

EXHIBIT B

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 Vigneiri et al.'s (2006) use of *post hoc* interpretation of results, speculative approach to on ecological uniqueness, and misrepresentation of facts. Our paper, titled: *Response to Vignieri et al. (2006): Should hypothesis testing or selective post-hoc interpretation of results guide the allocation of conservation effort?* (The panel cited this as if it were an unpublished report when it was peer reviewed, accepted for publication, and "in press" before the panel convened.) For the reasons detailed in Ramey et al. (2005 and 2006) we disagree with the approach of VEA, KEA, and the panel, all of which have relied on post-hoc interpretation of results in support of their conclusions.

Conclusion

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

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

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

Literature Cited

- Arbogast, B. S., J. P. Dumbacher, and S. J. Stepan (2006) Evaluation of scientific information regarding Preble's meadow jumping mouse. Sustainable Ecosystems Institute, Portland, OR.
- Baudouin, L., S. Pity, and J. M. Cornuet (2004) Analytical Bayesian approach for assigning individuals to populations. *Journal of Heredity* 95(3):217–224.
- Cegelski, C. C., L. P. Waits, and N. J. Anderson (2003) Assessing population structure and gene flow in Montana wolverines (*Gulo gulo*) using assignment-based approaches. *Molecular Ecology* 12: 2907–2918 .
- Conner, M. M., and T. M. Shenk (2003) Distinguishing *Zapus hudsonius preblei* from *Zapus princeps princeps* by using repeated cranial measurements. *Mammalogy* 84(4):1456-1463.
- Cornuet, J-M. S. Piry, G. Luikart, A. Estoup and M. Solignac (1999) New Methods Employing Multilocus Genotypes to Select or Exclude Populations as Origins of Individuals. *Genetics* 153:1989–2000.
- Crandall, K. A., O. R. P. Bininda-Emonds, G. M. Mace, and R. K. Wayne (2000) Considering evolutionary processes in conservation biology. *Trends in Ecology and Evolution* 15:290-295.
- Crandall, K. A. and J. C. Marshall (2005) An assessment of the threatened subspecific status of the Preble's meadow jumping mouse (*Zapus hudsonius preblei*) based on current molecular datasets. Report prepared by Genoma LLC - for the state of Wyoming, Office of the Attorney General.
- Jones, G. S. (1981) The systematics and biology of the genus *Zapus* (Mammalia, Rodentia, Zapodidae) unpublished Ph.D. dissertation. Indiana State University, Terre Haute.
- Krutzsch, P. H. (1954) North American jumping mice (genus *Zapus*). *University of*

Kansas Publications, Museum of Natural History 4: 349-472.

Pritchard, J. K., M. Stephens and P. Donnelly (2000) Inference of Population Structure Using Multilocus Genotype Data. *Genetics* 155: 945–959.

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

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

Ramey, R. R., J. D. Wehausen, H. P. Liu, C. W. Epps, and L. Carpenter (2006b) Is the Preble's meadow jumping mouse and evolutionarily distinct subspecies? Comments on the report by King et al. (2006). Comments submitted to the U.S. Fish and Wildlife Service on 18 May 2006.

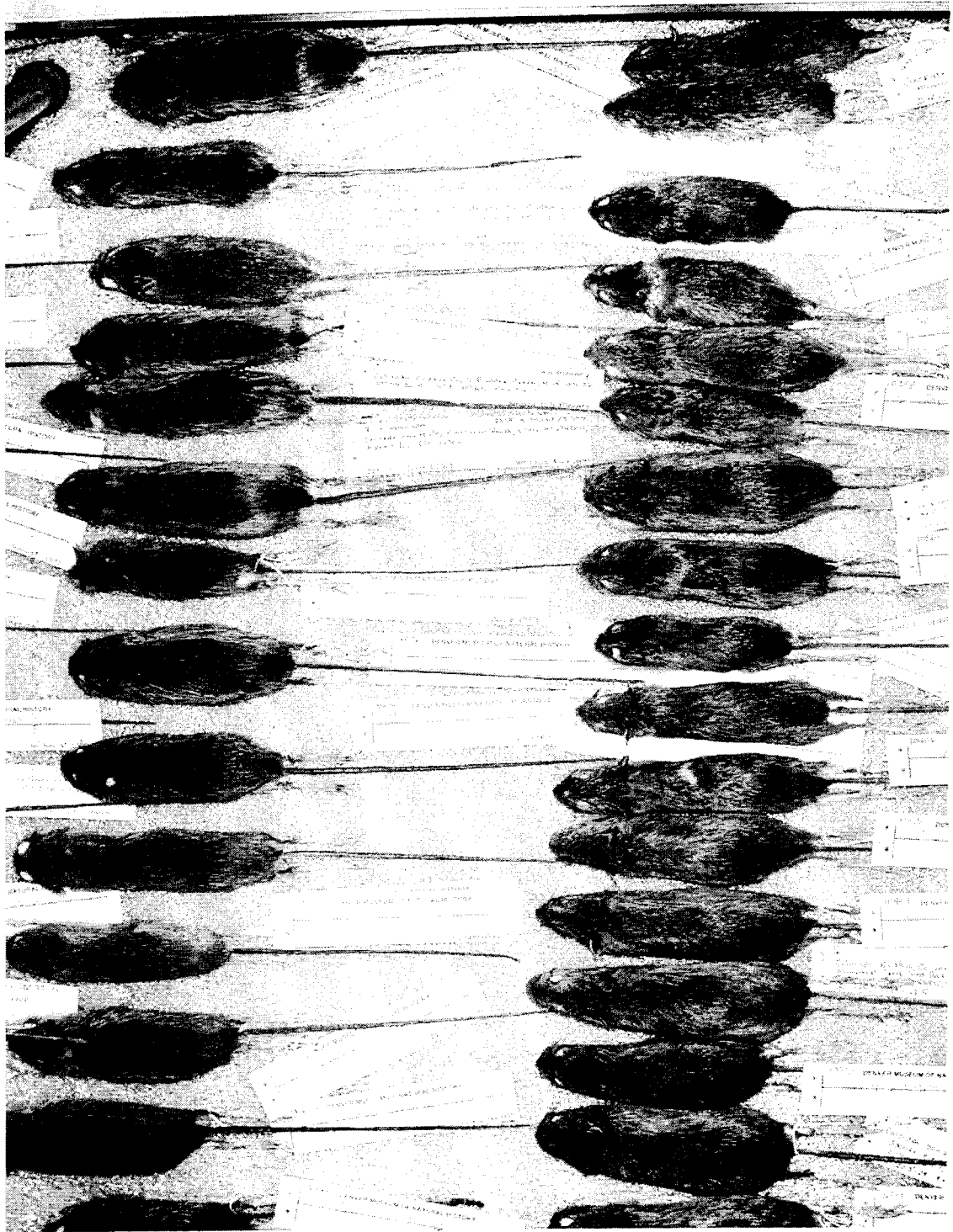
Riggs, L. A., J. M. Dempcy, and C. Orrego (1997). Evaluating distinctness and evolutionary significance of Preble's meadow jumping mouse: Phylogeography of mitochondrial DNA non-coding region variation. Final Report submitted to Colorado Division of Wildlife. 13 pp. + appendices.

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

Vignieri, S. N., E. M. Hallerman, B. J. Bergstrom, D. J. Hafner, A. P. Martin, P. Devers, P. Grobler, and, N. Hitt, (2006) Mistaken view of taxonomic validity undermines conservation of an evolutionarily distinct mouse: a response to Ramey *et al.* *Animal Conservation*. Published on-line May 2006.

Waples, R. S., and O. Gaggiotti (2006) What is a population? An empirical evaluation of some genetic methods for identifying the number of gene pools and their degree of connectivity. *Molecular Ecology* 15:1419–1439.

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



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

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

Abstract:

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

Background and conceptual approach:

"Both Preble (1899) and Krutzsch (1954) based their classifications on museum skins and skulls. The latter author relied heavily upon size and pelage coloration. Problems with this classification have become apparent. Utilizing Krutzsch's (1954) characters, Davis and Ernst (1971) were unable to determine whether a Minnesota population was *Z. hudsonius* or *Z. princeps*. A large number of specimens of *Z. hudsonius* collected in Tompkins 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 is 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 NC

***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.