

24 March 2004

TO: Rob Roy Ramey III and Gary Skiba »

FROM: David M. Armstrong

RE: Ramey *et al.* Report on *Zapus hudsonius preblei*

What follows are observations on a report by Ramey, Liu and Carpenter (2004) entitled "Testing the Taxonomic Validity of Preble's Meadow Jumping Mouse (*Zapus hudsonius preblei*)" dated 12 March 2004.

I have taken literally the invitation of the senior author (by telephone and on p. 15 of the Report) to provide comments and constructive criticisms; that has been my intent. I trust that the comments to follow will be taken in the collegial spirit with which they are offered. Obviously, as with any peer review, a little country wisdom may be salutary: consider the source, remember how much it cost you, and take it or leave it.

Caveat lector: my comments may reveal a some annoyance or otherwise appear to be hypercritical. I believe that this is annoyance with the process and not with the substance of the report or its authors.

The background is this: I have a fair amount of experience (> 35 years) providing reviews of technical manuscripts for professional journals. In that process one presumes that comments can be considered and if appropriate and relevant can be incorporated to improve a paper. The "industry standard" is either an informal, collegial "preview," before a manuscript is finalized and submitted for publication, or it's a collegial peer review, at the request of a professional journal, intended to fine-tune a submitted paper and/or to recommend for or against publication.

You'll find that I continue to suggest changes in spelling, typography, grammar, and the like although I realize that this report is *fait accompli*.

In the present process, the report apparently has been submitted and publicized, so it is beyond the point of useful, collegial, constructive input. Still, I will make editorial comments as well as substantive comments (1) because I can't help myself and (2) because the two are not unrelated. The medium is part of the message. Further, the comments may be useful as this report is re-written as a manuscript for possible publication.

For what it's worth, I had the same trouble reviewing (at the request of the USF&WS) various notices in the *Federal Register* concerning *Zapus hudsonius preblei*; I had the conflicted feeling that, on one hand, I wanted to do the right thing for colleagues or the mice, but—on the other hand—from any practical standpoint the review was irrelevant. In that case, I did the "reviews" anyhow

because they were required by some bureaucratic and political process. In the present case I decided to do the reviews anyhow because they were requested by friends and I suppose there is the chance that they will influence an eventual published paper on this topic or eventual conservation success in local and regional ecosystems.

The substantive, take-home message from the report is that the authors performed sophisticated molecular genetic studies, tested statistically some *a priori* hypotheses, conducted a multivariate statistical assessment of the quantitative measurements used by Krutzsch (1954), and evaluated the logic of qualitative descriptions and comparisons in Krutzsch's original description of *Zapus hudsonius preblei*. They concluded that the supposed subspecies is not worthy of subspecific recognition, by standards promulgated in part by the senior author in a cited paper. Further, they concluded that the population of *Zapus hudsonius* of central Colorado and adjacent southeastern Wyoming is not worthy of conservation attention.

These are interesting and important conclusions, important to a variety of human stakeholders (including—but not limited to—scientific, management, legal, political, and economic interests) and also to a peripheral and apparently disjunct population of meadow jumping mice.

In addition to the conclusions, there are some lingering questions implicit in the report, the answers to which should be critical to policy-makers. Specifically, the report mentions but does not explore in depth the possibility of hybridization between *Zapus hudsonius* and *Zapus princeps*. Depending on the nature and extent of that hybridization (which is not detailed), taxonomists might wish to re-evaluate the validity of *Zapus princeps* as a species. Also, the report suggests the possibility that *Zapus hudsonius preblei* is a peripheral isolate, but does not explore the possible implications of a disjunct range in any particular depth.

Turning to general comments on the report *per se*, I suspect that part of the problem I have with the report may be simply that it is, in fact, a report, not a manuscript intended for publication. A manuscript intended for actual publication would be double-spaced, with room for editorial remarks. A manuscript intended for publication probably would reflect or represent the usual style or tone of the systematic literature. This report, by contrast, is sometimes repetitive, argumentative, and dismissive of the methods of at least one systematist of an older generation; excursions into "the scientific method" and pleading about standards of critical thinking have no place in a professional taxonomic article (although they might be appropriate in a philosophical or methodological essay). A taxonomist does not have to talk about critical thinking; s/he just has to do it.

I am not competent to provide peer review of the methods and procedures of molecular systematics. Although I will raise some questions about the assumptions of the analysis and conclusions, my comments are mostly confined

to taxonomic issues (both substantive and "stylistic") and geographic issues and timescales—especially lingering questions.

By the way, in a report addressed (and by now submitted) to a high elected official and a Federal agency I would have expected a very high standard of presentation. Therefore I was surprised by the number of grammatical and typographic errors, and the general laxity of style (it not being obvious with which, if any, professional stylesheet the report was intended to conform).

I have made some editorial remarks on the report itself, as agreed with Ramey. Minor suggestions are made directly on the report. More extensive or substantive remarks are listed below, keyed to marginal numerals on the report.

Thanks for the opportunity to look at this report. I do hope that some of my comments will be of value. If there are questions about specifics, let me know. E-Mail would be the most expeditious means of communications; to increase the probability of timely response, please address both mausmann@aol.com and david.armstrong@colorado.edu.

Narrative Comments on Report by Ramey *et al.* (2004)

1. Given the quotation marks, the phrase “unsupported opinion” feels like a term of art and must stem from some source with which I am not familiar. I found the use of this phrase within quotation marks distracting. Would it not be more informative simply to say that qualitative characters used by Krutzsch (1954) apparently represent erroneous observations or extrapolation from too few specimens and are not supported by examination of the larger samples now available?
2. Where is the controversy? Cite some literature to indicate the history. Who said what when and where? Citation of representative papers from the taxonomic (or other) literature on either side of the allegedly controversial issue would seem like minimal requisite justification for the statement. If this is only a political or administrative controversy, and not a taxonomic one, that should be explicit. Again, citation is critical, to give credit where due and to allow readers the opportunity to confirm (or deny) assertions.
3. We learn only later what those “modern standards” are. Out of fairness to the intellectual process and to the spirit of science as a cumulative, self-correcting enterprise, if it really is necessary to judge Krutzsch as a scientist, here and elsewhere, he should be judged by the standards of 1954, not those of today. That is not to say that his conclusions cannot be re-evaluated and perhaps rejected, based on new techniques, newly available specimens, or even re-examination of old specimens. Errors are errors, and if they are identified they certainly should be corrected. Old Darwin was right (I paraphrase): false facts [i. e., errors of observation] are highly injurious to the progress of science, for they often endure long, but false theories [and a taxonomic judgment is an hypothesis or a theory, after all] are beneficial to science because everyone takes such salutary pleasure in proving them wrong.
4. Is this statement consistent with the alleged controversy noted earlier in this paragraph? If the taxonomy was not questioned critically by the scientific community, then where was or is the supposed controversy?
5. In the spirit of giving clear credit where due, I would note that Krutzsch (1954) proposed that *Zapus hudsonius* is subdivisible into 11 subspecies, two of which (*Z. h. preblei* and *Z. h. intermedius*) were named by Krutzsch himself. The 12th subspecies, *Z. h. luteus*, was recognized by Hafner *et al.* (1981) as representing *Z. hudsonius* rather than *Z. princeps*, with which it had been arrayed by Krutzsch. I would cite Hall (1981) as a more recent source of a map of the geographic range of *Z. hudsonius*. I believe that is the most recent such map based on examination of all of the literature; if I recall, Whitaker's (1972) map was based on Hall and Kelson (1959).

6. I think the statement that "The range of *Z. h. preblei* is restricted to the base of the Front Range in Colorado and into southeastern Wyoming" is equivocal. What does "the base of the Front Range" mean? In popular parlance, the "Front Range" seems to be the I-25 corridor. To a physiographer, the Colorado Front Range is that easternmost range of the Southern Rockies, extending from Pikes Peak north to the Poudre River. *Z. hudsonius* apparently ranges north beyond the Colorado Front Range in the "Laramie Foothills" and along the front of the Laramie Mountains of Wyoming as well.
7. I question the phrase, "the presumed cause of its uniqueness is the retreat of moist riparian habitat." If the population is distinct, then the cause of uniqueness is not habitat change but changes in gene frequencies, presumably due to natural selection. This needs to be clarified. Is the statement about proximate cause, ultimate cause, direct environmental influence, or what?

By the way does this sentence imply that the populations now known as *Z. h. preblei* are geographically isolated from the conterminous range of the species? I think that is an important consideration, from standpoints of both evolutionary genetics and conservation.

8. What is the relevance of this statement? Perhaps the intent is to underscore the need for the present study. The facts are that although Connor and Shenk (2003) used discriminant analysis of cranial measurements to distinguish *Z. hudsonius* from *Z. princeps*, to date there has been no thorough study of infraspecific variation of *Z. hudsonius* in Colorado and adjacent Wyoming, and no modern study (at the level of either morphology or molecules) comparing *Z. h. preblei* with other named populations of meadow jumping mice.
9. I find this confusing. If *Zapus hudsonius* is found to "freely hybridize" with *Z. princeps*, then *Z. hudsonius* is not a separate species (or *Z. h. preblei* has been allocated to the wrong species). If hybridization exists but is less than "free," then more subtle analysis would be called for. Either way, this statement raises major, unanswered questions.
10. I think that one can test genetic distinctiveness but not "taxonomic validity." (Also see my suggested changes to the title.) In the end, "taxonomic validity" is a judgment call, made by the community of systematic biologists in a dynamic, iterative, self-correcting process. A taxonomist makes a judgment, and other specialists either do or do not follow her or his arguments.

The first level of this process is peer review of a manuscript. Then the manuscript is published and becomes part of the formal record of science.

Even having been published, a taxonomic assertion is not an absolute but a sort of trial balloon, a testable hypothesis if you will, which may or may not be generally accepted in the long run.

In practice, for most groups of organisms, the taxonomic assertions of one author will remain the standard until another researcher revises the taxon in question. Sometimes this standard persists for many decades. Krutzsch (1954) is such a standard. It will remain the basic reference on North American "zapodids" until someone re-examines the material available to Krutzsch, adds to the analysis all of the material (or a statistically reliable sample thereof) that has accumulated since that time, adds an analysis of characters not available to Krutzsch (molecular data for example), and reaches new conclusions.

11. This sentence may include too many thoughts to be readily understandable. You used modern methods of genetic and phylogenetic analysis AND you used modern concepts of subspecies and distinct population segments. These are two quite different things and I would suggest that they be kept separate. You are using new techniques and you are also suggesting a change in the standard, in effect, raising the bar. New techniques can lead to new biological insights; that's great, I think. New standards represent a new judgment call. This is not quite biology, I think, but a matter of taste.

In the context of standards, I keep remembering an old but sage piece of advice: "...a subspecies should be described only when to fail to do so would obscure more biological truths than would be lost by describing the subspecies" (*in* Jameson, D. L., *et al.* 1966. *Proc. California Acad. Sci.*, 4th Ser., 33:551-620).

12. This is a personal statement about a personal reaction. I get uncomfortable when I hear comparative biologists talking about "the scientific method," as if there were just one way of doing science. It sounds pretentious, for one thing, so may put off some readers. But more important, some philosophers of science would argue that comparative biology (in which phylogenetic analysis is included) cannot be scientific because it does not permit real experiments. Individuals and species are unique genetic entities at unique points in space and unique moments in time; they are not replicable, by definition.

This does not mean, of course, that a person cannot pursue phylogenetic analysis rigorously, honestly, and productively. I just would not glorify that

as "the scientific method." Tone down the rhetoric and avoid the unavoidable and necessarily fruitless philosophical discussion.

13. Does this rule-of-thumb provide a basis to put real time into some statements later on that are presently ill-defined? (e. g., comment #26 keyed to Report, p. 9)
14. Here and throughout, this quasi-formal notation of hypothesis-testing may be lost on a lay audience. Quotation from the proposal feels like overkill to me. In a report (although perhaps not in a journal, with its stylistic standards), one could use layout techniques like indentations, boxes, or even boldface to set these off from the general text.

By the way, this insistence on rigorous hypothesis-testing (however laudable in itself) is also "bait" for attorneys who may miss the zoology if drawn too strongly to the niceties of the argument.

15. I realize that this terminology is used in a variety of publications (official US Fish & Wildlife Service petitions, for example). But as a matter of convention I would definitely avoid using "Preble's" as if were the name of something. Since about the 1930s most mammalogists have not given vernacular names for most subspecies, except for game species like desert bighorns and Roosevelt elk. So I would not even say "Preble's meadow jumping mouse"; I would say *Z. h. preblei*.

I would never, ever say "Preble's"; that is a possessive, an adjective. An adjective without a noun is meaningless. One would never say "that's a big" and expect it to convey meaning. Even if mammalogists did use vernacular names for subspecies, "Preble's" is equivocal. Do the authors mean *Sorex preblei*, *Tamiasciurus hudsonicus preblei*, *Dipodomys microps preblei*, *Peromyscus truei preblei*, *Phenacomys preblei*, or *Lutra canadensis preblei*?

Now I realize that the meaning is clear from context, but that's not the point. The point is, this usage is not idiomatic "mammalogese," so it might well sound un-professional to the wrong ears.

16. What does this have to do with the central question of this report, the taxonomic status of *Z. h. preblei*?

The observation of possible hybridization raises the important and interesting question of whether or not *Z. princeps* J. A. Allen (1893) is a valid species, distinct from the earlier-named *Z. hudsonius* (Zimmermann, 1780). However, at this point in the report it does seem to me like a secondary consideration, perhaps deserving of a little more play but in a separate paragraph. Hybridization with *Z. princeps* has been raised as a

possibility by various authors and certainly deserves to be explored, but that is an issue separate from variation within a putative subspecies, and, of course, those who have suggested the possibility of hybridization should be cited.

17. Good. Does this method also raise the question of geographical continuity? I think this may be a critical consideration with *Z. h. preblei*, and I know of no published study to try to understand the degree to which *Zapus hudsonius* is more or less continuously distributed (as a metapopulation) across eastern Wyoming, connecting the range of *Z. h. preblei* with that of either *Z. h. pallidus* or *Z. h. campestris*.
18. I agree with the importance of tying molecular samples to museum specimens. I agree strongly with the value of museums as repositories of information. However, I also am disappointed that ear punch samples could not be included in some part of this study. Basing this analysis on the few available museum specimens greatly restricts its utility. Under USF&WS protocols during the "PMJM Campaign," specimens could be prepared only of inadvertent trap casualties; however, my understanding is that a fair number of ear-punch samples were taken at the direction of USF&WS and CDOW. I presume that these were tied with specific geographic localities. Being conservative, I assume that field identifications of species of jumping mice are to some extent unreliable. However, using modern statistical techniques, I presume that one could run all of the available tissues and use some kind of discriminant analysis to sort *Z. hudsonius* from *Z. princeps*—assuming that there are genetic differences. This material could also be quite useful in approaching the separate but fundamental question of hybridization between *Z. hudsonius* and *Z. princeps*. Further, I suspect that the ear-punch samples could expand the geographic range of the analysis, which could only be a benefit to the reliability of the study.
19. It feels like this suspicion could have been tested. It could be a useful contribution to the collections management literature. I would be surprised to learn that specimens as late as 1980—especially those preserved in the semi-arid West—had arsenic in them. I prepared a few thousand specimens before 1980 and none of them was "preserved" with arsenic. If there's a chemical culprit interfering with amplification, I wonder if maybe it is residual organics from the bad old days (extending beyond 1980 even to the present in some collections) when insect repellants (Vapona®, PDB) or toxicants (CS₂) were routinely used in collections.
20. How do we know the "most closely related subspecies to *Z. h. preblei*"? Wouldn't the conservative statement be something about the geographically nearest neighboring populations?

21. This needs clarification and more extensive justification; this is an important assertion. The implication is that these specimens represent *Z. princeps*. Admittedly, I have not worked in that area for a number of years and I may be behind on the distributional literature, but to my knowledge, *Z. princeps* has not been reported from either Carter County, Montana, or Custer County, South Dakota. I'd dig deeper here. This could be a big deal.
22. This is an interesting result, but most stylesheets probably would not allow the underlined statement for emphasis. If there is concern that this assertion might get lost in the detail, I would set it off as a separate, short paragraph to draw the reader's eye to it.
23. First, this feels like a matter for Discussion rather than Results. Back in my editorial days, the rule-of-thumb was that if authors need to cite literature, their statement is Discussion, not Results. Moreover, when this is moved down the page to the Discussion, it deserves more explication. Explain to the reader how one might have a founder effect—or even an expanding range—without having some restricted genetic exchange at some level in the population.

Also, this section raises again my query #17, above, about continuity of range across eastern Wyoming to contact either of two possibly contiguous subspecies, *Z. h. pallidus* in the Platte River drainage, or *Z. h. campestris* in the Black Hills (*sensu lato*).

24. Is lower genetic variability not consistent with isolation and a founder effect?
25. It might be wise to review the assertion (from p. 5) that "recent" is intended to mean within the past 10,000 years and "very recent" is intended to mean within the past few hundred years. Also, I'm not sure what "few" means, but it might be important. If "a few" is 300-400, for example EuroAmerican influence would have come to roost in the range of *Z. h. preblei*, if it's > 300-400, then one would want to think about other external causes that could have influenced the distribution of meadow jumping mice (to test against the null hypothesis of random!)

Also, speaking as a biogeographer (not as a molecular systematist) I wonder why the insistence on comparison and connection with *Z. h. campestris* rather than *Z. h. pallidus*. Based on simple geography, one might reasonably suspect that mesic-adapted mammals of the South Platte River drainage (which includes most of the range of *Z. h. preblei*) would be more likely to be ecologically and genetically continuous with conspecifics of the Platte River drainage than with those to the north in the

Cheyenne and White River watersheds and beyond. If the genes say otherwise, so be it, but the geography deserves mention.

26. What does "long-term resident" mean here in the context of previously used, somewhat more explicit timeframes, "recent" and "very recent"?
27. Here and elsewhere, I wonder what ecological evidence for divergence would look like? I would think that a much more important criterion for recognizing a population as distinctive would be geographic isolation and lack of (or greatly restricted) gene flow. This report presents some evidence that there is lack of gene flow, but does not address the question of geographic isolation. Have there not been studies in southeastern and east-central Wyoming to address this issue at least in broad terms? As a rough first approximation, where have folks looked for meadow jumping mice, following established USF&WS protocols, and not found them? (I realize that lack of captures does not prove that the mice are not present, but simply that they were not caught, but still those trapping results are a start.)
28. A couple of points are stimulated by this paragraph. First, an agency report is not a publication and is therefore an inappropriate place to synonymize two subspecies. This report is private communication, or at most "gray literature," and it has no standing in zoological taxonomy. Second, the report seems not to recognize that this is a fairly small piece of the puzzle of geographic variation in the meadow jumping mouse. Absent a thorough taxonomic revision of subspecies of *Zapus hudsonius*, for example, I am not sure why *Z. h. preblei* should be considered a synonym of *Z. h. campestris* and not of *Z. h. pallidus*. And the authors have not investigated the distinctiveness of either *Z. h. pallidus* or *Z. h. campestris* relative to *Z. h. intermedius*, which is ascribed a range downstream in the Missouri-Mississippi watershed. And so forth.

In other words, a restricted, targeted investigation of this kind, laid out in an unpublished report, is not an appropriate vehicle for a taxonomic decision of the kind proposed. Rather, changes in infraspecific taxonomy and nomenclature should be based on thorough restudy of the species across its range—in other words, a study on the scale of Krutzsch (1954), but using the methods developed over the past 30-50 years to allow new and more sophisticated insights into evolutionary and ecological processes that any taxonomy ought to reflect. And they must be published in the peer-reviewed literature.

This is not to say, of course, that genetic answers to some narrow might not influence the choices that managers (depending upon the latitude allowed them by prevailing laws and regulations).

29. I could not follow the argument here (or at least, in the absence of knowledge of reproductive continuity between *Z. h. preblei* and other populations of *Zapus hudsonius*, I cannot evaluate it). My simple guess would be that if a population is not only peripheral but isolated, then it can—given time—have its own distinctive evolutionary role and tendencies. Therefore, my own simple (ethical, not demonstrably “scientific”) conclusion is that it ought to have conservation attention.

This leaves me wondering what the law and subsequent regulations have to say in this matter. I will leave that to others. My question is, does it make any difference that the population is disjunct even though it is not—yet—distinct by the criteria that have been utilized in this report?

30. This gratuitous indictment of Krutzsch (1954) is not useful. Of course his work is representative of mid-20th Century systematics. Notice when it was published! In fact, one could go back to W. H. Osgood’s (1909) masterful revision of mice of the genus *Peromyscus* and assert that Krutzsch’s work is merely an extension of methods now nearly 100 years old. That observation would be equally true and equally misplaced. What systematists of the last two to three generations have done was to move beyond the pre-Darwinian, typological systematics of their own predecessors, just as the thoroughly modern systematists of today can—with appropriate diligence and nifty chemical and statistical analyses — move beyond the skin-and-skull taxonomy of their intellectual parents and grandparents. That kind of change is to be expected and to be encouraged, but the change can (and, I would opine, should) be made without demeaning one’s predecessors.

Do not misunderstand. If Krutzsch made mistakes in measurements or interpretations, they should be corrected. If his sample sizes were too small to accurately represent variability within a population, we can do a better job today and we should.

Old Isaac Newton was not your most humble of scientists, but even he was reported to have said (paraphrasing predecessors of his own) “if I have seen farther it is because I stand on the shoulders of giants.” In science as in real life, I think a little humility goes a long way. Obviously, that is a statement of taste and manners, of course, not science.

31. A personal observation: I agree that it would be wonderful if there could be a modern systematic revision (based on phylogeographic analysis of molecular and morphological data) of all of the taxa that might be proposed for listing under the ESA. To be relevant, these would need to be on the scope of thorough revisions of whole species, not just the peripheral or disjunct populations that tend to populate the list of endangered species (and subspecies). I have not taken time to guess the

numbers of taxa that would need to be evaluated but I suspect that the total enterprise (even at the rate of \$57k per subspecies) would be prohibitively expensive. I do note that Hall (1981), listed 3607 subspecies and monotypic species of mammals in North America. Perhaps half of those are in the USA, and one would expect similar numbers of lissamphibians, squamate reptiles, and perhaps twice that many kinds of birds and even more kinds of fishes—and that considers only vertebrates, of course, not the whole biota. The total cost could exceed \$1 billion.

Beyond the financial cost, there is a huge opportunity cost. Conservation delayed is conservation denied. I happen to agree with the authors that there is room for improvement in the ESA and the consequent regulations and procedures (and even habits of mind) of the USF&WS and its personnel. I think we should move beyond endangered species (or subspecies or other evolutionarily significant units) to consider an Endangered Ecoregions Act (because the integrity of most ecoregions in the US is threatened or endangered) and then move internationally to an Endangered Ecosphere Treaty. But I digress; these are supposed to be comments on a report on a particular subpopulation of meadow jumping mouse!

32. I agree with the general assertion here. But (see above) I am not sure such a report is the place to take on all of the opportunities to improve the Endangered Species Act and its administration, however.
33. I do not recall having any substantive conversation or correspondence about this project prior to the preparation of this report, either at first or second hand. Certainly I do not deserve or desire any acknowledgment. My name (and that of anyone else on this list who had as little to do with the study or report as I did) should be removed from the list. This is a simple matter of giving credit where due and not giving credit where it is not due.
34. The word "catalog" has technical meaning to a field collector or a museum curator. This is not a catalog, but a list. A list of "specimens examined" is a typical feature of a taxonomic paper. However, this is not a conventional list of specimens examined (which do take a fairly wide range of formats).

Here specimens are listed in the order they were examined (measured?); conventionally, specimens would be listed by locality, in some geographical order. Sometimes this order is alphabetical by county within states ordered alphabetically.

Old Joseph Grinnell (followed by the late E. R. Hall and some of Grinnell's other students and their students, etc.) went a step farther. He urged that we arrange specimens in the museum geographically, from north to south

and west to east. This is an elegant system because it allows one to look in a tray of specimens, or several trays of specimens laid out side-by-side, and possibly get a first impression of geographic variation in size and color. That Grinnellian convention has been followed in lists of specimens examined by some authors, and I would recommend it. It urges a geographic dimension that is important in any discussion of subspecies because subspecies—whatever their value or quantitative definition—are fundamentally geographic and genetic concepts. They are geographically continuous subdivisions of species.

Usually in a manuscript for publication or a technical report, appendices appear after other end-matter (figures, tables).

35. By convention, in most stylesheets, figure legends go beneath figures. Also, and more important, I note that Figure 1 was run in with text on p. 6, whereas this Figure 2 is at the end of the report, buried behind an appendix. In a manuscript for publication, tables, figures, and appendices all would be placed at the end of the manuscript. In a report, I probably would put figures and tables in place in text, in part because that is where lay readers would expect to find them. The end of the report seems curious placement for Figure 2, which presents the data at the heart of the argument.
36. I wonder if these data belong in a table (which, in a report, I would run in with the text for ease of availability) or in an appendix (which does belong back here in the end-matter). This feels like it might be a worthy appendix, but authors might feel this is more important to the argument than that. (Appendices tend to be ignored by all but the most earnest readers.)

More important, I found myself wondering if there were not some way to map these data. Because subspecies are geographic ("mappable") entities, the argument in this paper should be about the geography of evolution. However, geography is difficult to find in the report except by implication, and almost never in detail. Mapping the distribution of the distinctive sequences would help me (and perhaps other readers) to visualize any geographic pattern that might exist. In other words, I would appreciate a phylogeographic analysis and discussion.

Further, because the intent of this paper is to test the validity of the taxonomic concept *Z. h. preblei*, I would definitely call these "supposed subspecies," so that the reader is reminded of the fact that there is some question about their validity.

Here and elsewhere, I find listing specimens examined only to county inadequate. Some of the counties mentioned are larger than some eastern

states. Particularly in the absence of a map, some sense for the geography of the situation is critical.

In this table and Table 2, I noted two different misspellings of "*preblei*."

Also, the column heading "Subspecies as per museum tag" caught my eye. The name on a museum tag has no standing in a taxonomic work; the assumption in a taxonomic work is that the person who examined the specimen determined the identity of ("expertized") the specimen.

37. Here and in accompanying text, emphasis is on Krutzsch (1954) and not on mice. It's fine to point out inconsistencies in the original description, but it seems to me much more important to re-examine the mice, with the greatly expanded specimen base now available, and to see whether there is recognizable geographic variation. If a modern researcher is uncomfortable with the qualitative descriptions and comparisons of an earlier time, some of the comparisons can now be quantified—color and shape for example.
38. I was unable to associate these points with Table 4. Does this belong elsewhere? Or perhaps these were just someone's notes, erroneously left behind.

Evaluating
the subspecies
Testing the Taxonomic Validity of Preble's Meadow Jumping Mouse (*Zapus hudsonius preblei*)

Report to the Governor of Wyoming and U.S. Fish & Wildlife Service (Revised)

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Revised 12 March 2004

New in this report:

- Results of discriminant analysis using repeated skull measurements.
- Haplotype tables and phylogeny revised to be more informative

Abstract

We examined three lines of evidence to test the taxonomic validity of *Z.h. preblei*. These included: 1) phylogenetic and population genetic analysis of 176 mitochondrial DNA sequences, 2) morphometric analysis skull measurements of 80 individuals, and 3) a critical review of the basis of Krutzsch's qualitative description of *Z.h. preblei* as a subspecies. Phylogenetic analysis of mtDNA sequence data revealed that *Z.h. preblei* was not unique relative to *Z.h. campestris*, all *Z.h. preblei* mtDNA haplotypes were found within individuals of *Z.h. campestris*. *Z.h. luteus* is most closely related to *Z.h. pallidus*. Population genetic analysis revealed greater mtDNA variation within rather than among *Z.h. preblei* and *Z.h. campestris*. The lowest mtDNA variation was found within *Z.h. preblei*. Our morphometric analyses (analysis of variance and linear discriminant analysis of repeated skull measurements) refutes the quantitative morphological basis for Krutzsch's description of *Z.h. preblei* as a subspecies. Rather than being smaller in most skull dimensions than *Z.h. campestris*, *Z.h. preblei* was significantly larger for two measurements, smaller for one, and insignificant for 6 others. Discriminating ability with a jackknifed posterior probability of ≥ 0.95 was poor, with 48% (35 of 72) of the specimens correctly classified to each subspecies. The skull shape and pelage differences noted by Krutzsch have no quantitative basis and must be considered as "unsupported opinion". The lack of genetic, morphological, or published ecological evidence for distinctiveness of *Z.h. preblei* from *Z.h. campestris*, means that these subspecies should be synonymized (considered the same subspecies - *Z.h. campestris*). *Z.h. preblei* does not appear to be sufficiently unique to qualify as a Distinct Population Segment under the Endangered Species Act.

who says controversy?

Introduction:

There is some controversy surrounding the taxonomic validity of Preble's meadow jumping mouse (*Zapus hudsonius preblei*) and conservation efforts under the Endangered Species Act (ESA) based on the presumed genetic uniqueness of this subspecies. This controversy is based upon the apparent weakness of the original taxonomic inference (Kruttsch 1954) which was an important component to the listing of *Z.h. preblei* under the ESA. The weakness of the original taxonomic designation includes: limited numbers of specimens used to describe the subspecies (3 adult skulls, 4 adult skins, 7 juvenile skins), qualitative descriptions that would not meet modern standards, and similarity in physical appearance of *Zapus* species and subspecies. The taxonomy of Kruttsch (1954) was not critically questioned by the scientific community or the USFWS until this study was proposed by the Denver Museum of Nature & Science in August 2002 and the results released in December 2003.

related to...

7 is all alleged with preblei?

According to Kruttsch (1954) *Z.h. preblei* is one of 12 subspecies of the meadow jumping mouse (*Z. hudsonius*), a species whose range covers approximately half of North America. The range of *Z. hudsonius* extends from the Pacific Coast of Alaska eastward to the Atlantic Coast; from the northern limit of tree growth south into central Colorado, Nebraska, eastern Kansas, Missouri, Tennessee, and northern Georgia (Kruttsch 1954, Whitaker 1972). The range of *Z.h. preblei* is restricted to the base of the Front Range in Colorado and into southeastern Wyoming. The presumed cause of its uniqueness is the retreat of moist riparian habitat across the eastern plains of Colorado that occurred following the opening of the Holocene, approximately 10,000 years ago (Hafner 1981, 1987).

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6 presumed "isolation" 7

This is not a subspecies of *Z. hudsonius*

To date, most of the research has focused on distinguishing *Z. hudsonius preblei* from the western jumping mouse (*Z. princeps princeps*). Connor and Shenk (2003) used discriminant analysis of skull measurements to distinguish specimens of *Z. h. preblei* from *Z. princeps princeps*. An unpublished report by Riggs et al. (1997) claimed that based on mitochondrial control region sequences *Z. h. preblei* forms "a homogenous group recognizably distinct from nearby populations and adjacent species of the genus." However, these authors did not gather data in such a manner as to be able to rigorously test whether *preblei* formed a monophyletic group. Furthermore Riggs et al. did not provide any statistical tests to support their conclusions. The data set used in the unpublished report by Riggs et al. (1997) is privately held by Biosphere Genetics Inc, Berkeley, CA.

2 N/A

If *Z. hudsonius preblei* is found to be indistinguishable from other subspecies of *Z. hudsonius*, then conservation efforts under the Endangered Species Act are being directed toward an organism that is more common and widespread than previously thought. If *Z. h. preblei* is found to be unique, relative to other subspecies of *Z. hudsonius*, then it may deserve conservation attention under the ESA, so long as it does not freely hybridize with *Z. princeps*, a common species whose distribution may overlap the western boundary of *Z. h. preblei*.

9 affecting in it with rest of 7

We tested the genetic distinctiveness and taxonomic validity of the Preble's meadow jumping mouse relative to other subspecies of the same species that are found in

10 not feasible - taxonomic judgment?

neighboring

bordering states. Our comparisons included samples of *Z. h. luteus* (from New Mexico and Arizona), *Z. h. campestris* (from Wyoming, Montana, and South Dakota), and *Z. h. pallidus* (from Kansas and Nebraska). We used phylogenetic and population genetic methods to analyze DNA sequence data, as well as modern subspecies and distinct population concepts (Ball and Avise 1992, Crandall et al. 2000). We also retested Krutzsch's original conclusions regarding cranial differences between *Z. h. preblei* and *Z. h. campestris*, using larger sample sizes. And finally, we examined Krutzsch's qualitative descriptions of skull shape and pelage differences between *Z. h. preblei* and *Z. h. campestris*.

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1) Analysis of Mitochondrial DNA sequence variation

Methods:

Conceptual approach:

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

(12) new sounds better present more the scientific method?

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In our original research proposal "Testing the Taxonomic Validity of the Preble's Meadow Jumping Mouse" we asked the following question "Are Preble's meadow jumping mice a unique subspecies relative to other nearby *Z. hudsonius* subspecies?" We then laid out the following hypotheses and critical tests:

"Hypothesis 1A: Preble's is a unique taxon, distinguishable from other subspecies of *Z. hudsonius* using mitochondrial DNA sequence data. The alternative hypothesis (Hypothesis 1B) is that Preble's will not be unique or distinguishable.

Critical test: Mitochondrial DNA sequence data for all samples show a pattern of reciprocal monophyly, or greater molecular variance among subspecies than within subspecies (in pairwise comparisons involving *Z. h. preblei*.) If we find that Preble's

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cannot be distinguished on the basis of mitochondrial DNA sequences, it will be unlikely that it will be differentiated for nuclear microsatellite DNA. However, if Hypothesis 1A cannot be refuted, then screening all samples for microsatellite loci becomes crucial to test if hybridization occurs between *Z. h. preblei* and *Z. p. princeps*.”

What does this have to do with hybridity & subspecies

Following our initial test using the criteria above, we also applied the conceptual approach of Crandall et al. (2000). These authors propose a hypothesis testing approach for recognizing distinct population segments using the criteria of genetic and ecological distinctiveness on recent and historic timescales. They advocate that ecological differences among populations can drive adaptive change that would not be detected by molecular markers alone. Therefore, we examined the literature for evidence of ecological differences between subspecies. We applied the conceptual approach using the crosshair classification of Table 1 in Crandall et al. (2000). We define “recent” as within the past 10,000 years (Holocene) and “very recent” as within the past several hundred years.

also see Crandall et al. (2000)

Acquisition of samples:

DNA samples were obtained from specimens in museum collections at the Denver Museum of Nature & Science, the University of Kansas, the Nebraska State Museum, and the University of New Mexico. We included only two ear punch tissue samples from live captured animals because they were needed to fill in a sampling area and photographs of these individuals were available. By relying on museum specimens, our results are repeatable. Additional questions may also be asked about each specimen at a later date, such as morphological distinctiveness. Museum research collections have the advantage of being open to public inspection and scientific research.

We sampled across the range of each putative subspecies, in order to sample the maximum extent of genetic variation across subspecies. This meant that we sampled more locations but fewer individuals per location. We included a limited sample from each of the subspecies of *Z. princeps* for use as an outgroup for phylogenetic analyses. Previous work by J. Cook (unpublished data) revealed a broad separation and reciprocal monophyly between *Z. princeps* and *Z. hudsonius* utilizing cytochrome *b* sequences, making *Z. princeps* an ideal outgroup for phylogenetic analyses.

Laboratory Methods:

Genomic DNA was extracted from frozen liver tissue and museum skin samples (5-10mg) using Qiagen DNeasy Tissue kit (Qiagen Inc.). Two specimens were from ear punch samples provided by Pioneer Environmental that had accompanying photographs (virtual vouchers). For frozen tissues, we followed the protocol provided in the Qiagen DNeasy Tissue kit. For skin samples, we modified the protocol slightly – samples were incubated at ATL buffer with proteinase K overnight at 56°C. 510bp of control region were amplified via the polymerase chain reaction (PCR) using primer L15320 and ZAP5P1r. The amplification conditions were as follows: in a 25 µl total volume, containing 5 µl of Invitrogen optimizer buffer D (17.5 mM MgCl₂, pH 8.5) (Invitrogen, Inc.), 2.5 µl of dNTPs (2.5 mM each), 1.25 µl of each primer (10 µM), 1 unit *Taq* polymerase, 1µl of template (200-300 ng), and 13.8 µl of sterile water. The temperature

profile for the PCR reaction consisted of an initial 2 min denaturation step at 94°C, followed by 30 cycles of 1 min at 94°C, 1 min at 58°C, 2 min at 72°C, and a final extension step at 72°C for 7 min. Amplified DNA was resolved by electrophoresis on 1.5% agarose gel that was stained with ethidium bromide to check for length, quality and quantity.

Some DNA extracts, most notably those of older museum specimens (prior ^{to} 1980), did not amplify well or at all. We suspect that this occurred because the older museum specimens were treated with arsenic during skin preparation. We were able to amplify DNA from these older museum specimens using nested PCR. Two primers, L15398 and H16498 were designed to amplify ca. 430 bp control region fragment within the L15320/ZAP5P1r primer combination. The relative positions and priming directions of the control region primers are shown in Figure 1. Genomic DNA was first amplified using primer L15320 and ZAP5P1r. The PCR products were cleaned using the Exo/SAP method. The PCR products 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). Subsequently the cleaned PCR product was reamplified using primer L15398 and H16498.

Handwritten notes in the left margin: "10-20% of old specimens", "heraldic", "780 is false", "the 1st time", "3/11/2000".

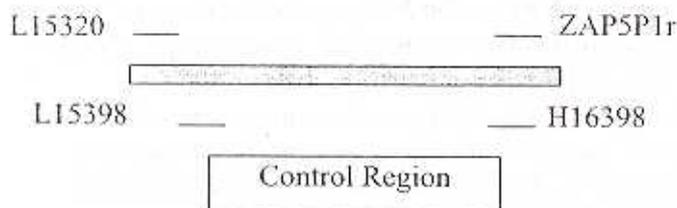


Figure 1. Location of primers used for PCR amplification of mitochondrial Control Region.

Automated Sequencing. The amplified PCR product was 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) to cleave nucleotides one at a time from an end of excess primers and to inactivate single nucleotides. Approximately 10-30 ng of cleaned PCR product was used as a template in a cycle sequencing reaction using the CEQ DTCS Quick Start Kit (Beckman Coulter, Inc.). The following cycling conditions were used: 96°C for two min, then 30 cycles of 96°C for 20 s, 50°C for 20 s, and 60°C for four min. The cycle-sequenced product was cleaned using the Beckman Coulter protocol. Fluorescent dye-labeled DNA was combined with 4 µl stop solution (equal volume of 100 mM EDTA and 3 M NaOAc pH 5.2), 1 µl glycogen (20 mg/ml), and 10 µl milli-Q H₂O, mixed well, and precipitated with 60 µl cold 95% (v/v) ethanol/water. Fluorescent dye-labeled DNA was recovered by centrifuging at 13,000 rpm for 20 min at 4° C. Pellets were washed with 100 µl 70% (v/v) ethanol/water, air dried and resuspended in 40 µl of dimethylformamide. Resuspended samples were added

to the appropriate wells of the CEQ sample plate, overlaid with mineral oil, and run on the Beckman Coulter CEQ8000. Sequences were determined for both strands and were edited and aligned using Sequencher™. All DNA sequences were determined by sequencing in the forward and reverse directions, with additional runs used to eliminate ambiguous base calls. Aligned and edited sequences were checked back against raw chromatograms to insure base calling accuracy.

Data Analysis. Consensus sequences were aligned using Sequencher and verified manually. Phylogenetic hypotheses based on distance and parsimony methods were conducted using PAUP* 4.0b10 (Swofford, 2002). A Bayesian analysis using MrBayes 3.04 (Huelsenbeck and Ronquist, 2001) was conducted as another means of estimating phylogeny. The HKY model with variable sites assumed to follow a discrete gamma distribution (e.g., HKY + I + Γ ; Hasegawa et al., 1985) was selected as the best fit for the dataset (Modeltest 3.06; Posada and Crandall, 1998).

Maximum-parsimony (MP) analyses were conducted with equal weighting, using the heuristic search option with tree bisection reconnection branch-swapping and 10 random additions. Bootstrapping with 1000 replications (as implemented in PAUP*) was used to evaluate node support. HKY distances were used to generate a neighbor-joining (NJ) tree based on the clustering method of Saitou and Nei (1987). Node support was assessed by completion of 1000 bootstrap replications (Felsenstein, 1985) in PAUP*, using the fast-search option. Bayesian analyses were performed based on the HKY model with invariable and variable sites with a discrete gamma distribution (e.g., HKY + I + Γ ; Hasegawa et al., 1985) model of evolution. Several short runs were first conducted using the default random tree option to determine when the log likelihood sum reached a stable value (by plotting the log-likelihood scores of sample points against generation time). Then metropolis-coupled MCMC simulations were run with four chains using the default random tree option for 1,000,000 generations and Markov chains were sampled at intervals of 10 generations to obtain 100,000 sample points. The last 95,000 sampled trees with branch lengths (the first 5000 trees having been removed as “burn-in”) were used to generate a 50% majority rule consensus tree. The percentage of samples that recovered specific clades on this topology represents that clade’s posterior probability; these are the P values, and $P \geq 95\%$ was considered evidence of significant support for a clade (Huelsenbeck and Ronquist, 2001).

20 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 most closely related subspecies to *Z. h. preblei* (Excoffier et al. 1992). Statistical significance of differentiation at these levels was quantified and tested using ARLEQUIN 2.0 (Schneider et al. 2000). ARLEQUIN 2.0 was also used to estimate mtDNA nucleotide diversity.

Results:

We sequenced mitochondrial control region from 58 *Z. hudsonius preblei*, 33 *Z. h. campestris*, 32 *Z. h. luteus*, 35 *Z. h. pallidus*, 7 *Z. princeps princeps*, 3 *Z. p. idahoensis*,

and 7 *Z. p. utahensis*. The alignment of 151 sequences (Table 1), excluding four specimens from Wyoming, one from Kansas, one from Montana, and one from South Dakota (see explanation below), of the partial mitochondrial control region from four *Zapus hudsonius* subspecies yielded 355 bp. Overall nucleotide composition was biased towards thymine (T)(34.3%) and adenine (A)(29.8%), followed by cytosine (C)(26.0%) and guanine (G)(9.9%).

Three variable sites (all transitions) were observed among 54 specimens of *Z. h. preblei* resulting in four haplotypes. [Note: four specimens of *Z. h. preblei* from Albany Co., Wyoming had almost identical sequences to *Z. p. princeps*. These four specimens were presumed misidentified and thus not included.] Twenty-nine variable sites (19 transitions, 8 transversions, and 2 indels) were observed among 31 specimens of *Z. h. campestris* resulting in sixteen haplotypes. Four sequences (two haplotypes) of *Z. h. campestris*, three from Lawrence Co., South Dakota and one from Crook Co., Wyoming, are more similar to sequences of *Z. h. luteus* and *Z. h. pallidus* than to other sequences of *Z. h. campestris*. One specimen of *Z. h. campestris* from Carter Co., Montana, and one specimen from Custer Co., South Dakota, has similar sequences to *Z. p. utahensis*. We presume they were misidentified and thus not included (Table 2).

Thirty variable sites were observed among 34 specimens of *Z. hudsonius pallidus* resulting in twelve haplotypes. Two sequences of *Z. h. pallidus* from Clay Co., South Dakota, are more similar to sequences of *Z. h. campestris* and *Z. h. preblei* than to other sequences of *Z. h. pallidus*. One specimen of *Z. h. pallidus* from Douglas Co., Kansas, has similar sequences to *Z. p. utahensis*. They are presumed misidentified and thus not included. Six variable sites were observed among 32 specimens of *Z. h. luteus* resulting in eight haplotypes.

Phylogenetic analysis of mtDNA sequences based on maximum parsimony, distance and Bayesian methods yielded concordant results that differed only in the positioning of terminal taxa (Figure 2, Table 1). Phylogenetic analysis of mtDNA sequence data revealed that *Z. h. campestris* is most closely related to *Z. h. preblei* and that *Z. h. luteus* is most closely related to *Z. h. pallidus*. These two clades had strong bootstrap support (Figure 2). *Z. h. preblei* and *Z. h. campestris* were not reciprocally monophyletic. All four of the mtDNA haplotypes found in *Z. h. preblei* were also found in *Z. h. campestris*. No unique mtDNA haplotypes were found in *Z. h. preblei*.

Genetic variation within subspecies as indicated by mtDNA nucleotide diversity was lowest in *Z. h. preblei* (0.0027, SD=0.0020) and approximately nine times higher in *Z. h. campestris* (0.0243, SD=0.0129). Nucleotide diversity in *Z. h. luteus* (0.0041, SD=0.0029) was twice that of *Z. h. preblei* but three times lower than in *Z. h. pallidus* (0.0135, SD=0.0075).

In a pairwise comparison between *Z. h. preblei* and *Z. h. campestris*, analysis of molecular variance revealed that most of the genetic variation was within (64%) rather than among these subspecies (37%), thus refuting hypothesis 1A and failing our test of genetic uniqueness. We did not include the highly divergent sequences of the 4 Albany Co.

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specimens in this analysis because it is likely that they are specimens of *Z.p. princeps* that were misidentified as *Z. h. preblei*.

Utilizing the criteria of genetic and ecological exchangeability as proposed by Crandall et al. (2000) for distinct populations, the mtDNA data does not refute the hypothesis of historic or recent genetic exchangeability (interbreeding) between *Z.h. preblei* with *Z.h. campestris*. This is because all four *Z.h. preblei* mtDNA haplotypes are found in *Z.h. campestris* from near the Black Hills of South Dakota. These mtDNA haplotypes that are shared between *Z.h. preblei* and *Z.h. campestris* span a range of up to 700km, from central Colorado to southeastern Montana. The fact all *Z.h. campestris* haplotypes are not found in the range of *Z.h. preblei* is consistent with founder effects and range expansion, not evidence of restricted genetic exchange. A review of the literature reveals that no quantitative evidence exists to reject the hypotheses of historic or recent ecological exchangeability (ecological similarity) between *Z.h. preblei* with *Z.h. campestris*. While it is possible that genetic exchange between these two putative subspecies is currently limited, this alone does not support them as being recognized as a distinct population segment (case 8, Crandall et al. 2000).

Discussion:

Our analysis of mtDNA sequence data refutes Hypothesis 1A, that *Z.h. preblei* is a unique taxon, distinguishable from other subspecies of *Z. hudsonius* (in this case *Z.h. campestris*) using mitochondrial DNA sequence data. The results of the mtDNA analysis reveal that *Z.h. preblei* is a less genetically variable population of *Z.h. campestris*.

The high level of mtDNA variation (nucleotide diversity) found in *Z.h. campestris* compared to *Z.h. preblei* does inflate the F_{ST} estimate, making these subspecies seem more diverged than the shared mtDNA haplotypes indicate.

While it is possible that the low level of mtDNA variation found in *Z.h. preblei* is the result of isolation and a northern migration into the range of *Z.h. campestris*, the pattern is more consistent with the hypothesis that the range of *Z.h. preblei* is the result of a recent southward colonization from the range of *Z.h. campestris*. Two observations support this later conclusion: first, no unique mtDNA haplotypes were found in *Z.h. preblei* and second, all of these haplotypes were closely related. The reduced mtDNA variation is consistent with a founder effect (e.g. population bottlenecks during a southern colonization). In contrast, if *Z.h. preblei* had been a long term resident along the Front Range and had evolved in isolation from *Z.h. campestris*, more unique mtDNA haplotypes would be expected – a situation found with *Z.h. luteus* compared to *Z.h. pallidus*. In either case, the shared mtDNA haplotypes indicate recent genetic exchange.

The failure of evidence to reject hypotheses of genetic and ecological exchangeability between *Z.h. preblei* with *Z.h. campestris*, using the approach of Crandall et al. (2000), means that *Z.h. preblei* with *Z.h. campestris* should be treated as a single population. If evidence from future trapping efforts supports a lack of current genetic exchangeability (e.g. genetic isolation) between *Z.h. preblei* and *Z.h. campestris*, these two subspecies

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would still be considered a single population for management purposes, using the criteria proposed by Crandall et al. (2000).

2) Morphometric analyses: Retesting Krutzsch's conclusions with larger sample sizes, analysis of variance, and discriminant analysis.

Methods:

To test the hypothesis that size differences in skull measurements reported by Krutzsch (1954) are representative of differences among subspecies, we compared 39 adult *Z.h. preblei* and 41 adult *Z.h. campestris* specimens using analysis of variance (ANOVA). Specimens were measured at the zoology collections at the Denver Museum of Nature & Science, and the University of Kansas Museum of Natural History. We utilized the same 9 skull measurements of Krutzsch (1954): occipitonasal length (from anteriormost projection of nasal bones to posteriormost projection of supraoccipital bone), condylobasal length (posteriormost part of exoccipital condyles to anteriormost projections of premaxillary bones), palatal length (anterior border of incisors to anteriormost point of postpalatal notch), zygomatic length (anteriormost point of zygomatic process of maxillary to posteriormost point of zygomatic process of squamosal), zygomatic breadth (greatest distance across zygomatic arches of ~~cranium~~ ^{at} right angles to long axis of skull), mastoidal breadth (greatest distance across mastoid bones perpendicular to long axis of skull), braincase breadth (greatest distance across braincase perpendicular to long axis of skull), interorbital breadth (least distance across top of skull between orbits), and upper tooth row length (anterior border of P4 to posterior border of M3). Our palatal length is larger than what Conner and Shenk (2003) reported due to differences in where measurements were taken.

Four repeated measurements (Conner and Shenk 2003) were taken with digital calipers and recorded to the nearest hundredth of a millimeter. Only adult skulls were measured, as determined by tooth eruption and wear. In several cases, fewer measurements were taken because of breakage or not taken because of previous breakage. Calipers were moved away from the skull and reset for each measurement. A single observer (L. Carpenter) measured all skulls in the study. We used the mean of the repeated measurements in both ANOVA and discriminant analysis (Connor and Shenk 2003).

We tested the cranial distinguishability of *Z.h. preblei* from *Z.h. campestris* from a multivariate perspective with linear discriminant analysis using SYSTAT 9.0. Forward, backward, and interactive stepwise procedures to develop the simplest discriminant models to eliminate statistically unimportant variables and to maximize the ratio of sample size to variables included in the model (Williams and Titua 1990). We used jackknifed estimates of posterior probabilities and classification ability for discriminant models (Afifi and Clark 1990). We used a previously published criterion for testing the hypothesis of distinguishability between subspecies: $\geq 90\%$ of specimens correctly classified at jackknifed posterior probabilities of $p \geq 0.95$ (Wehausen and Ramey 2000). This criterion was more discriminating than just the percentage of specimens correctly classified at a posterior probability of $p > 0.5$. Males and females were pooled in the analyses because of a lack of cranial sexual dimorphism in *Z. princeps* and *Z. hudsonius*

(Connor and Shenk 2003). This apparent lack of sexual dimorphism was also tested using stepwise discriminant analysis.

Results:

Analysis of variance

Our analysis of skull measurement data refutes the hypothesis above and the claim made by Krutzsch (1954) that *Z.h. preblei* is "averaging smaller in most skull measurements" than *Z.h. campestris*. A total of 3 measurement variables were found to be significantly different at a level of $p < 0.05$. Two of these measurements (zygomatic breadth and mastoid breadth, were significantly larger in *Z.h. preblei* than in *Z.h. campestris*, in the opposite direction to Krutzsch's claims that *Z.h. campestris* is larger. *Z.h. campestris* was only larger for one measurement (*interorbital breadth*) and it was only marginally significant (larger in *Z.h. campestris*) ($p = 0.037$). All other measurements were not significantly different (Table 3).

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Discriminant analysis

Four variables were determined to have the greatest discriminating power. These included: zygomatic breadth, mastoidal breadth, breadth of skull, and condylobasal length. A total of 33 *Z.h. preblei* and 39 *Z.h. campestris* were used in the discriminant analysis. The null hypothesis of equal covariances among subspecies was not rejected ($p = 0.147$). Discriminating ability with a jackknifed posterior probability of ≥ 0.95 was poor, with 48% (35 of 72) of the specimens correctly classified to each subspecies.

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Discussion:

Our morphometric analysis refutes the quantitative morphological basis for Krutzsch's description of *Z.h. preblei* as a subspecies. Krutzsch (1954) described *Z.h. preblei* as "averaging smaller in most skull measurements" but using ANOVA, we found only one out of nine variables to be significantly smaller in *Z.h. preblei*. The three significant differences that we did find should be viewed within the context of variation typically found among populations.

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Z.h. preblei failed the test of morphological distinguishability from *Z.h. campestris* using discriminant analysis of the same skull measurements as Krutzsch (1954) and a substantially larger sample size. The correct classification of specimens by the DFA was far less (48%) than the criterion that $\geq 90\%$ of specimens be correctly classified at jackknifed posterior probabilities of $p \geq 0.95$ (Wehausen and Ramey 2000). This is a refutation of Krutzsch's (1954) only quantitative basis for concluding that *Z.h. preblei* are morphologically distinguishable and therefore a unique subspecies relative to *Z.h. campestris*.

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a subspecies is considered a singular

As with other taxonomy papers of the period, Krutzsch's description in 1954 of *Z. h. preblei* as a newly recognized subspecies was based upon qualitative descriptions without statistical tests, and presumed geographic isolation. It represented the opinion of the author. The only quantitative comparison that Krutzsch (1954) used to support this "new" subspecies description, was based on measurements of only 3 adult specimens of

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Preble's that he compared to 40 specimens of *Z.h. campestris*. He examined the skin of a fourth adult specimen and the skins of 11 juveniles of *Z. h. preblei*. The three adult *Z. h. preblei* specimens were reported to be smaller in all skull dimensions.

3) A critical evaluation of Krutzsch's qualitative descriptions

We examined the basis of Krutzsch's qualitative differences in skull shape and pelage to determine the strength of the evidence that he used to infer that *Z.h. preblei* is a unique subspecies.

Three of the skull shape differences distinguishing *Z.h. preblei* and *Z.h. campestris* noted by Krutzsch (1954) had no reported measurements. Therefore the skull shape differences noted by Krutzsch have no quantitative basis and must be considered as "unsupported opinion". These shape descriptions include: "incisive foramina not truncate posteriorly; auditory bullae smaller, less well inflated; and frontal region usually more inflated". Additionally, one of the skull shape differences ("frontal region usually more inflated") did not have an accompanying qualitative description for either subspecies individually (Table 4).

When Krutzsch's pelage descriptions of each subspecies are listed side by side (Table 2), and compared to what he stated were distinguishing pelage differences, it is clear that two of the three pelage differences were made without a description of one or both subspecies. For example, one pelage difference ("upper parts generally dull, averaging lighter") had no comparative description for *Z.h. campestris*. The second pelage difference ("sides duller") did not have an accompanying description for either subspecies. The only pelage difference where there was a description for both subspecies was "less black tipped hair" on the dorsal band. These three differences in pelage between *Z.h. preblei* and *Z.h. campestris* noted by Krutzsch (1954) are entirely qualitative and must also be considered as "unsupported opinion". The underpinnings of Krutzsch's qualitative descriptions are without a quantitative basis, and fail the tests of falsifiability, comprehensiveness, repeatability, and sufficiency required by evidential reasoning (Lett 1990).

Conclusions:

Taxonomy

We examined three lines of evidence to test the taxonomic validity of *Z.h. preblei*. These included: 1) phylogenetic and population genetic analysis of mitochondrial DNA sequences, 2) morphometric analysis of skull measurements, and 3) a critical review of the logical basis of Krutzsch's description of *Z.h. preblei* as a subspecies. Our results failed to support the genetic distinctiveness of *Z.h. preblei* from *Z.h. campestris*. Our morphometric analysis refutes the quantitative morphological basis for Krutzsch's description of *Z.h. preblei* as a subspecies. The skull shape and pelage differences noted by Krutzsch have no quantitative basis and must be considered as "unsupported opinion".

The lack of genetic, morphological, or published ecological evidence for genetic distinctiveness (including adaptive divergence) of *Z.h. preblei* from *Z.h. campestris*, means that these subspecies should be synonymized (considered the same) and referred to as *Z.h. campestris*.

The lack of genetic, morphological, and ecological evidence supporting divergence of *Z.h. preblei* from *Z.h. campestris*, the weakness of the original taxonomic inference of *Z.h. preblei* being a subspecies (Krutzsch 1954), and the unsupported assumption that geographic isolation has driven genetic divergence between these putative subspecies, all point to *Z.h. preblei* being synonymous with *Z.h. campestris*. We therefore synonymize *Z.h. preblei* with *Z.h. campestris*.

28
what would you look like

29
why not w/ paludicola?

Does the evidence support consideration of Distinct Population Segment listing?

In a broader perspective, the range of *Z.h. preblei* represents less than 5% of the range of a species whose range is approximately half of North America (along streams and in meadows). This is not a compelling argument for *Z.h. preblei* to be a candidate for a distinct population segment designation (DPS). A DPS designation requires that a population be "discrete" and "of significance" (US Fish & Wildlife Service 1996). The "discrete" requirement, that a DPS is "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 & Wildlife Service 1996) is not supported by our genetic or morphological analyses.

29
what do they mean do with relative of ...

The "significance" requirement that, "evidence that loss of the discrete population segment would result in a significant gap in the range of a taxon" is not supported because of the broad distribution of *Z. hudsonius* (Figure 8). *Z.h. preblei* is a peripheral population of *Z. hudsonius* that does not rank as distinct using the criteria (spatial distance, life history, time, and ecology) proposed by Lesica and Allendorf (1994).

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this is the point. This also depicts out what does law + reg. help?

Hypothesis testing and peer review

Krutzsch's (1954) unsupported opinions about shape differences in skulls and coloration of skins, as well as skull measurement comparison based on a sample size of 3 *Z.h. preblei*, have carried the weight as the "best available science" in the listing of *Z.h. preblei*. However, the logical basis of these opinions was not critically evaluated by the USFWS, or others, during the listing process, despite the weakness of Krutzsch's (1954) inference by modern standards. The identification of *Z.h. preblei* specimens by museum curators or consultants similarly relied on Krutzsch (1954). The description of *Z.h. preblei* as a new subspecies is typical of the taxonomic work that appeared in the literature in the early to mid twentieth century. During that time, species and subspecies descriptions had little or no quantitative basis, relied on small sample sizes, and were based largely on opinion (Ramey 1993, Wehausen and Ramey 1993, 2000). Essentially, a species or subspecies was "what a good taxonomist said it was".

and before - at least since 1954 (1954)

30
of course this way taxonomists will!
precision!

(31)
a good point
to note is that
there is a
or not possible

The original review of the *Z.h. preblei* listing would have benefited from a critical peer review by more broadly trained systematic biologists and molecular/morphometric analyses to specifically test the taxonomic validity of subspecies. The Federal peer review standards proposed by the Office of Management and Budget (2003) are a good example of how peer review can strengthen the scientific justification for proposed ESA listings, delistings, and Biological Opinions. Also, genetic analyses with the specific goal of treating taxonomic categories as testable hypotheses (Ramey 1993, 1995; Wehausen and Ramey 1993, 2000) would have been appropriate in this case and others. In the case of *Z.h. preblei*, a genetic analysis was performed by Riggs et al. (1997) but not with the subspecies validity question in mind or critical hypothesis testing. Similarly, the listing rule (USFWS 1998) appeared to have accepted the taxonomy of Krutzsch (1954) without question. Our review differs from those previously (Riggs et al. 1997; Hafner 1997; USFWS 1998) because it involves hypothesis testing, utilizes multiple lines of evidence, and incorporates modern concepts of subspecies and distinct population segments. Our analyses suggest that a large expenditure of conservation effort under the ESA is being directed towards populations of a subspecies (*Z. h. campestris*) that are more widespread than previously thought.

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Scientific investigation involves critical thinking and evidential reasoning (Lipps 1999, Lett 1990, Platt 1964). Unsupported opinion and anecdotal observations are not scientific. In the case of endangered species management, facts (quantitative evidence) can be gathered in such a way as to answer specific questions, often at greater economy than courses of action whose basis is falsified later. Testing taxonomic classifications does not take as long, or cost as much, as one might initially think. The molecular data has taken approximately one year of part-time effort at a cost of approximately \$50,000. Our morphometric measurements, analysis, and write up has taken only three weeks of effort, at a cost of approximately \$7,000. Our analyses have benefited greatly from the availability of museum specimens in zoological research collections. Without these collections, this biodiversity research would not have been possible.

Point

In the future, we strongly urge the USFWS to work with the scientific community in developing incentives to apply both critical peer review and molecular/morphometric analyses to test the quantitative basis of all proposed subspecies and distinct population segment listings. To not do so invites a potential for misallocation of scarce conservation resources to populations that are not genetically or ecologically unique, and can erode public confidence in the implementation of the Endangered Species Act.

(32)

Acknowledgements:

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facility at Rocky Mountain Center for Conservation Genetics and Systematics at the University of Denver. Special thanks to Dr. Cheri Jones who expanded and curated the *Z.h. preblei* specimens at DMNS. We are grateful for discussions and constructive criticism throughout this project from the following people: Dr. David Armstrong, Dr. Laura M. Brown, Dr. Norm Clippinger, Dr. Joseph Cook, Clint Epps, Kristin Hintz, Dr. Cheri Jones, Dr. Carron Meany, Dr. Jeff Mitton, Bruce Roselund, Dr. Vern Stelter, and Dr. John Wehausen.

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33
don't
have any
substantive
conversations
prior to prep
a report

Comments and constructive criticisms on this report are appreciated. Please direct these to ramey@dmns.org. Thank you.

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Appendix I:

Catalog of specimens examined for skull morphometry. Specimens are listed in the order they were examined.

Denver Museum of Nature & Science, *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. Denver Museum of Nature & Science, *Z.h. campestris*: 8512. University of Kansas Natural History Museum, *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.

Figure 2. Neighbor-joining phylogram based on partial control region sequences using a HKY substitution model, depicting phylogenetic relationships among subspecies of *Zapus hudsonius*. One hundred seventy six sequences were obtained for this study (Table 1 and 2). In order to provide a reasonable size tree, one sequence from each haplotype was used. Bootstrap percentages are given when $\geq 50\%$. Other methods of phylogenetic analysis produced very similar trees. supposed

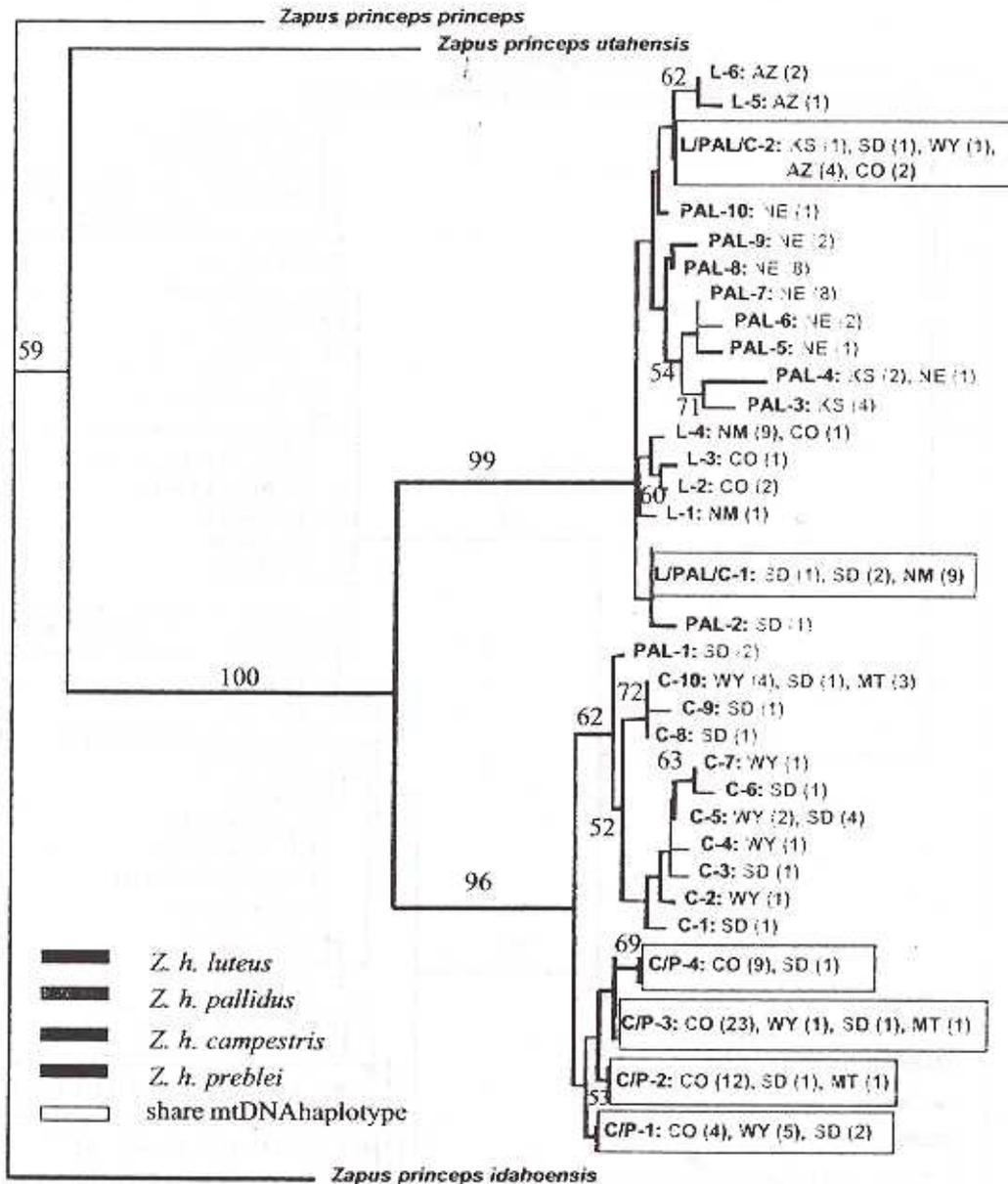


Figure 2. Neighbor-joining phylogram based on partial control region sequences using a HKY substitution model, depicting phylogenetic relationships among subspecies of *Zapus hudsonius*. One hundred seventy six sequences were obtained for this study (Table 1 and 2). In order to provide a reasonable size tree, one sequence from each haplotype was used. Bootstrap percentages are given when $\geq 50\%$. Other methods of phylogenetic analysis produced very similar trees.

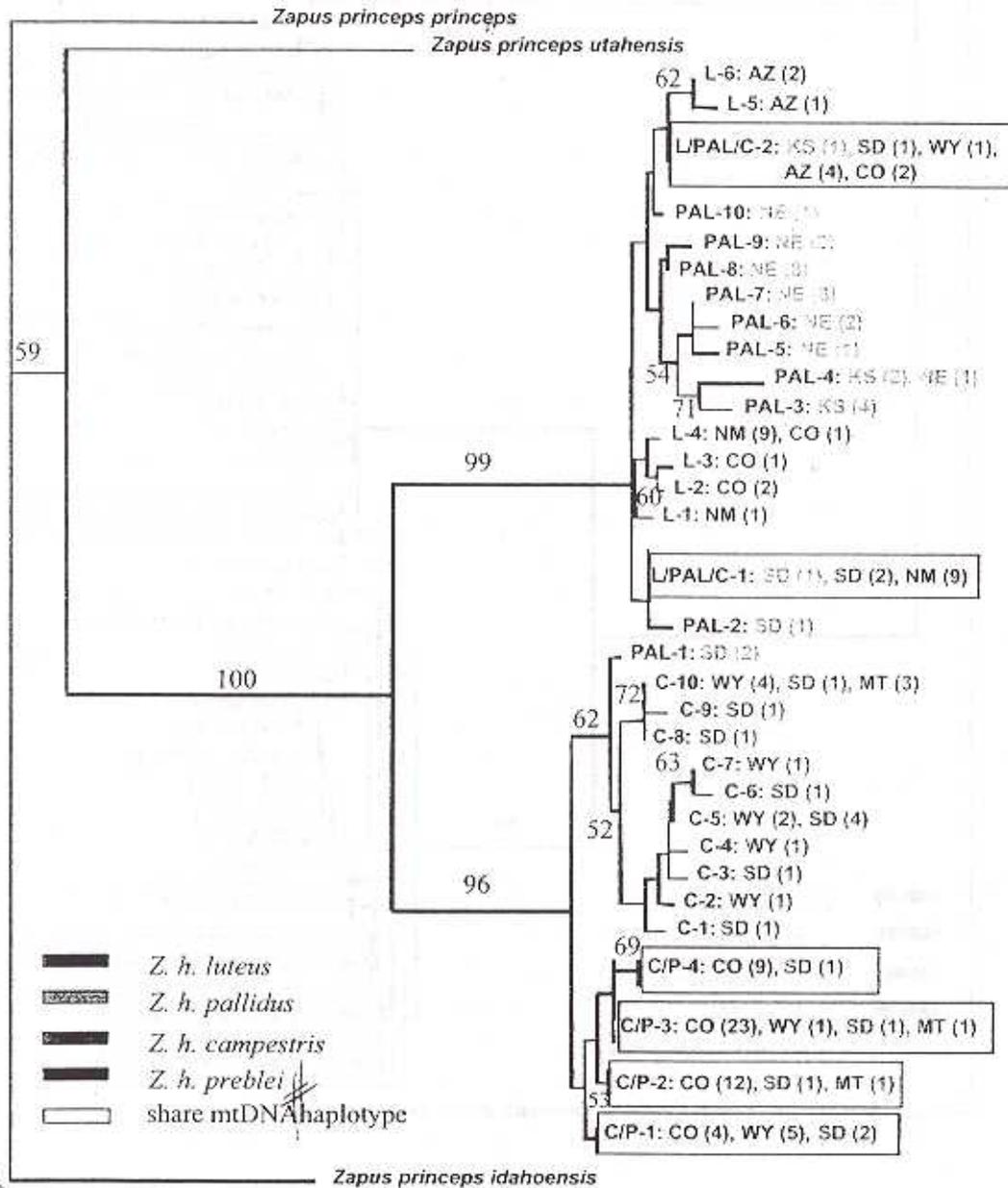


Figure 1 was run into the why not this one?

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Table 1. Specimens of *Z. hudsonius* used in phylogenetic analysis, listed by museum and tissue archive catalog number (DMNH = Denver Museum of Nature & Science; TK = Texas Tech (tissue archive); KU = University of Kansas; UNSM = University of Nebraska State Museum; MSB and NK (Tissue archive) = Museum of Southwestern Biology; PIONEER = Pioneer Environmental Services.)

36
 authority?
 how determined?

Representative individuals used in phylogenetic analysis	Additional specimens with identical mtDNA haplotype: ID, state, and county	subspecies	haplotype
MSB40951, AZ:Apache	MSB40994, AZ:Apache	Z.h. luteus Z.h. luteus	L6
MSB89194, AZ:Navajo		Z.h. luteus	L5
MSB86344, AZ:Apache	MSB91627, AZ:Navajo MSB91675, AZ:Apache NK1584, AZ:Apache DMNH8635, CO:Las Animas DMNH8633, CO:Las Animas KU41451, WY:Crook KU153706, KS:Leavenworth KU112661, SD: Lawrence	Z.h. luteus Z.h. luteus Z.h. luteus Z.h. luteus Z.h. luteus Z.h. campestris Z.h. pallidus Z.h. campestris	L/PAL/C2
UNSM20596, NE:Buffalo		Z.h. pallidus	PAL10
UNSM26492, NE:Buffalo	UNSM20879, NE:Buffalo	Z.h. pallidus Z.h. pallidus	PAL9
UNSM13217, NE:Cherry	UNSM12980, NE:Garden UNSM12991, NE:Garden UNSM26316, NE:Hall UNSM20744, NE:Hall UNSM20747, NE:Hall UNSM26462, NE:Merrick UNSM13067, NE:Thomas	Z.h. pallidus Z.h. pallidus Z.h. pallidus Z.h. pallidus Z.h. pallidus Z.h. pallidus Z.h. pallidus	PAL8
UNSM17482, NE:Antelope	UNSM17495, NE:Antelope UNSM17498, NE:Antelope UNSM17499, NE:Antelope UNSM13084, NE:Dixon UNSM14008, NE:Dodge UNSM13118, NE:Holt UNSM13343, NE:Lancaster	Z.h. pallidus Z.h. pallidus Z.h. pallidus Z.h. pallidus Z.h. pallidus Z.h. pallidus Z.h. pallidus	PAL7
UNSM13119, NE:Holt	UNSM13065, NE:Thomas	Z.h. pallidus Z.h. pallidus	PAL6
UNSM17727, NE:Boyd		Z.h. pallidus	PAL5
UNSM20600, NE:Buffalo	KU109633, KS:Osage KU109634, KS:Osage	Z.h. pallidus Z.h. pallidus Z.h. pallidus	PAL4

KU153597, KS:Macon	KU153598, KS:Macon KU153784, KS:Douglas KU153707, KS:Leavenworth	Z.h. pallidus Z.h. pallidus Z.h. pallidus Z.h. pallidus	PAL3
MSB37154, NM:Otero	MSB61696, NM:Otero MSB61684, NM:Otero MSB61690, NM:Otero MSB61693, NM:Otero MSB61712, NM:Otero MSB58369, NM:Rio Arriba NK871, NM:Otero NK884, NM: Socorro DMNH8630: CO:Las Animas	Z.h. luteus Z.h. luteus Z.h. luteus Z.h. luteus Z.h. luteus Z.h. luteus Z.h. luteus Z.h. luteus Z.h. luteus Z.h. luteus	L4
DMNH8631, CO:Las Animas		Z.h. luteus	L3
DMNH8632, CO:Las Animas	DMNH8634. CO:Las Animas	Z.h. luteus Z.h. luteus	L2
NK9976, NM:Bernalillo		Z.h. luteus	L1
MSB58370, NM:Rio Arriba	MSB56980, NM:Sandoval MSB56986, NM:Sandoval MSB56987, NM:Sandoval MSB56991, NM:Sandoval MSB56993, NM:Sandoval MSB62096, NM:Sandoval MSB62103, NM:Valencia NK856, NM:Sandavol KU112665, SD:Lawrence KU109963, SD:Lawrence KU110033, SD:Bennett	Z.h. luteus Z.h. luteus Z.h. luteus Z.h. luteus Z.h. luteus Z.h. luteus Z.h. luteus Z.h. luteus Z.h. luteus Z.h. campestris Z.h. campestris Z.h. pallidus	L/PAL/C1
KU110022, SD:Bennett		Z.h. pallidus	PAL2
UNSM27388, SD:Clay	UNSM27389, SD:Clay	Z.h. pallidus Z.h. pallidus	PAL1
DMNH10638/TK86190, WY:Weston	DMNH10639/TK86191, WY:Weston KU101558, SD:Pennington KU123593, MT:Carter KU123598, MT:Carter KU123599, MT:Carter	Z.h. campestris Z.h. campestris Z.h. campestris Z.h. campestris Z.h. campestris Z.h. campestris	C10
KU112663, SD:Lawrence		Z.h. campestris	C9
KU101564, SD:Pennington		Z.h. campestris	C8
KU20839, WY:Crook		Z.h. campestris	C7
KU83559, SD:Harding		Z.h. campestris	C6
KU20844, WY:Crook		Z.h. campestris	C5

	KU123597, MT:Carter	Z.h. campestris	
DMNH9579/XM1166, CO:El Paso	DMNH9313/XM875, CO:El Paso DMNH9315/XM879, CO:El Paso DMNH10380/TK86093, CO:El Paso DMNH9565/TK86106, CO:El Paso DMNH9563/TK86107, CO:El Paso DMNH9566/TK86118, CO:El Paso DMNH9573/TK86120, CO:Douglas DMNH9572/TK86121, CO:Douglas DMNH9571/TK86122, CO:Douglas DMNH9574/TK86166, CO:El Paso DMNH10607/TK86167, CO:El Paso KU109978, SD:Custer KU123592, MT:Carter	Z.h. prebleii Z.h. campestris Z.h. campestris	C/P2
DMNH10405/TK86095, WY:Albany	DMNH10258/TK86074, WY:Laramie DMNH10270/TK86081, CO:Larimer DMNH10404/TK86094, WY:Platte DMNH10406/TK86096, WY:Albany DMNH10407/TK86097, WY:Albany DMNH9568/TK86117, CO:Larimer PIONEER9A43, CO: Larimer PIONEER9B89, CO:Larimer KU109984, SD:Custer KU109985, SD:Custer	Z.h. prebleii Z.h. prebleii Z.h. prebleii Z.h. prebleii Z.h. prebleii Z.h. prebleii Z.h. prebleii Z.h. prebleii Z.h. campestris Z.h. campestris	C/P1

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Table 2. Specimens of *Z. princeps* used as outgroups in phylogenetic analysis and specimens that have an identical mtDNA haplotype or are on the same clade as the mtDNA haplotypes of representative individuals. Only the mtDNA haplotypes of the three representative *Z. princeps* individuals were used in phylogenetic analysis. Note that some individuals previously identified as *Z. hudsonicus* have mtDNA haplotypes identical to *Z. princeps*. These individuals were presumed to be misidentified and not included in phylogenetic or population genetic analyses.

see # 21

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

the no. bases for 21 years estimate the rate of evolution

spelling! correct!

*Sister taxa on the same clade as representative individual, with strong bootstrap support. For computation simplicity, these individuals were not used in phylogenetic analysis.

21

Some of these features will be features of *Z. campestris*

Table 3 Summary statistics for mean of repeated cranial measurements for *Z.h. campestris* and *Z.h. preblei*. Using ANOVA, 3 of the cranial measurements were significantly different ($p < 0.05$) between subspecies: zygomatic breadth ($P=0.0071$), mastoidal breadth ($P=0.012$), and interorbital breadth ($p=0.022$). *Z.h. preblei* was larger for both zygomatic breadth and mastoidal breadth, while *Z.h. campestris* was larger for interorbital breadth. Using single measurements from three adult specimens of *Z.h. preblei*, Krutzsch (1954) stated that *Z.h. preblei* was "averaging smaller in most cranial measurements" compared to *Z.h. campestris*. Our results refute this claim.

whereas

Subspecies/ Measurement	Number	Mean	S.D.	Min.	Max.
<i>Z.h. campestris</i>					
Occipitonasal length	37	23.046	0.609	21.623	24.048
Condylbasal length	39	19.944	0.571	19.083	20.92
Palatal length	39	10.105	0.305	9.313	10.635
Zygomatic length	40	9.548	0.338	8.678	10.163
Zygomatic breadth	39	10.972	0.377	10.055	11.728
Mastoidal breadth	39	10.261	0.292	9.53	10.82
Braincase breadth	40	10.321	0.263	9.765	10.7
Interorbital breadth	38	4.326	0.176	3.863	4.833
Upper tooth row length	40	3.689	0.14	3.365	3.945
<i>Z.h. preblei</i>					
Occipitonasal length	37	22.941	0.445	22.065	23.933
Condylbasal length	35	19.858	0.457	18.55	20.823
Palatal length	40	10.057	0.272	9.323	10.645
Zygomatic length	40	9.454	0.254	8.82	9.993
Zygomatic breadth	37	11.193	0.31	10.52	12.113
Mastoidal breadth	38	10.4282	0.28	9.62	10.855
Braincase breadth	38	10.345	0.211	9.81	10.838
Interorbital breadth	40	4.24	0.145	3.9	4.495
Upper tooth row length	39	3.725	0.112	3.418	3.97

meaning of bolded

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Table 4. Qualitative morphological comparisons made by Krutzsch (1954). The left column lists the descriptions for *Z.h. preblei* and the right column list descriptions for *Z.h. campestris*. The center column (bold italics) lists the differences Krutzsch (1954) used to distinguish *Z.h. preblei* from *Z.h. campestris*.

<i>Z.h. preblei</i>	<i>Z.h. campestris</i>
	<i>From topotypes of Z.h. campestris, Z.h. preblei differs as follows:</i>
Size medium	Size large
Color dull	(no description)
	<i>Upper parts generally dull, averaging lighter</i>
Back from <u>near Clay color to near Tawny-olive</u> with admixture of <u>black hair</u> forming <u>poorly defined</u> dorsal band	Back from <u>near Ochaceous-Tawny to near Ochaceous-buff</u> with admixture of <u>black-tipped hair</u> forming <u>distinct</u> dorsal band
	<i>less black tipped hair</i>
Sides lighter than back from <u>near Clay color to near cinnamon-buff</u>	Sides lighter than back, from <u>near Ochaceous-buff to near yellow Ocher</u> with <u>black hair interspersed</u>
	<i>Sides duller</i>
Lateral line distinct and clear Ochaceous-Buff	Lateral line <u>usually</u> distinct, of clear Ochaceous-buff
Belly white – sometimes with <u>faint wash of clear</u> Ochaceous-Buff above	Belly white, usually with <u>moderate suffusion</u> of near Ochaceous-buff
Tail bicolored, brownish to <u>light brownish-black</u> above, grayish-white to yellowish-white below	Tail bicolored, brownish to brownish-black above, grayish-white to yellowish-white below
Ears dark, <u>narrowly</u> edged with color of sides	Ears dark, edged with <u>Ochaceous-buff</u>
Feet grayish-white above	Feet grayish-white above
	<i>Averaging smaller in most cranial measurements</i>
Incive foramia <u>relatively narrow and elongate</u>	Incive foramia <u>long and usually truncate at posterior border</u>
	<i>Incisive foramia narrower, not truncate posteriorly</i>
Auditory bullae <u>moderately</u> inflated	Auditory bullae <u>well</u> inflated
	<i>Auditory bullae smaller, less well inflated</i>
Pterygoid fossae <u>relatively</u> broad	Pterygoid fossae broad
Postpalatal notch broadly rounded	(no description)
Interorbital region relatively narrow	(no description)
	<i>Least interorbital constriction narrower</i>
Zygomatic arch <u>not widely</u> bowed	Zygomata <u>relatively wide-spread and long</u>
Frontal region well inflated	(no description)
	<i>Frontal region usually more inflated</i>
Distance from incisors to postpalatal notch relatively short	(no description)

(no description)

Large medial projection on inferior ramus of zygomatic process of maxillary

(no description)

Condylbasial length and occipitonasal length relatively great

(no description)

Mastoid region and palatal region relatively broad

(no description)

Interparietal bone usually broad

Hypotheses to explain the pattern of shared mtDNAs across the range of Z.h. preblei and Z.h. campestris

1) Range of Z.h. preblei is a recent colonization from Z.h. campestris (mtDNAs represent a northward range expansion and hybridization)

2) Z.h. preblei evolved in isolation and spread north colonizing the range of Z.h. campestris (mtDNAs represent a northward range expansion and hybridization)

Reduced gene flow has led to the pattern of reduced gene flow among the range of Z.h. preblei and Z.h. campestris.