

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

R. R. Ramey II¹, J. D. Wehausen², H.-P. Liu³, C. W. Epps⁴ & L. M. Carpenter⁵

¹ Department of Conservation Biology, Denver Zoological Foundation, Denver, CO, USA

² University of California, White Mountain Research Station, Bishop, CA, USA

³ Department of Biological Sciences, University of Denver, Denver, CO, USA

⁴ Department of ESPM, University of California, Berkeley, CA, USA

⁵ Department of Zoology, Denver Museum of Nature & Science, Denver, CO, USA

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Correspondence

Rob Roy Ramey II, Department of Conservation Biology, Denver Zoological Foundation, 2300 Steele St., Denver, CO 80205, USA.
Tel/Fax: 303 258 9535. Email: ramey@spot.colorado.edu

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

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

Morphometric analyses

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

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

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

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

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

Ecological analysis

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

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

Molecular genetic analyses

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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