the current baseline."

but realizes this is a policy level discussion.

Ren

Renne Lohoefener/ARL/R9/FWS/DOI@FWS
02/01/2006 02:52 PM

To Julie MacDonald/ASFW/OS/DOI@DOI
cc Dale Hall/ARL/R9/FWS/DOI@FWS, David Bernhardt/SIO/OS/DOI@DOI, Matt J Hogan/ARL/R9/FWS/DOI@FWS, Renne
bcc
Subject Re: Kootenai Sturgeon Rule

History: This message has been replied to.

Julie

We are in agreement except for #1. Our biologists believe that water depths of at least 5 meters are needed for successful production of young sturgeon. There is no upper boundary that we know of. I believe you will be okay with this clarification since it narrows the conditions where this PCE would be present.

The rule was changed to reflect "greater than 5 meters" throughout. Please call immediately if this is a problem.

tks

Ren
Julie MacDonald/ASFW/OS/DOI@DOI

Julie MacDonald/ASFW/OS/DOI@DOI
02/01/2006 02:46 PM

To Matt J Hogan/ARL/R9/FWS/DOI@FWS
cc Renne Lohoefener/R2/FWS/DOI@FWS, Dale Hall, David Bernhardt/SIO/OS/DOI@DOI
Subject Kootenai Sturgeon Rule

This email is to confirm the telephone conversation I had with Renne. We agreed that the following changes are appropriate and the field will make them:

1. PCE for depth would be from 5 to 16 feet and conforming changes would be made throughout the rule.
2. The velocities and flows throughout the braided reach would be intermittant, seasonal, and the actual occurance would be somewhat scattered (Renne will work with solicitors to craft the language)
3. The rule will not refer to spawning habitat, as spawning is only one of several specific steps required to successfully generate sturgeon young, Renne will work with the field to come up with an appropriate phrase to capture the idea of habitat that supports all of the lifestages of spawning, incubation, and mobile embryo survival.
4. Edits to the version provided this morning will be accepted unless they affect the biology, Renne will be notified immediately to address any issues related to biology and will contact me if any other changes are needed.
5. Renne will double check with Solicitor's office to ensure this morning's edits are ok.

There won't be sufficient time for me to actually review the document prior to it going to the Register, but based on Renne's assurances listed above, I am comfortable the package will be ready for the Register and recommend signature.

Renne, If I missed any of the issues you raised, please supplement with another email, I think I got them all, but may have missed one or two.

ATTACHMENT 4 – Document requests

Director Hall asserted that DAS MacDonald made unreasonable document requests of staff. The Administrative Procedures Act requires the Service to make the research supporting the rule and the comment letters from the public available. For the past several years, it has been the practice of the Assistant Secretary's Office to review comment letters and research on most major rules. While there are many of these document requests, it appears that the specific request that Director Hall references is related to the rule making on the red-legged frog.

1. **DAS MacDonald requested 164 not 4,000 comment letters on the Red-legged frog.** In an email Director Hall asserted there were 4,000 documents contained in the request. The federal register notice for the red-legged frog notes there were 164 comment letters received (88 in the first request for comment and 76 in the second request for comment).
2. It is important to note that under the APA, the documents requested must be provided to anyone as part of the administrative record of the rulemaking. Staff was not requested to provide anything out of the ordinary.
3. It is also important to note that the Assistant Secretary's office had a longstanding practice of requesting comment letters and cites, and that the Service was free to provide just the substantive comments when the volume was large, such as when numerous form letters had been sent. This practice is documented in a number of emails over the years. The information was readily available for the investigators.

- **Email from Dale Hall asserting several thousand comments.**

Dale_Hall@fws.gov
03/01/2006 12:27 PM

To "Matt Hogan" <matt_hogan@fws.gov>
cc Julie MacDonald/ASFW/OS/DOI@DOI
bcc
Subject Fw: re some of your questions

Matt:

I received the request below from Julie and cannot agree to have our staff copy several thousand comments for a policy review. I'm afraid that, between the unacceptable treatment of my staff (highlighted last Friday), and astonishing requests like this that I must ask yoy to understand that I will no longer honor requests unless they come directly from you. This is truly unfortunate, but while I'm aggressively working with the Directorate and our employees to heighten professionalism and accountability, there seems to be an endless stream of unusual requests and hostility towards my staff that undermine timelines and workload. I'll be back in the office on Friday if you'd like to talk about it. Our employees deserve one chain of comand that hands out assignments and gives instructions. For us, that's me to you to Lynn to the Secretary. I realize your staff advisor is important to you, but I simply have to get control if I'm to be held accountable.

Thanks for all your support and dedication.

Dale

Sent from my BlackBerry Wireless Handheld

From: Julie MacDonald
Sent: 02/28/2006 01:39 PM
To: Dale Hall
Cc: Matt Hogan
Subject: Fw: re some of your questions

I am sorry to have to raise these issues to you, but as you asked me to on Monday, I have several requests. Thank you for your help in advance.

I requested copies of the comment letters on this listing on the 24th of February (see attached email). I have heard nothing regarding the request. Today, at the endangered species staff meeting, I asked if the request for comment letters had been made to the field. It had not, and Marge told me that she did not know if the request had gone to you yet. So I am formally requesting that these documents be made available for my review as soon as possible. These are public documents that anyone off the street can request under FOIA and the Service would provide them. In order to properly review the final rule, I need these documents. The later I receive them, the more difficult it becomes for me to complete my review in time to surname the rule. I appreciate your consideration in this matter.

In addition, the delisting for the marbled murrelet DPS was due here at the end of January. Renne and I discussed this schedule some months ago. It is now over a month past the time it was expected. Could you please let me know what is happening with this package? I am anxious that we move

impacts from critical habitat designations challenge these designations. The cycle of litigation appears endless, and is very expensive, thus diverting resources from conservation actions that may provide relatively more benefit to imperiled species.

The costs resulting from the designation include legal costs, the cost of preparation and publication of the designation, the analysis of the economic effects and the cost of requesting and responding to public comment, and in some cases the costs of compliance with the National Environmental Policy Act (NEPA) (42 U.S.C. 4321 et seq.). These costs, which are not required for many other conservation actions, directly reduce the funds available for direct and tangible conservation actions.

Background

It is our intent to discuss only those topics directly relevant to the designation of critical habitat in this rule. For more information on the California red-legged frog, refer to the revised proposed critical habitat designation published in the Federal Register on November 3, 2005 (70 FR 66906).

Previous Federal Actions

Previous Federal actions for the California red-legged frog can be found in our revised proposal of critical habitat for the California red-legged frog published in the Federal Register on November 3, 2005 (70 FR 66906). That information is incorporated by reference into this final rule. On November 23, 2005, the federal district court in the Eastern District of California granted a motion to extend the deadline for publication of the final critical habitat until March 31, 2006. This final designation is being completed and published in the Federal Register in compliance with that court order.

Summary of Comments and Recommendations

We requested written comments from the public on the proposed designation of critical habitat for the California red-legged frog published on April 13, 2004 (69 FR 19620). We also requested written comments from the public on the revised proposed designation of critical habitat for the California red-legged frog published on November 3, 2005 (70 FR 66906). We also contacted appropriate Federal, State, and local agencies; scientific organizations; and other interested parties and invited them to comment on both the proposal and the revised proposed rule. The comment period for the initial proposed rule opened on April 13, 2004, and closed on June 14, 2004. We extended the period from June 14, 2004 to July 14, 2004 (69 FR 32966). Comments and new information received in response to the first proposed rule which were relevant to the revised proposal and final designation were incorporated in the final rule as appropriate and are summarized with the comments received in response to the revised proposed rule below.

During the comment period for the initial proposed rule, we received a total of 98 comment letters from Federal, State, and local governments, and private individuals. Of these comment letters, 38 commenters generally supported the initial proposed designation of 4.1 million acres (1.6 million hectares) or provided specific information pertaining to the subspecies or habitat, and 58 commenters generally did not support the initial proposed designation as written or did not support the inclusion of certain lands. Of the 98 total comment letters, 39 comment letters focused on Land areas that we later determined to be nonessential to the conservation of the subspecies and that we are no longer including in this final designation. In summary, in our revised proposed rule and in this final designation, we used the best scientific information available in determining the areas essential for the California red-legged frog and removed all areas that we determined are not essential for the conservation of this subspecies and therefore do not meet the definition of critical habitat. We re-examined all initially proposed areas and removed any areas that do not contain one or more of the PCEs or that were determined to be nonessential for the conservation of the subspecies because: (1) The area is highly degraded and may not be restorable; (2) the area is small, highly fragmented, or isolated and may provide little or no long-term conservation value; and/or (3) other areas within the geographic region were determined to be sufficient to meet the subspecies needs for conservation.

We also considered several criteria in the selection of areas that contain the features essential for the conservation of California red-legged frog and focused on designating units: (1) Throughout the current geographic, elevational, and ecological distribution of the subspecies; (2) that would maintain the current population structure across the subspecies' range; (3) that retain or provide for connectivity between breeding sites allowing for the continued existence of viable and essential metapopulations, despite fluctuations in the status of subpopulations; (4) that possess large continuous blocks of occupied habitat, representing source populations and/or unique ecological characteristics; and (5) that contain sufficient upland habitat around each breeding location to allow for sufficient survival and recruitment to maintain a breeding population over the long term. We proposed critical habitat units in areas that have the highest likelihood to contain self-sustaining populations of California red-legged frogs based on the presence of the PCEs, the density of California red-legged frog occurrences, and the kind, amount, and quality of habitat associated with these occurrences. We believe this strategy allowed us to narrow our initial focus down to the habitats that meet the definition of critical habitat and are essential to the conservation of the subspecies.

During the comment period associated with the revised proposed rule that opened on November 3, 2005, and closed on February 1, 2006, we received 76 comments directly addressing the revised proposed critical habitat designation and the draft economic analysis. Of these comments, three were from peer reviewers, one from a Federal agency, and 32 from organizations. Five commenters supported the designation of critical habitat for the California red-legged frog, and 55 opposed the designation. Sixteen letters included comments or information, but did not express support or opposition to the revised proposed critical habitat designation. Comments received were grouped into 15 general issues specifically relating to the revised proposed critical habitat designation for the California red-legged frog, and are addressed in the following summary and/or incorporated into the final rule as appropriate. We did not receive any requests for a public hearing, however, we did receive one request for a public workshop from the Calaveras County Farm Bureau. On January 19, 2006, we held a public workshop in San Andreas, California, and on January 27, 2006, we held an additional public workshop for the Calaveras County Board of Supervisors and the general public.

Peer Review

In accordance with our policy published on July 1, 1994 (59 FR 34270), we solicited expert opinions from five knowledgeable individuals with scientific expertise that included familiarity with the subspecies, the geographic region in which the subspecies occurs, and conservation biology principles. We received responses from two of the peer

ATTACHMENT 5

Attachment 5

BODY OF THE INVESTIGATIVE REPORT

Pg. 3 Employee 1

Assertion: Bypassed staff and discussed with Field regularly.

Response: All discussions with staff were the result of the Washington Office and then the Regional Director's request.. This was the process followed until Director Hall required that ASFWP staff only interact with senior staff. That request was honored.

Assertion: DAS MacDonald determined the economic cost of designating the area as a critical habitat was unacceptable and ordered revision of the report.

Response: The determination to exclude the areas was that of the Assistant Secretary, and authorized under Section 4(b) (2) of the ESA. Revision of the report was ordered upon direction by the Assistant Secretary.

Pg. 4 Employee 1

Assertion: Interference minimized the ESA and ensnared it in litigation.

Response: The ESA program was already ensnared in litigation when the Bush Administration took office. All the work done by ASFWP was designed to improve the regulatory packages in order to assure their ability to survive a court challenge. Often rules were poorly written and appeared to be arbitrary and capricious because of the FWS's failure to elucidate the reasons for final determinations.

Assertion: Based the Vernal Pools analysis on incorrect information and business development figures.

Response: The information used was from the State of California's Department of Finance. Population, gross annual product, and unemployment rates were used to assess the fiscal health of the affected counties. The mathematical error was real, but because the comparisons involved were relative comparisons, it did not change the relative values which were the basis for the determination. Section 4(b)(2) of the Act is designed to provide an opportunity for the Secretary to make critical habitat designation decisions based on considerations other than the biology. It is specifically designed to take into account economic issues.

Pg. 4 Employee 2

Assertion: MacDonald would not accept the FWS scientific findings and applied alternative outside sources and insisted on changes.

Response: The ESA requires that the best available data be used in decision making. The FWS did not always consider all the data, and often 'cherry picked' for sources and reviewers which supported their position. I looked at all the data available, particularly that which was provided through the comment process. Where a study appeared to be newer/better than that which the FWS relied upon, I asked for an explanation of why the newer work was not used. The comment period required by the APA during rulemaking is there for a purpose. FWS had a tendency to ignore or discount any information received during the comment period unless it supported the rule.

Pg. 4 Employee 3

Assertion: Required scientific proof to regulate

Response: The statement is partially accurate. Data was required to support assertions and assertions supporting data were deleted from the text. However, proof was not required, just a basis in fact evidenced by data. The ESA requires data to support findings. If there is no data, the FWS does not have the authority to act under the law.

Pg. 4 Employee 4

Assertion: Preamble language undermined position of FWS.

Response: The preamble language was the product of work between the Assistant Secretary who is a former judge and a lawyer himself, and the Solicitor's office, not DAS MacDonald.

Assertion: California Tiger Salamander: allegedly changed science to force FWS to proposed listing as one population.

Response: The science was not changed. The DPS policy requires that differences be significant. At question was how to define significance. Tiger salamanders as a whole show a 7% range in genetic differences within the population. California Tiger Salamanders had only a 2% difference, well within the normal range. As a matter of policy, the ASFWP determined that 2% was not sufficient difference to be significant. That determination was a policy decision.

Pg. 6 Employee 5

Assertion: There is still an ongoing lawsuit against FWS for not delisting the delta smelt.

Response: To my knowledge, there is no lawsuit pending on this issue. But even if there was, the email explicitly supported the FWS determination that the smelt was still threatened.

Assertion: DAS MacDonald challenged the accuracy of the delta smelt population index that was used in the Delta Smelt status review.

Response: The challenge was correct. The index is not accurate. It is an amalgamation of sampling efforts at different times, using different protocols. Currently, there is no accepted protocol for sampling smelt, because biologists cannot decide how to accurately count them. The fish appear randomly, so it is not possible to be certain whether populations are dropping, or that they are just found in areas where no sampling is taking place. The FWS was directed to identify the inadequacies in the index and the weaknesses in the science so as to avoid giving the impression that our state of knowledge regarding the biology of the smelt was good. This direction was given with the knowledge and consent of the Assistant Secretary.

Assertion: DAS MacDonald said the USGS peer review of the Delta Smelt status review was 'no good'.

Response: While the exact wording of the statement regarding the peer review is unknown, DAS MacDonald did take issue with the fact that most of the peer reviews appeared to be a synopsis of whether the reviewer agreed with the FWS determination rather than a critique of the analysis and science used to arrive at the answers. Much of the language in the peer reviews was intemperate and merely stated the reviewers position as to whether the smelt should be listed.

Assertion: The email makes FWS appear confused about its own stance on the smelt's delisting.

Response: That statement is inaccurate. The email explicitly supports the FWS determination that the species remain listed. The issues raised are related to the characterization of the science involved in the determination. At issue was the fact that the FWS did not accurately convey to the public the profound level of uncertainty related the science used to list the smelt.

Assertion: JM was pressuring him to make subtle changes to his report or research.

Response: I did not pressure him to make changes to the research. I most definitely wanted the final determination to provide far more daylight as to the uncertainties around the science being used to support the listing. The proposed status review asserted certainty where none existed. In the case of the smelt, where very little is fully understood, an assertion of certainty in a federal rule results in rigidity in regulatory actions that is not supported by the science, makes the FWS vulnerable to lawsuits related to regulatory actions, and ultimately limits our ability to take actions designed to assist the species.

Page 7 Employee 6

Assertion: JM would curse and yell at her.

Response: I do not yell at people. I have cursed at times. Generally, I match the tone of the person I am speaking to.

Page 8 Employee 7

Assertion: MacDonald wanted to hire an outside consultant other than SEI

Response: Employee had no knowledge of what I did or did not want. I did not discuss issues with this employee. In fact, I recommended SEI as a consultant to Director Hall based on the work they had done on the Northern Spotted Owl.

Page 7 Employee 8

General observation about Employee: Employee does not agree with the Administration policy goals. Every time employee was in a meeting, employee monopolized the time with a running diatribe on the Administration's policies and why they were wrong. While employee is entitled to his opinions, as long as the President is in office, his policies are the policies of the Department. The employee's continued intransigence on this issue was brought to the attention of the Solicitor.

Assertion: PECE policy statement that ...'*MacDonald wanted to use attorneys to stomp down FWS staff and lie that the states were doing a better job than they actually were...*'

Response: The statement is purely false. The PECE policy is designed to provide a method for evaluating conservation actions in order to determine whether to include them in a listing decision. One of the requirements is that the actions be effective. In the case of the Sage grouse, states and private landowners were taking conservation actions based on the verbal and written advice they received from the FWS.

The FWS evaluation of the various state and landowner actions was predicated on a very narrow reading of the PECE policy by the FWS. That narrow reading resulted in a determination by the FWS that unless states and others could provide published peer reviewed documentation that their particular actions were effective, those actions could not be considered conservation actions in the listing determination. As a result, actions recommended by the FWS were not considered effective conservation actions by the FWS! This result was completely contradictory to the purpose of the PECE policy which was to encourage states and private landowners to take conservation actions prior to listings, in order to avoid species declining to the point that listing was necessary. In addition, that narrow reading gave lie to FWS recommendations. Finally, that narrow reading imposed an effectiveness standard that was beyond any that the FWS itself used

in listing, biological opinions, and recovery activities.

Apparently, the employee took exception to the ASFWP pointing out the inconsistencies in approach.

Page 15 Employee 9

Assertion: DAS MacDonald asked to have several employees removed.

Response: Supervisor was not requested to remove any employees. However, there are two employees that were specifically mentioned to the supervisor, as a result of complaints that were more serious and specific in nature than normal. DAS MacDonald relayed the complaints to the supervisor who had the authority and information to determine whether the complaints were valid and should be acted upon. This was done to fulfill the ASFWP's responsibility to the public and the Department to relay complaints received to those who are able to judge the merits of the complaint.

In the case of the first employee, on two separate occasions, two different people recounted conversations in which the employee threatened them. Because of the seriousness of the allegations, the comments were relayed to the supervisor. Both complainants were known to the supervisor and would have been considered reliable as they work often with the FWS in the region.

With respect to the second employee, continued complaints about the employee's professional integrity led to the suggestion that perhaps replacing the employee with a staff member better able to interact with the community (this was a cooperative conservation project) would be constructive and result in faster completion of the project. Again, it was a suggestion and no other action was taken. It is the responsibility of Executive staff to make managers aware when there are complaints and if appropriate I make suggestions. It is not inappropriate to suggest a response. Clearly, from the fact that the supervisor did not act on either complaint, the supervisor was neither intimidated nor did he feel he had received a directive.

Page 15 Dale Hall

Assertion: Has a *'running battle'* with her attempts to go outside the chain of command.

Response: Director Hall has misrepresented the facts. Since Mr. Hall became Director and requested that DAS MacDonald only interact with senior staff, that request has been respected. Prior to Director Hall's tenure, DAS MacDonald routinely interacted with all staff when directed by the AD for Endangered Species. Mr. Hall's characterization of a 'running battle' is his own. The business of the Assistant Secretary's office was conducted in a manner consistent with Director Hall's preferences.

Assertion: In Southwest Willow Flycatcher MacDonald decided that 1.8 not 2.1 miles was more accurate for nesting radius.

Response: The information provided by Director Hall is inaccurate. Based on the text of the proposed rule, the data the FWS had indicated:

> ...' *Most recorded between-year movements occurred within the same drainage from 1.6 to 29 km (1 and 18 mi), but movements as far as 40 km (25 mi) were recorded (Luff et al. 2000; Kenwood and Paxton 2001; E. Paxton, USGS, e-mail).*'

The initial draft proposed to base the designation on the farthest range of 25 miles. This appeared to be excessive, from a policy perspective. Generally speaking, regulation is not based on the exception. Instead, 18 miles was used because it represented the high range of where most of the movements occurred. This determination followed a discussion with FWS Director Steve Williams regarding the appropriate range as well as a more thorough discussion of the fact that many miles of habitat lacking the physical and biological characteristics of critical habitat were included, based on these overlapping radii. I asked then Director Williams biologically speaking how one could develop a decision rule that properly identified habitat without also including developed areas such as towns and cities (which was the case for the draft rule). Director Williams indicated that generally, overlapping ranges would not be used for species like the flycatcher, but that instead, the data would be examined to find where densest concentrations of sightings were found and those areas designated. I relayed that information to the AD for Endangered Species and also the field. It was ignored.

Assertion: Forced staff to change to 1.8 miles because it would avoid listing in California

Response: Director Hall has misrepresented the facts. Avoiding listing in California was not a goal, and in fact, both the proposed and final rule includes lands in California. In addition, at no time was property owned by DAS MacDonald's family even close to being affected by a flycatcher listing. Had the family's property been affected, I would have immediately recused myself from working on the rule. I would note that the IG had to have been fully aware of this fact, as the action implied would have been a violation of the government's conflict of interest prohibitions.

Assertion: DAS MacDonald had a particular interest in all California work because of a family ranch and previous work experience.

Response: Director Hall's statement is false. Most of the ESA listings and critical habitat designations are in California. At times other staff in the Assistant Secretary's office worked on ESA rule makings generally, because of familiarity with the regions. But virtually all ESA regulatory documents were reviewed and approved by DAS MacDonald. The statement that DAS MacDonald focused on California actions because of family property is not accurate and further, is misleading in that it implies improper activity on my part. Had family property been affected by any rule making, DAS MacDonald would not have been able to work on the listing because of conflict of interest prohibitions.

Assertion: Facedown on Kootenai Sturgeon. Hall challenged DAS MacDonald assertions and requested that she put in writing her position. DAS MacDonald relented and kept the 2.3 to 5.9 range.

Response: Director Hall's statement is inaccurate. At no time did DAS MacDonald require any particular range; rather the DAS pressed the FWS to clarify the rule and the scientific basis for its contents.

The entire discussion on the Kootenai sturgeon occurred between 11/2005 and 2/2006. The Service prepared a draft rule that was internally inconsistent and basically incoherent. ASFWP repeatedly requested information, resolution and clarification of the inconsistencies. FWS was non-responsive. There are numerous emails documenting the apparent inconsistencies, and requesting clarification. Draft language was provided by the DAS and the DAS repeatedly asked for confirmation as to whether the language was correct. In a 1/22/2006 email to the AD

Endangered Species DAS MacDonald stated:

> *' It is becoming increasingly troubling that the regional director, the regional solicitor, and everyone else with an interest in producing a defensible rule does not seem to be troubled by this apparent inconsistencies. The region seems unable to construct a rule that lays out the fact and logically proceeds to a conclusion. I am still unclear as to what the actual facts of the matter are, I have cobbled together what I think I am reading, but again, the apparent contradictions and muddiness of the statements in the rule, lead me to wonder if I might still not have gotten it quite right.'*

DAS MacDonald requested a meeting for clarification, and received no response from FWS. A final rule was delivered to ASFWP. DAS MacDonald refused to surname the rule because it was not possible to recommend to the Assistant Secretary that the rule was adequate. At that point, the FWS AD for Endangered Species resolved the outstanding issues and clarified the points necessary.

Assertion: DAS MacDonald circumvented the chain of command and went directly to the field for documents.

Response: The statement is inaccurate. DAS MacDonald interacted with the Service in the manner preferred by the Director of the FWS. Under the tenure of Director Williams DAS MacDonald worked directly with staff. Once Director Hall requested that interactions only occur with senior staff that request was honored. Director Hall's characterizations of DAS MacDonald circumventing the chain of command is a disingenuous effort to confuse the issue and impose his preferred method of management on another Director.

ATTACHMENT 6 – DOCUMENT RELEASE

The report notes there was no criminal activity and then asserts that DAS MacDonald violated the Code of Federal Regulations. The report asserts DAS MacDonald gave preferential treatment to individuals by releasing non-public information. The Inspector General apparently takes the position that only a FOIA Office can make a determination to release documents. DAS MacDonald had the authority to release the documents. No rule was violated in the release. Further, had the investigators reviewed the practice of the FOIA office in releasing documents requested, they would have found many similar documents released under FOIA.

1. EPA draft rule (2002): A draft EPA rulemaking was transmitted to a chemist working for Chevron. It was sent because there were a number of technical terms that were unclear. It is not clear that the person ever received the draft. No response was received, and no action was ever taken on the rule by EPA.

This, of all the allegations in the report, is most troubling. While the release of the document was not illegal, it was foolish. Relative inexperience is the only explanation. DAS MacDonald was a Special Assistant and had been working for the Department only a short time.

2. Draft Critical Habitat Guidance: The document was an early theoretical draft. DAS MacDonald had the authority to release the draft. As a practical matter, the draft contained nothing that provided PLF information upon which to base a suit. The fact that no suits were filed predicated on the contents is *prima facie* evidence that this is case, and your investigators were given that information. Had I released the document to the Center for Biological Diversity, who was regularly suing the Service on critical habitat, it would have been more arguable. The document primarily laid out the legal basis upon which practical guidance for developing critical habitat would be based.

3. Delta Smelt Email The document was released by DAS MacDonald at the request of the Farm Bureau. The document was not pre-decisional and was released by the FOIA office on July 7, 2004. The release was based on a formal FOIA request from the Farm Bureau. The report implies that there was an improper relationship and that the relationship generated the release of the document. In fact, a review of the FOIA requests received by ASFWP will demonstrate that the request, the specificity of the request, and the nature of the request were not unusual. The impropriety is implied by the report and does not exist in fact.

ATTACHMENT 7

Staff Interactions

The report contains a number of statements regarding negative interactions with staff. These allegations are virtually impossible to refute as they address verbal communication. However, your staff did review the numerous emails written over the past 5 years. This email correspondence demonstrates a uniform level of professional courtesy. It contains a multitude of polite requests for briefings and information that are replete with phrases like 'please could you...', 'would it be possible to...", as well as thanks and recognition of staff when a job was well done, or a compliment received. The comments on draft rulemakings that have been the subject of discussion were generally prefaced by a cover letter noting they could be wrong, and requesting staff review them. Finally, a review of draft and final responses to my comments demonstrate that staff routinely rejected my comments and suggestions. This written record does not support the assertions of intimidation made by those interviewed.

Criticism of fitness to review biological documents

Your report implies that an absence of a degree in biology makes one unfit to review and comment on FWS rulemakings. That perspective if applied to virtually any supervisory position, would result in many executives being found unfit. The review of the rules provided to the ASFWP consists of a check to ensure that the science is accurately represented, that the statements contained in the rules are supported by data, as required by the statute, and that the rules are internally consistent and consistent with other Service publications. All this can be done by reviewing the documentation supporting the rule. It requires no specialized knowledge.

In order to ensure the rules accurately represent the science, the cited literature is reviewed to ascertain whether it supports the statements in the rule. This does not require any expertise in biology. It merely requires the ability to read and confirm that research findings support the Service's statements.

The ESA requires that final rules be supported by data. Again, it does not require a degree in biology to understand whether there is data available to support a statement made by the

Service. If the data is available, the Service should be able to produce it, which is what was required.

Rulemakings must be internally consistent, or they are subject to challenge. For example, in the Bull Trout draft critical habitat, the Service identified virtually pristine water quality as being essential to the conservation of the species. That means, in order to designate any occupied lands as critical, the water quality must be pristine. The Service then proposed to designate as critical habitat stream reaches which had such poor water quality they were superfund sites. If pristine water quality is required for designation, then a designated site may not have poor water quality. By designating a site with poor water quality and requiring pristine water quality, the Service would have left the government open to a suit that either challenged the biological requirement of pristine water quality, or the designation of the superfund reach. My review was designed to avoid such situations, and in this case, the Service chose to remove the streams from the designation, rather than change the water quality requirement.

Exhibit Y

GREAT OUTDOORS COLORADO

NEWS RELEASE

FOR IMMEDIATE RELEASE
June 27, 2007

CONTACT: Chris Leding
303-226-4530
303-981-7900 (cell)

GOCO AWARDS $8 MILLION IN LOTTERY GRANTS

RECORD $100 MILLION INVESTMENT SLATED FOR PROJECTS IN FY 2008

DENVER – The Great Outdoors Colorado Board has approved a $100 million spending plan – the largest in its history – for Fiscal Year 2008 to, in part, address urgent open space protection needs in the state.

The Board also closed out FY 2007 by awarding $8 million in grants to 46 projects that will help preserve 4,000 acres of land and improve outdoor recreation in 34 counties:

- Among the open space projects receiving an award is the **260-acre Grange Family Ranch Project sponsored by Pitkin County** in partnership with Eagle County and the Town of Basalt. **GOCO awarded $822,828** to help purchase a conservation easement and partial fee title acquisition on the ranch, which lies in the heart of the Roaring Fork Valley. The ranch provides scenery enjoyed by thousands daily along the Rio Grande and Emma trails, Highway 82, and the Roaring Fork River and has significant riparian wildlife habitat. New trail easements will connect the ranch to the Emma Trail and BLM lands. The ranch also connects to a State Wildlife Area.

- **Brighton will get a new skate park with the help of a $200,000 GOCO grant.** The City's existing skate park is unsafe and doesn't meet the demand for a facility in this rapidly growing area. The new skate park will be constructed on City-owned land. Community youth will work closely with the skate park design firm to create an area accessible to skateboarders, in-line skaters and BMX riders.

- **The City of Steamboat Springs will use $30,000 from GOCO to create an Implementation Plan for property recently acquired on Emerald Mountain.** The acquisition was completed by BLM in a lengthy and complex land swap resulting in 4,139 acres designated as a Special Recreation Management Area. The acquisition is adjacent to properties currently owned by the City and the Division of Wildlife totaling 1,200 acres and all three parcels will be included in the Implementation Plan for over 5,300 acres of public access and recreation. Planning will assess trail connectivity between all three properties and the City and the need for recreation facilities.

-more-

$100 Million Available for FY 2008

The GOCO Board also approved the largest spending plan in its 15 year history by making $100 million in Lottery funds available for grants beginning July 1, the majority of which will be for land protection. In addition, the Board invited 20 projects to compete for $66 million of the $100 million for Legacy grants – GOCO's multi-year, multi-million dollar commitments.

The GOCO Board has continued to assess whether it can meet urgent land protection needs in the state with cash or whether it needs to exercise bonding authority granted it by voters in 2001. It has concluded, as it did in 2004 when it approved a $97 million spending plan, that projected Lottery proceeds make the use of cash -- without incurring the debt associated with bonding -- the wisest choice. In formulating this plan, the Board has made certain it retains its ability to bond should an unforeseen, urgent project come forward needing a large infusion of funds from GOCO. The structure of the spending plan is:

- $34 million will be awarded for the projects the Board funds annually – open space, community park and outdoor recreation, trails, and Colorado State Parks and Division of Wildlife projects.

- $66 million will be for Legacy grants with the majority of the 20 projects invited to compete seeking to protect large landscapes. These grants will be awarded in December 2007. Invited projects include:

    - **The Northeast Greenway Project sponsored by Adams County**, a proposed urban greenway and open space network covering approximately 150 miles of trails and adding approximately 1000 acres of parks and open space surrounding the Rocky Mountain Arsenal Wildlife Refuge. The project area extends from Denver to Brighton and Commerce City to Aurora.

    - **The Rio Grande Initiative** to protect land along the Rio Grande River in **Rio Grande, Alamosa, Conejos and Mineral counties.** The first phase of this effort targets the protection of 14,000 acres. The Rio Grande Headwaters Land Trust is the project sponsor.

    - **The Upper White River Watershed Project**, an effort by the Yampa Valley Land Trust to protect 11,000 acres of land in **Rio Blanco County**, an area under increasing pressure because of energy and mineral development.

Applications for the 20 projects will be due in August. During the Fall, GOCO Board members will tour the projects. Grants will be awarded in December. None of the projects that have been invited to apply are guaranteed funding.

3-3-3/Statewide

Great Outdoors Colorado is the result of a citizens initiative passed in 1992. It receives up to half of Lottery proceeds. GOCO's Constitutional mission is to preserve, protect, enhance and manage the state's wildlife, park, river, trail and open space heritage.

LISTING OF GRANTS AWARDED $8 MILION TO CLOSE OUT FY 2007

County	Project Sponsor	Project Title	Grant Award
Open Space Projects			
Archuleta	The Conservation Fund	Navajo River Watershed-Slash Bar Ranch, Phase III	$220,000
Garfield	Aspen Valley Land Trust	Rowe Ranch Conservation Easement	$600,000
Gunnison	Crested Butte Land Trust	The Kikel Parcels	$575,000
Pitkin, Eagle	Pitkin County	Grange Family Ranch Conservation Project	$822,828
Rio Grande	Rio Grande Headwaters Land Trust	Rio Grande Initiative	$368,750
Routt	The Nature Conservancy	Wolf Mountain Ranch, Phase III	$452,000
Saguache	Gunnison Ranchlands Conservation Legacy	Irby Ranch, Phase III	$375,092
San Miguel	San Miguel County	San Miguel Land Heritage Program: Bray Ranch Phase II	$245,400
Teller	The Conservation Fund	Stone Ranch, Phase II	$550,000
Local Government Park & Outdoor Rec Projects			
Adams	City of Northglenn	Croke Reservoir Improvement Project	$199,925
Adams	City of Brighton	Brighton Skate Park	$200,000
Arapahoe	Arapahoe Park and Recreation District	Spring Creek Park Expansion – Phase Two	$200,000
Arapahoe	Sundance Hills Metro District	Sundance Hills Playground Renovation	$8,945
Cheyenne	Town of Cheyenne Wells	Cheyenne Wells Preschool Playground Project	$13,195
Cheyenne	Cheyenne County	Field of Dreams Project	$184,823
Clear Creek	Clear Creek County	Blue Hill Acquisition	$90,000
Crowley	Crowley County	Crowley County Recreational Field Enhancement	$173,272
Custer	Town of Westcliffe	Custer County Preschool Playground	$27,053
Dolores	Town of Rico	Rico Town Park Playing Field	$29,415
Eagle	Town of Red Cliff	Renovation of the Town of Red Cliff Playground	$31,500
El Paso	Town of Calhan	Calhan Elementary/Community Playground - 'Step Up' Project	$200,000
El Paso	City of Manitou Springs	Soda Springs Park Renovation, Phase I	$109,358
Grand	Town of Kremmling	Roof The Rink, Chuck Lewis Rink	$63,390
Gunnison	City of Gunnison	Charmer Tennis Court Rebuild	$118,000
Jefferson	City of Lakewood	Ray Ross Park Phase One Development	$200,000
Morgan	Town of Hillrose	Town of Hillrose Bathroom Project	$15,391
La Plata	Town of Bayfield	Mesa Park Playground Equipment	$22,200
Las Animas	Las Animas County	Primero RE-2 School District Recreation Area	$153,136
Larimer	City of Fort Collins	Heritage Park Improvements	$110,000
Mesa	Town of DeBeque	DeBeque Town Park Playground Improvements	$23,000
Mesa	City of Fruita	Little Salt Wash Park	$200,000

Morgan	City of Fort Morgan	Baker Field Renovation	$165,220
Otero	Town of Manzanola	Manzanola Train Depot	$186,852
Otero	Town of Fowler	Girard Park and Playground Improvement	$200,000
Park	South Park Parks & Recreation District	South Park Skate Park, Phase III - Safety	$25,961
Phillips	Phillips County	Phillips County Fairgrounds Restroom Facility	$91,317
Prowers	Prowers County	Prowers County Open Air Pavilion	$200,000
Pueblo	Pueblo County	Pueblo County - Addition of baseball field at Runyon Sports Complex	$200,000
Rio Grande	City of Monte Vista	Chapman Park Improvement Project	$113,075
Washington	Town of Otis	Town of Otis Picnic and Playground Improvement	$31,500
Washington	Washington County	Woodlin School Athletic Lights	$31,371
Weld	Town of Platteville	Platteville Community Complex Enhancement Project	$128,688
Weld	Town of Frederick	Milavec Park Playground	$20,000

Planning Projects

Grand	Town of Hot Sulphur Springs	Pedestrian Bridge Planning	$15,050
Routt	City of Steamboat Springs	Emerald Mountain Implementation Planning Project	$30,000
Routt	Town of Hayden	Hayden Parks and Recreation Master Plan	$30,000
TOTAL			$8,050,706

-end-