



Smithsonian Institution

Genetics Program
3001 Connecticut Avenue NW
Washington DC 20008-22598
202-633-4198

Friday, June 3, 2005

Dr. Seth Willey
U.S. Fish and Wildlife Service
Ecological Services
P.O. 25486
Denver Federal Center
Denver, Colorado 80225

Dear Seth:

Thank you for allowing me to participate in the review process of this important and complex issue. I have finally completed the review of the report "Testing the uniqueness of *Z. h intermedius* relative to *Z. h. campestris*". I have considered all of the materials that you provided in order to make my evaluation.

Attached please find my comments and an updated version of my CV.

I hope that you find them useful and please let me know if have any questions or if you need any additional information.

Sincerely,

Dr. Jesus E. Maldonado
Genetics Program
National Zoological Park
Smithsonian Institution
3001 Connecticut Ave. NW
Washington DC, 20008
maldonadoj@nzp.si.edu
202-633-4198

1) Analyze the techniques used in morphometric, the population and the phylogenetic evaluation, and the maximum likelihood of recent gene flow analysis (MDIV) of subspecies of *Zapus hudsonius*. Were the appropriate methodologies and markers used? Do you support the author's mtDNA standard for delineating valid subspecies (greater variation among subspecies than within subspecies).

In order to study “*morphometric distinguishability*” the authors used a multivariate statistical analysis “linear discriminant analysis (LDA)” and found poor discriminating ability between *intermedius* and the pooled *preblei/campestris* sample. The statistical techniques used are appropriate however; since the authors made the *a priori* decision to group *preblei* and *campestris* then the test is limited to a two-group comparison. The analysis should be done using on all three samples separately. Furthermore, I suggest that if groupings have to be decided *a priori* that different combination of groupings (populations) besides subspecies be tested.

The methods for population genetic and phylogenetic analysis appear adequate. The Tajima's test of neutrality suggests that their sample of mtDNA is selectively neutral.

The AMOVA (analysis of molecular variance) is commonly used to deduce the significance of geographic divisions among local and regional population groupings (Excoffier *et al.* 1992). However, AMOVA is supposed to be used as a hierarchical approach analogous to analysis of variance (ANOVA) in which haplotype distances compared at various hierarchical levels are used as *F*-statistic analogs, designated as F_{ST} statistics. Again in this test, the authors only explore the subspecific grouping and do not perform any other population level comparisons. By doing this, the authors are not really attempting to identify what constitutes the different genetically homogeneous groups in the sample.

MDIV is a program that has been used to simultaneously estimate divergence times and migration rates between two populations under the infinite sites model and under a finite sites model (HKY). The program can be used to test if there is evidence for migration between two populations or evidence for shared recent common ancestry. You can also get maximum likelihood estimates of the demographic parameters. The program assumes that there is no recombination and is appropriate for mtDNA data such as the one used in this study.

2) Based on the data presented in the report do you support the author's conclusions regarding synonymizing *Z. h. preblei*, *Z. h. campestris*, and *Z. h. intermedius*?

While I support the taxonomic interpretations of the authors based on the data they presented, I would strongly suggest that they consider analyzing microsatellite data to corroborate their findings (See reply to question 5 below)

3) Based on the MDIV data presented in the report, do you view *Z. h. preblei* and *Z. h. campestris* as a single connected population?

The MDIV analysis of the mtDNA data strongly suggests that there is gene flow between *Z. h. preblei* and *Z. h. campestris*. All of the haplotypes present in *Z. h. preblei* are also found in populations considered to be *Z. h. campestris*.

4) Are there possible alternative interpretations of the data? How likely are these possibilities?

This study uses museum specimens for most of the sampling. While in the report, little mention was done as to the precautions that are commonly taken when dealing with ancient DNA, I suspect that the authors are well aware of the problems with contamination that are magnified when dealing with ancient DNA samples. Because this is a report and not a publication, I also assume that the authors will carefully document the number of replicates and confirmation in their publication. I should point out that all of the *Z. h. preblei* haplotypes were also found in *Z. h. campestris* samples (although in very low frequencies). Although the possibilities that these haplotypes are all the product of contamination is low and very unlikely if the investigators have a Laboratory facility that is equipped to deal with problems with ancient DNA extraction and processing.

5) What additional analysis, if any, is needed to verify the study's assertions and why?

I would strongly recommend that they add a microsatellites to their study. This is an important and worthwhile conservation genetics issue to pursue and will perhaps set precedence as to how we determine the management of an endangered species with a controversial taxonomy. We need at least this additional evidence and while I agree that microsatellite development is expensive and time consuming, I am aware that there are microsatellite primers already published for *Zapus* and that they may be useful to look at finer scale patterns of gene flow and population structure. (See S. N. Vignieri. 2003. Isolation and characterization of eight highly variable microsatellite markers in the Pacific jumping mouse (*Zapus trinotatus*). Molecular Ecology Notes 3:638-640.)

In the ancient DNA literature, the need for confirmation of sequences in other labs to rule out contamination is not uncommon and the authors (if they have not already done so) may consider testing the few individuals that appear in the *Z. h. campestris* that appear in the *Z. h. preblei* clade in an independent lab.

Of course it would be ideal to have more data than just genetics and morphology and in particular more detailed ecological, physiological, behavioral, and geographic and habitat data.

6) Has this new information changed your conclusions regarding the synonymizing of *Z. h. preblei* and *Z. h. campestris* as proposed in Ramey et al. 2004a? Please elaborate as necessary.

I think that the addition of the *Z. h. intermedius* samples and the additional morphometric and genetic analysis has made this a stronger and more interesting study. However, I feel that evidence from additional genetic markers (i.e. microsatellite data) is needed to confirm their conclusions. Although these markers are not likely to provide a signature of substantial evolutionary subdivision they might allow one to detect finer-scale population structure than MtDNA.