

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

Updated 2/14/08

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01/22/2008 04:31 PM

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cc
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Subject Preble's comments

Attachments: 8

As an initial matter, I provide the following excerpts from my July 31, 2007 written Congressional Testimony (full text is attached). It provides an excellent summary of the larger issues with the Proposed Rule and is fully supported by the publicly available record.

"The fundamental distinction between science and non-science is the criterion of falsifiability. In other words, all hypotheses must be testable. When clear-cut criteria are laid out in advance of data collection and all information considered (the scientific method), then there is less room for bias through the selective interpretation of the information. For the Endangered Species Act (ESA), which relies on scientific information, that means that data must be publicly available, conclusions open to question, and all information considered - including contrary information. If ESA decisions are not made in such an open and transparent way, then the moral authority of the ESA is compromised and valuable resources are diverted away from conservation."

"In the case of the Preble's mouse (listed as an endangered subspecies), the record will ultimately show that special interest groups, individuals, and academics with vested financial interests, and some U.S. Fish and Wildlife Service (USFWS) staff, have managed to maintain an invalid

subspecies as an ESA-listing by obfuscation, intimidation, and ignoring contrary evidence. I have five years of experience on this issue because I was the scientist who led the work that questioned the validity of the Preble's mouse subspecies and its presumed rarity, and concluded that it was not a valid subspecies.

Obfuscation

The USFWS erroneously reported twice in the Federal Register that the Preble's delisting petitions relied primarily on the results of our study. That is contrary to the fact that our research was only mentioned on half a page of the 106-page delisting petitions. The delisting petitions provided abundant information that these mice are more common and widespread than previously thought. Yet the USFWS has still failed to address these data over three years later.

The USFWS Denver office organized two sets of peer reviews of our research prior to publication. However, they had failed to rigorously review the weak evidence that was used previously in support of the listing.

After our original research refuting the validity of the Preble's mouse as a subspecies was published in 2005, the FWS at Region 6 went looking for another study that would support the listing. Shortly thereafter, a report came out by a USGS biologist that concluded that Preble's was a valid subspecies and made a wholesale portrayal of our work as inaccurate. This USGS report was leaked to the press by a pro-listing

environmental group amidst much media fanfare. Most of the press did not bother to read any of the original papers, or our responses. The key difference between these studies was how the problem was approached. We set criteria in advance of data collection and measured to those thresholds, whereas the USGS study relied on post-hoc interpretations and used a level of divergence so low that almost any population could be listed as endangered under the ESA, effectively removing such decisions from the realm of science.

In March of 2006, the staff at Region 6 sought to rush through approval of a peer review panel composed largely of agency biologists and scheduled for a time when I could not attend. After their efforts failed, another peer review panel was organized. The lead author of the USGS study, as well as environmental groups, influenced the structure and composition of the panel. A double standard was applied to evaluating panelist's conflicts of interest and to evaluating the evidence itself. Instead of reviewing all of the available science, the panel arbitrarily created its own burden of proof, which it then unilaterally applied only to our study. Rather than focus on the real issue of appropriate thresholds that can be used to define subspecies, they diverted attention by focusing criticism on results from a handful of specimens in our study. The panel failed to acknowledge that reanalysis of our data without these specimens, did not alter the overall results or conclusions of our study. Ultimately, if this panel's recommendations are followed and applied to other cases, it would mean that many inadequately defined subspecies would not be potentially falsifiable (i.e. could never be questioned).

This effectively puts ESA-listed subspecies evaluations outside the realm of scientific investigation.

We respectfully disagreed with the conclusions of the USGS study and prepared a response paper. That paper was accepted for publication in February 2007, however, the lead author of the USGS study managed to delay publication of our paper for months.

Intimidation

Over the course of two years I was harassed and intimidated by USFWS Denver staff, most notably, the leader of the Preble's Recovery Team who cursed me in harassing telephone messages, wrote fallacious slander about me to my supervisors, and threatened to withhold research funding for the project. A Preble's mouse consultant, representing a coalition of environmental groups, USFWS staff, and academics, all of who have financial stake in the Preble's listing or others like it, put pressure on my employer.

Ignoring contrary evidence

Most contrary information to the Preble's listing is absent from the USFWS Preble's Meadow Jumping Mouse Home Page. The USFWS gives dismissive treatment to contrary information in Federal Register notices, or does not provide it to peer reviewers. This speaks volumes about the selective use of information by this agency. For example:

- *The USFWS has not acknowledged that this supposed subspecies was originally based on measurements of only three specimens, nor have they acknowledged that the original scientist who described this subspecies in 1953 went on record in 2004 rejecting the validity of the subspecies.*

- *The USFWS has not acknowledged that an earlier (1997) genetics study that was used in support of the listing was never published and the data were never made publicly available, despite repeated requests. In short, that study was never subjected to a rigorous review.*

- *The USFWS has not acknowledged that the 1995 distribution study that was used in support of the listing was based on minimal effort and never published.*

- *The USFWS kept over a decade of Preble's trapping data in their files but never analyzed them. Independent analysis of those data showed that the supposedly rare Preble's mouse subspecies was far more common and widespread than previously thought.*

- *Contrary information missing from the USFWS website includes:*

1) *A 1981 dissertation that examined 9,000 specimens of jumping mice and concluded that there were no subspecies of meadow jumping mice.*

2) *A series of five papers in the journal Animal Conservation*

that followed our original study, including a 2006 response paper by my coauthors and myself. The only paper from this series that appeared on the website was the paper which supports the continued ESA listing.

3) A 1986 experimental study that showed that another species of rodent, the meadow vole, out-competes the meadow jumping mouse. In other words, when meadow vole numbers are high, meadow jumping mouse numbers are low and they are hard to catch.

4) An independent quantitative analysis of both the raw genetic data from our 2005 paper and the data from the USGS study. That quantitative analysis used thresholds from the literature and found no support for Preble's as a subspecies, let alone as an Evolutionary Significant Unit (ESU) or Distinct Vertebrate Population Segment (DPS).

5) Our August 2006 response to the Preble's review panel report that we provided to the USFWS.

6) Our response paper to the USGS study that we provided to the USFWS.

7) A 2003 study published in Conservation Biology that revealed that the Preble's subspecies ESA listing actually encouraged landowners to take steps that were counterproductive to conservation.

"Obfuscation, intimidation, and ignoring of contrary evidence have contributed to the continued ESA-listing of the Preble's mouse subspecies. As shown with the second and third examples [coastal California gnatcatcher and Peninsular bighorn sheep], the Preble's case is not an isolated incident; it is symptomatic of deeper problems within agencies charged with administration of the ESA. While there are many competent and dedicated staff within these agencies, there are neither adequate safeguards nor oversight to prevent other staff from cherry-picking, engaging in subjective interpretations, or completely ignoring contrary information altogether. There are scant few with the expertise or the time needed to detect such occurrences."

Regrettably, the Proposed Rule is proof that nothing has changed at the USFWS. In this Proposed Rule, the USFWS seeks to salvage an untenable ESA subspecies listing by proposing an even more arbitrary sub-subspecies population listing in Colorado and defending it through selective use of information.

For the record, I have attached copies of our published papers (Ramey et al. 2005, 2006, 2007) on the subject, our review of the SEI panel report, and documentation on some previously unreported issues. As I reported in my written Congressional testimony, the USFWS has consistently failed to post this and other contrary information on the Preble's home page. Rather than duplicate effort here, I will encourage the USFWS to fully consider this and accurately report on that information and focus

my following comments on some key issues that have not been addressed by the USFWS.

Agency Handling of the Data Quality Act Challenge

Imagine if a drug company produced a new drug and touted it as an alternative to an existing drug - citing supposed flaws with the other drug. Later, questions are raised as to whether the data used in support new drug can actually support its claims. A review of the data is commissioned because of potential legal challenge. The review ends up supporting the new drug, a little too wholeheartedly. However, it is discovered that the former CEO of the drug company who commissioned the new drug now heads up the very subsidiary company that was chosen to perform the supposedly independent review of the new drug.

This describes situation with how the Department of Interior handled review of King et al. in response to the Data Quality Act (DQA) challenge by attorney, Kent Holsinger. In that situation, the former regional Director of US Fish and Wildlife Service Region 6, Ralph Morgenwick, both commissioned the King et al./USGS study and was also the lead author on the agency's review of that study for the DQA response. These clearly compromise objectivity and introduce bias into a process that was designed to ensure objectivity and prevent bias!

Specifically, both DQA and the Office of Management and Budget (OMB) Guidelines require agencies to "ensure and maximize" the quality, objectivity, utility, and integrity" of

information disseminated by federal agencies. DQA §515(a), OMB Guidelines, § 11(2), 67 Fed. Reg. at 8458. The DQA and the Guidelines require agencies to issue guidelines ensuring and maximizing the "objectivity" of all information they disseminate. The OMB guidelines implementing the legislation define "objectivity," and that definition includes a requirement that information be "unbiased" in presentation and substance. "Objectivity," along with "unbiased," is considered to be, under the OMB guidelines, an "overall" standard of quality. 67 Fed. Reg. 8452, 8458 (Feb. 22, 2002).

Revisionist science by King

King et al. released their initial report in January 2006 (2006a). Subsequently, they added data and analyses from an additional 28 samples of *Z. h. preblei* from southern Wyoming. The combined data and analyses were accepted for publication, presented before the Sustainable Ecosystems Institute (SEI) panel, and resulted in the publication of King et al. (2006b) appearing in the journal *Molecular Ecology* .

However, a comparison of trapping notes and an accompanying photograph from researcher Rene Taylor with a spreadsheet provided by Mary Jennings of the USFWS (Zapus_King.xls) show that King had clearly misidentified a hispid pocket mouse (sample TMC9901) as *Z. h. preblei* in mtDNA and microsatellite analyses, and chose not to report that result in King et al. (2006b). The attached spreadsheet shows that mtDNA and microsatellite data indicated the sample was a The

spreadsheet shows results from 29 samples of *Z. h. preblei* while King et al. (2006b) show results from 28 samples of *Z. h. preblei* . The missing sample is TMC9901, a hispid pocket mouse--an entirely different genus of mice (*Perognathus hispidus*) than meadow jumping mice (*Zapus hudsonius*).

During early summer 2006 (before the SEI panel), Taylor, Jennings, and other researchers had been working together, using King's molecular analyses (Zapus_King.xls and other data) to plot *Z. hudsonius* and *Z. princeps* locations in Wyoming. Taylor noticed the error in King's results because she had captured TMC9901 and identified it as a hispid pocket mouse in 1999. After capturing TMC9901, Taylor had labeled it the "mystery mouse" in her notes because she was initially unsure of its identification. Fortunately she had photographed the mouse, kept the mouse until it was properly identified, and then released it back into the wild. In 2006, after Taylor shared the misidentification with the USFWS, results of that sample failed to appear in King et al. (2006b). No mention was made in King et al. (2006b), or elsewhere, about the misidentification.

This is an important issue for the following reasons:

- 1) King et al. (2006b) made a number of allegations about data used in Ramey et al. (2005) and sought a wholesale portrayal of that work as inaccurate. At the same time, King et al., the USGS, and USFWS sought to promote his work as flawless. However, King's result for sample TMC9901, as well as other actions detailed below, clearly call into question the accuracy of his work. King's failure to report this and any unfavorable

results is consistent with his alteration of the methods in the page proofs stage of King et al. (2006b). In that case, King had altered his methods section after we pointed out to the SEI panel that he did not use the same primers or PCR conditions as Ramey et al (2005), yet claimed to obtain the same results. The details of the changes that King made in proofs can be found in Ramey et al. (2007).

2) As we pointed out in Ramey et al. (2007): "*The publicly available record shows that KEA [King et al.] changed their interpretation of results at least twice, from subspecies being 'weakly differentiated' to 'evolutionarily distinct'; and the number of potential DPS's of *Zapus hudsonius preblei* changed from two, to three, to none.*" In Ramey et al. (2007) we did not include the specific references to each of King's changes because the editor of Molecular Ecology asked us to remove references to unpublished reports by King et al.. After King obtained his initial results and analyses, he sent out an internet posting describing the subspecies as "weakly differentiated" in mtDNA (see Data Quality Act challenge by K. Holsinger for details). Subsequently, this was changed to "evolutionary distinction" of subspecies in King et al. (2006a, b). The number of potential DPS also changed from two (in King et al. 2006a), to three (King's statements before the SEI panel), to none in final publication (King et al. 2006b).

As we pointed out in Ramey et al. (2007): "*This subjectivity [by King et al.] is symptomatic of an approach that lacks clearly defined thresholds, and epitomizes the problem that REA attempted to address. We believe that few if any*

subspecies or DPSs proposed for US-ESA listing or delisting will be falsifiable under the general approach and low level of genetic differentiation that KEA used to accept subspecies. General application of that approach may move the allocation of conservation effort outside the realm of scientific inquiry ." Continued reliance on King et al. (2006b) as "best available science" in the Proposed Rule, shows that this threshold has already been crossed by the USFWS.

3) Rather than publish their response to Ramey et al. (2005) in the same journal (*Animal Conservation*), which is typical for response papers, King chose to publish their response in a different journal (*Molecular Ecology*). In this way, King positioned himself as if King et al. (2006b) were an "original article" rather than a "response article" (to Ramey et al. 2005). This is an important difference because authors of original articles are typically allowed by editors to have the last word to any responses written about their article. So, by publishing in *Molecular Ecology* , King maneuvered into a more favorable position, one that potentially allowed him to have the last word. King's strategy also allowed him to delay publication of our response article, by claiming to the associate editor of *Molecular Ecology* that he was submitting a rejoinder to our response article and needed more time. Eventually, the Associate editor set a deadline, which King failed to meet, and our article (Ramey et al. 2007) was released for publication.

4) The publicly available record shows that King manipulated the composition of the SEI panel by lobbying and increasing time allotted to him and outside supporters 2:1 in his favor.

King also pushed for, and succeeded in, elimination of a panelist whom he worried would be sympathetic to Ramey et al. (2005) but made no such complaints about a panelist who was a collaborator with one of the primary critics of Ramey et al. (2005). This is detailed in the July 30, 2007 letter from Senator Allard to Secretary Kempthorne and in my July 31, 2007 written Congressional testimony (attached).

5) King's actions are not isolated incidents unique to the Preble's case. In the case of the Atlantic Salmon in 2000, the State of Maine had to file suit against the USFWS and NMFS in order to obtain genetic data from King about the uniqueness of salmon populations. Eventually, Maine prevailed and data were handed over along with what was discovered to be an altered data analysis file.

6) Now, in the Proposed Rule, there is reference to an unpublished article by King et al. (in review). However, requests to the USFWS Region 6 have shown that they do not have it in their possession. A recent request in writing from Congresswoman Musgrave to Secretary Kempthorne also failed to produce a copy of the paper. Yet my correspondence with the associate editor at *Molecular Ecology* suggests that there may have been a paper and that was the reason why our publication was delayed. In light of King's previous manipulation of the publication process and alteration of methods, results, and interpretations, this is a disturbing development.

If the King et al. (in review) paper (or data) does not exist, then

why is it cited by the USFWS in the Proposed Rule? This cannot be attributed to an error on behalf of the USFWS because they conceded King had this paper in his possession and that he had been unwilling to release it to the USFWS. Will this paper suddenly appear in print after the comment period closes? If so, it would preclude any sort of rebuttal and suggest that the USFWS and USGS prefer to wait until potential criticisms are logged-in, make changes to their "best available science", and seek delay in order obtain the last word on the subject.

I challenge the USFWS and USGS to defend the post-hoc manipulative approach, exemplified by King et al., as either objective or scientific.

**Application of the Solicitor's opinion on the meaning of:
*"In danger of extinction throughout all or a significant portion of its range"***

In the Proposed Rule, the USFWS seeks to apply the Solicitor's opinion in support of its Proposed Rule to retained ESA-listed status for *Z. hudsonius* populations in Colorado. The Solicitor's opinion is used to justify a sub-species listing, based on a subjective, non-quantifiable, and non-falsifiable approach. In short, it lacks a sound scientific and epistemological basis.

The central issue with the Solicitor's opinion is that it relies on a subjective approach rather than scientific one. ESA decisions are required to rely on a best available science standard; and the practice of science requires observation, measurement, and

potential falsifiability of conjectures (Popper 1963). However, the Solicitor's opinion avoids any use of thresholds, even for terms that lend themselves to measurement, such as quantifying "significant portion of the range", "current range", or "reasonably foreseeable future". Instead, the Solicitor relies on a subjective use of vague terminology. Decisions are to be made on a case-by-case basis, based on a non-scientific, subjective approach.

Rather than acknowledge that there are quantitative thresholds or consistent criteria that can be used to aid such decisions, the Solicitor relies on Chevron deference. That removes all such decisions from the realm of science and makes them more dependent upon the ideological gatekeepers that are in power at the time. This neither serves conservation in general nor the intent of Congress and The People in the passage of the ESA. In large measure, the failure of the ESA to recover more than a handful of species is directly a result of its lack of consistent standards with which to set priorities.

The Solicitor's opinion also allows any population of organisms qualify as an "endangered species". Specifically, viewing "significance" in the context of a subspecies or DPS, is a self-serving, reductionist approach that guarantees a limitless supply of endangered "species" to be regulated by the USFWS. It is simple logic that if a line is drawn around a population of a full species, the subset of individuals contained within that line will be more limited and occupy a smaller geographic range than the full species. If these are further subdivided into significant and non-significant portions of their range,

population numbers fall further within each unit. So, if endangered "species" are defined in such a way, there is no hope for recovering full species, because there are not enough resources to go around. It is the law of economics applied to conservation: inflate the number so-called species and you devalue the effort that can be allocated to them.

An even more extreme version of this approach is exemplified by the USFWS's suggestion in the Proposed Rule that: *"Another possibility to consider is whether smaller [listable] units might be appropriate. For example, one could consider each individual drainage or each individual county."* In his 2002 Senate Testimony, Steven Quarles clearly laid out the issues with this sub-species listing approach and the subjective 'standardless-standards' used by the USFWS and NMFS to define them. He also proposed some potential solutions to these issues. I would encourage the USFWS to consider these.

I would also encourage the USFWS to avoid inventing new vacant terminology to hide the fact that measurement has been effectively abandoned by that agency. For example, the proposed Rule suggests: *"The terms "resiliency," "redundancy," and "representation" are intended to be indicators of the conservation value of portions of the range."* These terms have no definitional basis.

What does "evolutionarily distinct" really mean?

The Zoological Society of London recently developed a quantitative approach to ranking species conservation priorities based on measures of their phylogenetic (evolutionary) uniqueness. It is called the Evolutionarily Distinct and Globally Endangered program (EDGE). This approach gives a higher ranking to species that are member of monotypic genera (one species in the genus) and lower priority of species that have many members within a genus. In ranking of 4,173 mammals, top ranking evolutionary distinct species included: Attenborough's long beaked echidna, Yangtze River Dolphin, pygmy hippopotamus, bactrian camel, slender loris, Java rhinoceros, and red panda. All of these are evolutionarily distinct (no closely related species and millions of years of divergence to nearest relatives) and globally endangered.

In contrast, consider the "evolutionary distinction" claimed by the King et al. (2006b) about *Z. h. preblei*, and upon whose "evolutionary distinction" the USFWS plans to invest upwards of a half a billion dollars in effort over the next 20 years (Ramey et al. 2006). Yet this is one of 12 putative subspecies of a species that ranges across half of North America, and overlaps with two other species in the genus *Zapus*. In the Zoological Society of London, EDGE method, the meadow jumping mice (*Zapus hudsonius*) ranks 2,114. If subspecies were added to this scheme, *Z. h. preblei* would drop off the chart as a conservation priority.

It should be clear from this example that local populations of *Z. h. preblei* that the USFWS is considering retaining as ESA-listed in Colorado are diverged on a scale of decades to

several thousand years. That is clearly not an "evolutionary distinction", it represents a shallow level of recent population divergence.

Thank you for this opportunity to provide comments.

Rob Roy Ramey II, Ph.D.
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 ✓ Ramey_Testimony.doc  ✓ Allard_to_Kemphom.pdf  ✓ Ramey_S1367943005002313a.pdf
 ✓ ResponseToVEA.pdf  ✓ Ramey_Response_to_panel_report.pdf  ✓ Ramey_et_al_response to KEA.pdf  ✓ Zapus_King1.xls  ✓ TMC_9901.doc

Testimony of
Rob Roy Ramey II, Ph.D.
Committee on Natural Resources
United States House of Representatives
July 31, 2007

My qualifications

As a field biologist and conservation geneticist, I have 27 years of experience in conservation, research and management of threatened and endangered wildlife. I have worked with: peregrine falcons; California condors; goshawks; rainforest birds; desert, Sierra Nevada, and Rocky Mountain bighorn sheep, argali sheep of Asia, meadow jumping mice, and African elephants. I have studied parasites and pathogens including: Psoroptic scabies mites; respiratory bacteria, and HIV. I earned a Ph.D. from Cornell University in Ecology and Evolutionary Biology; a master's degree from Yale University in Wildlife Ecology; and a bachelor's degree in Biology and Natural History from the University of California Santa Cruz. My postdoctoral experience included research at University of Colorado, Boulder and as a visiting scientist at the Center for Reproduction of Endangered Species at the San Diego Zoo. I was Curator of Vertebrate Zoology at the Denver Museum of Nature & Science and served as a consulting Science Advisor to the Office of the Assistant Secretary of the Interior in Washington, D.C. I am member of the Caprinae Specialist Group at the International Union for the Conservation of Nature (IUCN). I presently consult on endangered species scientific issues and conduct scientific research with Wildlife Science International, Inc.

Introduction

This hearing is focused on questionable actions of the current administration relative to science. However, to avoid science falling prey to partisan politics, there is a need to focus briefly on the larger question of what distinguishes science from non-science. The fundamental distinction between science and non-science is the criterion of falsifiability. In other words, all hypotheses must be testable. When clear-cut criteria are laid out in advance of data collection and all information considered (the scientific method), then there is less room for bias through the selective interpretation of the information. For the Endangered Species Act (ESA), which relies on scientific information, that means that data must be publicly available, conclusions open to question, and all information considered - including contrary information. If ESA decisions are not made in such an open and transparent way, then the moral authority of the ESA is compromised and valuable resources are diverted away from conservation.

I write today because there does appear to be a "Crisis in Confidence" with some of the "science" used in Endangered Species Act decisions. This is an issue that crosses administrations and sides of the aisle. The examples below show that there is a "crisis" occurring, for reasons other than what you may have been led to believe. There can also be serious consequences for those who dare to ask questions about information used in some ESA decisions.

Case 1: The Preble's Mouse Jumping Mouse

In the case of the Preble's mouse (listed as an endangered subspecies), the record will ultimately show that special interest groups, individuals, and academics with vested financial interests, and some U.S. Fish and Wildlife Service (USFWS) staff, have managed to maintain an invalid subspecies as an ESA-listing by obfuscation, intimidation, and ignoring contrary evidence. I have five years of experience on this issue because I was the scientist who led the work that questioned the validity of the Preble's mouse subspecies and its presumed rarity, and concluded that it was not a valid subspecies.

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applied only to our study. Rather than focus on the real issue of appropriate thresholds that can be used to define subspecies, they diverted attention by focusing criticism on results from a handful of specimens in our study. The panel failed to acknowledge that reanalysis of our data without these specimens, did not alter the overall results or conclusions of our study. Ultimately, if this panel's recommendations are followed and applied to other cases, it would mean that many inadequately defined subspecies would not be potentially falsifiable (i.e. could never be questioned). This effectively puts ESA-listed subspecies evaluations outside the realm of scientific investigation.

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Over the course of two years I was harassed and intimidated by USFWS Denver staff, most notably, the leader of the Preble's Recovery Team who cursed me in harassing telephone messages, wrote fallacious slander about me to my supervisors, and threatened to withhold research funding for the project. A Preble's mouse consultant, representing a coalition of environmental groups, USFWS staff, and academics, all of who have financial stake in the Preble's listing or others like it, put pressure on my employer.

Ignoring contrary evidence

Most contrary information to the Preble's listing is absent from the USFWS Preble's Meadow Jumping Mouse Home Page. The USFWS gives dismissive treatment to contrary information in Federal Register notices, or does not provide it to peer reviewers. This speaks volumes about the selective use of information by this agency. For example:

- The USFWS has not acknowledged that this supposed subspecies was originally based on measurements of only three specimens, nor have they acknowledged that the original scientist who described this subspecies in 1953 went on record in 2004 rejecting the validity of the subspecies.
- The USFWS has not acknowledged that an earlier (1997) genetics study that was used in support of the listing was never published and the data were never made publicly available, despite repeated requests. In short, that study was never subjected to a rigorous review.
- The USFWS has not acknowledged that the 1995 distribution study that was used in support of the listing was based on minimal effort and never published.
- The USFWS kept over a decade of Preble's trapping data in their files but never analyzed them. Independent analysis of those data showed that the supposedly rare Preble's mouse subspecies was far more common and widespread than previously thought.

- Contrary information missing from the USFWS website includes:

- 1) A 1981 dissertation that examined 9,000 specimens of jumping mice and concluded that there were *no subspecies* of meadow jumping mice.
- 2) A series of five papers in the journal *Animal Conservation* that followed our original study, including a 2006 response paper by my coauthors and myself. The only paper from this series that appeared on the website was the paper which supports the continued ESA listing.
- 3) A 1986 experimental study that showed that another species of rodent, the meadow vole, out-competes the meadow jumping mouse. In other words, when meadow vole numbers are high, meadow jumping mouse numbers are low and they are hard to catch.
- 4) An independent quantitative analysis of both the raw genetic data from our 2005 paper and the data from the USGS study. That quantitative analysis used thresholds from the literature and found no support for Preble's as a subspecies, let alone as an Evolutionary Significant Unit (ESU) or Distinct Vertebrate Population Segment (DPS).
- 5) Our August 2006 response to the Preble's review panel report that we provided to the USFWS.
- 6) Our response paper to the USGS study that we provided to the USFWS.
- 7) A 2003 study published in *Conservation Biology* that revealed that the Preble's subspecies ESA listing actually encouraged landowners to take steps that were counterproductive to conservation.

Case Two: The Coastal California Gnatcatcher

Two peer reviews of the coastal California gnatcatcher taxonomy were conducted by the USFWS (listed as an endangered subspecies). One internal peer review by federal agency biologists omitted substantial contrary information that was in the public record. The omitted contrary information included six technical reports reanalyzing the original data used to describe the subspecies, one peer-reviewed paper on gnatcatcher taxonomy, and a deposition by the scientist who described it as a new subspecies. In that deposition, the scientist recanted the reliability of key measurements, admitted to substituting estimates for missing data, and told of destroying original copies of his data *before* he finished his dissertation and published the results. Despite these revelations, the scientists who conducted the internal agency peer review then made a Powerpoint presentation to senior decision makers at the Department of Interior in Washington, D.C. That presentation

made no mention of the omitted contrary information and thus the subspecies listing of the coastal California gnatcatcher was maintained.

Case Three: Critical Habitat of Desert Bighorn Sheep in the Peninsular Ranges of California

The recovery plan for desert bighorn sheep in the Peninsular Ranges of southern California (listed as an endangered DPS) specifically called for a quantitative habitat analysis. Consequently, an extensive database of 21,055 bighorn sheep observations was compiled. However, Critical Habitat was subjectively defined by the USFWS and based upon the opinions of Recovery Team members rather than on a quantitative analysis of the observation data.

Several colleagues and I published a scientific paper on the determination of Critical Habitat for this population. We had to obtain the bighorn observation data under a Freedom of Information Act request because the local USFWS office would not release the data when requested. When we obtained the data, we found that it had been stripped of many attributes. When I asked for these additional data, I was told by the USFWS to go to the individual researchers. When I went to the individual researchers I was told: *"The USFWS data was deliberately provided in a format that would not facilitate a detailed analysis by those unfamiliar with the manner in which it was collected."*

In our subsequent analyses, we found that over 60 percent of designated Critical Habitat in the northern Santa Rosa Mountains had a near zero probability of bighorn sheep use. Critical Habitat for this DPS has been vacated in part and remanded for new rulemaking by the Court. In this case, both our analysis and the Court did not agree with the USFWS staff's so-called "science".

Conclusion

Congress and the Department of Interior could ask: "Why don't we ask the right questions in the first place *before* questionable subspecies and populations are added to the Endangered Species list?"

Obfuscation, intimidation, and ignoring of contrary evidence have contributed to the continued ESA-listing of the Preble's mouse subspecies. As shown with the second and third examples, the Preble's case is not an isolated incident; it is symptomatic of deeper problems within agencies charged with administration of the ESA. While there are many competent and dedicated staff within these agencies, there are neither adequate safeguards nor oversight to prevent other staff from cherry-picking, engaging in subjective interpretations, or completely ignoring contrary information altogether. There are scant few with the expertise or the time needed to detect such occurrences.

There are productive steps that could be taken to ensure that ESA decisions are based upon science rather than opinion and politics, while ensuring that priority for conservation effort goes to truly endangered species. I have suggested a number of these in previous Congressional testimony and publications.

Briefly, these include:

- 1) Take steps to ensure that all information, including contrary information, is considered in peer reviews, listing/delisting decisions and biological opinions. Consistent questions and standards in these peer reviews would serve conservation. Rather than internal agency peer reviews, require external/independent reviewers.
- 2) Require that data used in peer reviews, listing/delisting decisions, and biological opinions be publicly available.
- 3) Establish legally-definable minimum thresholds for the uniqueness of taxa that can be listed. Set the bar at a quantifiable and biologically meaningful level of distinctiveness.
- 4) Establish quantitative thresholds for "significance" used in DPS listings. This could be quantified in terms of percent range and/or census numbers.
- 5) Establish a quantitative approach for designating Critical Habitat.
- 6) Require compliance with priority rankings in order to allocate listing and recovery effort.
- 7) Take steps to eliminate financial and other conflicts of interest in Recovery Teams and peer reviews.
- 8) Evaluate hypothetical threats using a well-defined problem analysis approach.

In conclusion, I urge this Committee to pursue this reasonable and science-based path to protecting endangered species.

Thank you for the opportunity to write to you about these issues.

United States Senate

WASHINGTON, DC 20510-0606

July 30, 2007

The Hon. Dirk Kempthorne
Secretary
U.S. Department of the Interior
1849 C St., NW
Washington, D.C. 20240

Dear Secretary Kempthorne:

I am writing in regards to correspondence I requested from you on the proposed delisting of the Preble's meadow jumping mouse in August of 2006. Upon careful review of these correspondences, the information paints what appears to be a troubling picture of a coordinated effort on behalf of U.S. Fish and Wildlife Service (FWS) staff to retain the listed status of the Preble's regardless of what the best available science tells us.

The delisting petitions on Preble's were based on significant increases in known numbers and range. Both the States of Colorado and Wyoming have called for the immediate delisting of Preble's based on this data and taxonomic error. Many local governments also support delisting. It appears that FWS has chosen to ignore population and range data despite roughly 100 pages of data on the subjects submitted in the delisting petitions and publication in a peer-reviewed journal. This is illustrated in an e-mail correspondence between FWS officials in March of 2005. I have not included the specifics of this correspondence at this time because the Department has claimed this correspondence is privileged, but I would be happy to provide further details upon request.

The original delisting rule was based solely on the genetic and taxonomic review of Dr. Rob Roy Ramey. It appears that following the original delisting recommendation that Fish and Wildlife Service staff then embarked upon an aggressive campaign to discredit Dr. Ramey, ignoring that listing was based largely on the review of only four adult specimens of mice. With help from interested parties in academia, and perhaps environmental groups, FWS employed and funded an agency ally, USGS researcher Dr. Tim King, to protect Preble's listed status. After this, FWS staff influenced what was to be an "independent review" of the genetic and taxonomic issues related to Preble's.

It appears that FWS staff set their minds on rebutting Ramey whatever the cost. FWS staff were threatened and angered by Ramey's results. This is displayed in a Jan. 21, 2005 email from Preble's Recovery Team Leader Bruce Rosenlunt to University of Colorado Professor Andrew Martin: "I was going to include something with the e-mail on Ramey, but I did not want to make it seem I was mad as hell. To lower my blood pressure, I wrote a letter and sent it to the recovery team. Most of the Preble's Recovery Team was also mad, but Rob has a very strong following north of Cheyenne."

On October 28, 2004 Rosenlund also e-mailed Dr. Ramey's superior at the Denver Museum of Nature and Science (DMNS) complaining about national press embarrassing the FWS. In this email Rosenlund seemed to imply Ramey was pro-development, blustered that Ramey was not meeting deliverables and threatened to withhold funds from the DMNS. By spring, the FWS was writing press releases for the DMNS on the publication of Ramey. "Here is my suggestion of what I would like to get out today. Appreciated your help on this and want to maintain a positive public image on this." Rosenlund stated in a email to Stuckey in May of 2005. Later, in June of 2005, Rosenlund admitted that Ramey's manuscript met the FWS target "ahead of schedule."

Based on my research it appears that FWS staff may have encouraged others to exert pressure on the DMNS about Ramey's work. In e-mail correspondence to a consultant often employed to do Preble's trapping, FWS staff said, "Carron: Thanks again for your time and effort you have devoted to the DMNS Preble's issue. . . ." (Rosenlund to Carron Meaney, July 3, 2006.) Meaney had previously threatened Ramey in e-mail correspondence, "there are a lot of people who question your approach and have concerns about working with the museum in the future. I love the DMNS, and am very concerned to watch the alienation your behavior has wrought between the museum and the biology community." This concerning behavior was noted in the Vincent Carroll article, *On Point: The mouse that roared*, Published in April of 2006 in the Rocky Mountain News.

Following this the FWS turned to sympathizers in academia for help justifying the Preble's listing. "Sorry to hear there is so much bad news. Thanks for the Excel info. I can't advocate for one, or two or three or however many species based on mtDNA and a poorly designed morphological study." (Andrew Martin responding to Rosenlund, Jan 21, 2005.) Three days later, Martin again wrote to Rosenlund, "Hi any chance agency or non-profit folks are considering funding a genetic study of zapus that is independent of the Ramey group? . . . If this is on the burner, please consider us."

Less than two weeks after the above correspondence, Region 6 FWS staff communicated, "Since the Preble's has now published and the reality of what we need to accomplish is now coming into focus, we're starting to think more seriously about this USGS study." To perform the study the FWS enlisted, Dr. Tim King of the USGS, to refute the previously published work. Dr. King's one-sided history of splitting into subspecies and distinct population segments (DPSs) has been seriously questioned by his peers, in particular as it related to Maine Atlantic salmon. Newspaper reports suggest Dr. King refused to release, and may have altered, crucial data to support his findings. Here, Dr. King's work has been hotly criticized for bias in sampling, misrepresentations and inexplicable conclusions.

This situation is problematic for numerous reasons.

I see a waste of tax payers dollars:

I have reviewed correspondence between FWS staff that shows them scrambling to reallocate funds from other programs to cover the cost of King's review. These emails show willingness by staff to go to almost any lengths to provide funding for the unnecessary review. In addition I understand that this review went far beyond original

cost estimates eventually costing taxpayers hundreds of thousands of dollars. I have not included the specifics of these correspondences at this time because the Department has claimed the correspondence is privileged.

I see a violation of Interagency Policy on Peer Review:

Soliciting King's review was in violation of the FWS's Interagency Policy on Peer Review. The FWS violated its own peer review policy by commissioning Dr. Tim King to conduct, at public expense, yet additional review of Ramey outside of the comment period of the proposed listing.

I also see items that some could view as collusion with outside environmental interest groups:

As King's budget escalated, so too did the communications between FWS staff, environmental groups, academia and biologists with vested interests in Preble's listed status. In November of 2004 FWS employee Wiley passed along the Ramey work to environmental litigants, the Center for Native Ecosystems. Then on August 9, 2004, Jacob Smith of the Center for Native Ecosystems requested a meeting with the FWS regarding a 12-month finding for Preble's. Wiley replied that the FWS would set something up.

It appears Wiley may have gone so far as to have arranged for King to update the FWS's allies on King's progress. "What is the audience seeking an update?" asked King of Wiley, Aug. 16, 2005. Preble's Recovery Team Leader Bruce Rosenlund alluded to a meeting with undisclosed "parties," and offered to send a misleading request for Preble's samples to the recovery team. Rosenlund to Mary Jennings, May 19, 2005.

FWS kept the USGS study under wraps. But on January 3, 2006, Wiley writes to King: "[T]he word is out!! I'm amazed it stayed under wraps this long." Later in May of 2006 it appears that Tim King actually solicited positive comments on his views on Preble's from other splitters. Environmental groups were in touch with King too. On July 21, 2006 Sylvia Fallon with NRDC corresponded with King about the possibility of genetic standards in listing decisions.

I am also concerned about misleading request for Preble's Samples:

"Seems like this could be a real bombshell as written. On the other hand, may be a good way to open the door on the USGS genetics study." (FWS employee Rosenlund to Mary Jennings, May 19, 2005.) Alluding to a meeting with undisclosed "parties," Rosenlund offered to send a misleading request for Preble's samples to the recovery team under the guise that there had been some confusion about certain Preble's samples.

Upon review of numerous correspondences I am concerned that outside influences may have been exerted in the Preble's Review Panel.

The FWS campaign culminated in what was supposed to be an independent panel review of Ramey and King's work. But FWS staff seems to have colluded with King and

academia to influence even the review process. FWS staff had behind-the-scenes contact and communication with the panel chosen to review Ramey's work. In April, the Sustainable Ecosystems Institute (SEI) seemed to be ringing alarm bells with FWS staff over a high-level meeting held in Washington on genetics and listing decisions. (Wiley to Mary Henry, April 26, 2005). Wiley didn't want to get his SEI "contact" "in trouble" for spreading the word.

Perhaps using their "contacts" at SEI, the FWS tried to push through a stacked panel review of King's work compared to Ramey. "Per Ralph's [Morgenweck] direction, please let our panelists know that they should stand down." This from a Oct 20th 2005 email between Julie Lyke to P. Plage, S. Wiley. There is further evidence of collusion in a email between Plage and Hopi Hoekstra on Jan. 20, 2006. It looks as though some interested parties in academia wanted to influence the Preble's decision. Hoekstra, one of the researchers that works on listed subspecies of beach mice, suggested a kindred spirit, Sacha Vignieri, that CU's Martin had also blessed. This was indicated in a communication from Hoekstra to Plage on Januray 22, 2006. On March 3, 2005, Alabama researcher Michael Wooten asked the FWS for information on the status of delisting Preble's and noted that the people that work on listed subspecies of beach mice were watching closely. Later, Martin wrote to Vignieri, copying King and Hafner about the SEI panel and his desire to get one of them to represent "our arguments." Martin to Vignieri, June 20, 2006.

In addition FWS staff crafted an agenda for the SEI meeting and passed it along to Tim King. (Wiley to King, June 7, 2006.) Perhaps in response to the FWS agenda, the SEI panel changed it's agenda from equal time on the agenda to almost 2:1 in favor of the critics of Ramey. The panel also applied a double standard as to who could participate in the review with more deference to the critics of Ramey.

Apparently due to complaints from Dr. King, a panelist was removed from the SEI panel based on fears he would be sympathetic to the Ramey work (Dr. Eric Routman of San Francisco State University). But another panelist, Dr. Scott J. Steppan, remained on the panel despite his history of collaborator with Dr. Jim Patton.

The panel review on Preble's was a model of selective interpretation. The SEI panel claimed Ramey's work was "based on insufficient data to support their suggestions for taxonomic change," yet ignored the weak inference and small sample size used originally by Krutzsch (1954) to describe this subspecies (measurements of only 3 skulls and comparisons of only 4 skins). The panel criticized Ramey for using museum samples, but King admitted, "we have previously extracted DNA from 60-year old samples . . . and from numerous dried [Preble's] ear punches provided by the Colorado Natural Heritage Program." King admitted this in a correspondence to Robert Mark Timm on Sept. 13, 2005.

The SEI panel also failed to acknowledge that Dr. Krutzsch no longer supports his original subspecies description; ignored that a study across the entire *Zapus* genus had already been conducted by Jones (1981) that examined specimens from 123 collections, totaling almost 9,900 individuals and concluded that: "There is no evidence of any population of Zapus hudsonius being sufficiently isolated or distinct to warrant

subspecific status" (pages V and 303 from Jones 1981). Finally, SEI ignored a review of both Ramey and Kings work commissioned by the State of Wyoming that heavily favored Ramey's work. Crandall and Marshall (2006).

Perhaps the SEI panel was sensitive to academia's fear of the Crandall work. On the first of June, 2006, Andrew Martin wrote to King, and copied Vignieri, Hafner & Wooten, "[T]he Crandall report apparently commissioned by the State of Wyoming is interesting and contradicts, in very specific terms, the King et al. study. I have two questions: First, why was the Crandall report commissioned? And what the @#\$%\$#@ is going on?" Martin to King, June 1, 2006. This independent review of the Ramey and King data sets was conducted by internationally known population geneticists.

Prominent on the FWS Preble's web page is Dr. King's work and the SEI panel review. Crandall and Marshall (2006) is nowhere to be found. Also conspicuously absent from the FWS web page are:

- Crandall, K.A. (2006) Advocacy dressed up as scientific critique, Animal Conservation. 9:250-251
- Ramey, R.R., J.D. Wehausen, H.P. Liu, C.W. Epps, and L. Carpenter (in press). How King et al. (2006) define an "evolutionary distinct" mouse subspecies: a response. Molecular Ecology.
- Ramey, R.R., J.D. Wehausen, H.P. Liu, C.W. Epps, and L. Carpenter (2006) Response to the report: Evaluation of Scientific Information Regarding Preble's Meadow Jumping Mouse (prepared by the Sustainable Ecosystems Institute). Submitted the FWS (Aug. 2006).
- Ramey, R.R., J.D. Wehausen, H.P. Liu, C.W. Epps, and L. Carpenter. (2006) Response to Vignieri et al. (2006): Should hypothesis testing or selective post hoc interpretation of results guide the allocation of conservation effort? Animal Conservation. 9:244-247.
- Emma Marris, the species and the specious, Nature (Mar. 2007)

I am not sure why these items are absent but it would seem that these items should be available.

Perhaps emboldened by their ability to silence the best available information, FWS staff began to explore outsourcing their review of Preble's population and range to their allies at SEI. (Susan Linnear to M. Stempel, Aug. 3, 2006.) Wiley kept Tim King informed all the way. "See bold text below [proposing SEI review Preble's population, range and potential for DPS status] I think our folks are likely to be interesting [sic] in pursuing this more . . . let's keep talking." (Wiley to King, Aug. 3, 2006.)

Preble's was listed because the FWS concluded there was a loss of populations over a significant portion of its range. Post-listing surveys have shown Preble's to be quite

common. In fact, the number of sites known to be occupied by Preble's has increased over 400% (from 29 sites to more than 132 sites -- and counting).

Recently, the journal Nature published an article on controversy related to genetic and taxonomic status. Emma Marris, *The species and the specious*, Nature, 250 (Mar. 2007). Interestingly, the article explained that polar bears are not considered a species separate from grizzly bears. As you know, polar bears appear quite different than grizzly bears. They are located in dramatically different habitats and rely on different food sources. By contrast, Preble's (a listed subspecies) is physically indistinguishable from other subspecies of meadow jumping mice. In addition, the other so-called subspecies of meadow jumping mice reside in similar habitats, rely on similar food sources, and exhibit no known behavioral differences.

The contrast between Preble's and polar bears points to the need for sound policy for listing decisions. Accordingly, I urge you to use the disagreement on Preble's as an opportunity to ensure questionable subspecies with little or no quantifiable physical differences cannot be listed under the ESA.

State and federal governments are spending more on the Preble's meadow jumping mouse than over 1,135 species of wolves, whales, bighorn sheep, trout, tortoise, squirrels, snakes, birds, beetles and butterflies. "Funds for endangered species are very limited. Why would you want to spend these precious resources on taxa that are originally based on weak data and do not hold up to scientific scrutiny." K.A. Crandall, Advocacy dressed up as scientific critique, Animal Conservation (2006).

The FWS is long past its statutory deadline to act on the Preble's delisting. Now, as a result of a lawsuit filed by the State of Wyoming, the Department of Interior will issue a decision by October 31st. In addition I understand that the Department of Interior is looking into allegations that political influence was used when determining the listing status of several species including the Preble's Meadow Jumping Mouse. As the Department goes through this process I would hope that they look to see if political rationale was used to prevent the delisting of the Preble's Meadow Jumping Mouse.

The distribution, abundance and trends of Preble's support delisting regardless of taxonomic status. However, Crandall et al. (2006) constitutes the best available science on Preble's genetics and Jones (1981) constitutes the best available science on taxonomy. Accordingly, I urge you to issue a final rule delisting Preble's based on data error. I would also like you to look into any possible violations of Department of Interior policy as they relate to this case.

I appreciate your assistance with this matter.

Sincerely,



Wayne Allard
United States Senator

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

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

Abstract

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

INTRODUCTION

The United States Endangered Species Act (US-ESA) is intended to protect organisms that are threatened with extinction and promote their recovery. Organisms 'listed' for protection can include species, subspecies and distinct vertebrate population segments. Since the enactment of the US-ESA in 1973, 1851 organisms have been listed as threatened or endangered. Thirty-five organisms have since been removed from the list. Seven 'delistings' resulted from correction of taxonomic errors and six from recognition of other types of errors, while 14 organisms recovered and eight went extinct (http://ecos.fws.gov/tess_public/TESSWebpage). One of the criticisms of the US-ESA is that listings are

sometimes based on antiquated taxonomy or weak inference (National Research Council, 1995; Cronin, 1997; Gordon, Lacy & Streeter, 1997). It is in the best interest of biodiversity conservation to evaluate the systematics and taxonomy of candidates for listing and delisting. If defensible data are lacking and a protected organism is not distinguishable with a high degree of certainty from neighbouring, non-threatened relatives, considerable financial and logistical conservation effort may be misallocated at the expense of other endangered organisms. This applies to biodiversity laws globally.

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

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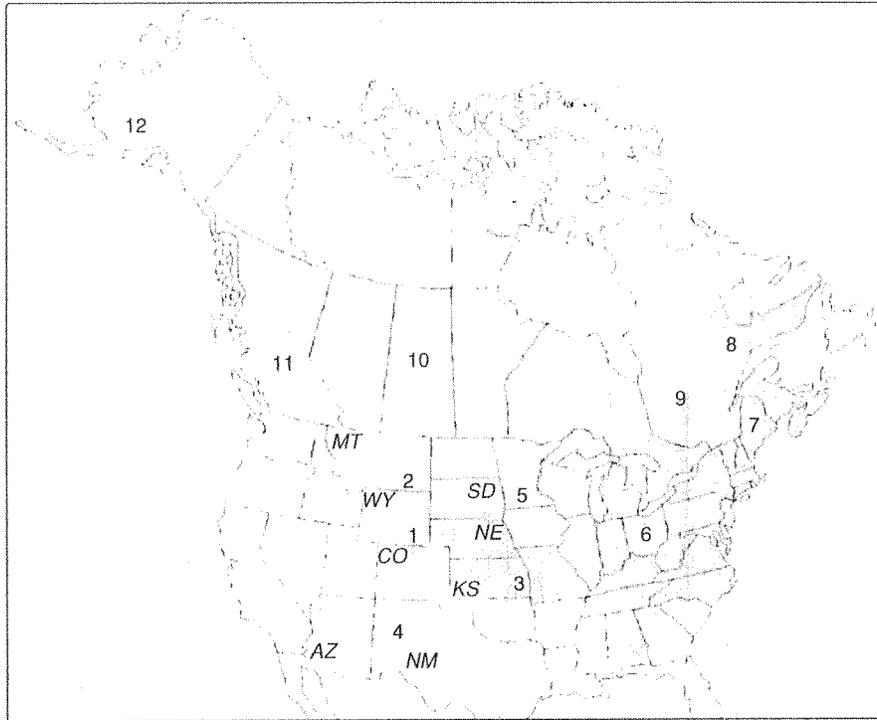


Fig. 1. Map of North America showing distribution and subspecies of *Zapus hudsonius* (Kruttsch, 1954; Hafner *et al.*, 1981). (1) *Z. h. preblei*, (2) *Z. h. campestris*, (3) *Z. h. pallidus*, (4) *Z. h. luteus*, (5) *Z. h. intermedius*, (6) *Z. h. americanus*, (7) *Z. h. acadicus*, (8) *Z. h. ladas*,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

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

Here, we test the uniqueness of *Z. h. preblei* relative to other nearby subspecies of *Z. hudsonius* using tests for multiple, genetically-based traits (Wehausen & Ramey, 2000; Pearse & Crandall, 2004). We treated taxonomic categories as testable hypotheses and used critical tests laid out in advance of data collection to provide an objective evaluation of the genetic distinctiveness of *Z. h. preblei* from nearby subspecies of *Z. hudsonius*. First, we retested

the original quantitative basis of Kruttsch's (1954) conclusions to split *Z. h. campestris* into three subspecies (*Z. h. preblei*, *Z. h. campestris* and *Z. h. intermedius*) using univariate and multivariate statistical analyses of skull measurements. Second, we used the conceptual approaches of Ball & Avise (1992), Avise & Johns (1999) and Hendry *et al.* (2000) as the basis of additional tests of *Z. h. preblei* as a subspecies. These authors and others (Crandall *et al.*, 2000; Zink, 2004) have sought consistency by suggesting that taxa or distinct populations be defined by congruence of multiple genetically-based traits. This is also important because phenotypic variation can reflect both genetic and environmental influences (Keita *et al.*, 2004). Third, we tested genetic and ecological exchangeability (Crandall *et al.*, 2000) of *Z. h. preblei* relative to other subspecies to determine if it should be considered a distinct population and, therefore, a conservation priority.

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

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

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

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

MATERIALS AND METHODS

Cranial morphometrics

We retested the quantitative basis of Krutzsch's (1954) conclusions regarding cranial differences between *Z. h. preblei* and *Z. h. campestris* using the same nine skull measurements: occipitonasal length, condylobasal length, palatal length, zygomatic length, zygomatic breadth, mastoidal breadth, braincase breadth, interorbital breadth and upper tooth row length. Skulls were from collections at the Denver Museum of Nature & Science (DMNS) and the University of Kansas Museum of Natural History (KU) (Appendix 1). Identity of samples was from museum tags, which relied upon geographical area from which a sample was collected and the current subspecies classification (Krutzsch, 1954; Hafner, Peterson & Yates, 1981). For each variable, four repeated measurements were taken using digital calipers and recorded to the nearest hundredth of a millimetre. Only adult skulls were measured, as determined by all molars being completely

erupted and having slight wear on M^3 (Krutzsch, 1954). Fewer measurements were taken for some specimens due to incomplete material. Calipers were moved away from the skull and reset for each measurement. One person (L.M.C.) measured all skulls (Palneirim, 1998). We used the means of the repeated measurements for 40 *Z. h. preblei* and 40 *Z. h. campestris* in ANOVA, PCA and LDA (Conner & Shenk, 2003). Those two subspecies were then combined for comparisons with 37 *Z. h. intermedius*.

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

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

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

MtDNA sequencing

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

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

Genomic DNA was extracted from frozen liver tissue, museum skin samples (5–10 mg) and ear punch specimens using Qiagen DNeasy Tissue kit (Qiagen Inc.). Approximately 460 base-pairs (bp) of the mitochondrial control region were amplified via polymerase chain reaction (PCR) using the primers L15320 (5'ATAAACAT-TACTCTGGTCTTGTAACC3') and ZAP5P1r (5'ATG-GCCCTGAAGTAAGAACCAG3'). Amplifications were conducted in a 25 μ l total volume, containing 5 μ l of Invitrogen optimiser buffer D (17.5 mM MgCl₂, pH 8.5) (Invitrogen, Inc.), 2.5 μ l of dNTPs (2.5 mM each), 1.25 μ l of each primer (10 μ M), one unit *Taq* polymerase, one μ l of template (*ca.* 50–100 ng double-stranded DNA) and 13.8 μ l of sterile water. Thermal cycling was performed with an initial denaturation for 2 min at 94 °C, followed by 30 cycles of 1 min at 94 °C, 1 min at 58 °C, 2 min at 72 °C, with a final extension of 10 min at 72 °C. Some museum specimens were amplified using nested PCR. We designed the nested primers, L15398 (5'ATCAGCACCCAAAGCTGATATTC3') and H16498 (5'CCTGAAGTAAGAACCAGATG3'), which amplified roughly 385bp within the first amplicon. Nested PCR was performed by using 1.0 μ l of the PCR product from the first reaction mixture as a template and reamplifying it with the nested pairs of primers. The remaining procedure was identical to the first PCR. Multiple negative controls were run with every PCR batch. The amplified PCR product was cleaned using the Exo/SAP method. Double-stranded DNA templates were incubated at 37 °C for 30 min and then at 85 °C for another 15 min with five units of Exonuclease I (ExoI, Amersham) and 0.5 unit Shrimp Alkaline Phosphatase (SAP, Amersham). For cycle sequencing reactions we used 1–5 μ l (20ng) of the cleaned PCR product as a template in a 10 μ l total volume with the CEQ DTCS Quick Start Kit (Beckman Coulter, Inc.). The following cycling conditions were used: 96 °C for 2 min, then 30 cycles of 96 °C for 20s, 50 °C for 20s and 60 °C for 2 min. The cycle-sequenced products were purified using an ethanol precipitation method following the Beckman Coulter protocol and separated by electrophoresis using a Beckman Coulter CEQ8000 sequencer.

Consensus sequences were aligned using Sequencher 3.1.1 (Gene Codes Corp., Ann Arbor, MI) and verified manually. Phylogenetic analyses based on distance, parsimony and maximum-likelihood methods were conducted using PAUP* 4.0b10 (Swofford, 2002). Modeltest 3.06 (Posada & Crandall, 1998) was used to evaluate 56 models of evolution in order to obtain an appropriate substitution model and parameter values for distance and maximum-likelihood analyses. Appropriate genetic distance (based on Modeltest results) was used

to generate neighbour-joining (NJ) trees based on the clustering method of Saitou & Nei (1987). Node support was assessed by completion of 10 000 bootstrap replications (Felsenstein, 1985) in PAUP*, using the fast-search option. Maximum-parsimony (MP) analyses were conducted with equal weighting, using the heuristic search option with tree bisection reconnection branch-swapping, 100 replications of random stepwise additions, gaps treated as missing, and MAXTREES set to 10 000. Bootstrapping with 10 000 replications (as implemented in PAUP*) was used to evaluate node support. The most likely model selected by Modeltest was used for maximum likelihood (ML) analyses. A neighbour-joining tree with appropriate genetic distance was used as the initial topology for branch-swapping. Node support was evaluated by 100 bootstrap pseudoreplicates. Split decomposition (SD) was calculated using SplitsTree version 2.4 (Huson, 1998) for all mtDNA data and for *Z. hudsonius* mtDNA data alone. Branch support was evaluated using 50 bootstrap replications.

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

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

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

MITOCHONDRIAL DNA (mtDNA) MDIV

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

Table 1. Dinucleotide microsatellite primers used in this study

Locus	GenBank accession no.	Primer sequence (5' to 3')	Annealing temp (°C)	Repeat of cloned allele	No. of alleles	Allele size range
Z.20	DQ063596	F:TCTTCCTCCCCAGACCTAC R:TCCCAAGGCCTAAACAGTGA	60	(CA) ₉	20	109–149
Z.48	DQ063597	F:GCTCATCTGCAATGGAGGA R:TTGTCTTTAGAAAACAAGATTACT	60	(CA) ₂₃	18	182–210
Z.52	DQ063598	F:CTCCAGCTCTGTCTTTGA R:TGGACAAGGCTACTGCTTCC	60	(GT) ₂₁	13	155–181
Z.7	DQ063599	F:CTTAGGCCTTGCAGTCAAGC R:TTAGCACCTCCAGCACATGG	60	(GT) ₇	20	154–190
Z.26	DQ063600	F:CATTTTACACCAGCAAACAGG R:TATTGGCTGCACATTCTTGC	60	(CA) ₁₆	19	141–171
Z.47	DQ063601	F:TGAAAAGAGCTAAATACTTGGGTAGA R:TGTCATTGCTCACTGTTTCCA	60	(CA) ₂₄	15	121–149

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

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

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

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

Microsatellites

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

were as previously described (Wehausen, Ramey & Epps, 2004).

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

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

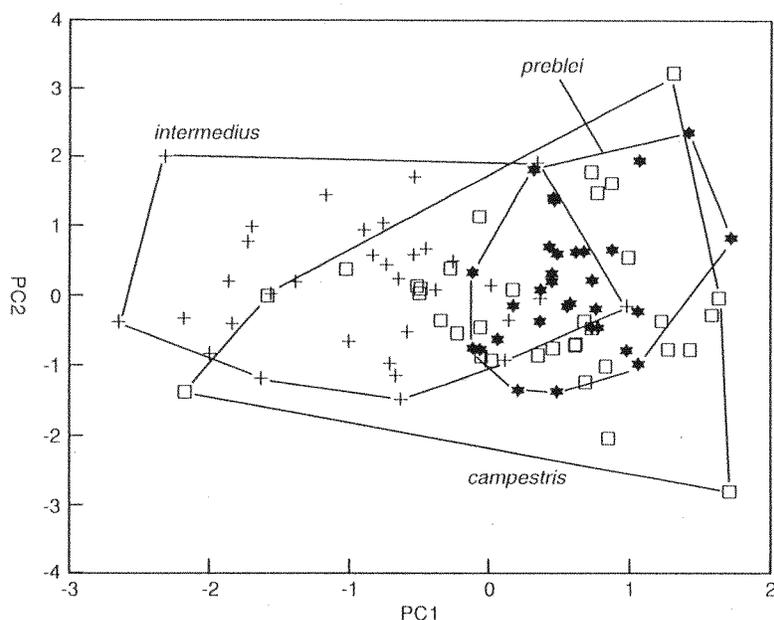


Fig. 2. Plot of Principal Component scores for PC1 and PC2. Subspecies are indicated by polygons.

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

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

RESULTS

Testing the original quantitative basis of taxonomic categories

Krutzsch (1954) stated that *Z. h. preblei* was smaller than *Z. h. campestris* in ‘most skull dimensions measured.’ However, our results revealed that *Z. h. preblei* was significantly ($P < 0.05$) smaller for only one measurement (interorbital breadth), but larger for two measurements (zygomatic and mastoid breadth) and insignificantly different for the six others. The significant differences

between subspecies were very small and of questionable biological significance relative to measurement resolution. The classification of *Z. h. preblei* as a separate subspecies therefore failed the test of uniqueness using the original criteria. When a combined sample of *Z. h. campestris* and *Z. h. preblei* was compared to *Z. h. intermedius*, they were significantly larger in all skull measurements. This is consistent with Krutzsch’s description of *Z. h. intermedius* being slightly smaller, although Krutzsch noted substantial intergradation with *Z. h. campestris* and *Z. h. pallidus*. Measurement data used in morphometric analyses were deposited with the Archivist at DMNS and are available online at www.dmns.org.

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

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

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

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

Testing putative subspecies: mtDNA analyses

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

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

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

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

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

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

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

Testing putative subspecies: microsatellites

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

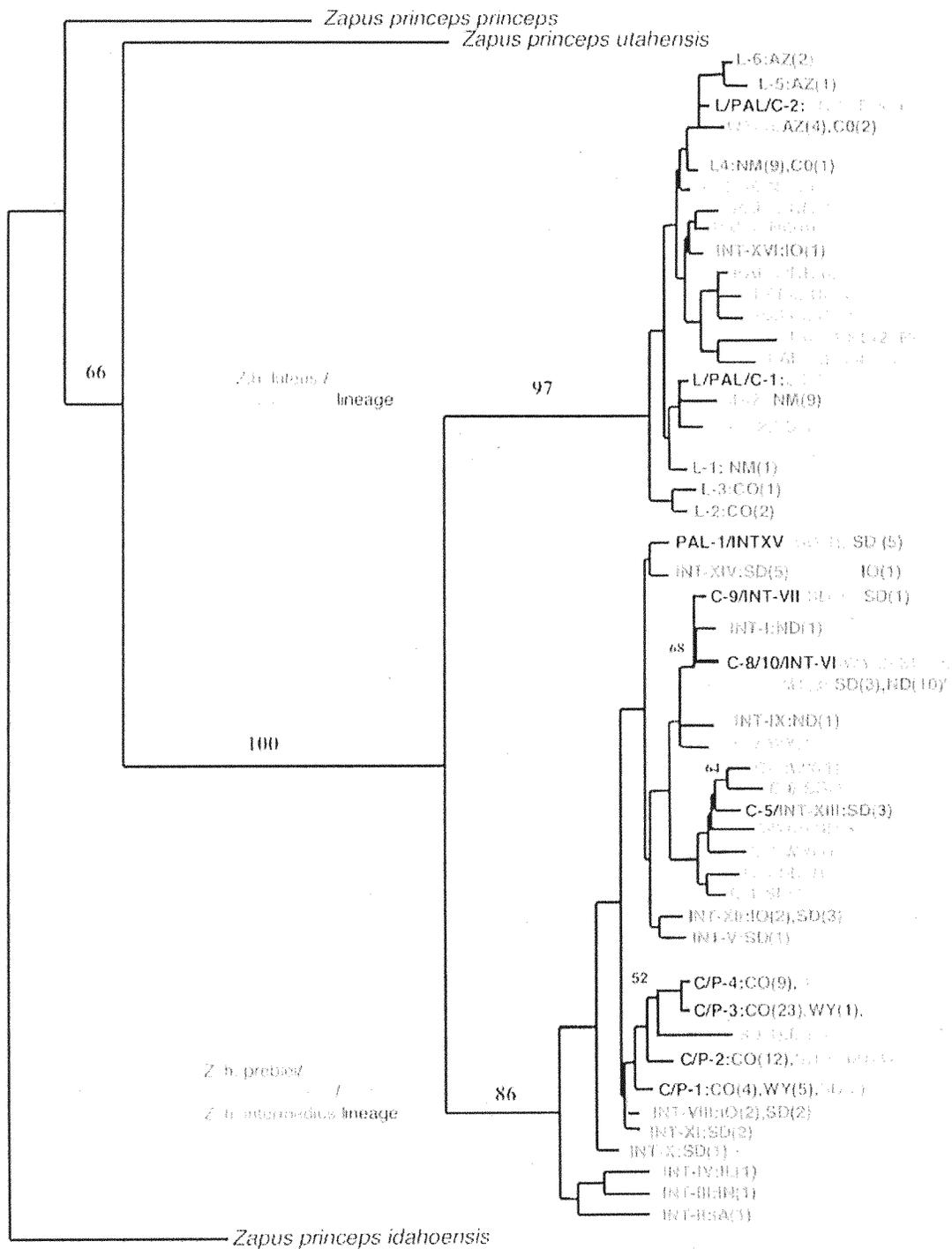


Fig. 3. Neighbour-joining phylogram inferred from partial mitochondrial DNA control region, depicting phylogenetic relationships between haplotypes of *Zapus hudsonius*. Bootstrap percentages are given when $\geq 50\%$. State and number of individuals with identical haplotypes are listed. Colours indicate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

values of F_{IS} (0.69–0.94) and consistently violated Hardy–Weinberg equilibrium, suggesting the presence of null alleles or selection at closely linked loci. It was dropped from subsequent analyses. When the data set was divided into five putative subspecies or into seven populations, no significant linkage disequilibrium ($P < 0.05$, after

correcting for multiple comparisons) was found in any population at any locus, or by locus across populations. F_{IS} for all populations was positive, with a pattern of heterozygote deficiency across most loci (Table 3). A probable explanation for the observed heterozygote deficiency is a Wahlund effect due to sampling of only one,

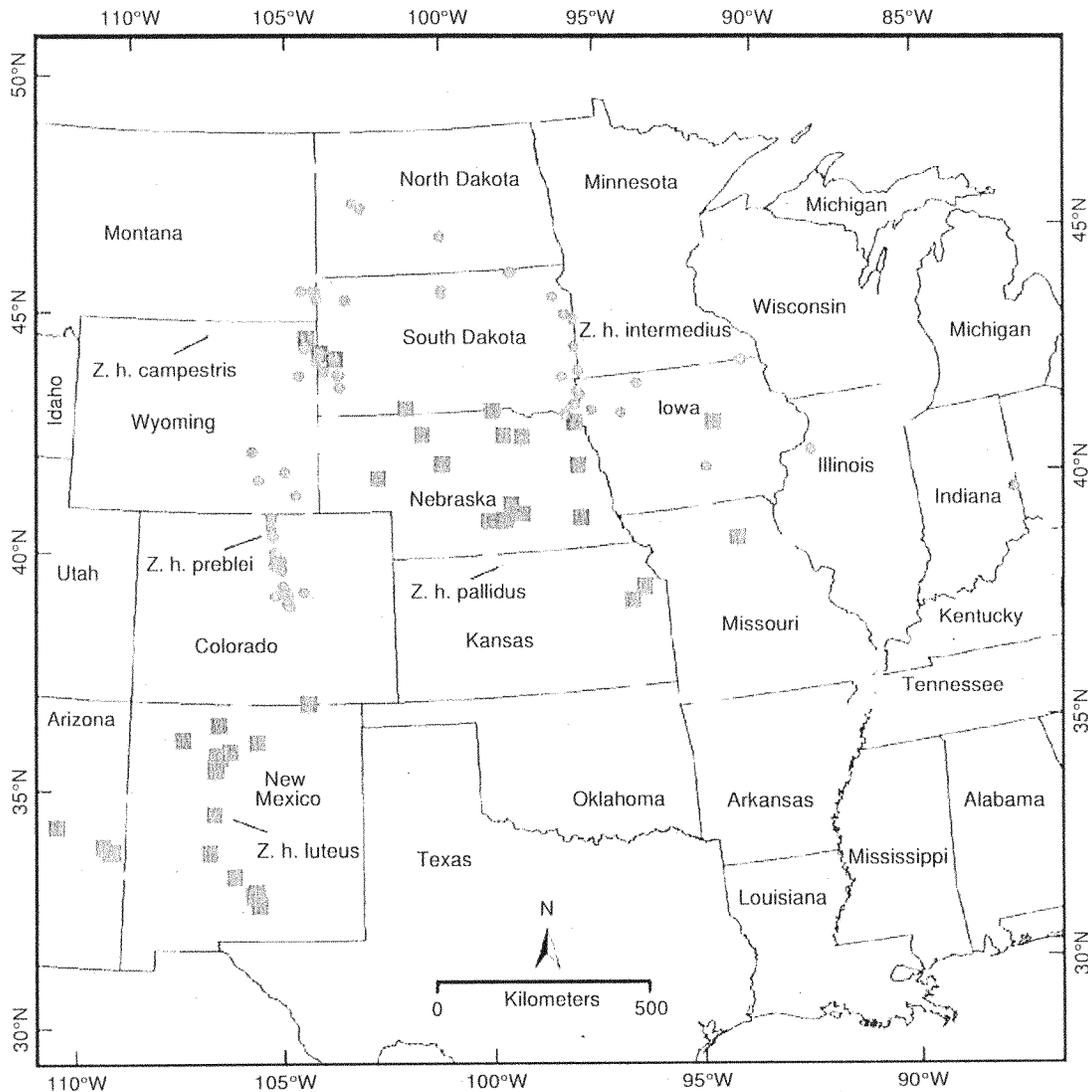


Fig. 4. Map showing collection locations of specimens used in mtDNA analyses. Multiple samples were taken from some locations. Grey outlines indicate subspecies ranges (Kruttsch, 1954; Hafner *et al.*, 1981). Pink circles indicate specimens on the *Z. h. preblei*/*Z. h. campestris* mtDNA lineage, green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

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

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

For analyses based on the seven populations, the northern and southern populations of *Z. h. preblei* both had lower allelic richness than any of the remaining subpopulations.

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

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

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

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

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

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

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

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

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

Testing genetic exchangeability

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

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

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

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

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

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

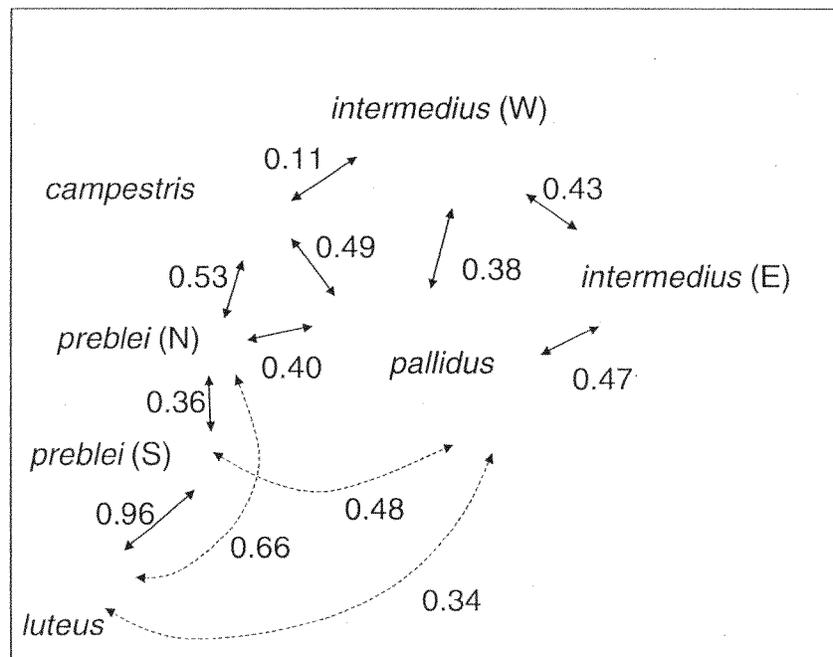


Fig. 5. Schematic diagram of seven subpopulations of *Z. hudsonius* showing Nei's genetic distances (D) between subpopulations as determined from five microsatellite loci. Comparisons between non-adjacent subpopulations are denoted by dotted arrows; comparisons between adjacent subpopulations are denoted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

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

STRUCTURE analyses indicated that $k=8$ was the most likely cluster number and this was used for assignment analyses, but the variability of the likelihood estimates was high, suggesting that clusters were poorly defined. This result is typical of situations reflecting isolation by distance (Worley *et al.*, 2004). STRUCTURE analyses supported a potential, very recent biogeographical separation of northern and southern populations of *Z. h. preblei*. Most individuals in each of these populations were assigned to unique clusters, although 24% of the northern samples were assigned to the cluster 5 (to which all but two of the southern samples were assigned; Table 6). Overall, our analyses suggested a pattern of greater genetic structure in the southwestern populations of *Z. hudsonius*: average values of q_{MAX} were highest in the southern population of *Z. h. preblei*,

followed by the northern population of *Z. h. preblei*, then *Z. h. luteus* (Table 7). Likewise, roughly 55% of individuals were assignable at $q > 0.90$ to the southern population of *Z. h. preblei*, whereas the northern population of *Z. h. preblei* had 42% and *Z. h. luteus* had 22% of individuals assignable at this level of q (Table 7). Other groups had few or no individuals assignable at $q > 0.90$ (Table 7). Thus, we could reliably assign only 55% of individuals to the most clearly defined population (the southern population of *Z. h. preblei*). We therefore cannot reject the hypothesis of recent genetic exchangeability, or the null hypothesis of historic genetic exchangeability (using results of the subspecies tests on mtDNA or microsatellite data).

Testing ecological exchangeability

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

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

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

'Zero' values are omitted for clarity.

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

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

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

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

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

DISCUSSION

Putative subspecies and taxonomic conclusions

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

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

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

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

Testing genetic and ecological exchangeability

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

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

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

In summary, we found no convincing evidence that would result in our rejection of the hypotheses of genetic or ecological exchangeability on recent or historic timescales for *Z. h. preblei*, *Z. h. campestris* and *Z. h. intermedius*. Therefore, these putative subspecies do not appear to be distinct populations (Crandall *et al.*, 2000). The results are consistent with the fact that *Z. h. preblei*, in particular

the southern population, is a peripheral population at the edge of the species range and subject to founder effects. *Zapus h. luteus* does not appear to have had much current or historic gene flow with *Z. h. preblei* based on mtDNA and microsatellite analyses. The extent to which very recent human development (e.g. the past 100 years) may have contributed to additional isolation and bottlenecks is unknown. Both of these would be expected to increase the degree of genetic distance from other populations (Hedrick, Gutierrez-Espeleta & Lee, 2001). Regardless of whether more relaxed criteria are used for testing recent genetic exchangeability, or if trapping studies confirm isolation, a rejection of recent genetic exchangeability would be insufficient to treat *Z. h. preblei* as a distinct population using the criteria proposed by Crandall *et al.* (2000). The results also suggest that *Z. hudsonius* from healthy nearby populations could be used to augment or re-establish populations within the range of *Z. h. preblei*, should this become a management objective.

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

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

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

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

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

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

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

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

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

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

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

DMNS: *Z. h. campestris* 8512.

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

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

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

These are listed by museum or tissue archive catalog number.

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

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

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

APPENDIX 2. Continued

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

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

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

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

Response to Vignieri *et al.* (2006): Should hypothesis testing or selective *post hoc* interpretation of results guide the allocation of conservation effort?

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In their response to Ramey *et al.* (2005), Vignieri *et al.* (2006, this issue; hereafter VEA) claim that they are concerned about erroneous application and interpretation of morphometric, genetic and ecological data. We share this concern, which is why we used a consistent hypothesis-testing approach to test the taxonomic validity of Preble's meadow jumping mouse *Zapus hudsonius preblei* as a subspecies and its uniqueness as a distinct population segment. We used critical tests that were set in advance of data collection to avoid subjective *post hoc* interpretation of results. We also used multiple lines of evidence for our tests of uniqueness to avoid erroneous conclusions (Ramey *et al.*, 2005). We do not agree with VEA that four lines of corroborating evidence can be considered to be 'narrow in scope'.

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

Morphometric analyses

At the center of this debate is the separation of *Z. h. preblei* as a subspecies by Krutzsch (1954) based on measurements of only three skulls and comparisons of only four skins – sample sizes that no modern taxonomist would accept. In their attempt to defend this taxonomy, VEA try to discredit all of our morphometric analyses, while ignoring the work of Jones (1981) that found no morphological support for

any subspecies of *Zapus hudsonius*. VEA state that our analyses suffered from intercorrelated data because 26 of 36 correlations among the nine skull measurements were significant at $P < 0.001$. Yet, these were the same measurements used by Krutzsch (1954), whose conclusions they attempt to defend. Traditional frequentist statistical tests that emphasize P -values have come under strong criticism (Cherry, 1998; Johnson, 1999; Anderson, Burnham & Thompson, 2000). Indeed, the P -values that VEA cite for correlations in our data reflect the large cumulative sample size we used, rather than statistically important levels of correlation among variables used in multivariate analyses of shape variation.

Krutzsch's sample sizes precluded meaningful statistical tests, and he used none: yet, VEA concluded that his finding of a smaller interorbital breadth in *Z. h. preblei* was a 'definitive finding'. VEA claim that interorbital breadth was the only one of the morphometric variables we measured that Krutzsch (1954) found to distinguish *Z. h. preblei* and that our finding of a difference for that character confirmed Krutzsch's (1954) conclusion. What Krutzsch (1954) actually stated was that *Z. h. preblei* was smaller than *Zapus hudsonius campestris* in most of the nine skull dimensions measured, a hypothesis that our data clearly refuted. Such univariate tests that VEA appear to espouse were replaced decades ago in morphometric analyses by multivariate analyses of shape variation (Reyment, Blackith & Campbell, 1984), which was the approach we used.

VEA criticize us for ignoring unquantified characters that Krutzsch (1954) included as the basis of his taxonomy, describing these as 'shape differences noted by a trained morpho-taxonomist'. They fail to realize that this 'trained morpho-taxonomist' (Krutzsch) does not accept his

taxonomy and has publicly stated that our research 'clearly invalidates *Z. h. preblei* and demonstrates its relationship to *Z. h. campestris*'. He went on to state: 'Perhaps most significant is the model you provide to unequivocally establish the uniqueness of an organism and its relationships before declaring it in danger of extinction. Such an analytical approach would prevent implementation of a process to support an agenda or a point of view. I can think of other listed endangered species that could have benefited for a prior, detailed, scientific appraisal' (P. Krutzsch in email to R. R. Ramey, entered into the U.S. Congressional Record on 28 April 2004).

Ecological analysis

Contrary to VEA's claims, we did not deny that *Z. h. preblei* seems to be currently isolated. What we questioned was *how long* this isolation has existed. Nor did we 'present nothing' that could be interpreted as a test of ecological exchangeability. We cited the original morphological research of Krutzsch (1954) and Jones (1981) as well as the literature reviews of Whitaker (1972, 1999), Clark & Stromberg (1987) and Cryan (2004) in support of our claim that no adaptive differences have been described between *Z. h. preblei* and other subspecies. Although it is possible that some critical adaptive difference had been 'missed' in the 106 years of study, starting with Preble (1899), none seem to have been noticed.

VEA make the assertion that 'the potential for ecological differentiation among these populations (putative subspecies of *Z. hudsonius*) is high'. However, the evidence and rationale they provide is speculative. VEA base their claims on K uchler's (1964) potential natural vegetation (PNV) classifications. PNV classifications are based on hypothetical 'climax' vegetation that could potentially occupy a site without disturbance or climatic change (Zerbe, 1998). PNV classifications are not mutually exclusive categories. For example, each of the PNV classifications cited in VEA has overlap in plant species. PNV classifications are qualitative, generalized descriptions of vegetation communities that do not take into account the mosaic nature of natural landscapes, including successional stages, nor do they accurately characterize moist riparian habitat occupied by *Z. hudsonius* in the Great Plains. VEA ignore the fact that *Z. hudsonius* is a generalist species in its food habits (eating seeds, insects, fruit and fungi) and habitat preferences (Quimby, 1951; Jones, 1981), making adaptation to specific forage species less likely. VEA's assertion that the potential for ecological differentiation is high is therefore questionable. Most importantly, speculation is an inappropriate basis for definitions of subspecies or lower levels of population distinction (Ball & Avise, 1992; Crandall *et al.*, 2000; Cronin, 2006); yet VEA declared *Z. h. preblei* a 'habitat-specific subspecies group'.

Molecular genetic analyses

VEA have made a case on the small value of the unscaled migration rates (m) derived from our analyses of mtDNA

variation, mistakenly suggesting that these rates reflect the number of migrants per generation. In fact, the scaled migration rates ($N_e m$) reflect a theoretical number of migrants per generation of 0.09–0.87 among putative subspecies. Although this value is low and suggests the possibility of continuing divergence because of genetic drift, we consider the relative ranking of gene flow rates between putative subspecies as more informative. This analysis suggests that *Z. h. preblei* and *Z. h. campestris* have recently experienced gene flow at higher levels than any other comparison, except *Z. h. campestris* and *Zapus hudsonius intermedius*.

VEA inaccurately report that reciprocal monophyly was the sole criterion we used for accepting divergence among subspecies. VEA seek to explain away the shared haplotypes among subspecies by labeling them as 'contaminant' haplotypes rather than acknowledging that shared variation is a common biological phenomenon. They attribute this 'contamination' to incomplete lineage sorting. Their table 1 shows that 22.6% of *Z. h. campestris* mtDNA sequences were *Z. h. preblei* haplotypes. This is hardly incomplete lineage sorting. Even if the mtDNA results for these seven samples are excluded from analyses it does not change the results to a degree that would lead to the alteration of our conclusions (MDIV: range of M 0–0.32; AMOVA 0.52 between *Z. h. preblei* and *Z. h. campestris*; *Z. h. preblei* is paraphyletic with low bootstrap support).

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

Z. h. preblei and the US-Endangered Species Act (ESA)

VEA suggest a double standard in evaluating evidence used in ESA listings. They state that Ramey *et al.* (2005) 'should most certainly not be presented as an adequate basis for the making of taxonomic decisions regarding a (US-ESA) listed taxon'. Yet they ignore the fact that *Z. h. preblei* was US-ESA listed based on far fewer data – Krutzsch's (1954) study of just a few specimens and an unpublished qualitative mtDNA study for which the data were never made public (Riggs, Dempcy & Orrego, 1997).

VEA raise some important questions with regard to subspecies and populations relative to the ESA. How should conservation effort be allocated relative to (1) hypothesized adaptive uniqueness, (2) geographic isolation of recent

origin and (3) populations showing minor differentiation at a few neutral loci that may be due to recent anthropogenic population bottlenecks?

We agree with VEA that it is impossible to predict future patterns of speciation. However, the US-ESA is not a biodiversity law that mandates the protection of all potential pathways to speciation (e.g. weakly differentiated populations or hypothetical evolutionary trajectories). VEA's suggestion that the ESA should protect all potential speciation pathways is impractical, logically inconsistent and not a view supported by the courts. It is impractical because there is great uncertainty in predicting potential speciation pathways. It is logically inconsistent because the evolutionary potential for some species can only be realized through the extinction of other species (e.g. in cases where one species is limited by another), leading to conflicting listing and recovery goals. Lastly, VEA's approach is in conflict with a recent US Ninth Circuit Court ruling that while 'the USFWS can draw conclusions based on less than conclusive evidence, ... it cannot base its conclusions on no evidence' (National Association of Homebuilders vs. Norton, No. CIV-00-0903-PHX, 2001). In other words, US-ESA decisions cannot be based on speculation or hypothetical scenarios alone.

In listing *Z. h. prebleii* as 'threatened', the US Fish & Wildlife Service (USFWS) concluded that there was a loss of populations over a significant portion of its range (USFWS, 1998). Post-listing surveys have shown this conclusion to be erroneous. Historically (pre-1980), the range of *Z. h. prebleii* was thought to be restricted to 14 eighth-order hydrologic units along the eastern edge of the Rocky Mountains in Colorado and Wyoming (State of Wyoming, 2003; data from Wyoming Natural Diversity Database and Colorado Natural Heritage Program), of which nine were thought to be occupied at the time of listing based on minimal survey efforts (USFWS, 1998). This rodent is now known to occur in all historically occupied hydrologic units in both Colorado and Wyoming. In addition, it has been captured in three hydrologic units north and east of its presumed historic range: the Upper Laramie Hydrologic Unit in Wyoming as well as the Kiowa and Chico Hydrologic Units in Colorado (State of Wyoming, 2003; see tables 4 and 5). Although development and habitat alteration have certainly caused some local extirpations, the number of occupied locations within these hydrologic units has increased over fourfold with greater survey effort, to over 126. Consequently, it appears that data on taxonomic uniqueness and geographical distribution used for ESA listing were both questionable. Yet, VEA propose to maintain the status quo of *Z. h. prebleii* under the ESA. This raises fundamental questions regarding the allocation of conservation effort.

The US Government Accountability Office recently reported that the time and costs that are required to recover US-ESA listed species, subspecies and distinct vertebrate populations are largely unknown (US Government Accountability Office, 2006). With the costs and duration of most US-ESA listings unknown, it would seem that prioritization in the allocation of conservation effort would become imperative. However, this has not been the case.

Although a prioritization scheme was established in the 1982 amendments to the US-ESA, it was based on taxonomic uniqueness, and it has subsequently been found that there is no correlation between priority rank and conservation expenditure (Restani & Marzluff, 2001, 2002). In other words, expenditures on local populations of otherwise common species (like *Z. h. prebleii*) often exceed the expenditures for full species that are at greater risk of extinction. For example, in a ranking of US-ESA expenditures in 2004, *Z. h. prebleii* ranked 125 out of 1260 listed taxa (USFWS, 2006). That put spending for *Z. h. prebleii* well above that for blue whales – an endangered species (rank 391) and only slightly behind the California condor – an endangered monotypic genus (ranked 119).

In the case of *Z. h. prebleii*, the only verifiable figures on the cost for the 23 632 ha critical habitat designation were conservatively estimated by the USFWS at \$79 to \$183 million from 2005 to 2015 (USFWS, 2003). Virtually, all of these funds will be spent on consultations rather than more permanent protection, such as land purchases or conservation easements. The development of long-term regional habitat conservation plans accounts for less than 4% of the expenditures. The estimate does not include costs incurred between the time of the listing and the designation of Critical Habitat from 1998 to 2003. It is conceivable that the total allocation of conservation effort for this population could exceed half a billion dollars within the next 20 years.

The United States may be unique in its ability to allocate such resources to non-distinct but presumably threatened populations of common species. However, it is clear that this conservation approach comes at the expense of many full species that are far more endangered. With many full species endangered worldwide, and limited resources to save them, many nations may not find the US-ESA model to be a desirable or sustainable approach to conservation.

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Response to the report: Evaluation of Scientific Information Regarding Preble's Meadow Jumping Mouse (prepared by the Sustainable Ecosystems Institute).

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We respectfully disagree with the Preble's review panel (report by Arbogast et al. 2006) on a number of points and find their conclusions biased and inadequately founded in science. The panel opined that the lines of evidence in Ramey et al. (2005) are insufficient to overturn *Z. h. preblei* as a subspecies and that additional data need to be gathered to "clarify" the issue. Yet no thresholds were advanced by the panel that could be used to objectively test this subspecies with additional data. It is our contention that the stance taken by the panel amounts to support for an approach that precludes falsification of virtually any ESA-listed subspecies or DPS.

Instead of reviewing all of the available science, the panel arbitrarily created its own burden of proof, which it then unilaterally applied only to Ramey et al. (2005). Rather than critically analyze the underlying data upon which *Z. h. preblei* became a subspecies and was ESA-listed (e.g. Krutzsch 1954; Riggs et al. 1997), the panel only examined the most recent papers and correspondence while ignoring much contrary information. If all available scientific information are considered, the U.S. Fish and Wildlife Service currently has sufficient scientific information to conclude that *Z. h. preblei* does not qualify as a valid subspecies or DPS.

Cranial Morphometry

We do not agree with the panel's claim that "*two of the lines of evidence presented by REA (their analyses of cranial morphometrics and ecological exchangeability) are based on insufficient data to support their suggestions for taxonomic change.*" The description of *Z. h. preblei* by Krutzsch (1954) was based on far less data and small sample sizes that precluded statistical tests. As we noted in our response to Vigneiri et al. (Ramey et al.

2006): "At the center of this debate is the separation of *Z. h. preblei* as a subspecies by Krutzsch (1954) based on measurements of only 3 skulls and comparisons of only 4 skins. ... Krutzsch's sample sizes precluded meaningful statistical tests, and he used none." The panel's opinion that our morphometric analyses are inadequate shows bias in ignoring the weak inference and small sample size used originally to describe this subspecies in 1954.

Based on their statements, the panel is willing to accept poor discriminant analysis assignments that are in many cases differ little from flips of a coin in discrimination ability (posterior probabilities of >0.50) in support of this putative subspecies. Similarly, the panel is willing to accept principal components analysis plots (an exploratory tool lacking inferential statistical capabilities that is not designed for hypothesis testing) as a means to define subspecies. The panel is unwilling to accept the fact that the only quantitative basis for Krutzsch's original description of *Z. h. preblei* is not supported by univariate statistical analysis using much larger sample sizes ($n=40$ per subspecies). Additionally, the panel does not acknowledge the fact that the scientist who originally described *Z. h. preblei*, Krutzsch, no longer supports this subspecies description (Ramey et al. 2006). This should settle the issue but has been ignored by the USFWS and the panel (see Ramey et al. 2006).

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

- 1) Krutzsch did not actually measure the auditory bullae or the inflation of the frontal region; those characters were only described qualitatively, thus conclusions had no objective basis. The panel's opinion that these could somehow be "coded for systematic analysis", ignores the fact that Krutzsch never measured them in the first place. Furthermore, the panel did not provide specifics as to how this could be done, nor thresholds that could be applied to such data to test (and potentially falsify) the hypothesis of uniqueness for *Z. h. preblei*.
- 2) Although Krutzsch's (1954) methods indicated that he measured incisive foramina width and length, no values for those measurements were reported.
- 3) We measured and analyzed the same nine measurements for which Krutzsch (1954) reported actual measurement data (mean, ranges, and sample sizes in Table 5, Krutzsch 1954), and we did not measure those qualitative skull characteristics (discussed above) for which he did not report measurements.
- 4) After examining pelage in several hundred museum specimens with the pelage descriptions in hand (sides duller, less black-tipped hair on dorsal stripe), we concluded that these were subjective qualitative assessments that would not be repeatable. (The attached photograph gives some idea of the variation within, and overlap among, putative subspecies.) In fact, the species, *Z. princeps* and *Z. hudsonius*, cannot be reliably

distinguished on pelage alone, which was why Conner and Shenk (2003) developed their cranial morphometric test to distinguish them and noted the following : "*Furthermore, the taxa* [the species: *Z. princeps* and *Z. hudsonius*] *are ecologically and physically similar and no reliable technique exists to distinguish live specimens in the field.*" In other words, *those species* are not reliably distinguishable using pelage color or pattern. Based on our examination of several hundred museum skins, *the subspecies* are even less so.

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

Genetic Analyses

In their discussion of differences in mtDNA results of the two studies, the panel stated: "In this section we discuss how the mtDNA sampling, quantity and quality issues outlined above may have lead REA (Ramey et al. 2005) and KEA (King et al. 2006) to come to different conclusions regarding the taxonomic status of *Z. h. preblei*." However, the panel failed to notice that our critical tests did not rely on any sharing of haplotypes among putative subspecies; instead, our subspecies test relied on: morphological analyses to test the original quantitative basis of *Z. h. preblei*, mtDNA reciprocal monophyly and Analysis of Molecular Variance, and the proportion and frequency of unique microsatellite alleles and pairwise F_{ST} . Our tests for distinct populations used the approach of Crandall et al. (2000) and did not rely on sharing of mtDNA haplotypes among putative subspecies.

The panel presents a neighbor-joining tree but fails to tell the reader that this is one of a number of *potential* graphical representations of evolutionary relationships among mtDNA haplotypes. Many other trees that are equally parsimonious can be found (as discussed in Ramey et al. 2005). The reliability of the branches of the single tree presented by the panel was not indicated by any bootstrap support values. As both Ramey et al. (2005) and KEA both showed, the bootstrap support for all but the two major mtDNA lineages representing *Z. h pallidus/luteus* and *Z. h. preblei/intermedius/campestris* are not reliable. Therefore, none of the phylogenetic analysis to date support *Z. h. preblei* as being reciprocally monophyletic or even close to being so. This result was confirmed by Crandall and Marshall (2006) in their analysis of KEA's data.

The panel and KEA equate *statistical significance* with *biological significance* in their evaluation of microsatellite data. This is the same pitfall that basic statistic textbooks urge students to avoid: *statistical significance* should not be blindly equated with *practical significance*. In other words, while a difference among populations might be statistically significant, the actual difference in means can be small in magnitude and with substantial overlap in range of values. KEA's data shows this pattern with extensive shared microsatellite alleles with other subspecies, especially *Z. h. campestris* and *Z. h. intermedius*. Statistical significance can also be an artifact of sampling design. As several authors have shown that when a large number of individuals are sampled for a

large number of microsatellite loci, and modern statistical tests are applied (as in KEA), it is very likely that statistically significant differences will be found among populations even with high levels of interbreeding and there will be a high level of correct assignment of individuals to populations (Cornuet et al. 1999; Pritchard et al. 2000; Cegelski et al. 2003; Baudouin et al. 2004; Waples and Gaggiotti 2006). Application of such an approach to defining subspecies and DPSs, means that population-level differences could qualify as ESA-listable units. This would lead to an unlimited number of potential ESA-listable units. It also means that these designations (and subsequent ESA listings) could be acceptable even if they were an artifact of sampling design. This was clearly pointed out in Crandall and Marshall (2006), and by Crandall and Ramey to the panel. Please refer to our discussion of statistical significance vs. biological significance in our comments on KEA submitted to the USFWS and the panel: *Is the Preble's meadow jumping mouse an evolutionarily distinct subspecies? Comments on the report by King et al. (2006)* and to the technical report by Crandall and Marshall (2006).

Additional problems with equating statistical significance in genetic results with biological significance were pointed out to the panel but were ignored. These included: 1) the fact that very recent human induced genetic bottlenecks and isolation can increase apparent genetic divergence among populations (such as *Z. h. preblei* north and south of Denver as discussed in Ramey et al. 2005 but not acknowledged by KEA, Vigneiri et al. (2006), or the panel); 2) a low level of female dispersal relative to males can result in higher levels of mtDNA divergence than nuclear markers (trapping data show that females disperse less often and over shorter distances); 3) a faster mutation rate in small mammals (due to small body size and short generation time) can result in higher levels of genetic divergence over the same period of time than in larger mammals. If genetic results are accepted without taking these factors into account, then ESA listings could be based on statistical significance that is of little or no biological significance.

The Scientific Basis for Uniqueness under the ESA

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

The panel noted that "*However, we also note that Z. h. preblei appears to be at a stage in its evolution in which clearly determining taxonomic rank will not be easy to do [our emphasis], and that large groups of scientists are unlikely to reach a unanimous consensus concerning its status.*" It would appear from this statement that this (and other) subspecies could be listed indefinitely if different conclusions can be reached by different authors depending upon how the results are "interpreted", while more data are continually called for. The panel's statement underscores the central conceptual issue that we addressed in Ramey et al. (2005): consistent thresholds for defining conservation units below the level of species have been lacking. Until such thresholds are established, both subspecies and Distinct Vertebrate Population Segments (DPS's) will remain subjectively defined. We have consistently argued that unless reasonable thresholds are set in advance and consistently applied, these classifications will continue to be based on opinion rather than scientific hypothesis testing (please refer to Ramey et al. 2005, 2006a, and the conclusions of 2006b).

The panel goes on to recommend an extensive research program to "*further clarify this issue*"; however, the panel failed to provide any critical tests that could be used to potentially falsify *Z. h. preblei* as a taxon. We do not agree with the panel's subjective approach and contend that it will *clarify nothing* because no thresholds or critical tests are proposed. Furthermore, we find that the absence of these (and their poorly defined *burden of proof*) makes this subspecies not falsifiable and therefore, makes the whole inquiry into its taxonomic status a moot point.

The panel presented *Z. h. preblei* as a "*valid, formally recognized subspecies*"; however, it is important to recognize that there was no standard for describing subspecies in 1954, nor is there currently. Scientists and amateurs alike have been free to use whatever approach they wanted. The International Code of Zoological Nomenclature (or *The Code*) only sets forth procedural guidelines for taxonomic description and not thresholds of uniqueness, data quality, or evolutionary basis. For example, *The Code* requires a type specimen (a body or parts thereof, Timm et al. 2005) as well as publication of the taxonomic revision and its distribution to at least four libraries. *The Code* does not require that the publication of taxonomic revision be peer-reviewed. The Preble's subspecies description in Krutzsch (1954) is a half page in length. The repeated citation of it in books on mammal taxonomy is simply a restatement (or summary) of the original description (see Appendix 2). Under *The Code*, *Z. h. preblei* and *Z. h. intermedius* are officially synonymous with *Z. h. campestris* with the publication of Ramey et al. (2005).

While additional geographic and taxonomic sampling could be expanded so that "*evolutionary and biogeographic history, as well as the taxonomic status of Z. h. preblei could be evaluated more critically within this broader framework*", we have consistently argued that this putative subspecies can be (and was) synonymized based on available scientific information. Such a genus-wide study across the entire *Zapus* genus has already been conducted by Jones (1981). That study involved morphological, ecological, and evolutionary information (including the fossil record), and examined specimens from

123 collections, totaling almost 9,900 individuals. Jones concluded that: "*There is no evidence of any population of Zapus hudsonius being sufficiently isolated or distinct to warrant subspecific status*" (pages V and 303 from Jones 1981). We find it discouraging that despite the fact that a copy of Jones (1981) was personally handed to the panel, the panel chose to make brief mention of it (to criticize it) and otherwise ignore the results and conclusions of this critical study. If the USFWS had looked more closely at this study before the listing of *Z. h. preblei* in 1998, this subspecies would never have been listed.

As we have pointed out in Ramey et al. (2006) and to the panel, while additional work could be done on the Kansas Museum of Natural History specimens in question (there are multiple explanations for differences), these mtDNA sequences can simply be excluded from analyses and the same basic result is obtained. In fact, as we presented to the panel, if all nested PCR results are excluded from analysis and KEA's mtDNA data from *Z. h. campestris* are substituted for ours, the same basic results are obtained.

The panel did not address the substantive issues raised by Ramey et al. (2006) about Vignieri et al.'s (2006) use of *post hoc* interpretation of results, speculative approach to on ecological uniqueness, and misrepresentation of facts. Our paper, titled: *Response to Vignieri et al. (2006): Should hypothesis testing or selective post-hoc interpretation of results guide the allocation of conservation effort?* (The panel cited this as if it were an unpublished report when it was peer reviewed, accepted for publication, and "in press" before the panel convened.) For the reasons detailed in Ramey et al. (2005 and 2006) we disagree with the approach of VEA, KEA, and the panel, all of which have relied on post-hoc interpretation of results in support of their conclusions.

Conclusion

In the field of conservation biology, there is a nearly universal tendency to err on the side of protection, even when the data in support of it are questionable. The panel's conclusions suggest that they accept any geographic isolation (no matter how recent) and genetic divergence (no matter how minor) to be sufficient to defend a subspecies (no matter how weak the evidence was to describe the subspecies in the first place). As such, we find that the panel uncritically equates statistical significance with biological significance. We do not think that this serves conservation or the public's best interest when applied to local populations of very common and widespread species, like meadow jumping mice which range over *half of North America* (see map in Ramey et al. 2005).

In our experience, it is a common occurrence that ESA-related peer reviews are not held to any quantitative threshold of uniqueness that could be consistently used to test the validity a subspecies or DPS. Without the application of thresholds, peer reviews essentially become *de facto* opinion surveys, with the inherent value-laden perspectives of any opinion survey. If reviewers do not fully give consideration to all the relevant evidence, it will compromise the completeness, and therefore the outcome, of any review. If this panel's recommendation is followed and applied to other cases, it would mean that

many inadequately defined subspecies would not be potentially falsifiable. This effectively puts ESA-listed subspecies evaluations outside the realm of scientific investigation. Probably the greatest twentieth century contribution to epistemology was Popper's (1958 or whatever citation you prefer) criterion that falsifiability separates science from non-science. It is our opinion that the panel abrogated their responsibility in presenting a biased interpretation of the available information that failed to recognize the basic implications of their conclusions relative to falsifiability and the application of science to the ESA. Our analysis of their conclusions finds that they amount to advocating that listings under the ESA do not need a scientific basis.

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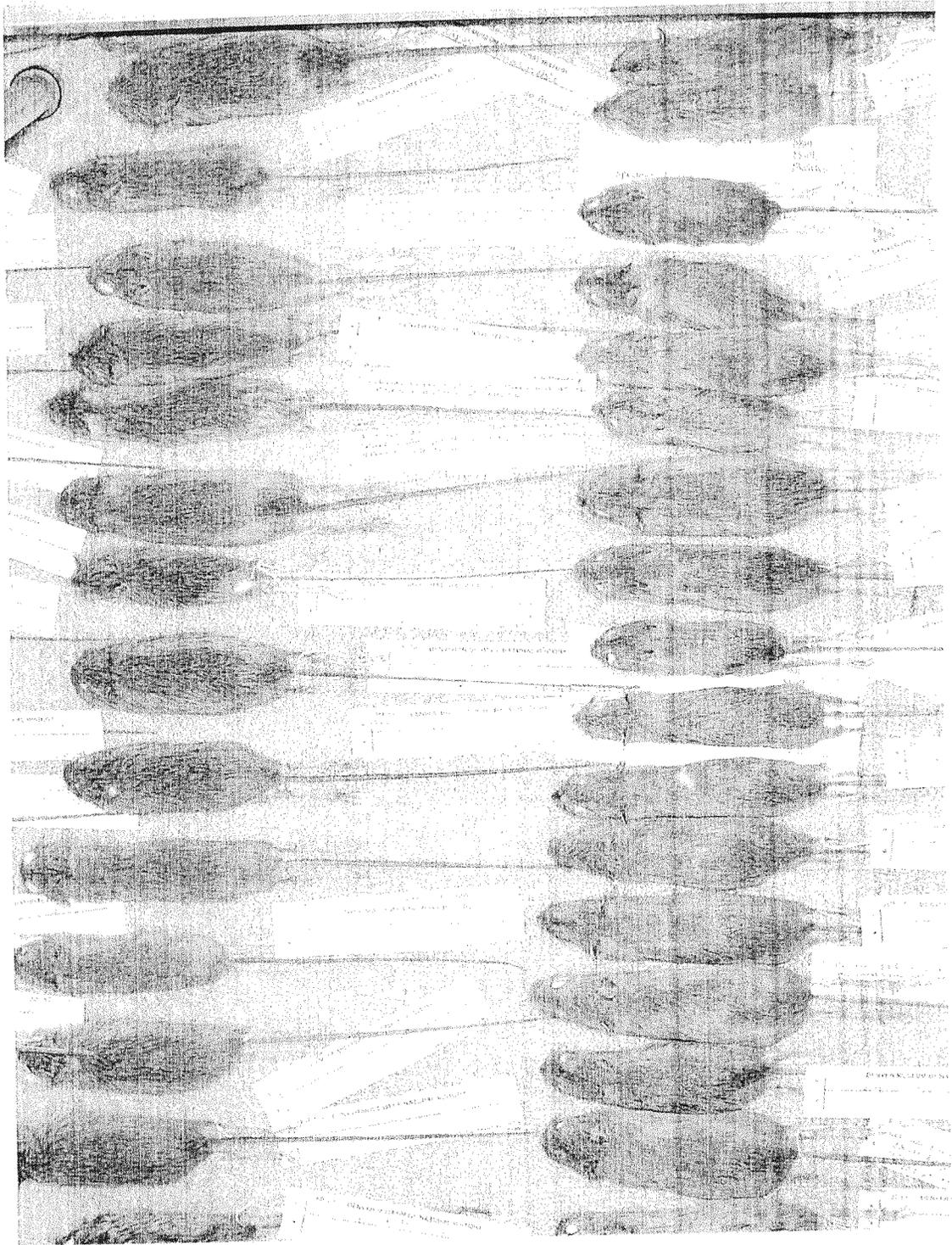
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Figure 1. A tray of *Z. h. preblei* specimens at the Denver Museum of Nature & Science, with two *Z. h. intermedius* specimens placed among them. (Hint: the *Z. h. intermedius* specimens are the ones on the yellow loan tags.) This photo gives some idea of the variation in size and pelage found within and among subspecies of *Z. hudsonius*.



Appendix 1. Relevant results and conclusions from Jones 1981 to the taxonomic validity of *Z. h. preblei*.

Jones 1981 examined thousands of *Zapus* specimens and traveled all over its' range in North America in the most extensive study of morphology and ecology of *Zapus* to date. Below are quotations from Jones (1981) on *Z. hudsonius* subspecific taxonomy, text in **bold** is provided for emphasis.

Abstract:

"There are two dental phenotypes of *Z. hudsonius*, one in northwestern (e.g. Alaska, British Columbia) and the other in eastern North America. This suggests an isolated population in the unglaciated portion of Alaska, in a manner similar to that theorized in the Southeast. **There is no evidence of any population of *Zapus hudsonius* being sufficiently isolated or distinct to warrant subspecific status.**" (From Page V of Jones 1981)

Background and conceptual approach:

"Both Preble (1899) and Krutzsch (1954) based their classifications on museum skins and skulls. The latter author relied heavily upon size and pelage coloration. Problems with this classification have become apparent. Utilizing Krutzsch's (1954) characters, Davis and Ernst (1971) were unable to determine whether a Minnesota population was *Z. hudsonius* or *Z. princeps*. A large number of specimens of *Z. hudsonius* collected in Tompkins County, New York, by John O. Whitaker, Jr., exhibits much of the color and size variation attributed to this species throughout its range, thus challenging recognized subspecific division.

These questions, the challenge of the new systematics to consider all neontological and palentological evidence, and the need to consider the relationships of the various populations in light of the biological species concept (Whitaker, 1970) prompted this author to examine the classification of the genus *Zapus*. The present study was conducted in an attempt to develop a classification which would more acceptably reflect the relationships in the genus *Zapus*.

In pursuing this goal, specimens and other biological materials were collected during two trips through western North America, specimens in numerous museums were studied, and biological information was gathered from the literature. The biological materials and information were gathered with the intent that they might reveal relationships among the various forms and might expose primary isolating mechanisms (i.e., barriers to dispersal) or secondary isolating mechanisms (i.e., barriers to reproduction).

Specimens from 123 collections (museums, university and college collections, and personal collections), totaling almost 9,900 individuals, were studied." (From pages 2-4 of Jones 1981)

Adaptations:

"Barry (1976, 1977) described the morphology of the small and large intestines and caecum of *Z. hudsonius* and concluded that this species had evolved structurally as an omnivore." (From page 258 of Jones 1981)

"That *Zapus* is an omnivore is substantiated by the foods it consumes. Whitaker's (1963) data indicate that it is an opportunist, taking advantage of readily available foods, with a preference for seeds (i.e., when seeds are in abundance, *Z. hudsonius* is primarily a granivore)." (From page 258 of Jones 1981)

Intraspecific systematics of *Z. hudsonius*:

"Discussion of INTRASPECIFIC SYSTEMATICS: A number of subspecies of *Zapus hudsonius* have been named (Appendix G). Krutzsch (1954) recognized eleven but the present study recognizes none. No named subspecies if geographically restricted by a barrier, with the possible exception of *Z. h. preblei*. Whether or not islands such as Martha's vineyard or Prince Edward Island harbor undescribed subspecies is as yet unstudied. Only recently has a relatively large collection from Prince Edward Island been made (HHT).

"Krutzsch (1954) named *Z. hudsonius preblei* on the basis of 4 adults and 7 non-adults, stating that it averaged smaller than adjacent *Z. h. campestris* (his classification) in most cranial measurements, including least interorbital constriction, smaller auditory bullae, and narrower incisive foramina. Table 41 includes these measurements, some additional ones, and comparative measurements from other portions of the species' range (extracted from tables presented elsewhere in this paper). It is evident from the table that *Zapus hudsonius* in Colorado is generally the same size as specimens from North Dakota, Pennsylvania, New England, and British Columbia. In fact, four measurements are larger in the Colorado sample than in specimens from North Dakota which are equal to Krutzsch's *Z. h. intermedius* which he described as being smaller than *Z. h. campestris* -- length of incisive foramina, width of auditory bullae, length of upper tooth row, and breadth of M3-M3. As stated above, however, the Colorado populations appear to be isolated along with those in south-eastern Wyoming. This arid land which may isolate these populations from those in northwestern Wyoming (Lowers, 1974) and Nebraska (Berry, 1974). Armstrong (1972) suggested that *Zapus hudsonius* in this area is a relict of a previously occurring humid grassland or savanna association. **Although they are isolated, no characteristics indicate that these populations have evolved into a separate taxon.**" (pages 288-289 of Jones 1981)

Evolutionary mechanisms:

"As pointed out in the discussion of speciation, *Zapus* was present and presumably isolated in what is now the south-eastern United States. With the recession of the glacier, *Zapus* moved northward, as did other species (Hadley, 1971). Prior to that range expansion, the Mississippi River and the glacier were presumable effective barriers; it was hypothesized earlier that isolation during the early Pleistocene may have resulted in speciation of *Zapus hudsonius*. But with the absence of the glacier there were and are no barriers to gene flow between populations east of the Great Plains. There is essentially

continuous distribution in the East (Fig. 31; Appendix E). The eastern subspecies which were recognized by Krutzsch (1954) occurred in successive northwest to southeast bands sharing long borders with adjacent subspecies. The Great Lakes and St. Lawrence River divided these bands in the East. An example of the status of these subspecies is Krutzsch's *Z. h. intermedius*. It crosses the Mississippi River, which assuredly would be a barrier if it were not for its northern terminus around which gene flow occurs. Further, the southwest border of *Z. h. intermedius*, which is continuous with the northeast border of *Z. h. pallidus*, extends from west central South Dakota to east central Missouri. *Zapus h. intermedius* was ambiguously distinguished: "All characters differentiating *Z. h. intermedius* from any contiguous subspecies are not present in every specimen, even in type series." (Krutzsch, 1954). This variation suggests a variable taxon which cannot be distinguished except by isolation. Briefly, specimens are generally lighter on the prairie than they are in the Northeast; this difference is the recognized phenomenon of populations evoking darker pelage on darker substrate, often correlated with increased rainfall. It was noted above that the Alaskan *Zapus* has the same pelage characteristics as the northern and northeastern populations...In conclusion, to distinguish subspecies where there are no barriers to gene flow and subsequently no distinct morphological differences would violate the premise that the subspecies is a genetic unit evolving towards the species." (From page 301 of Jones 1981)

Conclusions:

"There is no evidence that any population of *Zapus hudsonius* has been isolated long enough or under a set of circumstances to allow subspeciation." (From page 303 of Jones 1981)

Appendix 2: The full extent of Hall's (1981) authoritative treatment of *Z. h. preblei* in Mammals of North America.

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MAMMALS OF N

Zapus hudsonius preblei Krutzsch

1954. *Zapus hudsonius preblei* Krutzsch, Univ. Kansas Pub., Mus. Nat. Hist., 7:452, April 21, type from Loveland, Larimer Co., Colorado.

MARGINAL RECORDS (Krutzsch, 1954:453).—Wyoming: Springhill, 12 mi. N Laramie Peak, 6300 ft.; Chugwater; Cheyenne. Colorado: type locality; 5 mi. E Boulder; Semper; 3 mi. E Boulder.

Location	Code	County	State	Organization	Date collected	Sample #	Species ID		Elevation (ft)	Quad name	Township	Range	Trap location	
							mtDNA	microsatellite					Section	1/4-1/4 Sec
Chadwick Reservoir - Lodgepole Creek	CR3	Laramie	WY	Dave Young - WEST	9/11/1998	CR39801	Zapus princeps	Zapus princeps	6600	Isley	16N	69W	29	SW-NE
Lone Tree Creek	HEX	Laramie	WY	Dave Young - WEST	7/12/2000	HEX0001	Zapus princeps	Zapus princeps	6600	Granite	13N	69W	18	SW-NW
Y-Cross Ranch site A (S. fork of Horse Crk)	YCA	Laramie	WY	Dave Young - WEST	9/3/1998	YCA9801	Zapus princeps	Zapus princeps	6480	Horse Creek	17N	69W	35	E half
Y-Cross Ranch site A (S. fork of Horse Crk)	YCA	Laramie	WY	Dave Young - WEST	9/3/1998	YCA9802	Zapus princeps	Zapus princeps	6480	Horse Creek	17N	69W	35	E half
Y-Cross Ranch site A (S. fork of Horse Crk)	YCA	Laramie	WY	Dave Young - WEST	9/4/1998	YCA9803	Zapus princeps	Zapus princeps	6480	Horse Creek	17N	69W	35	E half
Y-Cross Ranch site A (S. fork of Horse Crk)	YCA	Laramie	WY	Dave Young - WEST	9/4/1998	YCA9804	Zapus princeps	Zapus princeps	6480	Horse Creek	17N	69W	35	E half
Y-Cross Ranch site A (S. fork of Horse Crk)	YCA	Laramie	WY	Dave Young - WEST	9/4/1998	YCA9805	Zapus princeps	Zapus princeps	6480	Horse Creek	17N	69W	35	E half
Y-Cross Ranch site A (S. fork of Horse Crk)	YCA	Laramie	WY	Dave Young - WEST	9/4/1998	YCA9806	Zapus princeps	Zapus princeps	6480	Horse Creek	17N	69W	35	E half
Y-Cross Ranch site A (S. fork of Horse Crk)	YCA	Laramie	WY	Dave Young - WEST	9/4/1998	YCA9807	Zapus princeps	Zapus princeps	6480	Horse Creek	17N	69W	35	E half
Y-Cross Ranch site B (Horse Creek)	YCB	Laramie	WY	Dave Young - WEST	9/3/1998	YCB9801	Zapus princeps	Zapus princeps	6480	Horse Creek	17N	69W	35	E half
Y-Cross Ranch site B (Horse Creek)	YCB	Laramie	WY	Dave Young - WEST	9/4/1998	YCB9802	Zapus princeps	Zapus princeps	6480	Horse Creek	17N	69W	35	E half
Y-Cross Ranch site B (Horse Creek)	YCB	Laramie	WY	Dave Young - WEST	9/4/1998	YCB9803	Zapus princeps	Zapus princeps	6480	Horse Creek	17N	69W	35	E half
Y-Cross Ranch site B (Horse Creek)	YCB	Laramie	WY	Dave Young - WEST	9/4/1998	YCB9804	Zapus princeps	Zapus princeps	6480	Horse Creek	17N	69W	35	E half
Chugwater Creek	CHG	Laramie	WY	Dave Young - WEST	7/20/1999	CHG9901	Zapus princeps	Zapus princeps	6480	Horse Creek	19N	69W	20	NE-NE
Cottonwood Creek at North Cottonwood	CTN	Albany	WY	True Ranches	8/12/1999	CTN9901	Zapus princeps	Zapus princeps	6480	Horse Creek	27N	71W	28	SE-SE
Duck Creek at Pole Creek	DUC	Albany	WY	True Ranches	8/18/1999	DUC9901	Zapus princeps	Zapus princeps	6480	Horse Creek	23N	72W	6	NE-NE
Little Bear East of I-25	ELB	Laramie	WY	True Ranches	7/9/1999	ELB9901	Zapus princeps	Zapus princeps	6480	Horse Creek	18N	67W	27	NE-NW
Little Bear East of I-25	ELB	Laramie	WY	True Ranches	7/9/1999	ELB9902	Zapus princeps	Zapus princeps	6480	Horse Creek	18N	67W	27	NE-NW
East Paulson Branch of Bear Creek	EPB	Laramie	WY	True Ranches	7/14/1999	EPB9903	Zapus princeps	Zapus princeps	6480	Horse Creek	17N	68W	1	SE-NW
East Paulson Branch of Bear Creek	EPB	Laramie	WY	True Ranches	7/14/1999	EPB9901	Zapus princeps	Zapus princeps	6480	Horse Creek	17N	68W	1	SE-NW
East Paulson Branch of Bear Creek	EPB	Laramie	WY	True Ranches	7/14/1999	EPB9902	Zapus princeps	Zapus princeps	6480	Horse Creek	17N	68W	1	SE-NW
Horse Creek	HRC	Laramie	WY	True Ranches	6/29/1999	HRC9901	Zapus princeps	Zapus princeps	6480	Horse Creek	17N	68W	1	SE-NW
Little Bear Creek	LBC	Laramie	WY	True Ranches	7/17/1999	LBC9901	Zapus princeps	Zapus princeps	6480	Horse Creek	17N	68W	1	SE-NW
Luman Creek	LUM	Platte	WY	True Ranches	8/3/1999	LUM9901	Zapus princeps	Zapus princeps	6480	Horse Creek	17N	68W	1	SE-NW
Luman Creek	LUM	Platte	WY	True Ranches	8/3/1999	LUM9902	Zapus princeps	Zapus princeps	6480	Horse Creek	17N	68W	1	SE-NW
Luman Creek	LUM	Platte	WY	True Ranches	8/3/1999	LUM9903	Zapus princeps	Zapus princeps	6480	Horse Creek	17N	68W	1	SE-NW
Murphy Canyon / Wyman Creek	MCW	Albany	WY	True Ranches	8/13/1999	MCW9901	Zapus princeps	Zapus princeps	6480	Horse Creek	26N	71W	30	SE-SE
Murphy Canyon / Wyman Creek	MCW	Albany	WY	True Ranches	8/13/1999	MCW9902	Zapus princeps	Zapus princeps	6480	Horse Creek	26N	71W	30	SE-SE
North Bear Creek	NBC	Laramie	WY	True Ranches	7/17/1999	NBC9901	Zapus princeps	Zapus princeps	6480	Horse Creek	18N	69W	11	SW-NW
North Laramie River	NLR	Albany	WY	True Ranches	8/12/1999	NLR9901	Zapus princeps	Zapus princeps	6480	Horse Creek	26N	71W	19	NE-NE
North fork of South fork of Bear Creek	NSB	Laramie	WY	True Ranches	7/6/1999	NSB9901	Zapus princeps	Zapus princeps	6480	Horse Creek	18N	68W	19	NE-NE
North fork of South fork of Bear Creek	NSB	Laramie	WY	True Ranches	7/6/1999	NSB9902	Zapus princeps	Zapus princeps	6480	Horse Creek	18N	68W	19	NE-NE
North fork of South fork of Bear Creek	NSB	Laramie	WY	True Ranches	7/6/1999	NSB9903	Zapus princeps	Zapus princeps	6480	Horse Creek	18N	68W	19	NE-NE
Rabbit Creek	RBC	Platte	WY	True Ranches	8/3/1999	RBC9901	Zapus princeps	Zapus princeps	6480	Horse Creek	24N	70W	16	SE-SW
South Bear Creek	SBC	Laramie	WY	True Ranches	7/11/1999	SBC9901	Zapus princeps	Zapus princeps	6480	Horse Creek	18N	68W	29	NE-NE
South Bear Creek	SBC	Laramie	WY	True Ranches	7/11/1999	SBC9902	Zapus princeps	Zapus princeps	6480	Horse Creek	18N	68W	29	NE-NE
South Bear Creek	SBC	Laramie	WY	True Ranches	7/11/1999	SBC9903	Zapus princeps	Zapus princeps	6480	Horse Creek	18N	68W	29	NE-NE
South Bear Creek	SBC	Laramie	WY	True Ranches	7/11/1999	SBC9904	Zapus princeps	Zapus princeps	6480	Horse Creek	18N	68W	29	NE-NE
South Bear Creek	SBC	Laramie	WY	True Ranches	7/11/1999	SBC9905	Zapus princeps	Zapus princeps	6480	Horse Creek	18N	68W	29	NE-NE
Sturgeon, Siebolt and ? Creek	SSC	Albany	WY	True Ranches	8/18/1999	SSC9901	Zapus princeps	Zapus princeps	6480	Horse Creek	25N	71W	7	NE-NE
Sturgeon, Siebolt and ? Creek	SSC	Albany	WY	True Ranches	8/18/1999	SSC9902	Zapus princeps	Zapus princeps	6480	Horse Creek	25N	71W	7	NE-NE
Sturgeon, Siebolt and ? Creek	SSC	Albany	WY	True Ranches	8/18/1999	SSC9903	Zapus princeps	Zapus princeps	6480	Horse Creek	25N	71W	7	NE-NE
Sturgeon, Siebolt and ? Creek	SSC	Albany	WY	True Ranches	8/18/1999	SSC9904	Zapus princeps	Zapus princeps	6480	Horse Creek	25N	71W	7	NE-NE
Sturgeon, Siebolt and ? Creek	SSC	Albany	WY	True Ranches	8/18/1999	SSC9905	Zapus princeps	Zapus princeps	6480	Horse Creek	25N	71W	7	NE-NE
Styble Creek	SYB	Platte	WY	True Ranches	8/7/1999	SYB9901	Zapus princeps	Zapus princeps	6480	Horse Creek	24N	69W	19	NE-NE
Three Mile Creek	TMC	Laramie	WY	True Ranches	7/28/1999	TMC9901	Zapus princeps	Zapus princeps	6480	Harris Peak	19N	69W	19	NE-NE
Cottonwood Tributary A	CTA	Laramie	WY	True Ranches	8/26/1998	CTA9802	Zapus princeps	Zapus princeps	6200	Harris Peak	27N	71W	22	SE-SW
Friend Creek	FRC	Albany	WY	USFS	9/2/1998	FRC9801	Zapus princeps	Zapus princeps	7470	Laramie Peak	26N	72W	4	SW-NW
Friend Creek	FRC	Albany	WY	USFS	9/2/1998	FRC9802	Zapus princeps	Zapus princeps	7470	Laramie Peak	26N	72W	4	SW-NW
Middle Crow	MCC	Albany	WY	USFS	7/16/1998	MCC9801	Zapus princeps	Zapus princeps	7380	Sherman mountain East	14N	71W	24&13	SE-NE
South Lodgepole	SLP	Albany	WY	USFS	7/15/1998	SLP9801	Zapus princeps	Zapus princeps	7700	Green top mountain	15N	71W	13	SW-SW
City of Douglas?	DOU	Converse	WY	USPWS	8/5/1999	DOU9901	Zapus princeps	Zapus princeps	4700	Chalk Buttes	32N	71W	17	NW-SW

Demographic info as follows:
Sex - female (F), male (M), unknown (U)
Age - adult (A), juvenile (J), subadult (SA), young (Y)
Reproductive status - reproducing (R), lactating (L), non reproducing (NR), pregnant (P)
Weight in grams.

COMMENTARY

How King *et al.* (2006) define an 'evolutionary distinction' of a mouse subspecies: a response

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King *et al.* (2006; hereafter KEA) produced a second independent set of genetic data on the Preble's meadow jumping mouse (formerly *Zapus hudsonius preblei*) and their analyses thereof led to the opposite conclusions of Ramey *et al.* (2005) (hereafter REA) regarding the uniqueness of that subspecies. KEA argued that their different conclusions result from sampling design, tissues used (museum specimens vs. fresh ear punches), amount of molecular genetic data used [longer and more mitochondrial DNA (mtDNA) sequences, more microsatellite loci], analytical methods used and criteria for defining subspecies. We respectfully disagree with their interpretation of differences between the studies and their wholesale portrayal of our work as inaccurate. We find the difference between conclusions is largely a function of basic conceptual and philosophical differences. While the KEA sampling represented a notable effort, it fell short in both sampling design and strength of inference that they attempted to present so forcefully.

Morphometric considerations

KEA chose not to use several key sources of contrary information that did not support their conclusions. These included a range wide study of over 9000 specimens of *Zapus* (Jones 1981), which did not find support for recognizing any subspecies of *Zapus hudsonius*. Also not cited was a literature review conducted by one of the coauthors of KEA (Cryan 2004), which could not find any published literature or reports suggesting adaptive or ecological differences among putative subspecies; a literature that spans 107 years of study. KEA also ignored the fact that we retested the original quantitative basis of Krutzsch's (1954) description

of *Z. h. preblei* and found no support for his results (that were based on measurements of just three skulls). That alone would be sufficient basis to reject the taxonomic separation of *Z. h. preblei*. Rather than acknowledging that finding, KEA dismissed the use of morphology in general because it might not reflect genetic differences, an argument they attempted to support by selective use of references. However, by that same argument, these mice should never have been listed under the United States Endangered Species Act (US-ESA) — a listing that KEA now strive to defend. Of some significance to this issue is the fact that Krutzsch, who originally described *Z. h. preblei*, no longer accepts it as a valid subspecies (Ramey *et al.* 2006).

Conceptual basis and thresholds for uniqueness

We based our analyses on a definition of subspecies provided by Ball & Avise (1992) to avoid the long history of taxonomic subspecies decisions having no definitional basis (Cronin 2006). Ball & Avise (1992) proposed that subspecies should represent major subdivisions in the gene-pool diversity of species. By that definition, subspecies are similar to evolutionary significant units (ESUs) as discussed by Moritz (1994a) in requiring deeper historical phylogenetic separation, an important criterion of Crandall *et al.* (2000), for recognizing distinct populations. Although KEA claimed to use the same conceptual basis, they actually employed a far lower threshold for subspecies than REA that appears to be equivalent to what Moritz (1994b) defined as management units. Their null hypothesis was that subspecies of *Zapus hudsonius* comprise a single homogeneous unit. The evidence that KEA considered adequate to reject that hypothesis was 'significant phylogeographical separation of mtDNA alleles between subspecies combined with congruent phylogeographical structure for nuclear loci'. The issue is: what constitutes a significant difference?

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KEA failed to acknowledge the ways in which the molecular results of REA were similar to their results. These include: (i) shallow levels of evolutionary divergence found among putative subspecies for mtDNA and microsatellites; (ii) support in mtDNA analyses for a *Z. h. pallidus/luteus* clade and a *Z. h. preblei/campestris/intermedius* clade; (iii) not even near reciprocal monophyly among putative subspecies; and (iv) few unique microsatellite alleles in *Z. h. preblei* despite a larger sample size for this putative subspecies. These similarities are important because of their bearing on how different conceptual approaches to subspecies affected differences in conclusions.

Statistical significance versus biological significance

In their null-hypothesis test of genetic homogeneity, KEA equated statistical significance with biological significance, an analytical approach that deviates from REA. It is well known that with larger sample sizes it is possible to find statistical significance in almost any comparison, especially when intervening geographical variation is ignored. As pointed out by Hedrick (2001): 'the statistical power for determining differentiation between groups is closely related to the number of independent alleles, so that even for a few highly variable microsatellite loci, there can be extremely high statistical power. When there is such high statistical power, extremely small molecular genetic differences between groups become statistically significant.'

Although KEA found a high level of statistical significance in their comparisons (using 27 microsatellites), the degree of differentiation among *Zapus hudsonius preblei*, *Z. h. campestris* and *Z. h. intermedius* were the lowest of any of the pairwise comparisons for mtDNA and microsatellites. The low degree of differentiation is illustrated by the fact that only four unique alleles were reported in *Z. h. preblei* (out of 279 in total), the lowest number of unique alleles for any subspecies sampled.

KEA reported high levels of correct assignment to subspecies using the program STRUCTURE. These authors attribute this to *Z. h. preblei* having 'considerable evolutionary differentiation' from other putative subspecies and to shortcomings of REA. However, KEA failed to acknowledge that this high level of correct assignment could also be an artifact of sampling design and number of loci surveyed (Rosenberg *et al.* 2005). KEA's findings raise a valid critique of all such studies — use of assignment tests such as STRUCTURE may not be an appropriate tool for evaluating taxonomic separation because of the sensitivity of these tests to the number of loci employed. Future efforts employing these types of analyses may need to establish threshold assignment probabilities for a set number of loci with a given amount of variation per locus to allow comparability between studies.

Sampling distribution

KEA sampled seven populations of *Zapus hudsonius preblei* but only one or two populations from each of the other putative subspecies. In contrast, REA sampled many populations, but few individuals per population, across the range of each putative subspecies. An ideal study design with unlimited resources would incorporate both approaches, as well as sampling across the entire species; however, this is not often practical because of logistical and funding constraints. Given the choice, which strategy provides the most objective test of subspecies uniqueness?

KEA claim their sampling strategy allows more appropriate statistical testing, but they do not acknowledge that their approach created artificial gaps in the distribution of genetic variation, leading to an 'isolation-by-sampling design' effect among all five of the subspecies. This sampling strategy predisposes the results to an exaggeration of genetic distances among putative subspecies. This assertion is supported by the fact that genetic distances from KEA were strongly correlated with geographical distances for the *Z. h. preblei/campestris/intermedius* lineage ($R^2 = 0.82$), including a sample from southeastern Wyoming that is intermediate between *Z. h. preblei* and *Z. h. campestris* (see KEA Fig. 3).

Sources of material

KEA criticized REA for using museum specimens, claiming a wide variety of problems associated with such tissue, based mostly on literature for ancient DNA samples, not museum specimens collected within the past 45 years. In truth, Ramey *et al.* (2005) used a mixture of museum specimens and frozen tissue. However, all of the *Zapus hudsonius preblei* specimens used by KEA are subject to the same issues they raise regarding museum specimens because these ear punches were obtained without bleaching the ear punch between samples or wearing laboratory gloves to reduce cross contamination (R. Taylor, personal communication entered into the record on 7/6/06; Riggs *et al.* 1997).

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

mtDNA Sequences

KEA assert that the shared mtDNA haplotypes found by REA between *Zapus hudsonius preblei* and *Z. h. campestris* are the result of contamination of museum specimens, and they present a reanalysis of these specimens in support of their assertion. While this may be a point well taken, KEA did not consider any alternative explanations (e.g. nuclear paralogs, heteroplasmy, different amplification primers

and conditions, and template quality). Additionally, the use by KEA of different primers, annealing temperatures (48 rather than 60 degrees) and buffers suggests conditions that could lead to different amplification success and results.

Despite efforts to portray our work as inaccurate, KEA changed several key methods and details of results at the proof stage *after* we pointed out those errors. First, KEA originally claimed that we amplified with primers L15926 and H16498, then changed this in the proofs to L15320 and ZAP5PLr (which they claimed did not work for disputed museum specimens). We actually used L15320 and ZAP5PLr, and we then used nested primers L15398 and H16498 for nested polymerase chain reaction (PCR) for a subset of weakly amplifying specimens (Ramey *et al.* 2005). Second, Table 1 of Appendix B in KEA clearly mixed up REA's mtDNA haplotypes and sample numbers but was changed by KEA in the proofs.

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

Are KEA's mtDNA sequences 'diagnostic'?

KEA claim their mtDNA results are 'diagnostic' in support of *Zapus hudsonius preblei* as a subspecies and as it being on 'its own independent evolutionary trajectory'. Although they present additional mtDNA sequence data, *Z. h. preblei* remains paraphyletic with low bootstrap support.

Standardization between studies

KEA misrepresented that they had not obtained necessary samples from the Denver Museum of Nature and Science. The submission of a proposal along with a request is a standard requirement for destructive sampling of any museum specimens. KEA's request was fulfilled *after* King provided a proposal for the use of specimens.

AMOVA

We concur with KEA that the AMOVA criterion that we proposed for mtDNA may not be an ideal measure with

which to test the uniqueness of subspecies or distinct populations. As found by KEA, if there are slight differences among mtDNA haplotypes, but those haplotypes are fixed or nearly fixed in populations, this will have a substantial effect on the value of Φ_{ST} . That could lead to the erroneous designation of a weakly differentiated population as a subspecies or distinct population segment (DPS).

Conclusions

As noted in REA, thresholds for identifying subspecies and DPS's below the level of subspecies have been lacking. It is legitimate to debate thresholds, but the need for them is obvious — there are many endangered taxa and not enough resources to conserve them. If conservation effort is allocated to nondistinct or weakly differentiated populations, other more unique taxa (e.g. full species) will lose out. Hypothesis testing relative to these thresholds can provide objective assessments of degree of uniqueness and a basis for prioritizing the allocation of conservation effort.

The publicly available record shows that KEA changed their interpretation of results at least twice, from subspecies being 'weakly differentiated' to 'evolutionarily distinct'; and the number of potential DPS's of *Zapus hudsonius preblei* changed from two, to three, to none. This subjectivity is symptomatic of an approach that lacks clearly defined thresholds, and epitomizes the problem that REA attempted to address. We believe that few if any subspecies or DPSs proposed for US-ESA listing or delisting will be falsifiable under the general approach and low level of genetic differentiation that KEA used to accept subspecies. General application of that approach may move the allocation of conservation effort outside the realm of scientific inquiry.

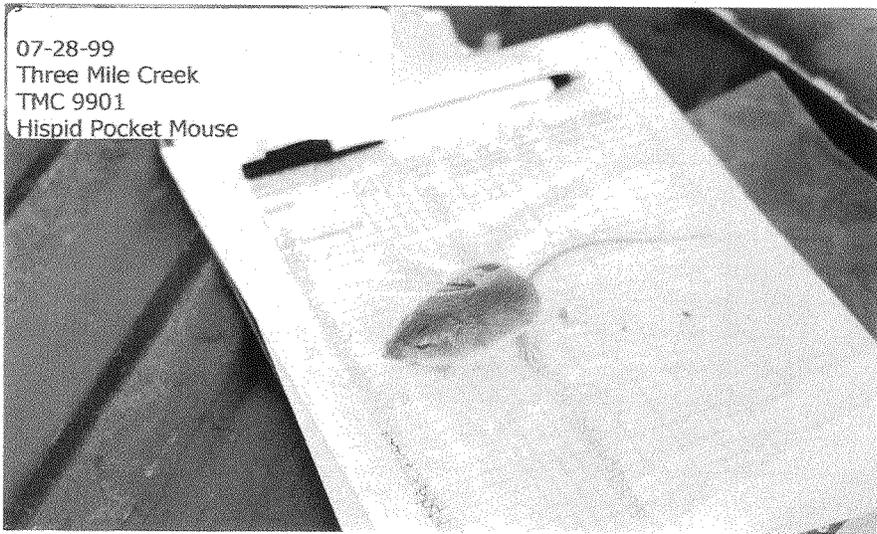
Keywords: conservation, Endangered Species Act, subspecies, taxonomy, *Zapus*

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07-28-99
Three Mile Creek
TMC 9901
Hispid Pocket Mouse

