

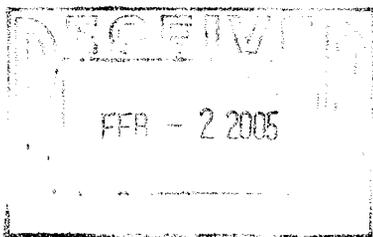
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Comments on Preble's Meadow Jumping Mouse Delisting Proposal

1. 1/31/05 Robert B. Hoff Colorado Springs, CO
2. 2/2/05 Nathan Arentsen Simpson College Progressive Action Coalition
Indianola, Iowa
3. 2/2/05 Maria DeLeon
4. 2/5/05 Miranda Mockrin Graduate Student
Dep. of Ecology, Evolution, and Environmental Biology
Columbia University, NY
5. 2/14/05 B. Scahau Florham Park, NJ
6. 2/10/05 Robert B. Hoff Green Valley, AZ (see 1 above)
7. 2/16/05 Christopher T. Massey Mountain States Legal Foundation
Lakewood, CO
8. 4/8/05 Andrew Martin Dep. Of Ecology and Evolutionary Biology
University of Colorado, Boulder, CO
9. 4/28/05 Pat Devers Department of Fisheries and Wildlife Sciences
Paul Grobler Virginia Polytechnic Institute and State University
Eric Hallerman Blacksburg, VA
Nataniel Hitt,
10. 4/29/05 Melissa I. Young Regulatory Specialist
Colorado Rock Products Association
Centennial, CO
11. 5/2/05 Ken Hamilton Executive Director
Wyoming Farm Bureau Federation
Laramie, WY
12. 5/2/05 Renee C, Taylor Environmental Coordinator
True Ranches
Casper, WY
13. 5/2/05 Jim Bensberg Chairman
Board of County Commissioners of El Paso County, CO
Colorado Springs, CO
14. 5/3/05 John A. Kolanz Office of the City Attorney
City of Greeley

- Greeley, CO
15. 5/3/05 Leah Berkman Denver, CO
16. 5/3/05 Jerry Sonnenberg President
Coloradoans for Water Conservation and Development
Denver, CO
17. 5/3/05 Dr. Tom W. Quinn Associate Professor
Codirector, Rocky Mountain Center for Conservation
Genetics and Systematics
Department of Biological Sciences
University of Denver
Denver, CO
18. 5/3/05 Ken Faux CO
19. 5/3/05 Cheryl Matthews Director
Douglas County
Division of Open Space and Natural Resources
Castle Rock, CO
20. 5/3/05 Mark Maslyn Executive Director, Public Policy
American Farm Bureau Federation
Washington, DC
21. 5/3/05 Dave Freudenthal Governor
State of Wyoming
Office of the Governor
Cheyenne, WY
22. 5/3/05 Dr. Mark Bakeman
Craig Hansen
Dr. Andrew Martin
Dr. Carron Meaney
Dr. Ann Ruggles
Ryon Thomas
23. 5/3/05 Erin Robertson Center for Native Ecosystems, Denver, CO
Jeremy Nichols Biodiversity Conservation Alliance, Denver, CO,
Nichol Rosemarino Forest Guardians, Santa Fe, NM
Brian Brademeyer Native Ecosystem Council, Rapid City, SD
24. 5/3/05 Ann Bonnell 2nd Vice President, Audubon Society of Greater Denver
Polly Reetz Board Member and Conservation Chair
Littleton, CO

25. 5/3/05 Richard C. Stem Deputy Regional Forester, Resources
Forest Service
Rocky Mountain Region
Lakewood, CO
26. 5/4/05 Guy N. Cameron President
American Society of Mammalogists
27. 5/4/05 Paul Kruse Albany, Converse, Goshen, Laramie, Platte counties, WY
28. 5/4/05 Russell George Executive Director
Department of Natural Resources
State of Colorado
Denver, CO



ROBERT B. HOFF
2500 NORTH CIRCLE DRIVE
SUITE 100
COLORADO SPRINGS, CO 80909
(719) 638-2277

Jan. 31, 2005

Field Supervisor
US Fish & Wildlife Service
Colorado Field Office
Ecological Services
755 Parfet Street, Ste. 361
Lakewood, Colorado 80215

Per your recent announcement of a proposal to remove the Preble's Meadow Jumping Mouse from the Endangered Species List, I wish to make comment.

Dr. Ramey's report seems to be quite clear as to the supposed Preble's status and it has been vetted now in an independent peer review and it has been endorsed by the gentleman who originally classified the supposed Preble's as a distinct sub-species. The science would therefore seem to support a delisting.

I see, however, that an analysis is to be made as to whether the supposed Preble's is a Distinct Population Segment. This grasping at straws will not work. Many, many thousands of supposed Preble's have been found in the years intervening between the original listing and today. A draft recovery plan for the supposed Preble's envisioned 20,000 as a population sufficient to recover the animal. The Critical Habitat Plan also envisioned 20,000 Preble's as sufficient to prevent extirpation. We now know that there may be a population of 60,000 or more supposed Preble's in Colorado and Wyoming and it is difficult to give credence to the theory that it is a distinct population segment.

There comes a time in the lives of all rational adults when, confronted with a mistake, they say, "I made a mistake. I regret that it may have caused any difficulty. Please accept my apology." Can the Service summon up the courage to say that?

Very truly yours,

A handwritten signature in cursive script that reads "Robert B. Hoff".

Robert B. Hoff

Agency : FISH AND WILDLIFE SERVICE

Title : Endangered and Threatened Wildlife and Plants; 12-Month Finding on a Petition To Delist the Prebles Meadow Jumping Mouse (*Zapus hudsonius preblei*) and Proposed Delisting of the Prebles Meadow Jumping Mouse

Subject Category : Endangered and threatened species: Findings on petitions, etc.-- Prebles meadow jumping mouse

Docket ID :

CFR Citation : 50 CFR 17

Published : February 02, 2005

Comments Due : May 03, 2005

Phase : PROPOSED RULES

Your comment has been sent. To verify that this agency has received your comment, please contact the agency directly. If you wish to retain a copy of your comment, print out a copy of this document for your files.

Please note your REGULATIONS.GOV number.

Regulations.gov #: EREG - 2 Submitted Feb 03, 2005

Author : Mr. Nathan Arentsen

Organization : Simspson College Progressive Action Coalition

Mailing Address :

Attached Files :

Comment : Being an environmental science major at Simpson College in Indianola, Iowa, I find it extremely disturbing that the Fish and Wildlife Service has decided to pursue a regulation change on the narrowest of peer-reviewing approval margins (8-6). Consideration of the weakness of support of three reviews in that narrow majority is more disturbing still.

As well, and equally disappointing, is the fact many of the supporting peer-reviews are unusually brief, dismissive, and overall poorly written in their analyses-a rare practice for the usually moderate FWS regulators. The Preble's Meadow Jumping Mouse deserves more thorough consideration, regardless of if higher administrators feel compelled to appease builders in suburban Colorado.

For these reasons and many more that emphasize the best-science reasoning approach, I strongly urge the FWS to withdraw the regulation proposal 50 CFR Part 17.

Finally, I hope that the FWS has not become political in its actions and believe that reverberations within the environmental science will be strong if that is in fact what has happened. I deeply appreciate being taken into account in the reconsideration of this regulation and look forward to future constructive work by the Fish and Wildlife Service.

Agency : FISH AND WILDLIFE SERVICE

Title : Endangered and Threatened Wildlife and Plants; 12-Month Finding on a Petition To Delist the Prebles Meadow Jumping Mouse (*Zapus hudsonius preblei*) and Proposed Delisting of the Prebles Meadow Jumping Mouse

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Please note your REGULATIONS.GOV number.

Regulations.gov #: EREG - 1 Submitted Feb 02, 2005

Author : Ms. Maria DeLeon

Organization : n/a

Mailing Address :

Attached Files :

Comment :
Field Supervisor,

I would like to ask that you do not delist the Prebles Meadow Jumping Mouse. After reading the information provided, it seems clear that removing the mouse from federal protection will allow greater degradation of its habitat and will likely lead to its disappearance. Moreover, the majority of the objective, expert peer reviews opposed delisting the mouse. I would ask that you follow the advice of the experts and not contribute to the continuing degradation of the environment in the United States and the resulting endangerment and disappearance of animal species. Please continue to protect this mouse.

Thank you for your time,
Maria



Miranda Mockrin
<mhm2004@columbia.edu>
02/05/2005 05:43 PM

To FW6_PMJM@fws.gov
cc
bcc
Subject [FR Doc: 05-02020];[Page 5404-5411]; Endangered and
threatened species: Findings on petitions, etc.-- Prebles
meadow jumping mouse

Field Supervisor
Colorado Field Office
Ecological Services
755 Parfet Street, Suite 361,
Lakewood, Colorado 80215

To Whom it May Concern:

I am writing to express my concern for the conservation of the soon-to-be delisted population of Prebles meadow jumping mice. I believe one unpublished study is not sufficient evidence to establish that the Prebles meadow jumping mouse is genetically homogenous to other populations of meadow jumping mice. Without submitting this work to wide peer-review and allowing scientific consensus to build, I think the federal government should provide continued environmental protection for this population of meadow jumping mice. Even if the species is delisted, I think the federal government should fulfill its requirements for a five year monitoring period, although this species may have been listed in error. A five year study of the species' distribution and abundance after delisting would provide valuable information about the fate of such "mistakenly" listed species after delisting, and will allow the wider US population to judge if the Colorado state and county efforts will be sufficient to conserve this population after delisting.

Sincerely,

Miranda Mockrin

Graduate Student
Department of Ecology, Evolution, and Environmental Biology
Columbia University



jean public
<jeanpublic@yahoo.com>
02/14/2005 02:40 PM

To FW6_PMJM@fws.gov
cc
bcc
Subject public comment on federal register of 2/2/05 vol 70 no 21 pg
5404

usdoi usfws 50 cfr part 17
rin 1018 au 12
delist prebles meadow

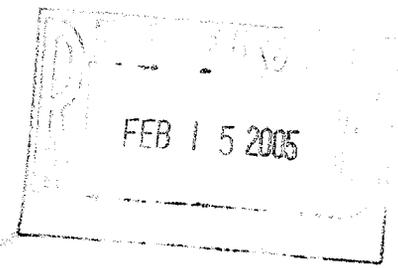
i oppose and object to delisting of this mouse. this
agency seeks to avoid protecting the environment and
allows it to be destroyed continually.

comment on page 7 - the reference to "peer reviewers"
- sometimes peer reviewers are all in the same "club"
so that you get absolutely no independence at all in
judgment. favors are traded back and forth and the
wildlife is the loser. Did that happen here? Do we
have truly independent per reviewers who do not rely
on this govt agency for their bread and butter and who
can make judgements for wildlife?

b. sachau
15 elm st
florham park nj 07932

Do you Yahoo!?
Yahoo! Mail - 250MB free storage. Do more. Manage less.
http://info.mail.yahoo.com/mail_250

**ROBERT B. HOFF
535 PASEO SOLAZ
GREEN VALLEY, AZ 85614
(520) 399-4344**



Feb. 10, 2005

Field Supervisor
US Fish & Wildlife Service
Colorado Field Office
Ecological Services
755 Parfet Street, Ste. 361
Lakewood, Colorado 80215

RE: Delisting of the Preble's Meadow Jumping Mouse

On the basis that one who complains of a given situation should offer solutions as well as criticism, I wish to expand on my comment of Jan. 31st.

The science involved in the listing was almost non-existent. The Service had no knowledge of past or present populations, had no goal for future populations and ignored the statements of a half-dozen or so biologists who warned that little was known of the Preble's. In order to avoid future action without information, I submit that any future listing protocol should include the following:

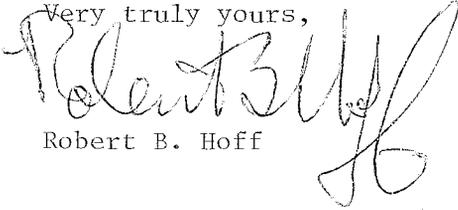
Provide an historic population estimate and a current population estimate. Explain why the current population is inadequate to prevent extinction. Describe the rate of decline of that population. Provide a minimum viable population estimate for the species. Provide a critical habitat plan and an economic analysis at the time of listing. Provide a realistic recovery plan with a realistic timetable. Provide sources and methodology for all of the above. Provide independent peer review prior to listing. (In the Preble's listing, some of the same people who did the studies also did the peer review. This is not independence.) Provide DNA testing for all sub-species prior to listing.

The Service has prosecuted, fined and jailed individuals for violating the Endangered Species Act. The Service, however, does not follow the law itself, presuming, one supposes, that there is one law for the citizen and another for the Service. Specifically, the Service routinely fails to respond to petitions to list or to delist with the prescribed time periods. The Service routinely fails to designate habitat and perform an economic analysis at the time of listing. The Service routinely fails to perform the mandatory five year reviews required by the Act. Congress must mandate that the Service follow the law.

One of the major causes of difficulty with the ESA is the taking of the use and value of private land without just compensation. This can be addressed in several ways. One, of course, is to pay the landowner for the land taken for habitat. Another can be the provision for a tax credit to the landowner commensurate with the value of the land taken. This would require Federal legislation. A third method would be for the local authority to provide a density bonus to a builder or developer who wishes to purchase a property subject to ESA restrictions. Such a bonus would prevent the down-valuing of the landowner's property.

What does not work is the present Act. It is not effective in recovering species, it is enormously costly to landowners and to state and local governments and it causes great distrust and disrespect for the Service and for the Federal Government as a whole. These changes should cut the volume of litigation down very sharply and will allow Service funds to be spent where they should be, in the protection of truly endangered or threatened species.

Very truly yours,

A handwritten signature in cursive script, appearing to read "Robert B. Hoff". The signature is written in dark ink and is positioned to the right of the typed name.

Robert B. Hoff

cc: Sen. Allard
Sen. Salazar
Rep. Hefley
Rep. Pombo
Mtn. States Legal
file



"Chris Massey"
<cmassey@mountainstatesle
gal.com>

02/16/2005 12:39 PM

To <FW6_PMJM@fws.gov>

cc

bcc

Subject Comments

Please find attached comments regarding the Proposed Delisting of the Preble's Meadow Jumping Mouse.

Thank you,

Christopher T. Massey
Staff Attorney
Mountain States Legal Foundation
2596 South Lewis Way
Lakewood, CO 80227
Tel: 303-292-2021, ext. 20
Fax: 303-292-1980



www.mountainstateslegal.com Proposed Delist.1.doc

February 16, 2005

VIA ELECTRONIC MAIL

Field Supervisor
Colorado Ecological Services Field Office
U.S. Fish and Wildlife Service
755 Parfat Street, Suite 361
Lakewood, Colorado 80215

Re: *12-Month Finding on a Petition to Delist the Preble's Meadow Jumping Mouse (*Zapus hudsonius preblei*) and Proposed Delisting of the Preble's Meadow Jumping Mouse*

To Whom It May Concern:

Mountain States Legal Foundation ("MSLF") respectfully submits the following comments on the *12-Month Finding on a Petition to Delist the Preble's Meadow Jumping Mouse (*Zapus hudsonius preblei*) and Proposed Delisting of the Preble's Meadow Jumping Mouse (70 Fed. Reg. 5404, February 2, 2005)*.

Introductory Remarks

MSLF is a non-profit, public interest legal foundation organized under the laws of the State of Colorado. MSLF is dedicated to the defense and preservation of individual liberty, the right to own and use property, limited and ethical government, and the free enterprise system. Many of MSLF's members reside or do business within areas affected by the listing of the purported Preble's Meadow Jumping Mouse (*Zapus hudsonius preblei*) as "threatened" under the Endangered Species Act ("ESA") and the subsequent designation of critical habitat for the rodent in Colorado and Wyoming. Many of these members own significant portions of land within the designated areas. Many of these members have used, and seek to continue to use, this land for agricultural, recreational, and residential purposes. Accordingly, these members strongly support the delisting of the Preble's Meadow Jumping Mouse under the ESA and, consequently, the removal of any critical habitat designation for the Preble's mouse in Colorado and Wyoming.

Delisting

Under the ESA, an endangered species is "any species [of animal] in danger of extinction throughout all or a significant portion of its range," as determined by the Secretary of the Interior. 16 U.S.C. § 1532(6). Threatened species are "any species which is likely to become an endangered species within the foreseeable future throughout all or a significant portion of its range." *Id.* § 1532(20). The U.S. Fish and Wildlife Service ("FWS") has concluded that the term species, including subspecies, should be

applied, “according to the best biological knowledge and understanding of evolution, specialization, and genetics.” 61 Fed. Reg. 4707 (January 18, 2001). The ESA further requires the Secretary to “make determinations” regarding the listing of species “solely on the basis of the best scientific and commercial data available.” 16 U.S.C. § 1533(b)(1)(A). The species of rodent now in question, the purported Preble’s Meadow Jumping Mouse, is not, and has never been, likely to become an endangered species in the foreseeable future in any part of Colorado or Wyoming. Indeed, the best scientific and commercial evidence available at the time of the original listing of the Preble’s mouse as “threatened” under the ESA in 1998 supported this conclusion.

However, the FWS ignored the language and requirements of the ESA in originally listing the rodent as “threatened.” The FWS failed to acquire the best available data and never conducted a scientifically competent review of the status of the species. Such a review, at a minimum, should involve a rudimentary compilation of the available trapping data. In public meetings regarding proposed “recovery plans” for the rodent, FWS officials most closely involved with the species made it abundantly clear that the available data on the species had never been compiled for analysis, not even after the species was originally listed. Since it has never been compiled, no conclusions regarding that data should have been possible and the initial listing should never have occurred. The administrative record of the listing supports this view; it is devoid of any indication that the FWS conducted a legitimate review of the status of the species and considered only the best available scientific and commercial data. On the contrary, better data and better science were not only “available” but also were in the possession of the FWS at the time it originally listed the species.

In fact, sound science available in 1998 indicated the purported Preble’s Meadow Jumping Mouse may not even be genetically distinct from other, abundant species of mice. Even the data upon which the FWS relied strongly indicated that the species was not threatened, or that the status of the species was indeterminate, or that the species does not exist throughout large areas now proposed as critical habitat.

For example, the FWS commissioned a study in the early 1990’s from contractors Stephen Compton and Roy Hugie. That study purportedly was instrumental in listing the species. In a memorandum to the FWS dated June 5, 1992, however, Compton and Hugie themselves declared that they were not sure they would be able to complete the study as proposed because they had no reliable method of distinguishing the Preble’s Meadow Jumping Mouse from other subspecies of mouse in the field, especially in Wyoming. In fact, they called their own anticipated field identifications “highly questionable,” despite that by their own account, “positive field identification . . . is an essential prerequisite for determining [the] current status of PMJM . . .” (emphasis by Compton and Hugie). Further, Compton and Hugie pointed out “little information is available concerning the traditional range of the PMJM in Wyoming [and] [t]herefore, changes or threats to PMJM status will be difficult or impossible to determine.” Compton and Hugie informed the FWS that because they and the FWS had and could obtain zero reliable baseline data on the species, the FWS could not possibly reach a defensible conclusion as to whether population was growing, static, or declining. The FWS was also on notice that they were looking for the species where it could not be

found and that, therefore, regulating land use in those areas was a wasted effort. The Wyoming Fish and Game Department, by letter to the Service dated May 9, 1995, pointed out:

surveys in Wyoming . . . may not be particularly useful because we were unable to locate *Zapus hudsonius preblei* in 2,500 trap nights . . . and were also unable to locate habitat we considered highly suitable to this species . . .

Professor David Armstrong of the University of Colorado added, in a letter to the FWS dated July 22, 1997, that the “best available” science relies on primary sources, but the FWS relied on secondary sources. Further, Professor Armstrong highlighted that the FWS relied heavily on data specific to the Western Meadow Jumping Mouse, which cannot be assumed to be accurate for the purported Preble’s Meadow Jumping Mouse.

Accordingly, the “best available science” that the FWS relied upon in originally listing the Preble’s Meadow Jumping Mouse strongly supports the conclusions of Ramey *et al.* (2003) and (2004) that Preble’s is not a discrete taxonomic entity, does not meet the definition of a subspecies, and was originally listed in error. MSLF and its members strongly support the delisting of the Preble’s Meadow Jumping Mouse under the ESA and, consequently, the removal of any critical habitat designation for the Preble’s mouse in Colorado and Wyoming.

Distinct Population Segment

A complete and thorough review of the current “best available science” further provides that the *Z. h. preblei* (Preble’s Meadow Jumping Mouse) portion of *Z. h. campestris* does not qualify as a Distinct Population Segment (“DPS”) in need of protection under the ESA. The discreteness of the population segment of *preblei* throughout Colorado and Wyoming in relation to the remainder of *campestris* to which it belongs is not markedly significant. The populations of both have not been found to be markedly separated as a consequence of physical, physiological, ecological, or behavioral factors. Furthermore, the conservation status of the population segment of either *preblei* or *campestris* is not threatened or endangered. Conservative estimates available suggest that in excess of 60,000 purported Preble’s mice inhabit Colorado and Wyoming. This number alone far exceeds the 20,000 mouse population requirement to prevent extinction and the 32,500 mouse population purportedly within designated critical habitat – both figures established and relied upon by the FWS.

Moreover, the FWS must follow the accepted guidelines to ensure that any listing decision under the ESA is made upon the “best scientific and commercial data available.” 59 Fed. Reg. 34271 (July 1, 1994). These guidelines direct the FWS to:

- 1) Require the evaluation of all scientific information used in making a listing decision;
- 2) Gather and impartially evaluate the biological, ecological, and any other information that is contrary to the official position taken by the FWS;

- 3) Ensure that the evaluation of all information supporting or contrary to any position proposed by the FWS is documented;
- 4) Use primary and original sources of information as the basis for listing decisions or recommendations;
- 5) Adhere to the time frames established in the ESA for listing decisions; and,
- 6) Conduct management level review of any documentation developed by the FWS to verify and assure the quality of the science used in the establishment of official agency positions.

Accordingly, MSLF and its members believe the current “best available science” concludes that the *Z. h. preblei* (Preble’s Meadow Jumping Mouse) portion of *Z. h. campestris* does not qualify as a Distinct Population Segment (“DPS”) in need of protection under the ESA.

Respectfully Submitted By:

MOUNTAIN STATES LEGAL FOUNDATION

Christopher T. Massey
Staff Attorney
2596 South Lewis Way
Lakewood, Colorado 80227
303-292-2021
303-292-1980 facsimile



Seth Willey/R6/FWS/DOI
04/08/2005 11:27 AM

To Peter Plage/R6/FWS/DOI@FWS, Mary E
Jennings/R6/FWS/DOI@FWS
cc Andrew.Martin-1@colorado.edu

bcc

Subject Preble's MJM public comment - Andrew Martin

Pete,

We should consider this public comments for the Preble's mouse rulemaking.

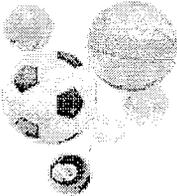
Seth

Seth L. Willey
Ecological Services, ESA Listing & Delisting
US FWS Region 6, Denver, CO
Seth_Willey@fws.gov
303-236-4257 (Fx) 303-236-0027

How you gonna have any fun in this life if you're
always doing what lawyers tell you to do?

-- Dr. Science

----- Forwarded by Seth Willey/R6/FWS/DOI on 04/08/2005 11:22 AM -----



Jean Clemens/R6/FWS/DOI

04/08/2005 10:50 AM

To Seth Willey/R6/FWS/DOI@FWS

cc

Subject Fw: How and where to submit a public comment

Hi Seth, This is for your response. Thanks for your help.

Jean Clemens

U.S. Fish & Wildlife Service
Region 6 * External Affairs
(303)236-7905



----- Forwarded by Jean Clemens/R6/FWS/DOI on 04/08/2005 10:53 AM -----



Andrew.Martin-1@colorado.e

du

04/08/2005 10:45 AM

To Jean_Clemens@fws.gov

cc

Subject Re: How and where to submit a public comment

I have re-analyzed Ramey's data using the same analytical approach that he used

in his most recent report and have summarized the results and described why his conclusions are wrong. I intend to submit a second comment regarding the limitations of his morphometric analysis. Thanks for considering my comment.

Regards,

Andrew Martin
Dept of Ecology and Evolutionary Biology
University of Colorado

Quoting Jean_Clemens@fws.gov:

>
>
>
>
> Mr. Martin,
>
> Send me your comment and I will forward it on to the proper program.
>
>
>
>
> Andrew.Martin-1@c
> olorado.edu To:
> MountainPrairie@fws.gov
>
> 04/05/2005 09:26 cc:
> submit a public comment Subject: How and where to
> AM
>
>
>
>
>
> Hi
> I'd like to submit a comment on the delisting of Preble's meadow jumping
> mouse
> based on an analysis of the genetic data. What is the best way to do this?
>
> Andrew Martin
> University of Colorado
>
>
>



PreblesDelistResponse.pdf

Response to petition to de-list Preble's meadow jumping mouse

The issue of how we pursue the science of delineating species has important and real implications beyond debates about the reality of species. An endangered species carries significant legal protection often times with profound economic ramifications. Consider the case of the Preble's meadow jumping mouse, *Zapus hudsonius preblei*. Currently, this taxon is recognized as a distinct subspecies and is afforded protection under the ESA in part because its range overlaps with the spread of humans along the riparian corridors of the Front Range of Colorado. Based on genetic data, the closest related subspecies to the Preble's mouse is the Bear Lodge meadow jumping mouse, *Zapus hudsonius campestris*.

Recently, Ramey et al. sequenced a small region of the mitochondrial genome, referred to as the control region because the particular sequence is involved in the regulation of the replication and expression of the mitochondrial genome, for a collection of mice that included several subspecies of *Zapus*, including Preble's and Bear Lodge meadow jumping mice. (The control region turns out to be a convenient molecular marker for studying genetic relationships. Indeed, the idea that all human beings are closely related and derived from African ancestry stems from analyses of control region sequences.) Based on the similarity of sequence between individuals of the two subspecies, the scientists advocated that the Preble's and Bear Lodge meadow jumping mice be considered a single subspecies.

How were they able to arrive at such a "clean" and certain conclusion? Ramey et al. wrote "We used the scientific method to provide an objective test of the genetic distinctiveness of the Preble's meadow jumping mouse. Using hypotheses laid out in advance of data collection, we used the criteria of Ball and Avise (1992) and Moritz (1994) to test the taxonomic uniqueness of *Z. h. preblei* relative to other subspecies of *Z. hudsonius*. These authors were the first to provide a conceptual basis for recognizing subspecies (which are generally equated with evolutionary significant units or ESUs) that has both an evolutionary and quantitative basis. Ball and Avise (1992), and Moritz (1994) provided the following criteria for recognizing subspecies or ESU's: the subspecies or ESU must represent a major division in the diversity of the gene pool of a species based on concordant distributions of multiple genetically-based traits; it must have a plausible evolutionary mechanism for differentiation, and it must be on separate mitochondrial DNA lineages (reciprocal monophyly). The criteria of reciprocal monophyly for mitochondrial DNA requires that subspecies be separated long enough (e.g. generations since separation = 2 times the effective population size) for them to be on separate evolutionary pathways. While strict reciprocal monophyly is a clear-cut standard, it may be refuted if additional sampling reveals even one shared mitochondrial DNA type among subspecies. We prefer a less restrictive standard, specifically, there must be greater diversity among putative subspecies than within them. We previously used the approach outlined above in taxonomic revision of wild sheep (Ramey 1995, Wehausen and Ramey 2000, Tserenbatta et al. in press)."

Later, however, Ramey et al. wrote, in their description of the results from genetic analyses, that "...*Z. h. preblei* and *Z. h. campestris* showed low, but nonzero, levels of

very recent gene flow (m and M) (Table 2). 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 non-zero, therefore the null hypothesis of no very recent gene flow can also be rejected for these putative subspecies.”

My understanding of the scientific method and hypothesis testing is that the hypothesis is a statement about nature. In this case, the appropriate null hypothesis is that the two subspecies of *Zapus* (namely *Z. h. preblei* and *Z. h. campestris*) comprise a single taxonomic entity. A prediction of this hypothesis is that the two subspecies should exhibit little, if any, genetic differentiation. In terms of gene flow, we should expect extensive (homogenizing) gene flow between the two subspecies.

In formulating the correct null hypothesis, we can adopt published approaches for the expected predictions. Ramey et al. argued that Moritz’s criterion of reciprocal monophyly is appropriate; however, publications following Moritz’s Trends in Ecology and Evolution paper in 1994 showed that his criterion was overly restrictive, and Moritz, in a second 1994 paper, backed off and suggested that an appropriate prediction of the alternative hypothesis (namely that there are two distinct taxonomically-recognizable entities) is “...significant, but not necessarily absolute, phylogenetic separation of alleles between populations.” Moreover, Moritz (1994b) stressed that “...it is important to seek corroborating evidence from nuclear loci.” Thus, the prediction of the null hypothesis is that there are not significant differences in allele frequencies between subspecies, where significance is assessed using a randomization procedure. As Ramey et al. noted, however, “...there must be greater diversity among putative subspecies than within them.” This prediction is unconventional and will, in many cases, be overly restrictive in the same way that reciprocal monophyly is overly restrictive.

The crux, I think, of Ramey et al.’s “scientific method” is the postulate that subspecies should not show evidence of recent gene flow. Is the proposition that distinct subspecies do not engage in gene flow reasonable, and more to the point, do distinct subspecies exchange genes? Few studies of such phenomenon for *subspecies* have been carried out, although most experts, including O’Brien and Mayr (1991) recognize that gene flow can and does occur between subspecies. Studies of distinct *species* indicate that gene flow can occur between species, albeit rarely. Gene flow has been documented between species in nature for cichlid fishes (Hey et al. 2004), old world mice (Payseur et al. 2004), elephants (Roca et al. 2005) sunflowers (Reiseberg et al. 1999), trout (Young et al. 2001), *Drosophila* (Machado and Hey 2003) and a long list of other taxa. Moreover, species’ boundaries can be porous for some genes and impenetrable to others (Payseur et al. 2004). Mitochondrial genes fall into the group of genes that readily flow across species’ boundaries (Duvernell and Aspinwall 2001). Given that species are not impenetrable, discrete, independent units, we shouldn’t expect subspecies to be genetically isolated; in fact, we should expect some level of gene flow between subspecies. As O’Brien and Mayr noted (1991: 1187): “that is why they (subspecies) are not species.”

Given the tremendous difficulty and uncertainty of defining species, the best we can do as scientists without complete information about the nature of biological variation of

species or subspecies is to construct a null hypothesis that rests on a defensible understanding of nature. Rather than assume we can adequately define species' boundaries, we can ask whether some set of individuals that group into two subspecies comprise a single gene pool. Such a hypothesis makes several testable predictions. First, if all individuals are part of the same gene pool, then estimates of the timing of divergence between two subspecies will be zero, implying, of course, that there is only one population. Second, an analysis of variation will show insignificant differences between the two subspecies; in other words, the same genes are present in the two subspecies as a consequence of *significant* gene flow between the subspecies.

Using the same analytical tool employed by Ramey et al. (namely MDIV analysis [Nielsen and Wakeley 2001]) we can first ask what results that match the predictions of the null hypothesis look like. MDIV produces posterior probabilities distributions for two parameter values that describe the data: the time that two populations diverged in the past (in N_e generations, where N_e is the effective population size), and the number of migrants moving between the two populations each generation. Parameter values with the highest probability provide the best explanation of the data. An example of the first prediction of the null hypothesis is shown in figure 1 (left panel). In this particular case, the highest probabilities are for values of divergence time, T , that are near zero, indicating that the two populations are NOT divergent. Similarly, estimates of the number of migrants per generation for the same two populations (Figure 1, right panel) show that higher values have higher probabilities, corresponding to a view in which these two populations regularly exchange genes.

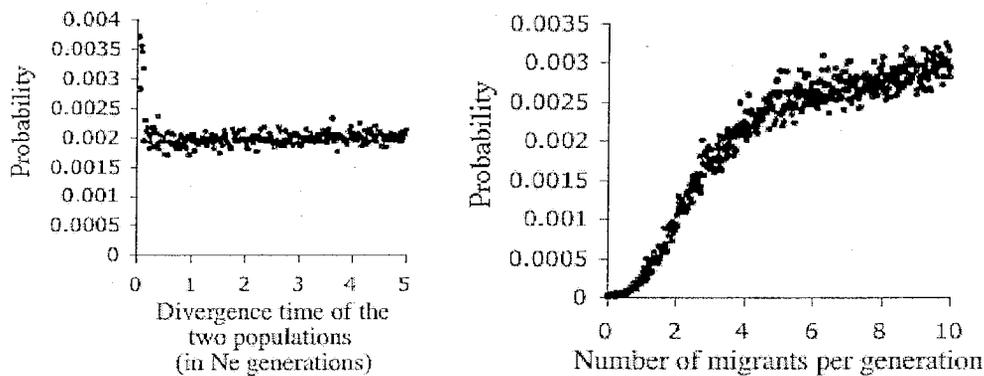


Figure 1. Estimates of divergence time and levels of gene flow between two geographically remote populations of fish (Martin, unpubl. data) illustrating an example in which the two populations are not genetically different (despite differences in the frequencies of different alleles in the two populations). *Left.* Posterior probability distribution of divergence times between two populations derived from MDIV analysis of the data. Each point represents a probability of a particular divergence time (in N_e generations). Note that the highest probabilities are near zero, indicating that the two putative populations have not diverged. This probability distribution is typical of situations in which the two samples of individuals (i.e. the two putative populations) comprise a single, panmictic gene pool. *Right.* Posterior probability distribution for the number of migrants between the two populations each generation indicating significant gene flow ($M > 10$).

The picture painted by figure 1 is one of two samples of individuals that can be considered, for all practical purposes, a single population. The divergence time is

indistinguishable from zero and gene flow is extensive. In terms of our hypothesis, we are unable to refute the null; namely, that two arbitrary samples of individuals are drawn from the same population. If these two populations were distinct subspecies (based on some criterion), then such results provide compelling evidence for taxonomic revision and the synonymization of the two subspecies because we could not refute the null hypothesis.

What do the data look like for the two focal subspecies of meadow jumping mice? In contrast to the expectations of the null hypothesis, we can reject that the two subspecies have a divergence time of zero—indeed, the two subspecies appeared to have diverged at least $2N_e$ generations ago (Figure 2, left panel). Second, gene flow is limited; the highest probability (i.e. the most likely estimate of the number of migrant per generation) suggests fewer than 1 individual per generation moves between the two subspecies (Figure 2, right panel). Moreover, perusal of the gene tree indicates that gene flow is unidirectional—from *Z. h. preblei* into *Z. h. campestris* (see original reports by Ramey et al.). (Note this is contrary to one of the reviewers [Jack Sites] interpretation of the data. The observation is that one or two *Z. h. campestris* individuals have a mitochondrial type that is common in *Z. h. preblei* and very different from other *Z. h. campestris* types. The only explanation for this is that a *Z. h. preblei* type flowed into *Z. h. campestris*.) If hybridization occurs between the two species, such unidirectional gene flow suggests that there may be barriers to reproduction, perhaps because a cross between a *Z. h. campestris* female and a *Z. h. preblei* male may fail due to some sort of pre- or postzygotic isolating mechanisms. Without additional information, it is impossible to evaluate the significant and extent of possible hybridization events.

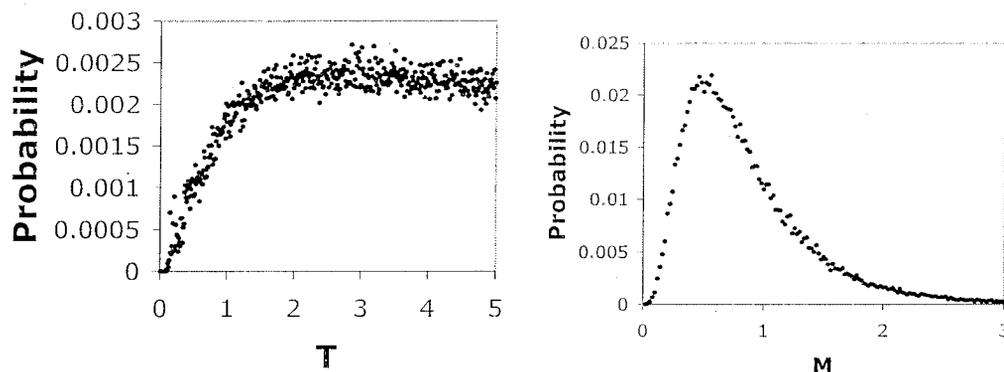


Figure 2. Left. Posterior probability distribution of estimated divergence time, T , between the two subspecies of meadow jumping mouse. Note that the highest probabilities are substantially greater than zero, indicating two distinct and divergent populations exist. Right. Posterior probability distribution for the number of migrants per generation, M , between the two species showing that the most probable value, M , is less than 1.0, suggesting very limited gene flow between the subspecies.

Thus, for these two subspecies of *Zapus* we can refute the null hypothesis that the two subspecies are part of the same underlying distribution of variation. We are left with the hypothesis that the two subspecies comprise significantly different assemblages of

genetic variation. Importantly, such an analysis does not refute or support the contention that there are two distinct subspecies in nature because such an assertion requires detailed information about the biology of the two subspecies, including aspects of reproduction, mate choice, genome characteristics, ecology, physiological performance under different conditions, etc. Such information is unavailable and difficult to obtain.

Differences in interpretation of genetic data between Ramey et al and myself are not dependent on the particular analysis employed—we both used the same analytical tools. I specifically adopted the same analytical approach as Ramey et al. to show that the difference in conclusions stems from how we set up the hypotheses for testing. Ramey et al. adopted an unconventional null hypothesis in which they explicitly define subspecies as groups of individuals that do NOT exchange genes with related groups of individuals. Having done so, their finding of gene flow and their inability to reject their null hypothesis makes dissolution of subspecies axiomatic. However, when the conventional approach is adopted—the null hypothesis is that the sampled individuals come from the same underlying distribution—we are left with the current state of affairs as the best explanation of the data; namely, that recognition of distinct subspecies is a better explanation of the genetic data than to synonymize the subspecies.

Such differences in scientific approach can have dramatic consequences. Ramey et al.'s conclusion precipitated a petition, filed by the State of Wyoming and the political lobby group Coloradans for Water Conservation and Development to lift Endangered Species Act (ESA) protection for the Preble's meadow jumping mouse (January 28th news release). Removal of protection would open up valuable riparian habitat for development—primarily conversion to housing subdivisions suitable for human and domestic animal habitation. Interestingly, the USFWS service noted that the decision to go ahead with the delisting process stemmed from peer reviews of the work in which a slight majority agreed with genetic analysis. Science as a way of knowing about nature does not proceed by a majority vote. The foundation of science is robust tests of appropriate hypotheses. Now, with the appropriate hypothesis tested, taking a vote is moot: the subspecies *Zapus hudsonius preblei*, a taxonomic designation that is NOT REFUTED by the genetic data, remains threatened with extinction and should continue to receive protection under the ESA.

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"Hallerman, Eric"
<ehallerm@vt.edu>
04/28/2005 11:09 AM

To <FW6_PMJM@fws.gov>
<Peter_Plage@fws.gov>, "Devers, Patrick"
cc <pdevers@vt.edu>, "PAUL GROBLER" <paulg@ul.ac.za>,
"Hallerman, Eric" <ehallerm@vt.edu>, "Nathaniel P. Hitt"
bcc
Subject

Department of Fisheries and Wildlife Sciences
Virginia Polytechnic Institute and State University
Blacksburg, VA 24061-0321

April 28, 2005

Susan Linner, Field Supervisor, Colorado Field Office
U.S. Fish and Wildlife Service, Ecological Services
755 Parfet Street, Suite 361
Lakewood, CO 80215

Dear Ms. Linner,

We write this letter to present our evaluation of the 2004 manuscript by R. Ramey, H.-P. Liu, and L. Carpenter, "Testing the validity of Preble's meadow jumping mouse (*Zapus hudsonius preblei*)". We are concerned that the authors reach conclusions that are not justified by the narrow scope of the study and by the data. We argue that a broader scope of work and a more circumspect analysis are needed in order to reach defensible conclusions.

Before presenting our critique, we feel it appropriate to present our qualifications for presenting our review. We are collectively affiliated with a leading department of fisheries and wildlife sciences. We include two mid-career population geneticists, a post-doctoral fellow, and a graduate student with background in ecology and population and molecular genetics. We read and discussed the manuscript at length before framing the critiques and recommendations below. We offer criticisms of the molecular genetic analysis, choice of criteria for reaching phylogenetic inferences from the data, lack of thorough consideration of hybridization, and aspects of hypothesis testing. We also note that the manuscript contains many vague and unsupported assertions, as well as grammatical and spelling errors. The discussion contains editorialization not strictly pertinent to the biological determination at hand, and inappropriately calls into question other listing determinations, many or most of which included hypothesis testing and peer review.

Molecular Genetic Analysis

We have two criticisms of the molecular genetic analysis, namely: (1) the length of mitochondrial DNA sequence was short (355 base-pairs [bp] of the control region), and may not have contained enough genetic information to delineate the two presumably closely related subspecies of meadow jumping mouse *Zapus hudsonius campestris* and *Zapus hudsonius preblei*, and (2) no nuclear genetic markers, e.g., DNA microsatellites or genic DNA sequences, were analyzed to corroborate results from the mitochondrial genome. These deficiencies are significant, especially considering that the data, analyses and conclusions reported in this study are being used by the U.S. Fish and Wildlife Service (USFWS) to delist a federally endangered subspecies. Our major concern is that the standards of molecular genetic evidence used to delineate these two taxa are unacceptably low, and will set precedence for future taxonomic studies of a similar kind. It is important that USFWS and other management agencies realize that many taxonomic studies have been conducted upon other organisms that apply higher standards of genetic evidence. For example, Roca et al. (2001) used 1732 bp from four nuclear DNA genes to separate African forest elephants from savannah elephants as separate species, Culver et al. (2000) used 891 bp of mitochondrial DNA and 10 DNA microsatellites to collapse 15 historically recognized subspecies of puma into six subspecies, and Jones et al. (2005) used 1900 bp of combined mitochondrial and nuclear DNA sequences and 10 DNA microsatellites to distinguish populations of endangered freshwater mussels as either species or subspecies. In addition, the authors of these studies used geography, life history, behavior, and morphology as additional lines of evidence to corroborate their findings. Thus, various examples of comprehensive and holistic genetic studies are available in the literature, and should be used by the USFWS as standards for delineating federally endangered species.

The results of the phylogenetic analysis by Ramey et al. (2004) show all individuals identified as *Z. h. preblei* grouping together in one distinct clade. This finding alone should signal to geneticists and managers that a real phylogenetic subdivision may exist between *Z. h. preblei* and *Z. h. campestris*, thereby triggering investigation of additional genetic markers. Yes, DNA sequences of some individuals of *Z. h. campestris* grouped with the *Z. h. preblei* clade. However, because such a short length of mitochondrial DNA sequence was used to construct the phylogenetic tree, we caution decision-makers to not feel overly confident that the proposed genealogical relationships will hold when more DNA sequences are analyzed. Furthermore, incomplete sorting of genes in phylogenetic appraisals is well documented in various studies, including taxa that are clearly separate biological species (Avice 2000). For example, hundreds of cichlid fish species have been described from Lake Victoria in East Africa that are morphologically, behaviorally and ecologically distinct, but very difficult to distinguish genetically using DNA sequences. Therefore, if one looks closely at the phylogenetic tree produced by Ramey et al. (2004), one will see that a few of the *Z. h. campestris* DNA sequence haplotypes [L/PAL/C-1:SD(2); L/PAL/C-2:SD(1), WY(1)] group with clades comprising *Z. h. luteus* and *Z. h. pallidus*, two subspecies that clearly are diverged genetically from the clade containing *Z. h. campestris* and *Z. h. preblei*.

Hence, a very plausible alternative hypothesis is that the ecological, geographical, and demographic history of *Z. h. campestris* has allowed that population to retain a large proportion of the ancestral genetic variation of the common ancestor of the *Zapus hudsonius* subspecies

complex. This hypothesis is supported by at least two lines of evidence, including: (1) the observation that the population of *Z. h. campestris* contained a high level of genetic variation – 16 haplotypes, the most observed among the subspecies investigated – for such a narrowly distributed subspecies, and (2) the population is centrally located geographically among other meadow jumping mouse species and subspecies, perhaps allowing more opportunities for hybridization and introgression to occur. Furthermore, based on the geographic data provided from the museum specimens used in the Ramey et al. (2004) study, it seems unlikely that *Z. h. campestris* and *Z. h. preblei* constitute a single, continuously distributed population. Specifically, the populations of these two subspecies appear disjunct, and seemingly little or no location data exist to suggest that they were historically sympatric. This distributional pattern should be another signal to managers that barriers to gene flow exist. We suggest that the Platte River and Laramie Mountains in southeastern Wyoming likely provide formidable barriers to dispersal between populations of these two subspecies.

To resolve the taxonomic uncertainty between populations of *Z. h. campestris* and *Z. h. preblei* at the molecular genetic level, we suggest: (1) sequencing additional regions of mitochondrial DNA (e.g., *cytochrome-b* and *16S*) to achieve a total analyzed sequence length of over 1000 bp, and (2) analyzing approximately 10 or more DNA microsatellite loci to test for levels of gene flow. Ramey et al. (2004) state on page 5 of their report that if the Preble's meadow jumping mouse cannot be distinguished on the basis of mitochondrial DNA, then it will be unlikely that it will be differentiated for nuclear microsatellite DNA. This statement is misinformed and misleading. Most geneticists who use DNA microsatellites will attest that these markers generally have much higher mutation rates than mitochondrial DNA, and therefore, are more likely to differentiate closely related taxa (Balloux and Lugon-Moulin 2002).

Criteria for determination of taxonomic groups

A basic problem in the Ramey et al. (2004) study concerns the application of ecological and genetic criteria for the delineation of taxonomic groups. We find it troubling that the authors acknowledge problems associated with Moritz's (1994) reciprocal monophyly criterion, but then proceed to base their conclusions largely on the basis of this criterion. By choosing Moritz's (1994) criterion, the authors increased the likelihood that they would find "no differences" between subspecies. This bias is compounded as a result of the relatively low number of mtDNA base pairs examined. We further note that the authors did not consider other definitions of evolutionarily significant units (e.g., Waples 1991) that might be pertinent to the issue of taxonomic validation of *Z. h. preblei*. We, therefore, believe that the approach utilized establishes poor precedence for the delineation of taxonomic groups.

The authors cite a published paper by Connor and Shenk (2003) with a well-discussed and peer-reviewed protocol for assessing morphometric data and analysis of subspecies comparisons. The study by Connor and Shenk used 12 cranial measurements to distinguish subspecies designations, whereas the Ramey et al. study used only nine to conclude that this subspecies is

synonymous with *Z. h. campestris*. Why didn't the authors use this peer-reviewed prototype to compare morphometrics? Another concern regarding the conclusion based on their morphometric analysis is that there is no way of distinguishing these two putative subspecies. That being the case, then, what level of certainty can be placed on the identification of the museum specimens used in this study? Doesn't this study have to assume that the reported ranges of these two subspecies are valid and allopatric (see hybridization discussion below), as the authors' acceptance of reported identifications were based totally on where the specimens were collected and not on identifiable characters? With no collecting on their own to validate museum records and perhaps assess range boundaries, we feel that this analysis is insufficient as presented to adequately support their conclusion.

We agree with the authors that ecological information should be evaluated to complement genetic data. However, we disagree with their use of morphological traits in museum specimens to serve as surrogates for ecological information. As described in Crandall et al. (2000), it is essential to consider behavioral, life history, metapopulation dynamics, and habitat use information to complement genetics data. Ramey et al. (2004) failed to test their assumption that morphological traits serve as a surrogate for ecological distinctiveness. Without the test of this key underlying assumption, we remain unconvinced that their morphological measurements and analysis are diagnostic of taxonomic groups.

Hybridization

Ramey et al. (2004) dismiss the issue of hybridization without sufficient conceptual or analytical treatment. The authors state that *Z. h. preblei* hybridization with *Z. campestris* would exclude *Z. h. preblei* from conservation under the ESA. We agree with leading population genetic and phylogenetic authorities (O'Brien and Mayr 1991) that hybridization does not necessarily preclude listing of a subspecies under the ESA. Hybridization takes many forms, some of which are natural and some of which are anthropogenic in origin (Allendorf et al. 2001). The USFWS currently lacks a comprehensive policy for the treatment of hybridized populations under the ESA (Allendorf et al. 2004), so it is inappropriate for Ramey et al. (2004) to conclude that hybridization would preclude consideration. Conservation of *Z. h. preblei* would depend on the distribution, abundance, and connectivity of non-hybridized populations, which are not considered or evaluated by Ramey et al. Therefore, a categorical decision about the treatment of hybridization under the ESA is unsupported and inappropriate.

Hypothesis testing

The authors indicated they used a hypothesis-testing framework to evaluate the distinctiveness of *Z. h. preblei* based on genetic and ecological criteria as recommended by Crandall et al. (2000). Notably, no null hypothesis was presented; the null hypothesis might be stated as there is no evidence of genetic or ecological differences between subspecies. Despite the authors' assertion, they failed to present ecological evidence that either supported or rejected a null

hypothesis. The authors (page 5) also stated “We examined the literature for evidence of ecological differences between subspecies”. However, they did not provide detailed methods for the selection and evaluation of articles or support their assertion with even a single citation. Important issues were not discussed, including: Which articles were available for consideration and during what time period? Which ecological characteristics were evaluated and how were they evaluated? Literature reviews are commonly printed in peer-reviewed journals and can provide useful information on a variety of topics. However, lack of details or supporting citations is such that concluding “lack ... of ecological evidence for genetic distinctiveness of *Z. h. preblei* from *Z. h. camprestris* ...” must be considered an unsupported opinion. Rather, any lack of peer-reviewed research on *Z. h. preblei* ecology – including life-history characteristics, population dynamics and viability, and habitat selection – suggests that current knowledge is deficient and more research is needed before a final ruling can be made as to the population’s status as a subspecies or distinct population segment.

Use and interpretation of AMOVA

The authors use AMOVA as a measure of the distinctiveness of *Z. h. preblei*, and set the criterion that “*there must be greater diversity among putative subspecies than within them*”. The results showed that most of the genetic variation was within (64%) rather than between (37%) subspecies. The authors did not present a significance value for the AMOVA test. However, the authors claim that *Z. h. preblei* fails their test of genetic uniqueness. There is a significant shortcoming in this approach: The within-population component of total genetic diversity often exceeds the between-population component, even when recognized separate species are compared. This is expected, considering the close genetic relationship and evolutionary history of congeneric species. For example, using mtDNA data, Liebers et al. (2001) found that only 26.8% of diversity among gull populations resides between acknowledged species. From microsatellite data, Grobler et al. (2005) found that only 29.2% of variation among blue and black wildebeest populations occurs among species. It is thus extremely unlikely that an AMOVA-based analyses of subspecies would reveal more diversity between than within subspecies. The criterion used is therefore dubious, and the conclusion drawn from failure to meet this criterion is not valid.

Conclusion

Against this background of lacking methods and unsupported inferences, we find that the conclusions of the Ramey et al. (2004) manuscript do not support the downlisting of *Zapus hudsonius preblei*. We urge further study of phylogeny of the species using a wider array of molecular genetic markers, morphological characters, and life history traits. Because of our strong misgivings about the study at hand, we further urge that it not be regarded as setting precedent for how the conservation status of a taxon of interest would be evaluated. We believe that Ramey et al. (2004) does not incorporate the best available science for the task of taxonomic delineation.

We are pleased to have had the opportunity to offer our evaluation of this study. We are, of course, willing to provide further feedback to the agency if desired.

Sincerely,

Pat Devers
Post-doctoral fellow

Paul Grobler
Associate Professor

Eric Hallerman
Professor

Nathaniel Hitt
Doctoral candidate

Att: Culver et al. (2000)
Roca et al. (2001)

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Culver et al 2000.pdf



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REPORTS

crease in the total continental flux of Sr or a change in its isotopic composition, or both. The contribution of G-B to the global cycle

$$\left(\frac{d\alpha_{Sr-SW}}{dt}\right) = \left(\frac{J_{GB}}{N_{Sr}}\right) \cdot (\alpha_{Sr-GB} - \alpha_{Sr-SW}) \quad (2)$$

is equal to $0.82 \times 10^{-4} \text{ My}^{-1}$ for the low estimate of $^{87}\text{Sr}/^{86}\text{Sr}$ and $1.86 \times 10^{-4} \text{ My}^{-1}$ for the high estimate of $^{87}\text{Sr}/^{86}\text{Sr}$ in Table 2. This rate of change is a factor of ~ 2.3 to 5.3 higher than the observed average value of $d\alpha_{Sr-SW}/dt \sim 0.35 \times 10^{-4} \text{ My}^{-1}$ for the past 40 My.

We also note that use of a $^{87}\text{Sr}/^{86}\text{Sr}$ value of 0.711 for global river and continental flux creates an imbalance in the Sr cycle. To rectify this situation, we need to lower the continental flux isotopic composition to about 0.71049 [similar to the value proposed in (7)]. Also, the additional global continental Sr flux from groundwater would cause a rise in $^{87}\text{Sr}/^{86}\text{Sr}$ of 0.0095 over 40 My if left unbalanced. This is higher by a factor of 7 than the observed rise over the past 40 My.

Thus, we conclude that the groundwater data have an enormous effect on the interpretation of the seawater Sr isotope balance. Although we do not claim that the new values presented in Table 2 should be considered as final, these data urge caution about overinterpreting Sr isotope data from a few local watersheds in this area. For example, trying to use the seawater Sr isotope curve to infer the detailed tectonic uplift history of the Himalayas as well as for estimating effects on global climate change still involves considerable uncertainty. Because of the highly variable nature of $^{87}\text{Sr}/^{86}\text{Sr}$ in the G-B river system, reliable average values are difficult to estimate.

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charged water and the groundwater flowing beneath the river bottom (>30 m) do not discharge to the G-B rivers and their tributaries; only the groundwater from the shallow part of the aquifer (<20 m) will discharge to the G-B rivers and contribute to the base flow of the rivers. Thus, the groundwater flux to the oceans is estimated here on the basis of the average groundwater travel time below 30 m.

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Genetic Evidence for Two Species of Elephant in Africa

Alfred L. Roca,¹ Nicholas Georgiadis,² Jill Pecon-Slatery,¹ Stephen J. O'Brien^{1*}

Elephants from the tropical forests of Africa are morphologically distinct from savannah or bush elephants. Dart-biopsy samples from 195 free-ranging African elephants in 21 populations were examined for DNA sequence variation in four nuclear genes (1732 base pairs). Phylogenetic distinctions between African forest elephant and savannah elephant populations corresponded to 58% of the difference in the same genes between elephant genera *Loxodonta* (African) and *Elephas* (Asian). Large genetic distance, multiple genetically fixed nucleotide site differences, morphological and habitat distinctions, and extremely limited hybridization of gene flow between forest and savannah elephants support the recognition and conservation management of two African species: *Loxodonta africana* and *Loxodonta cyclotis*.

Conservation strategies for African elephants have consistently been based on the consensus that all belong to the single species *Loxodonta africana* (1-3). Yet relative to African savannah elephants, the elephants in Africa's tropical forests are smaller, with straighter and thinner tusks, rounded ears, and distinct skull morphology (2-11). Although forest elephants are sometimes assigned subspecific status and designated *L. a. cyclotis*, their degree of distinctiveness and of hybridization with savannah elephants has been controversial and often ignored (2-12). Recently, a comprehensive morphological comparison of metric skull measurement from 295 elephants (10, 11) and a provocative molecular report limited to a single individual (13) noted ap-

preciable distinctions between forest and savannah specimens.

Here we report the patterns and extent of sequence divergence for 1732 nucleotides from four nuclear genes (14) among 195 African elephants collected across their range in Africa and from seven Asian elephants (*Elephas maximus*). African elephants were sampled, with biopsy darts (15, 16), throughout the continent, including individuals from 21 populations in 11 of 37 African elephant range nations (Fig. 1). Based on morphology (2-11) and habitat (17, 18), three populations were categorized as African forest elephants, whereas 15 populations in southern, eastern, and north-central Africa were categorized as savannah elephants (Fig. 1). DNA sequences from four nuclear genes, including short exon segments (used to establish homology to mammalian genes) and longer introns (which would evolve rapidly enough to be phylogenetically informative), were determined for all elephants (19). The genes include *BGN* [646 base pairs (bp)], *CHRNA1* (655 bp),

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¹Laboratory of Genomic Diversity, National Cancer Institute, Frederick, MD 21702, USA. ²Mpala Research Center, Post Office Box 555, Nanyuki, Kenya.

*To whom correspondence should be addressed. E-mail: obrien@ncifcrf.gov

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GBA (100 bp), and *VIM* (331 bp), with sequence from all four genes obtained for 119 individuals. An alignment of variable sites and the composite genotypes are presented in supplemental information (20). Among 1732 bp, 73 sites were variable and 52 were phylogenetically informative. These nucleotide variants defined nine unique savannah genotypes among 58 individuals and 24 unique forest genotypes among 24 individuals. We observed nine genetically fixed nucleotide site differences between Asian and African elephants (*BGN* 121, 155, 219, and 513 and *CHRNAI* 011, 079, 274, 301, and 548) and one that approaches fixation (*BGN* 505). There were five fixed site differences between African savannah and forest elephants (*BGN* 304, 485, 508, 514, and 569) and two that were nearly fixed (*CHRNAI* 251 and *GBA* 20) (20).

Three methods of phylogenetic analysis (minimum evolution, maximum parsimony, and maximum likelihood) (21–23) revealed a

concordant deep genetic division between the forest and savannah populations of African elephants (Fig 2). The forest elephants of Dzanga-Sangha, Lope, and Odzala grouped together, separate from 15 savannah populations, which formed a distinct phylogenetic clade or lineage. An estimated 94% of the observed genetic variation ($F_{ST} = 0.94$, $P < 10^{-5}$) (24, 25) was due to differences between forest and savannah elephants and 6% to intragroup differences. Mantel tests (26) revealed only marginal association of genetic versus geographic distance ($r = 0.19$, $P = 0.03$), and that association was attributed completely to forest versus savannah population differences ($P > 0.05$ for forest or savannah populations tested separately).

Although forest and savannah elephants formed two genetically distinct groups, sequences from populations within the two categories could not be distinguished hierarchical analysis of molecular variance (AMOVA) (24,

25). For example, we could not genetically differentiate the forest elephants in Dzanga-Sangha from those of Lope (F_{ST} $P > 0.05$). Despite the extensive geographic distances separating them, the savannah populations in southern, eastern, and north-central Africa were genetically indistinguishable (F_{ST} $P > 0.05$). Forest elephants are genetically more diverse than savannah elephants (Fig. 2). The average number of within-group pairwise differences among 24 forest elephants was 1.74 as compared with a value of 0.06 among 58 savannah elephants (24, 25, 27). Each forest elephant had a unique composite genotype, whereas the 58 savannah elephants defined only nine distinct genotypes (20). Forest elephants displayed larger numbers of heterozygous nucleotide sites than did savannah elephants (an average of 3.54 heterozygous autosomal sites per individual in forest elephants versus 0.39 for savannah elephants) (20). These observations suggest a recent founder event in the history of the savannah metapopulation. A potential time venue for the bottleneck is indicated by fossil evidence, which suggests that the savannah elephant's range greatly expanded at the end of the Pleistocene, after *Elephas iolensis*, the predominant African species, became extinct (3, 12).

The genetic and phylogenetic distinctiveness was evident without exception between 36 sampled forest elephants from three populations and 121 savannah elephants collected in 15 populations throughout sub-Saharan Africa. Each savannah population was genetically closer to every other savannah population than to any of the forest populations, even in cases where the forest population was geographically closer. Individuals from two "indeterminate" populations [Mount Kenya and Aberdares (Fig. 1)] contained exclusively savannah elephant genotypes (see Fig. 2, $F_{ST} = 0.88$, $P < 10^{-5}$ in comparing both populations to three forest populations). Genotypes found in the third "indeterminate" population, Garamba, were diverse and predominantly nested within the forest elephant clade in the phylogenetic analyses. The forest populations (including Garamba) were genetically closer to each other than to any savannah populations, including several that were geographically close. A single exceptional Garamba individual, GR0021, contained five signature sequence sites that were diagnostic for savannah elephants (*BGN* 304T, 485T, 508G, 514G, and 569C), as well as a single site (*GBA* 79T) that was diagnostic for the forest elephants.

The high level of genetic distinction is demonstrated by calculation of F_{ST} values among savannah, forest, and Asian elephant populations as well as by the computation of genetic distances (average pairwise differences) among them (24, 25, 27). Highly significant differentiation is evident between savannah, forest, and Asian elephants (F_{ST} $P < 10^{-5}$) but not between Garamba and forest elephants ($P >$

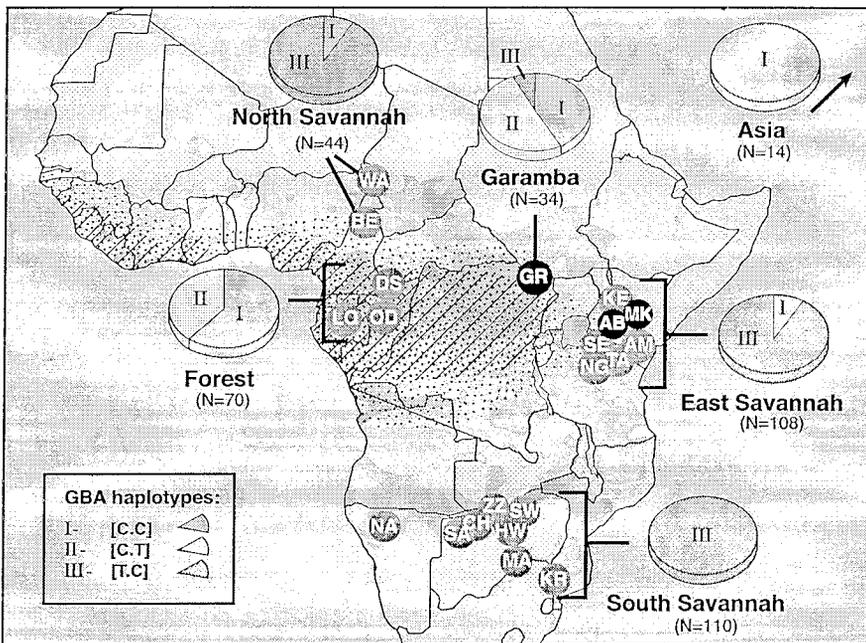


Fig. 1. Locations of sampled African elephant populations. Circles indicate sampling locations and population abbreviations. Green circles are forest populations (the number of elephants sampled is given here in parentheses after the location): DS, Dzanga Sangha (17); LO, Lope (16); and OD, Odzala (3). Red circles are savannah populations: AM, Amboseli (6); BE, Benoue (8); CH, Chobe (5); HW, Hwange (5); KE, Central Kenya (9); KR, Kruger (10); MA, Mashatu (7); NA, Namibia (14); NG, Ngorongoro (10); SA, Savuti (6); SE, Serengeti (7); SW, Sengwa (6); TA, Tarangire (7); WA, Waza (14); and ZZ, Zambezi (7). Black circles are three populations that were not classified a priori in either category: AB, Aberdares (17); GR, Garamba (18); and MK, Mount Kenya (3). Garamba is located in the Guinea-Congolian/Sudanian transition zone of vegetation in Congo, which includes a mixture of forest and secondary grasslands (17) suitable for both African elephant groups. Savannah, forest, and morphologically intermediate elephants have been reported in Garamba (11, 33). The forests of Mount Kenya and Aberdares are currently isolated by surrounding bush (18), and both have elephants that more closely resemble the savannah morphological phenotype. However, these forests may have recently been contiguous with other forest habitat (17, 18) and retained relict forest elephants. Orange indicates current African elephant range (7); historic range is in bright yellow (10). The dotted pattern indicates the extent of tropical forest (hatched) and forest/savanna transitional vegetation zones (17). Pie charts indicate the combined population frequencies of *GBA* haplotypes: I, [C.C]; II, [C.T]; and III, [T.C] for nucleotide sites 20 and 79, respectively, in Asian, forest, Garamba, and three savannah regional populations. N = number of elephant chromosomes.

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0.05). The genetic distance (average pairwise difference) between forest and savannah elephants is 9.0, which is 58% of the distance between Asian and African elephant genera (average = 15.5) (24, 25, 27). Tests for molecular evolutionary rate differentials did not reveal significant differences ($P > 0.05$) for the two African groups (24, 28, 29). Considering the estimation from fossil evidence for the divergence time between the two genera as 5 million years ago (12), the results suggest that forest and savannah elephants diverged approximately $2.63 (\pm 0.94)$ million years ago (24, 27, 29), which is comparable to species-level distinction in other mammalian taxa, including elephants (12, 30, 31). This estimate should be considered as a maximum age, however, because the more recent genetic homogenization of the savannah elephants would inflate genetic distance as a consequence of a recent founder event.

Genetic distinctiveness between forest and savannah elephants is also apparent when individual gene variation is examined. For *GBA*, two variable sites in African elephants define three haplotypes ([C.C], [C.T], or [T.C] for nucleotide sites 20 and 79, respectively) that have large forest versus savannah frequency differences (Fig. 1, exact test $P < 10^{-5}$ for forest versus savannah). The predominant haplotype in savannah elephants is [T.C] (frequency = 0.96), whereas alternative [C.C] and [C.T] haplotypes comprise 100% of the forest elephants, suggesting that reproductive isolation exists between the two groups (Fig. 1). For *VIM* and *CHRNA1*, complete and exact haplotypes could not be determined for individuals heterozygous at two or more nucleotide sites, because gametic phase cannot be assessed (for example, for a two-locus genotype, does a double heterozygote G/C,T/A individual contain GT + CA or GA + CT haplotypes?). However, among forest and Garamba elephants, polymorphisms occurred at six nucleotide sites in *VIM* that were genetically monomorphic in savannah elephants (20). Similar differences in the occurrence of polymorphic nucleotide sites were apparent within *CHRNA1*: All sites that were variable among forest and Garamba elephants were fixed in savannah populations, whereas the two sites that were variable in savannah elephants were fixed in forest and Garamba elephants (20). Likewise, both *CHRNA1* and *VIM* had an insertion/deletion variant limited to forest and Garamba elephants (20). The presence of these deletion variants in Dzanga-Sangha, Lope, and Garamba also is consistent with the recent occurrence of gene flow among these forest elephant populations across the Congolian forest.

The X linkage of *BGN* seen in other mammals (14) was affirmed in elephants by the presence of heterozygous nucleotide sites among females but not among the hemizygous males. Nineteen variable sites in *BGN*

were used to identify 169 haplotypes from 55 males and 57 females. A minimum spanning phylogenetic network of the nine unique *BGN* haplotypes observed (Fig. 3) showed clear differentiation of a single distinct Asian haplotype ($n = 13$ chromosomes), two African savannah haplotypes ($n = 103$ chromosomes; including Aberdares and Mount Kenya), and six African forest haplotypes ($n = 53$ chromosomes; including one Garamba in-

dividual). For *BGN*, the number of nucleotide changes separating forest from savannah elephant haplotypes (six steps) was nearly as large as that separating either from the Asian elephant haplotype (seven steps). The *BGN* haplotypes present in the forest elephant populations were not found among savannah elephants, whereas haplotypes seen in the savannah elephants were not present in the forest populations ($P < 10^{-5}$, exact test of

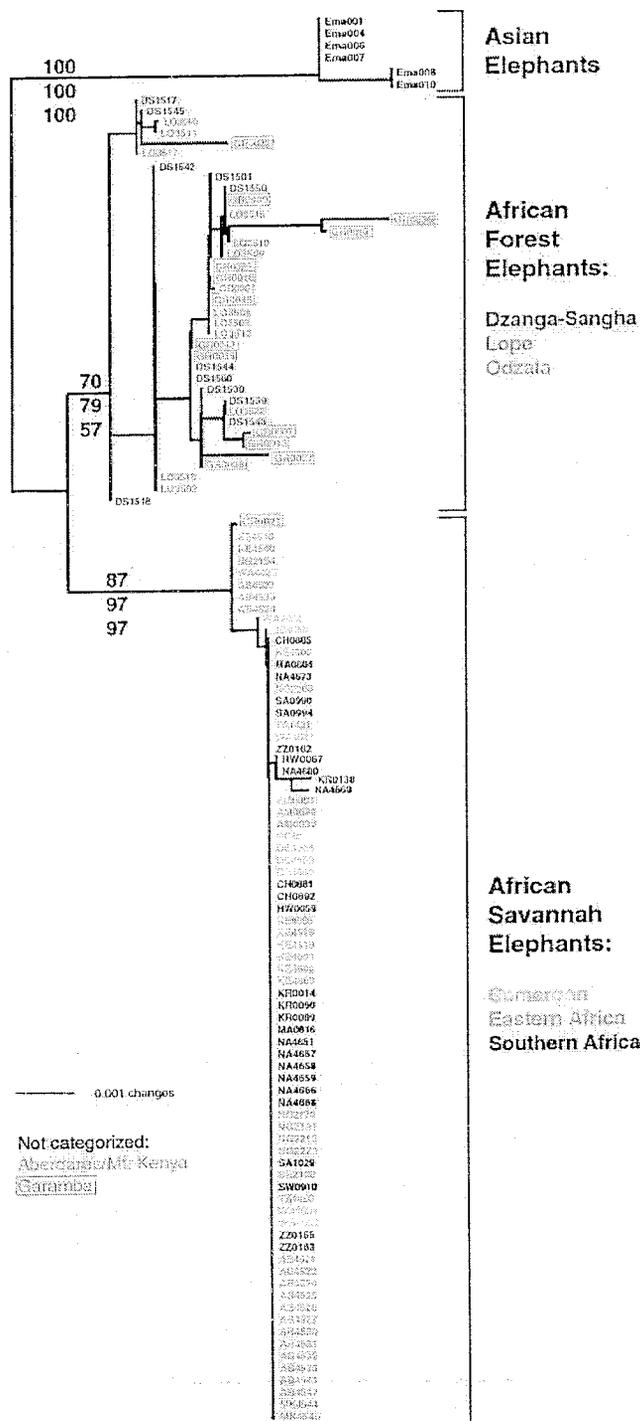


Fig. 2. Phylogenetic relationships for Asian, African forest, and African savannah elephants inferred from combined analyses (21–23) of 1732 bp (*BGN*, *CHRNA1*, *GBA*, and *VIM*); the two-letter codes for African elephant populations are given in Fig. 1. Asian elephant individuals are coded "Ema." The minimum evolution (NJ) tree is shown. Concordant trees were obtained by MP (tree length was 248 steps; CI = 0.927, RI = 0.934) and ML (-ln L = 2774.53539) analyses, which produced the same topology in defining the three groups. Bootstrap resampling support (100 iterations) is listed on branches for NJ (top), MP (middle), and ML (bottom) analyses for nodes supported by all three methods.

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the forest versus savannah haplotype frequencies) (24, 25, 32), suggesting a high degree of reproductive isolation between the forest and savannah populations. Taken together, the distinction affirmed by independent unlinked nuclear genes (Figs. 1 and 3) (20) offers strong support for the concept of appreciable genetic divergence between the African savannah and forest elephant populations.

There was no molecular genetic evidence of hybridization among 3 forest and 17 savannah elephant populations [defined a priori, plus Aberdares and Mount Kenya (Fig. 1)]. In Garamba, however, three individuals (GR0021, GR0035, and GR0037) showed genotypes with a combination of forest and savannah taxon-specific alleles, suggesting a history of limited hybridization in the ancestors of this population (20), as has been suggested by some (33), but not all (10, 11). GR0021 grouped with savannah elephants in the phylogenetic analysis, whereas animals GR0035 and GR0037 had largely forest genotypes (Fig. 2) except for the *GBA* [T.C] haplotype, which is absent in forest elephants but predominant in savannah elephants (Fig. 1). The paucity of gene introgression between forest and savannah populations even near regions of potential physical contact [that is, in north-central Africa or near Garamba (Fig. 1)] suggests that hybridization in nature is rare and perhaps minimized by behavioral or physiological reinforcement. In this regard, no

elephant from any population, including Garamba, displayed a predicted F_1 hybrid genotype (that is, heterozygous at the genetically fixed sites between savannah and forest elephants), affirming the lack of gene flow or hybridization among the sampled elephants.

The molecular results of a pan-African phylogeographic elephant survey reported here offer support for the idea that a long period of adaptive evolution (estimated at 2.63 ± 0.94 million years) separated the savannah and forest elephant lineages. As such, the results strongly support recognition of species-level distinctions between African elephant taxa (5-11). Although reproductive isolation is the principal criterion for species recognition according to the Biological Species Concept (34), local hybridization or even the presence of a "hybrid zone," as may have occurred in Garamba, would not preclude species recognition, because the genetic integrity of the parent species remains intact (34, 35). Hybrid zones that fail to spread or homogenize the genetic distinctiveness of contact species have been observed with scores of other species (35, 36). These considerations, along with the combined morphological, ecological, and molecular data, are cogent indicators that there should be species-level recognition for *Loxodonta africana* (Blumenbach 1797), the African savannah elephant (37) and *Loxodonta cyclotis* (Matschie, 1900) (4), the African forest elephant. Given the rapid deple-

tion of both forest and savannah elephant numbers in the past century and the ongoing destruction of their habitats, the conservation implications of recognition and species-level management of these distinct taxa are considerable (1, 10, 38).

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- DNA was extracted with a Qiagen Qiaamp kit. Previously described primer sequences (14) were used to amplify segments of four nuclear genes that in humans are on separate chromosomes: *BGN*, *CHRNA1*, *GBA*, and *VIM*. The following primers specific to the resulting elephant sequence were developed and used: *CHRNA1-F2* (5'-GCTCTGGG CTGGAATCC-3'), *CHRNA1-R3* (5'-CGCCTGGGAAAGAGG-3'), *VIM-F2* (5'-CGCA TCTGGAGTCCCTGG-3'), and *VIM-R2* (5'-TTGAACCAATGTTCAGGAA-3'). Polymerase chain reaction using Taq Gold (Perkin-Elmer) consisted of a hot start at 95°C for 9.75 min, then 40 cycles of 15 s at 95°C and 30 s of annealing at 60°C (cycles 1 and 2), 58°C (cycles 3 through 8), 56°C (cycles 9 through 14), 54°C (cycles 15 through 20), or 52°C (cycles 21 through 40), and 60 s of extension at 72°C, with a final extension at 72°C for 5 min. Products were purified with Centricon concentrators (Amicon). ABI BigDye Terminator sequences were resolved on an ABI 377 system. Homology of each elephant gene segment was established with the program NCBI BLAST 2.0 (39). All sequences for each gene segment were deposited in GenBank (accession numbers AY044919 through AY045493).
- Supplemental Web material is available on Science Online at www.sciencemag.org/cgi/content/full/293/5534/1473/DC1. It is also available at the Laboratory of Genomic Diversity Web site at <http://lgsd.nci.nih.gov>.
- DNA sequences were aligned with the software CLUSTALX (22) and visually inspected. Phylogenetic analyses of the concatenated data set (all four genes) were performed with heuristic searches (with random taxon addition and tree bisection-reconnection branch swapping) for maximum parsimony (MP), minimum evolution [neighbor joining (NJ)], and maximum likelihood (ML) methods implemented in PAUP*4.0b4 (23). MP analyses treated multistate characters as polymorphic and gaps as a fifth state. NJ analyses were performed with Kimura-2 parameter distances. ML analyses used empirical base fre-

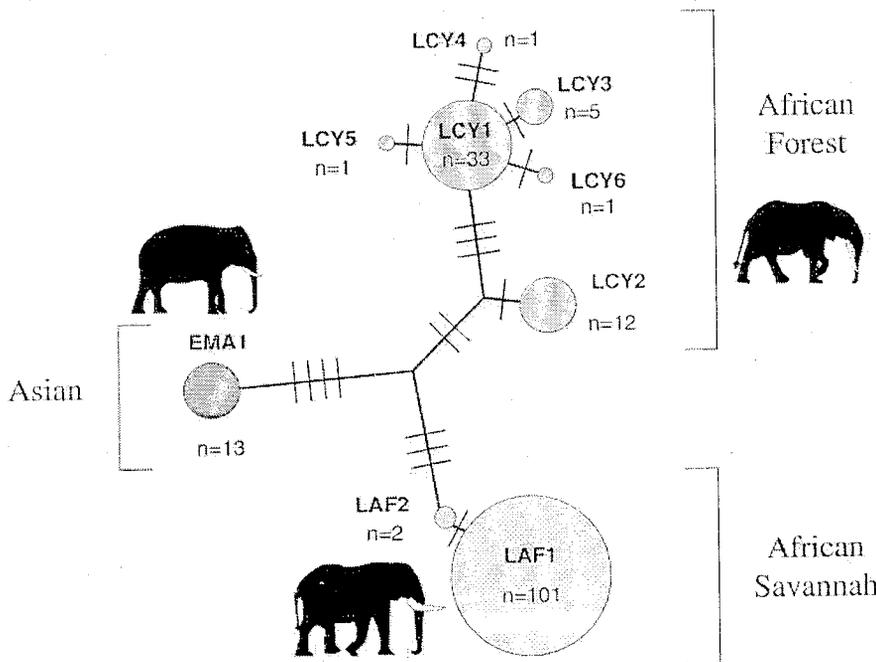


Fig. 3. Minimum spanning network depicting relationships among nine haplotypes observed for the X-linked *BGN* gene for Asian, African forest, and African savannah elephants. Hatch marks indicate the number of nucleotide differences separating each haplotype. Haplotypes were determined using 7 Asian (EMA), 74 African savannah (LAF) and 31 African forest elephants (LCY) for which the sex was known (a total of 55 males and 57 females). Haplotypes unique to each of the three taxa are identified by differences in shading; the number of chromosomes is indicated for each haplotype.

The Ground State of the Ventral Appendage in *Drosophila*

Fernando Casares* and Richard S. Mann†

In *Drosophila melanogaster*, the antennae, legs, genitalia, and analia make up a serially homologous set of ventral appendages that depend on different selector genes for their unique identities. The diversity among these structures implies that there is a common ground state that selector genes modify to generate these different appendage morphologies. Here we show that the ventral appendage that forms in the absence of selector gene activity is leglike but consists of only two segments along its proximo-distal axis: a proximal segment and a distal tarsus. These results raise the possibility that, during evolution, leglike appendages could have developed without selector gene activity.

Selector genes encode transcription factors that specify the identity of segments and appendages in insects and vertebrates (1, 2). The Hox genes are a subset of selector genes that are required for generating morphological differences along the antero-posterior axis of most animals. Studies in the fruit fly, *Drosophila melanogaster*, demonstrate that

altering Hox function can cause one body part to be transformed into another. Perhaps in large part because they govern the development of entire body parts, changes in how Hox genes, and selector genes in general, were used during evolution have led to modifications in animal body plans throughout the animal kingdom (2, 3).

quencies and estimated values for the shape parameter for among-site rate variation ($\alpha = 0.119$) and the transition/transversion ratio (1.89). Bootstrap resampling support was based on 100 iterations.

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24. Population genetic analyses were done with Arlequin 2.000 software (25); Hierarchical AMOVA for pairs of individual locales were used when both had $n > 4$; larger regional comparisons used all individuals from all sites. Kimura-2 parameter distances were used for AMOVA (with 16,000 permutations for significance tests), population pairwise F_{ST} 's (10,000 permutations), and average pairwise differences; exact tests of population differentiation based on haplotype frequencies used 100,000 steps in the Markov chain and 4000 dememorization steps (32). Mantel tests used 10,000 permutations for significance testing (26). Nucleotide sequence rate constancy was tested for the three elephant taxa with the method of Tajima in MEGA 2 (28, 29). The forest-savannah elephant divergence date was calculated with the use of the ratio of between-group averages determined in MEGA 2 (27, 29), using 70 individuals that had no more than two heterozygous sites in their concatenated sequence, with standard error estimated by the bootstrap method (500 replications).
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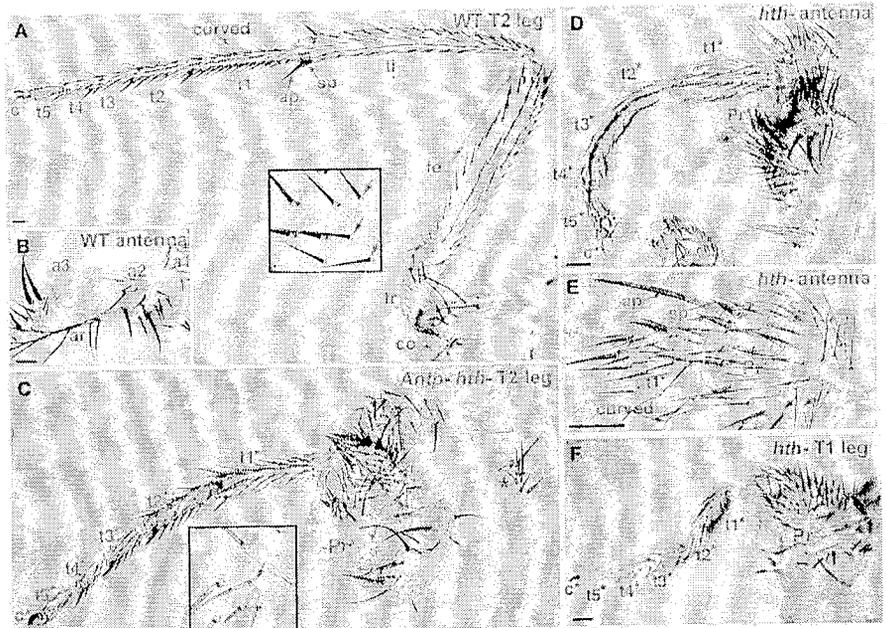


Fig. 1. The ground state ventral appendage is a leglike appendage with two segments. (A) A wild-type (WT) T2 leg has five segments from proximal to distal: coxa (co), trochanter (tr), femur (fe), tibia (ti), and tarsus, which is subdivided into tarsal subsegments 1 to 5 (t1 to t5) and a distal claw (c). Five bristle types are indicated: bracted (green arrows), unbracted (red arrows), curved, spurs (sp), and apical (ap). The inset shows a closeup of the proximal femur where both bracted and unbracted bristles are present. The inset comes from a different wild-type leg. (B) A wild-type antenna consists of four segments, from proximal to distal: antennal segments 1 to 3 (a1 to a3) and arista (ar). (C) *Antp⁻hth⁻* T2 leg. Most of this appendage is mutant (y^-). The recovered tarsal segments (t1* to t5*) and single proximal segment (Pr*) are indicated. The inset shows a region of a similar appendage with bracted and unbracted bristles. The asterisk [also in (D)] indicates a proximal plate with unbracted bristles that is typically associated with the ground state. (D) An *hth⁻* antenna results in an indistinguishable appendage morphology as seen in (C). Most of this appendage is mutant (y^-). (E) A high-magnification view of part of the t1* and Pr* segments of an *hth⁻ y⁻* antenna. The same bristle types are observed in *Antp⁻hth⁻* T2 legs. (F) An *hth⁻* T1 leg with proximal fusions. Transverse row bristles (arrow), which are indicative of a first leg identity, are observed.

Subspecific Affinity of Black Bears in the White River National Wildlife Refuge

J. Warrillow, M. Culver, E. Hallerman, and M. Vaughan

The black bear population of the White River National Wildlife Refuge (NWR) is adjacent to populations of black bear in Louisiana (*Ursus americanus luteolus*) which are listed as threatened under the U.S. Endangered Species Act. Wildlife management plans can pose restrictions on bear harvests and timber extraction; therefore the management plan for the White River NWR is sensitive to subspecific classification of its bear population. The objective of this study was to analyze genetic variation in the White River NWR and seven adjacent populations of black bears to assess the subspecific affinity of the White River NWR population. Here we report the variation at seven microsatellite DNA loci among eight black bear populations. The patterns of genetic variation gave strong support for distinguishing a southern group of black bears comprised of the White River, Arkansas; Texas River, Louisiana; Upper Atchafalaya, Louisiana; Lower Atchafalaya, Louisiana; and Alabama/Mississippi populations. Phylogenetic analysis of individual variation suggested that historical black bear introductions into Arkansas and Louisiana affected gene pools of certain southern receiving populations, but did not significantly change interpopulation relatedness. Phylogenetic inferences at both the population and individual levels support the hypothesis that the White River NWR population of black bears belongs to the *U. a. luteolus* subspecies.

The black bear (*Ursus americanus*) is comprised of 16 subspecies, including the American black bear (*U. a. americanus*) distributed throughout most of eastern North America, the Louisiana black bear (*U. a. luteolus*) in Louisiana and adjacent areas (Hall 1981), and the Florida black bear (*U. a. floridanus*) in Florida and adjacent areas. The Louisiana black bear was listed as threatened under the U.S. Endangered Species Act (ESA) in 1992. In December 1998, the U.S. Fish and Wildlife Service concluded in a final ruling that listing for the Florida black bear was not warranted under the ESA.

Although black bears once ranged throughout most of North America (Vaughan and Pelton 1995), recent fragmentation has isolated many populations, such as the White River National Wildlife Refuge (NWR) population in Arkansas. Genetic characterization of the bear population's subspecific affinity is important with regard to formulating the management plan for the refuge because the two subspecies in question (*U. a. americanus* and *U. a. luteolus*) differ in terms of ESA Protection. This is particularly important with regard to harvest regulations, and also

poses possible restrictions on timber extraction and on a river channelization proposal in areas adjacent to the White River NWR. Currently the subspecific affinity of the White River NWR black bear population is unclear. In a general synthesis of mammalian distribution and systematics, Hall (1981) classified the White River NWR population as *U. a. americanus*. Using DNA fingerprinting techniques to characterize variation of nuclear DNA, Miller (1995) tentatively concluded that the White River NWR population exhibited greater affinity to *U. a. luteolus* than to *U. a. americanus*. However, that study did not focus on the White River NWR population, and did not include all population-by-population comparisons relevant for reaching a firm conclusion regarding its subspecific classification. Kennedy et al. (1996) concluded that the White River NWR population belonged to *U. a. americanus*, based on morphometric comparisons on a small number of skulls ($n = 6$). Other black bear population genetic and phylogeographic studies, using either mitochondrial control region sequence (Wooding and Ward 1997) or microsatellite DNA markers (Paetkau and Strobeck 1994), did not in-

From the Department of Fisheries and Wildlife Sciences, Virginia Polytechnic Institute and State University, Blacksburg, VA. J. Warrillow is currently at the Department of Fisheries and Wildlife, Michigan State University, East Lansing, Michigan. M. Vaughan is currently at the USGS/BRD—Virginia Cooperative Fish and Wildlife Research Unit, Virginia Polytechnic Institute and State University, Blacksburg, Virginia. We thank the U.S. Fish and Wildlife Service (FWS) for funding this research, and W. McDearman of the U.S. FWS at Jackson, MS, for constructive comments on an earlier version of this manuscript. We also would like to thank the following individuals and agencies for assistance in trapping bears and collecting blood and tissue samples: D. Garshelis with the Minnesota Department of Forestry and Wildlife; D. Goad with the Arkansas Game and Fish Commission; M. Hurdle and N. Hunter with the U.S. FWS at White River NWR, AR; H. Jacobson and T. White at Mississippi State University; T. Edwards with the U.S. FWS at Texas River NWR, LA; R. Pace at Louisiana State University; K. Shropshire with the Mississippi Department of Wildlife, Fisheries, and Parks; K. Guyse with the Alabama Department of Natural Resources; J. Kasbohm with the U.S. FWS; and D. Miller with the Florida Game and Freshwater Fish Commission. Address correspondence to Melanie Culver at the address above or e-mail: culver@vt.edu.

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clude black bear samples from the southeastern United States (although Wooding and Ward included a single individual from Florida out of 258 total black bears).

Development of the management plan for the White River NWR and planning of economic activities adjacent to the refuge are sensitive to the subspecies classification of its black bear population. Hence the objective of this study was to analyze genetic variation in White River NWR and adjacent populations of black bears to assess the classification of the White River NWR population based on genetic evidence. The recent development of microsatellite DNA markers for bears (Paetkau et al. 1995; Paetkau and Strobeck 1994) offers the opportunity to screen allelic variation at particular genetic loci and to analyze departures from Hardy-Weinberg expectations. Here we report the results of analyses of allelic variation at seven microsatellite loci among eight populations of black bear.

Materials and Methods

DNA Purification

Samples of blood or other tissues were collected from 151 individuals representing eight black bear populations (Figure 1). Sampling included three populations of the American black bear (*U. a. americanus*): Ouachita National Forest, Arkansas (OU); Ozark National Forest, Arkansas (OZ); and Cook County, Minnesota (CC). The Cook County population was sampled because it represents the source population for many historic translocations into the other populations in this study. Individuals from Mobile River, Alabama ($n = 11$) and Red Creek Wildlife Management Area (WMA), Mississippi ($n = 2$) were taken as representing the Florida subspecies of black bear (*U. a. floridanus*) (AL). These populations are separated by only 60 km, although the Red Creek WMA falls within Hall's (1981) view of the range of nominal *U. a. luteolus* (see results below regarding individual phylogeny). Three populations of the Louisiana black bear (*U. a. luteolus*) included the Lower Atchafalaya River Basin, Louisiana (LA); Upper Atchafalaya River Basin, Louisiana (UA); and Tensas River NWR, Louisiana (TR). The White River NWR population (WR), the subspecific affinity of which is uncertain, also was sampled. DNA was purified from blood or other tissues following a proteinase-K digestion and phenol-chloroform extraction protocol (Miller 1995). DNA samples were frozen at -20°C in $1\times$ TE buffer.

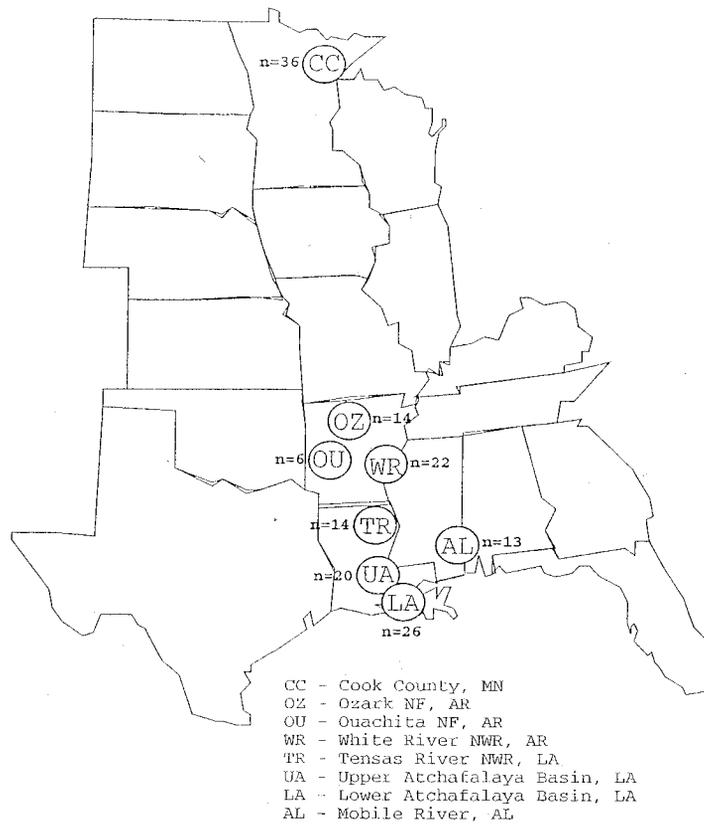


Figure 1. Locations of black bear populations sampled for genetic variability. n refers to the sample size for each population.

Microsatellite Markers

Dinucleotide microsatellite repeats of genomic DNA were amplified by the polymerase chain reaction (PCR) using seven primer pairs. Primer pair sequences were obtained from Paetkau et al. (1995) and Paetkau and Strobeck (1994). Primer pairs were custom-produced by Operon Technologies (Alameda, CA) or IDT (Coralville, IA). Primer sequences, PCR conditions, and reagents were used as described in Paetkau and Strobeck (1994). Amplification was performed using the following cycling conditions: 2 min at 94°C ; 30 cycles of 15 s at 94°C , 30 s at 55°C , and 15 s at 72°C ; and 5 min at 72°C . After amplification, the PCR products were subjected to electrophoresis through a 7% native TBE polyacrylamide gel (Hoefer SE 600 gel apparatus, Amersham Pharmacia Biotech, San Francisco, CA) and visualized by silver staining (Naish KA, personal communication). Amplification product sizes were estimated using a 10 bp molecular weight ladder (GibcoBRL, Life Technologies). Allele size estimates were not regarded as exact, but were standardized between gels by running samples of known genotypes on every gel, after every sec-

ond or third test sample. All primers produced light "shadow bands" (Hauge and Litt 1993) approximately 8 bp larger than the alleles. Homozygotes were distinguished by noting only one shadow band in the target area.

Genetic Diversity and Population Structure

Measures of genetic diversity were estimated at the individual level, as well as within and between populations. Average expected heterozygosity, average number of alleles, total number of alleles, number of unique alleles, average variance in number of repeats, and average range in number of repeats were estimated from microsatellite data using the program MICROSAT (version 1.5; Minch et al. 1999). Deviations from Hardy-Weinberg equilibrium (Guo and Thompson 1992) were tested for each microsatellite locus using the Arlequin program (version 1.1; Schneider et al. 1997). The degree of population differentiation among the eight populations was estimated using analysis of fixation indices. Two estimators, F_{ST} (number of different alleles; Michalakis and Excoffier 1996; Reynolds et al. 1983; Weir and Cock-

Table 1. Matrices of F_{ST} (below diagonal) and R_{ST} (above diagonal) values among eight black bear populations at seven microsatellite loci

	AL	LA	UA	OU	OZ	TR	WR	CC
AL		*0.22	*0.13	*0.32	*0.49	*0.23	*0.22	*0.24
LA	*0.35		0.02	0.09	*0.14	0.05	0.11	*0.09
UA	*0.25	*0.07		0.01	0.12	0.06	*0.10	*0.08
OU	*0.35	*0.13	0.05		0.01	0.14	0.13	0.00
OZ	*0.33	*0.18	*0.09	0.02		*0.23	*0.26	0.04
TR	*0.39	*0.22	*0.16	*0.18	*0.24		0.06	*0.14
WR	*0.56	*0.37	*0.31	*0.32	*0.37	*0.18		*0.14
CC	*0.25	*0.15	*0.09	0.00	*0.05	*0.18	*0.29	

* Indicates significant P value at $P < .002$ (after Bonferroni adjustment) based on 100 permutations. Population designations are as in Figure 1.

erham 1984) and R_{ST} (sum of squared size differences; Slatkin 1995), for microsatellite data, as implemented in the Arlequin program (version 1.1; Schneider et al. 1997), were used to quantify population subdivision (Table 1). The significance levels of F_{ST} were assessed after employing a Bonferroni adjustment (Weir 1996) for multiple comparisons. An analysis of molecular variance (AMOVA) for detecting subdivision (Excoffier et al. 1992) was performed among populations and within groupings of populations, using both F_{ST} and R_{ST} , for two grouping strategies: (1) with two groups comprised of the southern group (Upper Atchafalaya, Lower Atchafalaya, White River, Tensas River, Alabama/Mississippi) and the northern group (Cook County, Ouachita, Ozark); and (2) with three groups comprised of Alabama/Mississippi, the rest of the southern group (Upper Atchafalaya, Lower Atchafalaya, White River, Tensas River), and the northern group (Cook County, Ouachita, Ozark). Significant differences among populations for the average number of alleles per locus were assessed using analysis of variance (ANOVA), and grouped using the LSD multiple comparison procedure. Significant differences among observed average heterozygosities were tested using GENMOD, and significant pairs were found using the CONTRAST procedure in SAS version 6.12 (SAS Institute Inc., Cary, NC). In addition, the Assignment Calculator program (Brzustowski 2000) was used to determine the frequencies at which an individual's composite genotype could be used to successfully assign individuals to the population from which they were actually sampled (Paetkau et al. 1995, 1997). When an allele was missing from a population, instead of zero, a frequency of 0.01 was selected, 1000 randomized runs were performed; and randomization was accomplished by shuffling alleles at each locus within populations with no replacement.

Phylogenetic Analyses

The MICROSAT program (version 1.5; Minch et al. 1999) was employed to estimate pairwise genetic distances both among individuals and populations using the kinship coefficient (D_{kf}) and proportion of shared alleles (D_{ps} ; Bowcock et al. 1994) metrics; these metrics previously have been used to study several felid species (Culver et al. 2000; Johnson et al. 1999). D_{kf} and D_{ps} both measure the proportion of shared alleles between individuals or populations, but D_{ps} weights shared, rare alleles, whereas D_{kf} is sensitive to frequencies of shared alleles. In addition, Nei's standard distance metric (G_{ST} ; Nei 1987) was used to quantify relatedness among populations, as has previously been applied to populations of canids (Garcia-Moreno et al. 1996; Roy et al. 1994) and wombat (Taylor et al. 1994). The nomenclature of G_{ST} , used by the MICROSAT program, refers to Nei's identity, and as used here is equivalent to Nei's standard genetic distance. In this study, genetic distance among populations and individuals was estimated using several metrics (D_{kf} , D_{ps} , and G_{ST}), each with a particular strength. Rather than attempt to select one best metric, we chose to examine the variety of metrics and look for agreement among all results. The phylogenetic analysis of individuals was performed using data both for all individuals ($n = 151$) and for only individuals whose DNA supported amplification for six or more loci ($n = 145$). Phylogenetic trees were constructed from the D_{kf} , D_{ps} , and G_{ST} distance matrices using the NEIGHBOR option of the program PHYLIP (version 3.5c; Felsenstein 1993). The data were entered into NEIGHBOR both sequentially from the data file and also in randomized order. Phylogenetic trees were drawn using the program TREEVIEW (version 1.5; Page 1998).

Results

Diversity Measures

Seventy-one alleles were observed at seven microsatellite loci (Table 2). The degree of variation ranged from 8 to 14 alleles per locus, with an average of 5.6 alleles per population at each locus. Allele frequencies were different among the eight populations. Cook County and Ouachita black bear populations exhibited a more even distribution of allele frequencies, with maximum frequencies of 0.45 and 0.50, respectively, while the other populations showed some allele frequencies in the 0.61 to 1.00 range. Overall the Cook County population exhibited the greatest amount of genetic diversity relative to the other populations, quantified in terms of the average number of alleles per locus (8.71 versus a range of 2.43–6.14, $P < .0001$) (Table 3), total number of alleles (36 versus 14–32), number of unique alleles (4 versus 0–2), and average range in number of repeats (9.43 versus 4.14–8.00) (Table 4). The White River and Alabama/Mississippi populations consistently exhibited relatively low genetic variation at all seven microsatellite loci (Tables 3 and 4), notably in terms of the low average number of alleles ($P < .0001$). Observed average heterozygosities were statistically higher for the Cook County population ($H = 0.54$) relative to three southern populations (Alabama/Mississippi, Lower Atchafalaya, and White River, $H = 0.38$ –0.42) ($P < .0167$, $P < .0153$, and $P < .0022$, respectively).

Population-Level Relatedness and Phylogeny

Phylogenetic relationships were inferred from pairwise genetic distances using D_{kf} , D_{ps} (Bowcock et al. 1994), and G_{ST} (Nei 1987) distance measures. All metrics produced similar distance matrices, therefore only those for D_{kf} and D_{ps} are shown (Table 5). The greatest genetic distance estimate, for all distance metrics, was observed between two southern populations, Alabama/Mississippi and White River ($D_{kf} = 0.44$, $D_{ps} = 0.71$, $G_{ST} = 0.67$), that is, between populations of nominally different subspecies. Otherwise the greatest distances occurred between populations representing the *U. a. americanus* and *U. a. luteolus* subspecies. The smallest genetic distances were observed between Cook County and Ouachita ($D_{kf} = 0.04$, $G_{ST} = 0.00$), or White River and Tensas River ($D_{ps} = 0.30$) populations. Generally, smaller genetic distance values were ob-

Table 2. Allele frequencies at seven microsatellite loci among eight populations of black bears (population designations are as in Figure 1)

Locus <i>GIA</i>		Population	AL	LA	UA	OU	OZ	TR	WR	CC
No. of different alleles	10	No. of alleles	24	52	40	10	24	28	44	69
Allele	174	Frequency	0	0.02	0	0	0	0	0	0.01
	176		0	0	0.03	0	0	0	0	0
	178		0	0.02	0.1	0	0	0	0	0.06
	180		0	0	0.03	0	0	0.07	0	0
	182		0	0	0.03	0	0	0	0	0.12
	184		0.92	0.02	0.2	0.5	0.38	0.89	0.95	0.45
	186		0	0.27	0.1	0.2	0.21	0	0.05	0.06
	188		0.08	0.65	0.15	0.3	0.08	0	0	0.23
	190		0	0	0.2	0	0.33	0	0	0.04
	194		0	0.02	0.18	0	0	0.04	0	0.03
Locus <i>G10X</i>		Population	AL	LA	UA	OU	OZ	TR	WR	CC
No. of different alleles	14	No. of alleles	24	52	40	12	22	28	44	66
Allele	128	Frequency	0	0	0	0	0	0	0	0.08
	134		0	0	0	0	0.05	0	0	0.03
	136		0	0	0	0	0	0	0	0.03
	140		0	0.02	0	0	0	0	0	0.03
	142		0	0.62	0.33	0.33	0.18	0.29	0.3	0.32
	144		0.63	0.02	0.15	0	0	0	0	0
	146		0	0	0	0.08	0.32	0	0	0.09
	148		0.04	0.04	0	0.08	0.09	0.43	0.5	0.02
	150		0.08	0.12	0.08	0.17	0.18	0.07	0.05	0.15
	152		0.13	0.08	0.23	0	0	0	0	0.06
	154		0.08	0.04	0	0	0.09	0.04	0	0.03
	156		0.04	0.06	0.15	0.08	0	0.18	0.16	0.06
	158		0	0.02	0.08	0.17	0	0	0	0.08
	160		0	0	0	0.08	0.09	0	0	0.02
Locus <i>G1D</i>		Population	AL	LA	UA	OU	OZ	TR	WR	CC
No. of different alleles	9	No. of alleles	26	52	40	12	28	28	44	71
Allele	166	Frequency	0	0.02	0.08	0.08	0.18	0	0	0.13
	168		0	0	0.05	0	0.04	0.14	0	0.17
	170		0.38	0.71	0.63	0.42	0.43	0.39	0.52	0.23
	172		0	0	0	0.25	0.14	0	0	0.04
	174		0	0	0.03	0.08	0.11	0	0	0.08
	176		0	0	0.05	0.08	0.04	0	0.16	0.01
	178		0.62	0.27	0.18	0.08	0.07	0.29	0.32	0.34
	180		0	0	0	0	0	0.04	0	0
	182		0	0	0	0	0	0.14	0	0
Locus <i>G1OL</i>		Population	AL	LA	UA	OU	OZ	TR	WR	CC
No. of different alleles	14	No. of alleles	26	52	40	12	28	28	44	72
Allele	132	Frequency	0.12	0.62	0.35	0.17	0.04	0.71	0.57	0.17
	134		0.12	0.04	0.23	0.42	0.43	0	0	0.31
	136		0	0	0	0	0.32	0	0	0.06
	138		0	0	0	0	0.04	0	0	0.06
	140		0	0	0	0	0.04	0	0	0.01
	146		0	0	0	0	0	0	0	0.07
	148		0.77	0.15	0.05	0	0	0	0	0.08
	150		0	0.04	0.03	0.17	0	0.25	0	0.08
	152		0	0.12	0.25	0.25	0.14	0.04	0.34	0.11
	154		0	0.04	0.1	0	0	0	0.09	0.04
	158		0	0	0	0	0	0	0	0
Locus <i>G1OP</i>		Population	AL	LA	UA	OU	OZ	TR	WR	CC
No. of different alleles	12	No. of alleles	26	52	40	12	24	28	44	70
Allele	146	Frequency	0	0.04	0	0	0	0	0	0
	148		1	0.4	0.45	0	0	0.43	0	0.04
	150		0	0.06	0	0	0	0	0	0.04
	152		0	0.06	0.15	0.17	0	0.36	0.91	0.06
	154		0	0.02	0	0	0.13	0	0	0.03
	156		0	0.08	0.05	0.17	0.25	0.18	0.09	0.19
	158		0	0.19	0.23	0.33	0	0	0	0.4
	160		0	0.15	0.13	0.17	0.29	0	0	0.6
	162		0	0	0	0.17	0.08	0	0	0.11
	164		0	0	0	0	0	0.04	0	0.07
	166		0	0	0	0	0.17	0	0	0
	168		0	0	0	0	0.08	0	0	0
Locus <i>G1OC</i>		Population	AL	LA	UA	OU	OZ	TR	WR	CC
No. of different alleles	8	No. of alleles	24	48	39	11	26	28	44	68
Allele	104	Frequency	0	0	0	0	0.04	0	0	0.09
	106		0	0.02	0.15	0.27	0.04	0	0	0.13
	108		0.5	0	0	0	0	0	0	0
	110		0.17	0	0	0.09	0.15	0	0	0.09
	112		0.17	0.17	0.03	0.09	0.35	0	0	0.43
	114		0	0.69	0.56	0.36	0.31	1	1	0.25
	116		0.17	0.1	0.18	0.18	0.12	0	0	0.01
	118		0	0.02	0.08	0	0	0	0	0
Locus <i>G1OB</i>		Population	AL	LA	UA	OU	OZ	TR	WR	CC
No. of different alleles	8	No. of alleles	26	50	40	12	28	28	44	72
Allele	148	Frequency	0	0.02	0.05	0	0	0	0	0.01
	152		0.88	0.74	0.8	0.42	0.61	0.43	0.16	0.44
	154		0	0.02	0.03	0.25	0.07	0	0	0.11
	156		0	0.04	0	0.25	0.11	0.32	0	0.22
	158		0	0	0	0	0.04	0.07	0	0.1
	160		0	0.14	0.08	0.08	0.11	0.18	0.84	0.08
	162		0.12	0.04	0.05	0	0.07	0	0	0.03

(71 total alleles)

Table 3. Significantly different groupings for number of alleles in the eight black bear populations using the least significant difference (LSD) method

Grouping	Mean no. Alleles	Population
A	8.5714	CC
B	6.1429	LA
B	6.0000	OZ
B	6.0000	UA
C	4.8571	OU
C	3.5714	TR
D	2.8571	WR
D	2.5714	AL

Population designations are as in Figure 1.

served among populations of the same subspecies.

Phylogenetic associations among populations were inferred by the minimum evolution method as estimated by the Neighbor-Joining (NJ) algorithm (Felsenstein 1993) using *Dkf*, *Dps*, and G_{ST} distances. Bootstrap (BS) values of 70% or greater were regarded as significant; a 70% BS value corresponds to a probability of $\geq 95\%$ that the corresponding clade is real (Hillis and Bull 1993). All three distance measures resulted in construction of trees with similar topologies, with the G_{ST} and *Dps* trees identical; therefore only the *Dkf* and *Dps* trees are shown (Figure 2). All trees showed strong support for associations between the Tensas River and White River populations (BS values: *Dkf* = 100, *Dps* = 99, G_{ST} = 100), and between the Upper Atchafalaya and Lower Atchafalaya populations (BS values: *Dkf* = 93, *Dps* = 77, G_{ST} = 88). Two of the trees (*Dps* and G_{ST}) also gave strong support for a genetically distinct southern group comprised of the White River, Tensas River, Upper Atchafalaya, Lower Atchafalaya, and Alabama/Mississippi populations (BS = 98 and 71, respectively). The *Dkf* tree gave weak support for the association of these 5 southern populations. The Ozark and Ouachita populations were significantly grouped in the *Dps* and G_{ST} trees (BS = 87 and 71, respectively). For all three trees (*Dkf*, *Dps*, and G_{ST}), there was a relationship, although weak, of the Cook County population with the Ozark/Ouachita group (BS values: *Dkf* = 59, *Dps* = 45 and G_{ST} = 40), and the position of the Alabama population was uncertain within the southern group (BS values: *Dkf* = 36, *Dps* = 47, G_{ST} = 47). The structure of the trees was identical, as were significant bootstrap values, when the data were reentered using randomized input order.

Table 4. Genetic variability metrics among eight populations of black bear at seven microsatellite loci

Population	N	*H(exp)	*H(obs)	Average no. of alleles	Total no. of alleles	No. of unique alleles	Average variance in no. of repeats	Average range in no. of repeats
AL	13	0.35	0.39	2.88	17	1	3.43	4.14
LA	26	0.54	0.42	6.14	27	1	5.89	8.00
UA	20	0.66	0.55	6.00	30	1	7.65	7.57
OU	6	0.73	0.56	4.86	26	0	6.42	5.86
OZ	14	0.73	0.54	6.00	32	2	4.75	7.14
TR	14	0.48	0.53	3.57	18	2	5.42	6.14
WR	22	0.33	0.38	2.43	14	0	5.38	4.14
CC	36	0.77	0.54	8.71	36	4	7.23	9.43
TOTAL	151						11	

*H = Heterozygosity

Population designations are as in Figure 1.

Population Structure

Allele frequencies within the seven southern populations did not differ significantly ($P > .05$) from those expected under Hardy-Weinberg equilibrium. However, the Cook County population was not in Hardy-Weinberg equilibrium ($P < .05$).

Most pairwise comparisons using the F_{ST} and R_{ST} statistics reflected significant genetic differentiation ($P < .002$, following Bonferroni correction) among populations (Table 1). However, differences between Ouachita and Ozark, Ouachita and Upper Atchafalaya, and Ouachita and Cook County were not significant ($P > .002$) with either estimator. Ten additional comparisons were not significantly different ($P > .002$) using the R_{ST} estimator, particularly those involving the Ouachita population. Those pairwise comparisons that were significantly different from R_{ST} included all comparisons involving Alabama/Mississippi, all comparisons between Cook County and members of the southern group, and all comparisons between Ozark and the southern group (with the previously mentioned exception of Ozark and Upper Atchafalaya).

A hierarchical analysis of populations (AMOVA) within and between the two groups (northern and southern) consistently found significant subdivision among

populations (19.8% of variation, $P = .00$ for F_{ST} ; 7.7% of variation, $P = .00$ for R_{ST}) but not among groups of populations (2.7% of variation, $P = .12$ for F_{ST} ; 8.2% of variation, $P = .02$ for R_{ST}). However, when the Alabama/Mississippi population was removed from the southern group and considered separately, the subsequent three-group AMOVA (northern, southern, and Alabama/Mississippi) found significant subdivision both among populations ($P = .00$ for F_{ST} and R_{ST}) and among groups of populations ($P = .03$ for F_{ST} and $P = .00$ for R_{ST}), with a smaller percent of variation among populations (15.1% for F_{ST} and 5.1% for R_{ST}) and a larger percent among groups of populations (8.9% for F_{ST} and 10.8% for R_{ST}).

The Assignment Calculator program (Brzustowski 2000), correctly assigned 118 of 151 individuals (78%) to their population of origin, 11 (7%) were assigned to the closest neighboring population, and 22 (15%) were assigned incorrectly (Table 6). The closely neighboring populations were Upper Atchafalaya and Lower Atchafalaya, White River and Tensas River, and Ouachita and Ozark. Alabama/Mississippi and Cook County were not considered to have a close neighboring population. Four populations had >91% correct assignment rates: Alabama/Mississippi (100%), White

River (95%), Tensas River (93%), and Lower Atchafalaya (92%). None of the six Ouachita bears were correctly assigned and three of them were assigned to the Cook County population.

Individual-Level Phylogeny

Each black bear exhibited a unique composite genotype using seven microsatellite markers. Removing data for individuals lacking genotypes at two or more loci ($n = 145$) did not change the relationships among individuals; therefore only analyses using all individuals ($n = 151$) will be presented. Phylogenetic patterns were examined considering each individual as a taxonomic unit. Both genetic distance measures relevant for comparing individual genotypes, *Dkf* and *Dps* (Bowcock et al. 1994), resulted in construction of "correct" trees in which samples from the same geographic area tended to cluster together, although not surprisingly, with little bootstrap support (Figure 3). Because both trees yielded similar clustering, only the *Dps* tree is shown. The inferred minimum evolution (NJ) phylogenies (Felsenstein 1993) depicted several notable features. White River and Alabama/Mississippi bears formed two monophyletic groups, with two exceptions in the White River group (inclusion of OUI41 and exclusion of WR536); these two individuals also were incorrectly assigned using the assignment test. The two Red Creek WMA bears (*U. a. luteolus*) clustered very closely with individuals from Mobile River (*U. a. floridanus*), suggesting the close genetic relationship of bears in these respective geographic regions; although of nominally different subspecies, these regions are separated by only about 60 km. The Tensas River population formed a tight cluster, although not monophyletic, and the White River group branched from the Tensas River cluster. Individuals from the Cook County population consistently associated with Ozark, Ouachita, Upper Atchafalaya, and Lower Atchafalaya populations, but never associated with White River or Alabama/Mississippi populations. Ouachita individuals did not form a unique cluster in either tree. When the data were reentered using randomized input order, the same pattern of grouping was observed for all populations, except that the single Lower Atchafalaya group was split into two groups on the *Dkf* tree.

Discussion

Population-Level Relatedness

Genetic diversity within the eight black bear populations studied differed between

Table 5. Matrices of genetic distance metrics among eight black bear populations at seven microsatellite loci, kinship coefficient (*Dkf*) below diagonal and proportion shared alleles (*Dps*) above diagonal

	AL	LA	UA	TR	WR	OU	OZ	CC
AL								
LA	0.29	0.60						
UA	0.21	0.06	0.57					
TR	0.29	0.16	0.32	0.61				
WR	0.44	0.27	0.14	0.47	0.71			
OU	0.29	0.12	0.08	0.50	0.55	0.68		
OZ	0.28	0.16	0.10	0.21	0.55	0.41	0.66	
CC	0.24	0.13	0.09	0.16	0.30	0.53	0.64	0.65

Population designations are as in Figure 1.

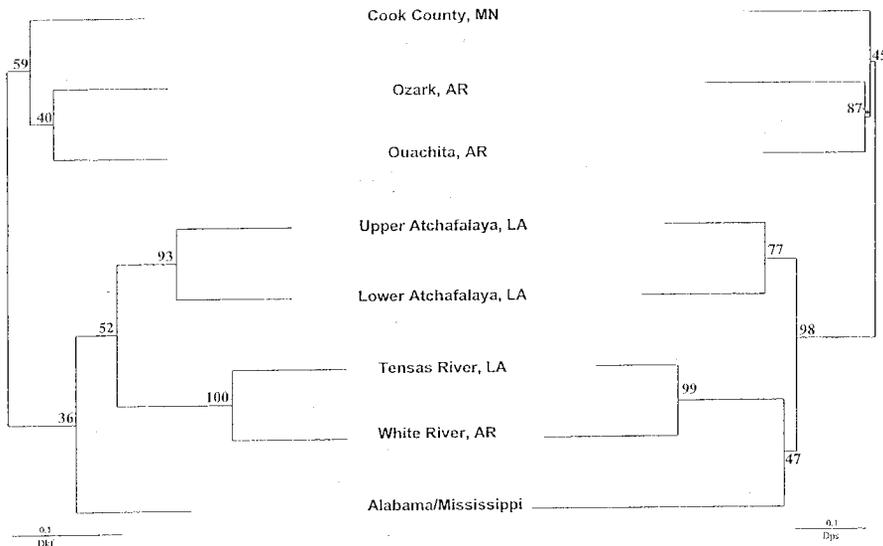


Figure 2. Phylogenetic relationships constructed from seven microsatellite loci among eight black bear populations, including 151 individuals. Bootstrap percentages are indicated at each relevant node. Neighbor-joining tree estimated using kinship coefficient distances (*Dkf*) with a $1 - kf$ transformation (left side), and using proportion of shared alleles distances (*Dps*) with a $1 - ps$ transformation (right side). Trees are rooted at the midpoint.

the *U. a. americanus* and *U. a. luteolus* subspecies. Diversity was greater within *U. a. americanus*, which corroborates previous results of Miller (1995). The Cook County population exhibited the highest level of diversity observed. The lowest variation was found in the White River and Alabama/Mississippi populations. Overall the level of microsatellite variation observed in this study is within the range observed for other black bear populations, where heterozygosities ranged from 0.35 to 0.80 and the average number of alleles ranged from 2.3 to 8.0 among populations (Paetkau and Strobeck 1994).

The observed patterns of genetic variation distinguished a southern group of black bear populations (White River, Tensas River, Upper Atchafalaya, Lower Atchafalaya, and Alabama/Mississippi), which differed from the northern populations using all three distance measures. Further, two of the distance measures (*Dps* and G_{ST}) provided significant statistical support for this relationship. Within this southern group, the White River and Tensas River populations were grouped with statistical significance, as were the Upper Atchafalaya and Lower Atchafalaya populations. All populations in the southern group are nominally *U. a. luteolus*, except White River is *U. a. americanus* and Alabama/Mississippi is *U. a. floridanus*. However, the genetic relationship between the White River and Tensas River populations was as close as those inferred for other consubspecific pairwise comparisons (e.g., UA/LA and OU/OZ) in this

study, leading to our inference that the White River and Tensas River populations are members of the same subspecies, and supporting reclassification of the White River population as *U. a. luteolus*. This is because of the close association of the White River population with the Tensas River population and other southern (*U. a. luteolus*) populations, and the nonassociation of the White River population with the Ozark and Ouachita (*U. a. americanus*) populations.

A larger genetic distance between Alabama/Mississippi and the other four southern populations (LA, UA, WR, and TR), significant group subdivisions using AMOVA when Alabama/Mississippi was not grouped with the other southern populations, and the inconsistent phylogenetic positioning of the Alabama population within the southern group suggest that the Alabama/Mississippi population may be genetically distinct from other southern populations. In order to be verified, this hypothesis requires further testing involving additional *U. a. floridanus* populations, which may associate with the AL/MS population. However, other work (Miller 1995) suggested that the distinction between *U. a. floridanus* and *U. a. luteolus* may be unwarranted.

Individual-Level Relatedness

Black bears from Cook, Lake, and St. Louis Counties, Minnesota, and from Manitoba, Canada were released into the Ozark, Ouachita, Upper Atchafalaya and Tensas River populations from 1958 to 1966 (Rog-

Table 6. Results of test assigning individual black bears to the population in which their genotype was most likely to occur.

Source population (sample size)	Assigned population (frequency)
AL (n = 13)	13 to AL/MS (100%)
LA (n = 26)	24 to LA (92%) 1 to UA (4%) 1 to OU (4%)
UA (n = 20)	12 to UA (60%) 5 to LA (25%) 1 to OU (5%) 1 to OZ (5%) 1 to CC (5%)
OU (n = 6)	1 to OZ (17%) 1 to LA (17%) 1 to WR (17%) 3 to CC (50%)
OZ (n = 14)	9 to OZ (64%) 2 to OU (14%) 3 to CC (21%)
TR (n = 14)	13 to TR (93%) 1 to WR (7%)
WR (n = 22)	21 to WR (95%) 1 to LA (5%)
CC (n = 36)	26 to CC (72%) 2 to LA (6%) 4 to OU (11%) 4 to OZ (11%)

ers 1973; Smith and Clark 1994). These reintroductions, and subsequent reproduction, may have contributed to the phylogenetic similarities of Cook County bears with Ozark, Ouachita, and Atchafalaya individuals. Cook County individuals, however, did not associate phylogenetically with Tensas River individuals using the *Dkf* metric; using the *Dps* metric, only one Cook County individual associated with Tensas River individuals. This is presumably because the Tensas River reintroduction was relatively small (35 individuals versus 130–200 individuals into the other areas of Arkansas and Louisiana) (Taylor 1971, cited in Pelton 1989), and introduced individuals may not have contributed considerably to the Tensas River gene pool. Had there been no genetic effect of reintroduction, we would expect to find that the individuals from the source populations would not have associated phylogenetically with individuals from the recipient populations. Further, since the source populations (Minnesota, Manitoba) are spatially and temporally separated from the recipient populations, a considerable degree of genetic differentiation would be expected between source and recipient. The White River, Tensas River, and Alabama/Mississippi populations appear to have unique evolutionary histories relative to the other populations. This is consistent with the fact that two of these populations were not augmented with Cook County individuals. In addition, the Alabama/Mississippi, White River, and Tensas

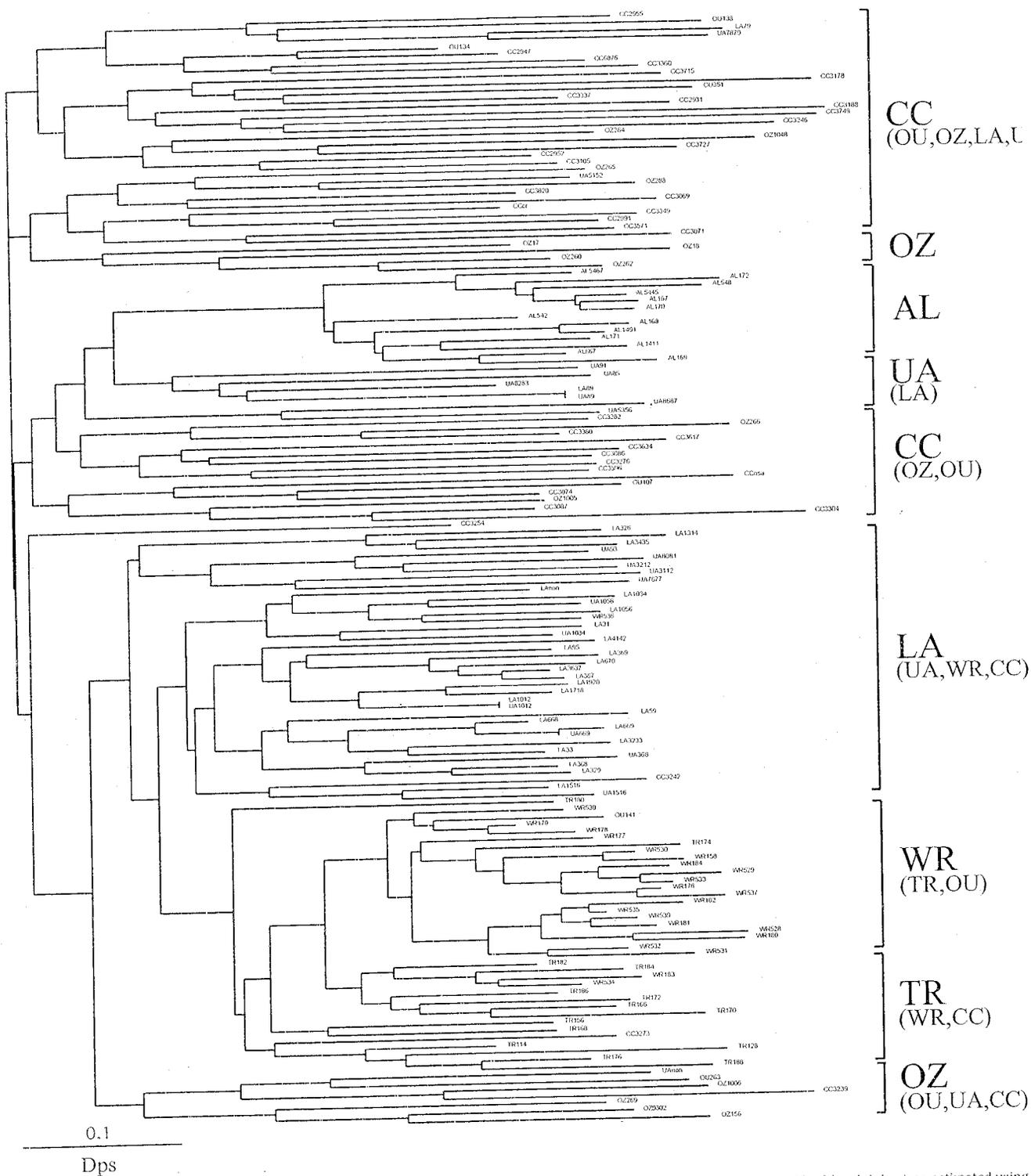


Figure 3. Phylogenetic relationships among 151 individual black bears constructed from data at seven microsatellite loci. Neighbor-joining tree estimated using proportion of shared alleles (*Dps*) with a $1 - ps$ transformation, and midpoint rooting

River populations were found to be more cohesive relative to other populations for two reasons: (1) 93–100% of individuals were assigned to their originating populations and (2) individuals assigned incor-

rectly from these three populations were still assigned within the southern group of populations. Thus the White River, Tensas River, and Alabama/Mississippi populations may be taken as more representative

of native southern black bear populations which have not been affected by introgression of northern genes. Ouachita is the only population not differentiated from all other populations, as evidenced

by several nonsignificant F_{ST} values; further, no Ouachita individuals were assigned correctly to the Ouachita population, and half were assigned to Cook County. This observation could be explained if the Ouachita population represents a natural or artificial intergrade population between the northern (*U. a. americanus*) and southern (*U. a. luteolus*) types. Hence we infer that while black bear introductions have affected the gene pools of several southern populations, the overall pattern of interpopulation relatedness was not altered. Earlier work using DNA fingerprinting and analysis of band-sharing frequencies (Miller et al. 1998) did not detect reintroduction effects. Greater sensitivity to interpopulation hybridization may have resulted from the use of allelic microsatellite markers in this study, as well as the larger number of interpopulation comparisons available.

Management Implications

Phylogenetic inferences at both the population and individual levels, based on variation at seven microsatellite loci, support the hypothesis that the White River NWR population of black bear belongs to the *U. a. luteolus* subspecies, which has threatened status under the U.S. Endangered Species Act. These inferences are being considered by the U.S. Fish and Wildlife Service to reach a finding regarding subspecific affinity of that black bear population. In particular, the issue of black bear harvest from the White River NWR is likely to be affected. Other data sets regarding genetic and morphologic characters might also be assessed to support or refute our inference regarding the subspecific status of the White River NWR population.

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Corresponding Editor: Stephen J. O'Brien



"Melissa Young"
<myoung@crpa.cc>
04/29/2005 10:36 AM

To <FW6_PMJM@fws.gov>
cc
bcc
Subject Colorado Rock Products Association Comments on Preble's
Meadow Jumping Mouse Delisting

U.S. Fish and Wildlife Service,

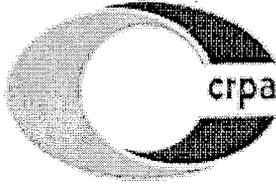
Please see the attached comments from the Colorado Rock Products Association supporting the delisting of the Preble's Meadow Jumping Mouse

Please reply stating the receipt of the comments and contact me if you have any questions

Sincerely,
Melissa I. Young, Esq.
Regulatory Specialist
Colorado Rock Products Association
6855 South Havana Street, Suite 540
Centennial CO, 80112
303-771-5290 direct
303-886-2178 cell
303-290-8008 fax
myoung@crpa.cc



PMJM Delisting Comments 4-29-2005.doc



Colorado Rock Products Association

April 29, 2005

Field Supervisor
Colorado Field Office
Ecological Services
755 Parfet Street, Suite 361
Lakewood, CO 80215

RE: RIN 1018-AU12 Endangered and Threatened Wildlife and Plants; 12-Month Finding on a Petition to Delist the Preble's Meadow Jumping Mouse (*Zapus hudsonius preblei*) and Proposed Delisting of the Preble's Meadow Jumping Mouse

U.S. Fish and Wildlife Service:

The Colorado Rock Products Association (hereinafter "CRPA") appreciates the opportunity to comment on the U.S. Fish and Wildlife Service's (hereinafter "Service") proposal to delist the Preble's Meadow Jumping Mouse (PMJM) from the List of Endangered and Threatened Wildlife. See 50 CFR 17.11. The CRPA commented on the Service's July 17, 2002 proposal to designate critical habitat and the January 28, 2003 proposal announcing the availability of the draft economic analysis and draft environmental assessment for the proposal to designate critical habitat for the PMJM. We submit these comments in support of the proposal to delist the PMJM.

The CRPA represents 33 producer members and 31 associate members throughout the state of Colorado who produce over 33 million tons of aggregates, crushed stone and sand and gravel, which are used in various forms of construction for highways, sidewalks, residential and commercial buildings, and water and sewage treatment plants. Many of our members' companies are vertically integrated, thus producing ready mixed concrete and asphalt, and many are family owned businesses. Our members employ more than 5,000 people and produce over 85 percent of all the aggregates used in the State and produce over 85 percent of the ready mixed concrete in the State. Thus, aggregates producers are major contributors to the Colorado economy.

The Service has completed a review of the best available scientific and commercial information and has determined that the removal of the PMJM from the List of Endangered and Threatened Wildlife is warranted. See 70 Fed. Reg. 5404, 5405 (February 2, 2005); See also Endangered Species Act, 16 U.S.C. §1533(b)(3)(A) and (B) (1973).

The CRPA supports the delisting of the PMJM based upon the peer reviewed study conducted by Dr. Rob Roy Ramey II (Ramey *et al.* (2004)). Dr. Ramey concluded that “based on the lack of genetic, morphological, or published ecological evidence for genetic distinctiveness between the Preble’s and the Bear Lodge meadow jumping mouse, these subspecies should be synonymized (considered the same subspecies) as *Zapus hudsonius campestris*.” 70 Fed. Reg. at 5407. In addition, Dr. Ramey concluded that the PMJM is not threatened by a loss of habitat, as its territory covers many Western states. See Ramey *et al.* (2004). Irrespective of the Federal listing status, the State of Colorado and local governments intend to continue conservation efforts to protect the PMJM. Private landowners will have to work with government entities in developing habitat mitigation plans and open space protection for the PMJM.

Many industries, including the construction materials industry, were negatively affected by the listing of the PMJM as threatened and the subsequent designation of critical habitat. As a producer of aggregates used to build infrastructure, our industry was affected by the cost to conduct PMJM habitat and trapping surveys (from \$2,800 to \$10,000 per site), mitigation (\$8,600 for one year at a site), and Section 7 consultations (approximately \$37,700 at a site) to obtain Section 404 permits. In addition, the State’s mired efforts to improve and repair existing infrastructure represented a lost business opportunity for our industry along with other potential customers who could not develop their land due to the listing and designation of critical habitat for the PMJM. These adverse effects could have been avoided by reforming the Endangered Species Act to include the use of “sound science” in determining whether or not a species should be listed as endangered or threatened.

Thank you for your consideration of the foregoing comments of the Colorado Rock Products Association. If you have any questions on the above, or would like to speak with us further about our comments, please do not hesitate to contact me at 303-771-5290.

Respectfully submitted,



Melissa I. Young, Esq.
Regulatory Specialist

cc: Paul Schauer, CRMCA/CRPA Managing Director
Sen. Wayne Allard
Sen. Ken Salazar
Rep. Diana DeGette
Rep. Mark Udall
Rep. John Salazar
Rep. Marilyn Musgrave
Rep. Joel Hefley
Rep. Tom Tancredo
Rep. Bob Beauprez
Russell George, Executive Director, CO Dept. of Natural Resources