

2 June 2005

Dr. Seth L. Willey
Ecological Services
ESA Listing & Delisting
US FWS Region 6,
Denver, CO 80225

Dear Dr. Willey:

This is our response to your request for a review of “Testing the uniqueness of *Z. h. intermedius* relative to *Z. h. campestris*” by Ramsey II, Liu, Carpenter, and Epps. This report was prepared by Robert Baker and Peter Larsen. Both have worked extensively with molecular data and associated museum voucher specimens to better understand the uniqueness of populations, species, and phylogeographic variation. Robert is a faculty member here at Texas Tech University and Peter Larsen is a graduate student who is working with Robert.

First, we should state that we are strong supporters of the Endangered Species Act. We do feel, however, that taxonomic units that are protected should be well defined either morphologically or genetically or both. With the limited resources that we have for conservation and protection of endangered species, it is a mistake to use them to protect something that is not uniquely divergent from all other populations.

In an overview, we believe that *Zapus hudsonius preblei* is not sufficiently differentiated from other populations of *Zapus hudsonius* to justify being recognized as a distinct subspecies. We think the support for the above conclusion is present in both the morphological analyses and in the molecular analyses.

Answers to specific questions are as follows: the numbers preceding each response correspond to the numbers on your letter dated 25 Mar 2005.

1. Appropriate methodologies and markers were used to a point. It would have been best of course to have had all 3 billion basepairs of the nuclear genome sequenced and compared for all specimens. In the absence of that, some information from the nuclear genome would've been helpful, like AFLP analysis (Vos, P. et al. 1995. AFLP: a new technique for DNA fingerprinting. *Nucleic Acids Res.* 23, 4407-4414). Realistically, however, if there's no more variation present in the mitochondrial D-loop than was present in *preblei* and the compared populations, it is very unlikely that variation in the nuclear genome is going to be more informative than sequence values in the D-loop. The problem with using AFLP is while there are some studies published; the wealth of data that is needed to make it a well understood and documented data set is just not there yet. The method is promising, but from a legal standpoint, we don't understand the biological variation across species.

2. Yes.

3. Yes. The fact that derived haplotypes are shared between the two populations is most parsimoniously explained as either a recent shared common ancestry or recent gene flow between the populations.
4. We suppose there are always alternative interpretations of the data, but it is difficult for us to formulate a different explanation for the data that we feel has reasonable probability of being valid.
5. A study of the nuclear genome as mentioned above. We suspect that it would be highly redundant to the information already presented.
6. No. We think synonymizing *intermedius*, *preblei* and *campestris* will be more inline with the systematic changes that need to be applied across most mammalian groups present in North America. If we used the level of divergence in *preblei* as a standard, then there would literally be thousands of more subspecies within the rodents of North America. In our opinion, this would be less than desirable as there are already more subspecies recognized than can be justified by the data.

Please contact us if you desire additional information or clarification.

Robert Baker and Peter Larsen