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## **Comments on Preble's Meadow Jumping Mouse Delisting Proposal**

(Listed in order received. Dates are those on comments.)

### *Reopened Comment Period*

29. 2/6/06 Mark Lusch, Cheyenne, WY
30. 2/18/06 Tom and Mary Ann Cunningham, Green Mountain Falls, CO
31. 2/18/06 Bruce Roberts, Monument CO
32. 2/20/06 Mitchell Baldwin
33. 2/21/06 Oliver A. Richardson
34. 2/22/06 Robert B. Hoff, Colorado Springs, CO (see 1 and 6 above)
35. 2/22/06 Colleen Miller
36. 2/21/06 Linda Samelson, Colorado Springs, CO
37. 2/26/06 Jennifer K. Frey, Frey Biological Research, Radium Springs, NM
38. 2/25/06 Nick Ordon, Falcon, CO
39. 3/1/06 Unsigned, Colorado Springs, CO
40. 3/9/06 Leslie Barstow, Golden, CO
41. 3/9/06 Peter Bray, Portland, OR
42. 3/9/06 Donna Miller, Golden, CO
43. 3/13/06 Daryl E. Mergen, Colorado Springs, CO
44. 3/31/06 Ronald W. Opsahl, Staff Attorney, Mountain States Legal Foundation, Lakewood, CO (See 7 above)
45. 3/31/06 C. J. Rapp, Littleton, CO
46. 4/4/06 Ken Faux, Greenwood Village, CO (see 18 above)
47. 3/31/06 Ken Hamilton, Executive Vice President, Wyoming Farm Bureau Federation, Laramie, WY

48. 3/31/06 Renee C. Taylor, Environmental Coordinator, True Ranches, LLC, Casper, WY (see 12 above)
49. 4/13/06 Robert E. Arlen, Science Faculty, University of Phoenix, Casper, WY
50. 4/17/06 Sandra A. Eddy, Aurora, CO
51. 4/18/06 Kent Holsinger, Hale Friesen, LLP, Denver, CO. On behalf of Colorado Water Conservation and Development
52. 4/28/06 Robert A. Schorr, Zoologist, Colorado Natural Heritage Program, Colorado State University, Fort Collins, CO
53. 4/28/06 Eric Hallerman, Professor, Department of Fisheries and Wildlife Sciences, Virginia Polytechnic Institute and State University, Blacksburg, VA
54. 5/11/06 Sacha Vignieri, Center for Study of Evolution, University of Sussex, Brighton, UK
55. 5/15/06 Jonathan Dowling, Assistant Vice President, Wyoming Contractors Association, Cheyenne, WY
56. 5/1/06 Sallie Clark, Chair, Board of County Commissioners of El Paso County, Colorado Springs, CO
57. 5/16/06 Sylvia M. Fallon, Conservation Genetics Fellow, Natural Resources Defense Council
58. 5/17/06 Don Britton, Manager, Wheatland Irrigation District, Wheatland, WY
59. 5/17/06 Dale Moore
60. 5/18/06 Carron Meaney (Meaney and Co.; Research Associate, DMNS; Curator Adjoint, University of Colorado Museum), Thomas Ryon (Wildlife Biologist and Certified Ecologist), Mark Bakeman (President, Ensign Technical Services Inc.) and Anne Ruggles (Bear Canyon Consulting), CO
61. 5/18/06 Tina Comerford, Wheaton, IL
62. 5/17/06 Niel A. "Mick" McMurry, Shareholder, Sybille Ranch LLC, Cheyenne, WY
63. 5/18/06 Rob Roy Ramey, II, Nederland, CO
64. 5/18/06 Jim Magagna, Executive Vice President, Wyoming Stock Growers Association, Cheyenne, WY

65. 5/18/06 Erin Robertson, Staff Biologist, Center for Native Ecosystems, Denver CO. On behalf of: Jeremy Nichols, Conservation Director, Biodiversity Conservation Alliance, Denver, CO and Nicole Rosario, Conservation Director, Forest Guardians, Santa Fe, NM (See 23 above)
66. 5/18/06 Patrick J. Crank, Attorney General, State of Wyoming, Cheyenne, WY
67. 5/19/06 Cheryl Matthews, Director, Douglas County Division of Open Space and Natural Resources, Castle Rock, CO (See 19 above)



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05/18/2006 04:27 PM

To FW6\_PMJM@FWS.gov  
cc  
bcc

Subject Wyoming Comments / Preble's Mouse

\*\* High Priority \*\*  
\*\* Reply Requested When Convenient \*\*

Field Supervisor  
Colorado Field Office  
Ecological Services  
P.O. Box 25486  
Denver, WO 80225

To Whom it May Concern  
Please see the attachments from the Wyoming Attorney General's Office  
re: Additional Comments on Proposed Delisting of the Preble's Meadow  
Jumping Mouse.

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Sue Petrie vcf



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May 18, 2006

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Re: Additional Comments on Proposed Delisting of the Preble's Meadow Jumping Mouse  
(*Zapus hudsonius preblei*)

To whom it may concern:

Thank you again for the opportunity to provide comments on the Proposed Delisting of the Preble's Meadow Jumping Mouse (Preble's Mouse). We appreciate the opportunity to be a part of the ongoing process, and hope that the U.S. Fish and Wildlife Service (Service) looks very carefully at the additional materials submitted with these comments. These comments are submitted as a supplement to the previous comments submitted by the State of Wyoming. We have expressed our deep concern over the transparent bias of Region 6 personnel in this review process and we continue to hold great reservations because of the manner in which this review has been conducted. It is our belief that the only possible way to obtain a fair review of the genetic data is for the Service to remove decision making authority from Region 6 and select qualified, non-bias individuals to complete this aspect of the delisting petition.

A week before the end of the 12 month rule review, Region 6 disclosed an unpublished study by the U. S. Geological Survey that was prepared at the specific request of Region 6. The study criticized the Service's basis for delisting the Preble's Mouse. The timing and content of the report was indicative of the predisposition of Region 6. To address the new report, and as part of our commitment to have the Service consider objective and factually driven science, we are attaching a professional report prepared by Dr. Keith Crandall and Dr. Jonathan Marshall, of

Genoma LLC, entitled, "*An Assessment of the threatened subspecies status of the Preble's meadow jumping mouse (Zapus hudsonius) based on current molecular data sets*" (Genoma Report). The Genoma Report represents the most accurate and comprehensive analysis of the combined genetic data used in the two existing Preble's Mouse genetic studies and addresses the precise issue the Service has submitted to the scientific review panel (the Panel). Thus, the Genoma Report represents the best available science in accessing if *Z. h. preblei* was properly categorized as a subspecies. We hope that the Service and the Panel find the report helpful in differentiating the two studies. We look forward to an active, participatory role with the Panel and a final resolution in this matter. With that in mind, please find below our supplemental comments concerning issues that have arisen since the Service extended the 12-month review of the proposed rule.

1. PROCEDURAL HISTORY:

On May 13, 1998 the Preble's Mouse was listed as threatened. 63 Fed. Reg. 26517 (May 13, 1998). On December 17, 2003, the State of Wyoming filed a petition to delist the Preble's Mouse. The Service issued a 90-day finding on March 31, 2004 finding a 12- Month review was warranted. 69 Fed. Reg. 16944 (March 31, 2004). On February 2, 2005 the Service proposed to delist Preble's Mouse from the list of threatened species. 70 Fed. Reg. 5404 (Feb. 2, 2005). The Service reported that delisting was based, to a large extent, on a report by Ramey, *et al.* (2005) (Ramey Study) which concluded that Preble's Mouse should not be considered a subspecies. 70 Fed. Reg. 8557.

On February 17, 2006 the Service extended the 12-month period in which to make a decision on the proposed delisting for an additional six months and re-opened the comment period for the proposed delisting of the Preble's Mouse. 71 Fed. Reg. 8556 (Feb. 17, 2006). The comment period was reopened because the Service received a "recently completed unpublished study [that] substantially disagrees with the determination contained in the proposed rule that Preble's is not a distinct subspecies." *Id.* This new study, by Tim L. King, *et al.* (King Study), was prepared by the U. S. Geological Survey (USGS) at the specific request of Region 6. Based on prior work performed by Dr. King, the result of the King Study was a foregone conclusion. Region 6 ordered the King Study to negate the Ramey Study and provide some basis for reversing its own 12-Month finding to delist the Preble's Mouse.

Initially Region 6 proposed appointing two expert panels to advise it on both the taxonomic issues and threats to the species and potential extinction risks. 71 Fed. Reg. 8556-57. The Service later cancelled the two panels, and instead issued a Request For Quotation and Statement of Work to retain a private contractor to conduct the scientific panel review. (Request For Quotation, April 3, 2006). The consultant-contractor will select the Panel that will evaluate and explain the Ramey and King studies and submit its findings in a report to the Service.

(Statement of Work, at p. 1-2). More specifics concerning how the Panel will operate, what questions it will be presented, and who will be selected to the panel have yet to be determined.

2. GENOMA, LLC - REPORT ON SUBSPECIES STATUS OF PREBLE'S MOUSE:

The State of Wyoming retained the services of Genoma, LLC, and Drs. Keith Crandall and Jonathon Marshall, to perform a review of the combined genetic data used in the Ramey Study and the King Study to obtain the most comprehensive analysis possible of the existing genetic data and to evaluate and comment on the inconsistencies between the two reports<sup>1</sup>.

To reconcile the Ramey Study and the King Study, Dr. Crandall combined the data sets from both studies to determine whether the Preble's Mouse is a valid subspecies of *Z. hudsonius*. The Genoma Report combined the microsatellite data from the Ramey and King Studies. It ran the program STRUCTURE which organizes individuals into clusters or populations. Using this program, it was determined that migration rates between *Z. h. Preblei*, *Z. h. campestris*, and *Z. h. intermedius* are comparatively high in relation to other rodents. These results, using the most recent and differentiating genetic splitting techniques, directly rebut the sub-specific designation based on microsatellite data as proposed by the King Study. That is, even if the Service chooses the most discrete genetic differentiation to split subspecies based on extremely minor, recent genetic fluxuations, *Z. h. Preblei*, is still synonymous to *Z. h. campestris*, and *Z. h. intermedius*.

Using the more conservative, historic assessments, and combining the data to estimate CR and CytB phylogenetic relationships demonstrated a well supported monophyletic group for *Z. hudsonius*, and no support for exclusive clustering of any of the *Z. hudsonius* subspecies. (Genoma Report, at 20). The Genoma report also concluded that the combined mtDNA and morphometric data indicate the taxon *Z. h. preblei* is simply not a valid taxonomic unit. Results from the report identify significant concerns in classifying any of the *Z. hudsonius* as subspecies. The findings of the Genoma Report are consistent with the conclusions in the Ramey Study and explain how the King Study did not use adequate sampling, failed to consider any morphologic data, and that King's findings are otherwise incongruous with the overall genetic data, as well as the generally accepted genetic threshold for delineating subspecies.

3. SCIENTIFIC REVIEW PANEL:

On April 3, 2006 the Service issued a Statement of Work for a scientific review panel to "analyze, assess, and weight the reasons why the data, finding, and conclusions of King *et al.*

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<sup>1</sup> The report, "An Assessment of the threatened subspecies status of the Preble's meadow jumping mouse (*Zapus hudsonius*) based on current molecular data sets" (2006), is attached as Exhibit 1 to the comments.

differ from the data, finding, and conclusions of Ramey *et al.*” (Req. For Quotation, Statement of Work, at 1-2, April 3, 2006). The Genoma Report should go a long way in resolving this issue. The findings of the Panel will obviously be subject to how the Panel is conducted, what information is presented to the Panel, and the selection of panelists. To assure that all points of view are represented and reviewed by the Panel, Wyoming strongly encourages the Service to allow active participation by the respective parties to the petition to delist, including Wyoming, in the panel review process. It is imperative that panel members be provided all the necessary data well in advance so each member can be thoroughly versed in all the issues and have a comprehensive understanding the scientific materials. Without adequate preparation the panel will not be able to develop complete, well reasoned responses or cogently discuss the issues.

To ensure that the panel conducts a “balanced, independent, and objective” review of the issue, the State of Wyoming must be able to actively participate in the Panel. The State desires to submit written materials to the Panel and have Dr. Ramey address the various issues raised as a result of the conflicting studies and critical reviews. Wyoming would also like the opportunity to direct questions to Dr. King concerning his work. The State believes it is essential that Dr. Crandall present his findings to the Panel and be available for questioning and discussion concerning the Genoma Report that is being submitted with these comments. In the Statement of Work, the Service delegates to the contractor the responsibility of who may participate in panel. (Req. For Quotation, Statement of Work, at 2, April 3, 2006). Such delegation is not appropriate, and Wyoming requests that the Service clarify that the parties may be present during deliberations.

The Service must make specific findings regarding DAT data and explain why there is such a variance between what was known to exist in 1998 and what exists today. Either the information relied upon to list Preble's as threatened was incorrect, or the subspecies has been successfully re-established throughout its entire original range and beyond. This issue is not whether the mouse is present. Rather, the issue is whether the data used in the Service's 1998 listing decision was erroneous or the Preble's Mouse was poorly trapped prior to 1998.

5. SCOPE OF REVIEW:

Rather than address the two issues raised in the Petition to Delist, namely whether the Preble's Mouse was incorrectly designated as a subspecies and, if not, are there sufficient numbers to warrant delisting, the Service has consistently chosen to side step these issues and expand its review to incorporate other *Z. hudsonius* subspecies. Using this strategy, Region 6 is attempting to direct the discussion back to protecting Preble's as a distinct population within whatever classification it falls into. This extra territorial inquiry simply muddies the issues and further accentuates the bias of Region 6 towards protecting their own special interests. In our May 2005 comments, Wyoming pointed out that examining the status of *Z. h. campestris* goes far beyond the

four corners of the petition to delist the Preble's Mouse. The Genoma Report further corroborates this position since *Z. h. campestris* cannot be distinguished from *Z. h. intermedius*. *Z. h. intermedium* is dispersed over at least 10 states. Wyoming submits that when considered in light of neighboring populations which the Ramey and Crandall Studies report as being synonymous with Preble's, the petition to delist is even more substantiated.

If the Service finds, based on the current population counts, that the Preble's Mouse was miscounted and based on those counts, it is not threatened with extinction, the exercise of reviewing other subspecies is moot.

6. RESPONSES BY RAMEY TO THE KING STUDY AND OTHER CRITICAL REVIEWS:

The unpublished King Report was not completed until January 27, 2006, a week prior to end of the 12-Month review deadline. The raw data was not made available until mid March 2006. As a consequence, Dr. Ramey and his associates have not had sufficient time to prepare a written response to the King Study. Dr. Ramey, at the request of Region 6, had begun preparing a written response which will be presented to the Panel. The State of Wyoming requests that the written response be incorporated into the record and considered by the Service.

Immediately after receiving the King Study, Region 6 distributed the study and solicited, from self selected peers, critical reviews that compared the Ramey and King Studies. Because of the severe time constraints, Dr. Ramey and his associates have not had an opportunity to address all of the reviews received by the Service. Attached to these Comments is one responsive paper submitted for publication by Dr. Ramey and others which respond to a critical review authored by Sacha N. Vignieri, *et al.* (2006). Dr. Ramey will submit additional responses to the Panel and provide testimony addressing the issues raised in the reviews. Again, because of the constricted time restraints imposed by the last minute submission of the King Study, it is requested that the Service allow for supplementation of the record specifically for Dr. Ramey's anticipated written responses and testimony before the Panel.

7. CONCLUSION:

Dr. Crandall, who is highly regarded by his peers within his field of study, is unequivocal in his conclusions. Based upon the best data currently available, the Preble's Mouse was incorrectly classified as a sub-species, and at a minimum, *Z. h. Preblei*, *Z. h. campestris*, and *Z. h. intermedius* are genetically the same sub-species. Morphometrically, the three subspecies are indistinguishable. Thus, not only is the Preble's Mouse more abundant in Wyoming and Colorado in more hydrological units than at any other time, it is currently thriving in no less than 14 states.

Wyoming Comments / Preble's Mouse  
U. S. Fish & Wildlife Service, Region 6  
May 18, 2006  
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We respectfully submit that for the Service needs to establish a workable threshold from which genetically based taxonomic classifications can be derived. Without creating a compass to guide subspecies classification, all the interested parties, as well as the Service, are left to the vagaries and whims of ideologically divergent geneticists and theorists who are more committed to prioritizing their respective points of view than establishing sound policies based on the best available science. As the final arbiter, the Service needs to articulate and promulgate universal policies regarding the methodology for classifying and reviewing subspecies designations under the ESA. The Service would be doing a great service to all if a rationale, scientific protocol could be established to facilitate the process which would allow for better utilization of our time and resources in protecting those species that are truly endangered.

Dated May 18, 2006

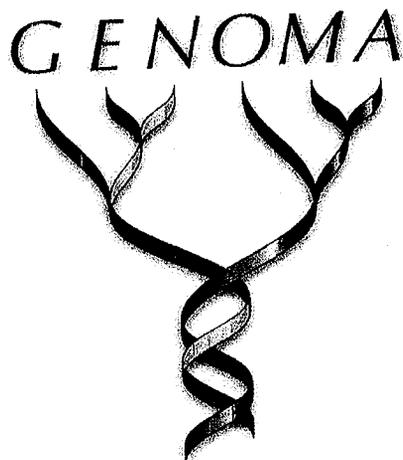
A handwritten signature in black ink, appearing to read 'Patrick J. Crank', written over a horizontal line.

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Enclosures

## REPORT

Prepared by Genoma LLC – Keith A. Crandall, Ph.D.<sup>1</sup> and  
Jonathon C. Marshall, Ph.D.

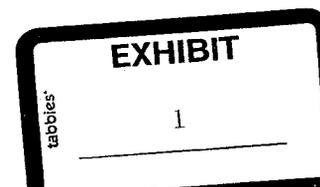


For the State of Wyoming, Office of the Attorney General

*An assessment of the threatened subspecific status of the  
Preble’s meadow jumping mouse (*Zapus hudsonius  
preblei*) based on current molecular data sets*

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### I Introduction



Preble’s meadow jumping mouse (*Zapus hudsonius preblei*) is one of twelve proposed subspecies within the *Zapus hudsonius* (meadow jumping mouse) species complex. *Zapus hudsonius* is found throughout North America ranging from West to East coast and as far north as Alaska and as far south as central New Mexico, Mississippi, and Alabama (see distribution map from Ramey et al. 2005, Figure 1). The distribution of the Preble’s meadow jumping mouse (PMJM) does not overlap with other meadow jumping mouse subspecies and corresponds to the Front Range corridor running from Colorado Springs, Colorado to Cheyenne, Wyoming (Ramey et al. 2005, Figure 1).

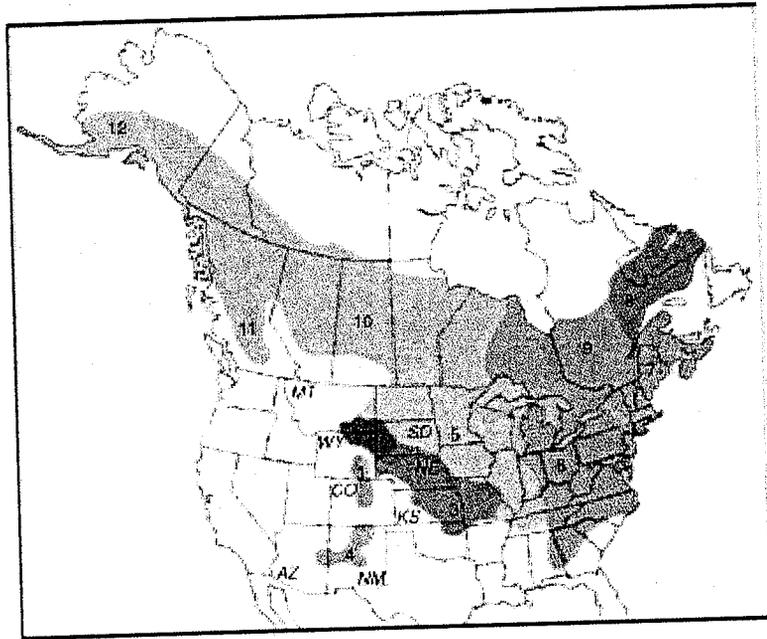


Fig. 1. Map of North America showing distribution and subspecies of *Zapus hudsonius* (Knutzsch, 1954; Hafner et al., 1981): (1) *Z. h. preblei*, (2) *Z. h. campestris*, (3) *Z. h. pallidus*, (4) *Z. h. luteus*, (5) *Z. h. intermedius*, (6) *Z. h. americanus*, (7) *Z. h. acadicus*, (8) *Z. h. luteus*, (9) *Z. h. canadensis*, (10) *Z. h. hudsonius*, (11) *Z. h. tenellus* and (12) *Z. h. ulascensis*.

On May 13, 1998 the PMJM was designated as a threatened subspecies by the U.S. Fish and Wildlife service (<http://mountain-prairie.fws.gov/preble/>). However, a recent study has called into question the appropriateness of such a designation (Ramey et al. 2005). The Ramey et al. (2005) study reanalyzed and expanded a previous morphological study (Knutzsch 1954) used in the listing process but found: 1) that Knutzsch's (1954) conclusions were not supported, and 2) there was no reliable multivariate morphometric discrimination between PMJM and other nearby *Z. hudsonius* subspecies. Additionally, Ramey et al. (2005) was unable to find significant underlying genetic differentiation between PMJM and the other subspecies using microsatellite and

mitochondrial DNA sequence data. Due primarily to the findings of Ramey et al. in February 2005 the U.S.F.W. service issued a 12-Month Finding on a petition to delist the PMJM as a threatened subspecies under the U.S. Endangered Species Act. In early 2006 a new study was released (King et al. 2006) that questioned the conclusions of Ramey et al. 2005 and called for a continuation of the threatened subspecies status for the PMJM. In their study, King et al. also analyzed microsatellite and mitochondrial DNA sequences but approached their study from a drastically different sampling scheme as compared to the Ramey et al. 2005 study. The Ramey et al. study had widespread and fairly dense sampling but few individuals were taken from each locality, on the other end of the spectrum, the King et al. study sampled few localities but large numbers of individuals from each locality.

In this study, we investigate the seemingly different conclusions of these two studies and consider them in light of the sampling schemes employed. We also combine the data sets where possible and extend the analytical approaches used to determine, according to the current data, if the PMJM represents a distinct subspecies within the *Z. hudsonius* species.

## II Ramey et al. 2005

### A. Microsatellite Data

We reanalyzed the Ramey et al. 2005 microsatellite data using all six microsatellite loci from five *Zapus hudsonius* subspecies, *Zapus hudsonius preblei*, *Zapus hudsonius campestris*, *Zapus hudsonius intermedius*, *Zapus hudsonius pallidus*, and *Zapus hudsonius luteus*. Figure 2 shows the localities of the samples taken in both the Ramey et al. and King et al. studies. Table 1 also provides the state and county names for each locality, the numbers of samples taken, and GPS coordinates for a central locality within the county. We converted the Ramey et al. microsatellite data into a format (Appendix 1) appropriate to run on the computer program STRUCTURE (Pritchard et al. 2000). STRUCTURE organizes individuals into clusters or populations that minimize Hardy-

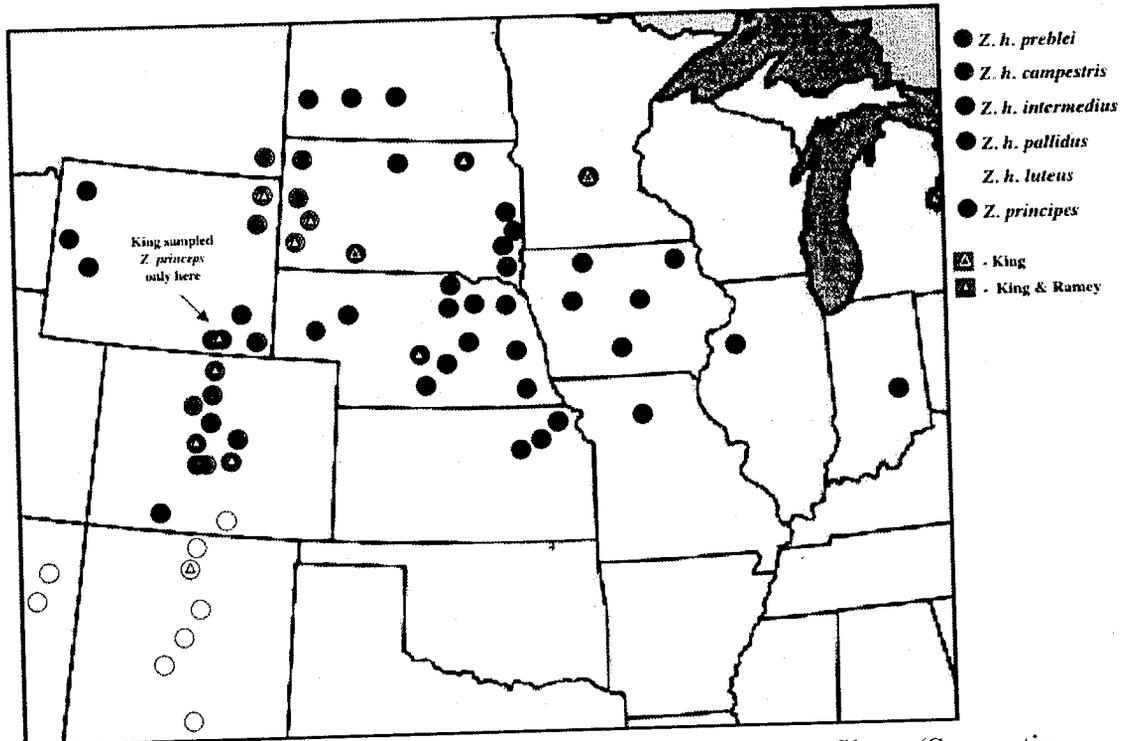
Weinberg and Linkage disequilibria. In this way researchers let the data determine the population boundaries rather than assigning individuals to populations based solely on geographic location. Our strategy was to allow for an ancestral admixture model in selecting the optimal number of population clusters (K) for the entire data set regardless of *a priori* subspecies determination. We selected an optimal K by adhering to the following suggestions from the STRUCTURE help files

“There are a couple of informal pointers which might be helpful in selecting K. The first is that it's often the situation that  $Pr(K)$  is very small for K less than the appropriate value (effectively zero), and then more-or-less plateaus for larger K ... In this sort of situation where several values of K give similar estimates of  $\log Pr(X|K)$ , it seems that the smallest of these is often “correct”. It is a bit difficult to provide a firm rule for what we mean by a “more-or-less plateaus”. I think that a sensible way to think about this is in terms of model choice. That is, we may not always be able to know the TRUE value of K, but we should aim for the smallest value of K that captures the major structure in the data. ... A corollary of this is that when there is no population structure, you will typically see that the proportion of the sample assigned to each population is roughly symmetric ( $\sim 1/K$  in each population), and most individuals will be fairly admixed. If some individuals are strongly assigned to one population or another, and if the proportions assigned to each group are asymmetric, then this is a strong indication that you have real population structure. ... In summary, you should be skeptical about population structure inferred on the basis of small differences in K if (1) there is no clear biological interpretation for the assignments, and (2) the assignments are roughly symmetric to all populations and no individuals are strongly assigned.”

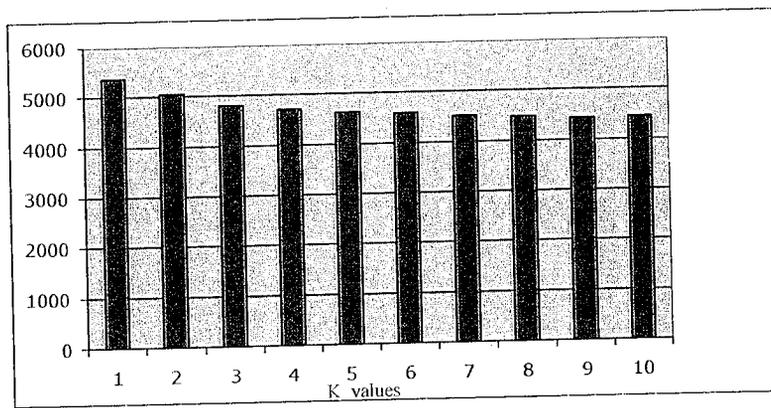
After an optimal K was selected, we then looked for evidence of admixture between clusters by identifying individuals that have similar assignment probabilities (inferred ancestry) to more than one cluster or no assignment probability greater than 0.80 to any cluster. We also looked for evidence of admixture between subspecies by identifying individuals from a subspecies that were assigned to clusters with individuals predominately from other subspecies. Like Ramey et al. 2005 and King et al. 2006, we

used a burn-in of 15,000 followed by 100,000 replicates and tested  $K = 1$  through  $K = 10$ . We performed these analyses 10 separate times in order to adequately search the likelihood space.

**Figure 2.** Distribution of all localities used in this study. Colored dots with no symbol indicate localities that were sampled only by Ramey et al. Color dots and symbols are explained in key below.



Extended results for all runs are given in support files (Supporting Documents/Data & Result Files/ Ramey MS Structure Results). Table 2 shows a summary of the likelihood scores for each of the 10 runs and the average likelihood scores for all runs at each  $K$ . A visual representation of average likelihood scores reveals a leveling-off of scores after  $K = 3$  (Figure 3). We also noted possible leveling-off points at  $K = 5$  and  $K = 9$ . In order to select a preferred  $K$  value we compared assignment probabilities for best score values at each alternative (see Supporting Documents/Data & Result Files/ Ramey MS Structure Results/Ramey Structure  $K = 3/5/9$ ) and determined that  $K = 3$  not only had the least admixed population assignments but also was the most concordant with “a clear biological interpretation for the assignments” or sets of subspecies designations. This result was also similar to the  $\Delta K$  ad hoc statistic result of the King et al. 2006 study discussed below. Unfortunately a statistical test for selecting  $K$  is not available in STRUCTURE. Table 3 shows the inferred ancestry for each sample



1g Mouse

**Figure 3.** A visual summary average likelihood scores for each K value estimated in STRUCTURE based on the Ramey et al. data, Purple bars represent absolute values of these scores, where the lower the bar the better the score. The optimal K value is the lowest value of K with a 'good' score or one that divides the individuals into populations that explain most of the variation.

for the best score at K=3. Population boundaries between clusters appeared to be semi-permeable as a number of individuals (13% of total) showed assignment probabilities < 0.80. Additionally a number of individuals (11% of total) had their highest probability of assignment to clusters of non-subspecific individuals (cluster 1 = *Z. h. preblei*, cluster 2 = *Z. h. pallidus* + *Z. h. luteus*, and cluster 3 = *Z. h. campestris* + *Z. h. intermedius*). These cases occurred most frequently in *Z. h. intermedius*, followed by *Z. h. campestris*, *Z. h. pallidus*, *Z. h. preblei*, and *Z. h. luteus*, in that order (Table 3). These results demonstrate that there is indeed limited gene flow among the three populations identified by the STRUCTURE analysis.

Our STRUCTURE results indicated that the five subspecies samples by the Ramey et al. study can be divided into three populations roughly equivalent to the three clusters identified above. To quantify the degree of admixture between *Z. h. preblei*, *Z. h. campestris* + *Z. h. intermedius*, and *Z. h. pallidus* + *Z. h. luteus*, we used coalescent-based methods to estimate relative measures of  $\Theta$  ( $4N_e\mu$ , a measure of effective population size and mutation rate) and interpopulation migration rates ( $Nm$ ) using the program MIGRATE (Beerli and Felsenstein 2001) based on the Brownian motion model. Appendix 2 shows the formatted infile. A summary of the results of this analysis is given in Table 4. Conditions of analysis and extended results for each run are given on accompanying disk (Supporting Documents/Data & Result Files/ Ramey MS Migrate Results). Table 4 (B) converts Migrate output into migration rates ( $Nm$ ) that can be compared across studies. One advantage that these likelihood-based estimates have over traditional estimates of gene flow via  $F_{st}$  statistics is that asymmetrical migration rates can be estimated between populations. When considering gene flow into and out of *Z. h.*

*preblei*, we see that the most restricted migration is from the *Z. h. campestris* + *Z. h. intermedius* cluster into the *Z. h. preblei* cluster (0.46) and the highest migration from the *Z. h. preblei* cluster into the *Z. h. campestris* + *Z. h. intermedius* cluster (2.14). The immigration and emigration into and away from the all clusters ranged from 0.46 to 5.76; comparing this to other migration rates in rodents shows that comparatively high rates are found in these *Z. hudsonius* ‘subspecies’. For instance in the African ground squirrel (*Xerus inauris*) migration rates (Nm) between populations within the species were estimated to be 0.64 to less than 0.001 (Herron et al. 2005), between population of common voles (*Microtus arvalis*) estimates ranged from 3.3 to 0.15 (Hamilton et al. 2006), between population of Tuco-tuco (*Ctenomys rionegrensis*) estimates ranged from 0.17 to less than 0.001 (Wlasiuk et al. 2003), between population of two deer mice species (*Peromyscus keeni* and *Peromyscus maniculatus*) estimates ranged from 1.00 to less than 0.001 and 4.74 to less than 0.001 respectively (Zheng et al. 2003). In all of these cases, we find migration rates lower than the lowest estimate between any of the *Z. hudsonius* populations and only a few higher, however, only in one of the above cases (*Peromyscus maniculatus*) have subspecies based on molecular data been described. This calls into question support of subspecific designation based on these microsatellite data.

## **B. Mitochondrial DNA Sequence Data**

Ramey et al. 2005 sequenced a 346 bp piece of the mitochondrial control region (CR) gene to test for reciprocal monophyly between *Z. h. preblei* and its neighboring subspecies. King et al. 2006 also sequenced this same region of the mtDNA and we have combined these data sets and performed various analyses with them below. Here we will only mention briefly a couple of interesting points noted when comparing Ramey et al.’s CR data with their microsatellite data.

In their study, Ramey et al. found that *Z. h. preblei* contained few unique CR haplotypes and most haplotypes were also found in low frequencies within the range of *Z. campestris* (Table 5). The low frequencies of these shared haplotypes within *Z. h. campestris* caused King et al. to question the quality of these data. King et al. 2006 (p22, line 666) states:

“For example, Ramey et al. (2005) reported the presence of *Z. h. preblei* haplotypes in DNA extracted from five dried museum skins of *Z. h. campestris* collected from Custer County, SD. The authors suggested this finding indicated recent gene flow and alluded to the presence of these haplotypes as a critical element in the decision to recommend synonymy of these subspecies. In the present study, 31 *Z. h. campestris* sampled recently from the same site in Custer County, SD used by Ramey et al. (2005), along with 30 additional specimens from neighboring Crook County, WY were subjected to mtDNA CR and CytB sequence analysis. All 61 individuals were determined to possess *Z. h. campestris*-specific mtDNA haplotypes. Moreover, the same conclusion was reached with the microsatellite loci, as no *Z. h. campestris* individual from either of these collections was assigned to *Z. h. preblei*. Given the prominent role the haplotypes obtained for the five museum skins from Custer County, SD and two additional specimens from Carter County, MT have played in the conclusions drawn by Ramey et al. (2005), it is unsatisfactory that an *a posteriori* analysis was not considered as part of a routine quality assurance/quality control effort. Since no attempts were made to reproduce the previous CR results, to confirm the findings with another region of mtDNA, or to apply an additional finer resolution technique such as microsatellite DNA analysis, combined with our failure to detect *Z. h. preblei* haplotypes among 61 *Z. h. campestris* from the same and an adjacent location, the conclusions drawn by Ramey et al. (2005) should be considered questionable.”

The point made above by King et al. is well taken and when much is dependent on these few samples assurances should be taken that these samples were not misidentified or that the DNA isolated from these samples has not been cross contaminated. One way to control against this is to look at the microsatellite profiles for each of the *Z. h. campestris* individuals that have a ‘*Z. h. preblei*’ CR haplotype. If these individuals were misidentified before DNA extraction or contaminated with *Z. h. preblei* DNA after extraction then their microsatellite genotypes should also show a ‘*Z. h. preblei*’ profile and have a high probability assignment to the *Z. h. preblei* cluster. Table 5 shows the groupings of all identical CR haplotypes from both studies. From Table 5

we see that ZhcaK110013, ZhcaK109984, ZhcaK109985, ZhcaK123592, ZhcaK109978, and ZhcaK109972, all have ‘*Z. h. preblei*’ CR haplotypes. However, assignment probabilities from Table 3 show that ZhcaK110013 is assigned to the *Z. h. campestris/intermedius* cluster with a probability of 0.988. Also, all the others samples in question have their highest probability assignment to the *Z. h. campestris/intermedius* cluster, ZhcaK109985 (0.969), ZhcaK109978 (0.937), ZhcaK109972 (0.694), with the exception of ZhcaK109982 which shows similar assignment probabilities to all three clusters and ZhcaK123592 for which no microsatellite data are given. This indicates that these samples were neither misidentified, miscataloged, nor cross contaminated. Conflicting nuclear and mtDNA signals could be the products of different levels of resolution targeted by the different markers and types of analysis. The fast evolving microsatellite markers coupled with the **population**-level STRUCTURE analysis illustrate the current interactions of these populations whereas the slower evolving shared CR haplotypes may be indicative of historical interactions or mitochondrial introgression. If the subspecific category is to represent historical isolation in addition to current population structure, high levels of concordance between these analyses should be required. If simple allele frequency differences (highly dependent on sampling scheme) were allowed to fill this requirement most if not all colonization and bottleneck events would also instantaneously spawn new subspecies, something many scientists would find discomfoting. An alternative explanation for different assignments based on mtDNA versus nuclear (microsatellite) markers is the potential for sex biased dispersal of mtDNA alleles (maternally inherited) given the different and asymmetric migration rates for the diagnosed populations.

A number of the CR mtDNA haplotypes from *Z. h. preblei*, *Z. h. campestris*, and *Z. h. pallidus*, individuals from the Ramey et al. study were identical or nearly identical to *Z. princeps* haplotypes (see Network 3 and 4 in Figure 10). Ramey et al. interpreted these results as cases of misidentification. Unfortunately, no microsatellite data were generated to test these in the same way the *Z. h. campestris* individuals with *Z. h. preblei* haplotypes were tested above. The four *Z. h. preblei* individuals that were ‘misidentified’ all came from Albany County, Wyoming. *Z. princeps* were also sampled from this county, which merits consideration of a different interpretation than ‘misidentification’. It

is possible that some gene flow is occurring at this much deeper interspecific level. If so, this may be indicative of a tradition of ‘over-splitting’ taxa by biologist within the *Zapus* genus. Results from previous studies indicate that gene flow between *Z. princeps* and *Z. h. preblei* and other subspecies is not only likely but probable. As summarized in Beauvais 2001:

“The relatively large zone of co-occurrence in southeast Wyoming raises the issue of potential hybridization between the 2 species [*Z. h. preblei* and *Z. princeps*]. Hybridization between related species in areas of co-occurrence is known to occur in several other free-ranging vertebrates (see examples in Pague and Grunau 2000). Hybridization between *Z. hudsonius* and *Z. princeps* in Wyoming is suggested by recent analyses of variation in mitochondrial DNA. Although these analyses can distinguish the 2 species in other parts of their ranges (e.g., the South Platte basin in Colorado), they are unable to reliably assign species identity to *Zapus* specimens from southeast Wyoming. **The general consensus among regional mammalogists is that *Z. hudsonius* X *Z. princeps* hybridization is the most parsimonious explanation for such results** (Riggs et al. 1997, Pague and Grunau 2000, Schorr 2001).”

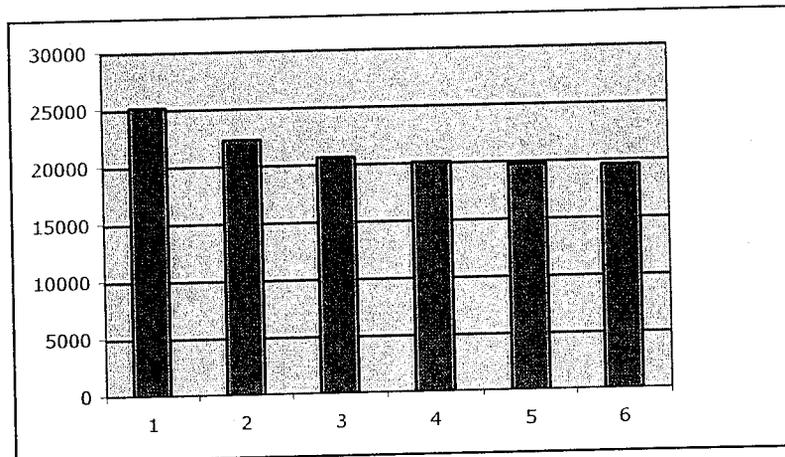
It may be that the genus *Zapus* may be suffering not only from a tendency to split taxa but also from non-rigorous delimitation of species boundaries. This makes any discussion of subspecies dubious. Biologists may be better served by preceding debate on subspecific classification with substantial and meticulous examinations of species boundaries. In other words ‘you can’t have cupboards if you ain’t got walls’ *Neil Young-Old Laughing Lady*. A detailed analysis of this potential hybrid zone that incorporates both nuclear (microsatellite) and mitochondrial markers would contribute substantially to clarification of our current issue.

### III King et al. 2006

#### A. Microsatellite Data

King et al. 2006 screened 320 samples for 21 microsatellite loci across the same five *Zapus* subspecies as above. We reanalyzed the King et al. microsatellite to verify their results and also to observe patterns in assignment probabilities for the optimal number of populations (K). Again, Figure 2 shows the localities of the samples taken in both the Ramey et al. and King et al. studies. Table 1 also provides the state and county names for each locality, the numbers of samples taken, and GPS coordinates. We converted the King et al. microsatellite data into a format (Appendix 3) appropriate to run on the computer program STRUCTURE (Pritchard et al. 2000). Conditions for the King et al. STRUCTURE analysis and method for selecting the optimal K were identical to the analysis of the Ramey et al. data and are given above. Resulting output files for all runs are given in support files (Supporting Documents/Data & Result Files/ King MS Structure Results).

We confirm the findings of King et al. 2006 and select an optimal K value of three (Table 6, Figure 4). The result is identical to the K value selected with the Ramey et al. data set only in the current analysis we see a more profound leveling of likelihood



**Figure 4.** A visual representation of the absolute values of likelihood scores from ten separate STRUCTURE runs for King et al. data set. K values range from 1 to 6. Little improvement of likelihood scores is evident after K = 3.

scores at K = 3. The composition of resultant clusters was also very similar to our reanalysis of the Ramey et al. data (*Z. h. preblei*, *Z. h. pallidus* + *Z. h. luteus*, and *Z. h. campestris* + *Z. h. intermedius*). Table 7 shows the inferred ancestry for each sample. Population boundaries between clusters appeared to be much more distinct than in the STRUCTURE analysis of the Ramey et al. data. For instance, few individuals showed assignment probabilities < 0.80 and all of these occurred in the *Z. h. campestris/intermedius* cluster. Additionally no individuals had their highest probability of assignment to clusters of non-subspecific individuals.

The differences in results and conclusion of these studies seem to be largely due to the sampling schemes employed by each study. King et al. rightly point out that sampling is critical in intraspecific studies and is distinct from systematic studies. King et al. argue for dense sampling at specific locations with sparse sampling across locations throughout the distribution of the subspecies. King et al. correctly point out that the basis of inference by Ramey et al. (frequency differences instead of evolutionary relationships) is highly dependent upon sampling individuals at a given location with the Ramey et al.

sampling design lacking in terms of individuals per site. Yet the conclusions reached by King et al. are also highly suspect in that leaving large geographic gaps between sampling sites when the taxon is known to range within those gaps leads to artificial inferences of population structure when, in fact, a gradient of variation may exist with gene flow across the gradient. Thus the optimal sampling strategy for such studies is a combination of the two approaches; broad geographic sampling with multiple individuals sampled per sampling location (Templeton et al. 1995; Sites & Crandall 1997; Templeton 2004; Morando et al. 2003). Below we attempt an approximation of this scheme by combining the CR data sets of Ramey et al. and King et al. Unfortunately, the scoring of microsatellite allele size on different machines can be tricky and the lack of generalized size standards run by these lab groups made it impossible to combine the microsatellite data into a single analysis. Ideally some of the samples scored in the first study (Ramey et al. 2005) should have been run in the second (King et al. 2006) to allow the combining and calibration of results.

Both studies have limitations in their sampling strategies. The conclusions by King et al. of population structure are particularly suspect given the sampling design of their study. For example, King et al. fail to sample in areas most likely to show gene flow between subspecies (Figure 5). These areas include, *Z. h. preblei* from southern Wyoming, where you would find individuals most likely to show evidence of gene flow between *Z. h. campestris*, *Z. h. pallidus*, and even *Z. princeps* based on geographic proximity and previous studies (see above), and *Z. h. pallidus* from western Nebraska. King et al. have just a single locality sampled for *Z. h. luteus* and just two sites sampled for the critical *Z. h. campestris* and *Z. h. intermedius*. This is particularly problematic with the widespread distribution of *Z. h. intermedius* across 11 states with sampling in only the NE corner of South Dakota and an adjacent site in central Minnesota. The central problem here is a taxonomic issue relative to the entire species complex and possibly sister species within the genus, thus the entire species complex should be sampled to resolve the issue.

Like our STRUCTURE analysis of the Ramey et al. microsatellite data set our analysis of the King et al. data indicated that the five subspecies samples can be divided into three populations equivalent to the three clusters identified above. Again to quantify

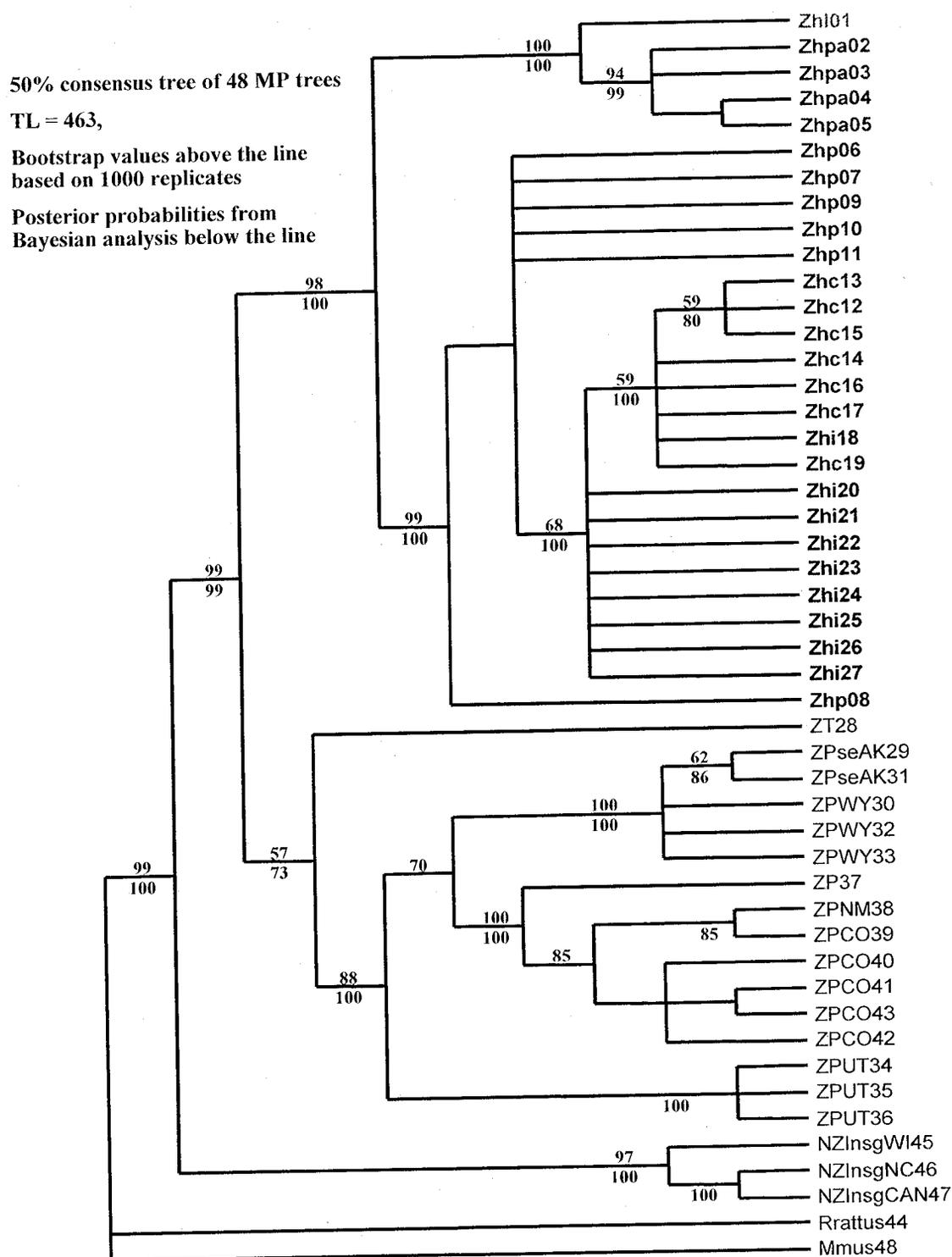
the degree of admixture between *Z. h. preblei*, *Z. h. campestris* + *Z. h. intermedius*, and *Z. h. pallidus* + *Z. h. luteus*, we used MIGRATE (Beerli and Felsenstein 2001) to estimate migration between these clusters. Conditions of analysis and extended results for each run are given in the supporting material (Supporting Documents/Data & Result Files/King MS Migrate Results). Table 8 shows the estimated genetic diversity ( $\Theta$ s) for each cluster as well as the migration rates ( $M_{xy}$ ) between all clusters (x and y). Table 8 (B) shows estimates of population migration rates ( $Nm$ ) between clusters. The results of Table 8 (B) are similar to those estimated from the Ramey et al. microsatellite data (Table 4). We see nearly equal rates of migration out of *Z. h. preblei* but interestingly an increase of migration into *Z. h. preblei* from the other two clusters (1.21 and 2.45 as opposed to 0.46 and 0.47). Conversely lower migration rate estimates between the *Z. h. campestris* + *Z. h. intermedius*, and *Z. h. pallidus* + *Z. h. luteus* clusters resulted in the analysis of the King et al. data. As a whole, we draw similar conclusions here as with the analysis of the Ramey et al. data with migration rates again on par with and even in a little excess of other within species comparisons where subspecies are not recognized (see above).

## B. Mitochondrial DNA Sequence Data

The King et al. studies generated sequence data from two mtDNA genes. Like Ramey et al. they sequenced a piece of the control region only slightly larger. We will discuss this below in the section on the combined analysis. King et al. also generated a ~1 Kb piece of the cytochrome b (CytB) mitochondrial gene for 292 individuals from 13 localities (Figure 2) representing the subspecies *Z. h. preblei*, *Z. h. campestris*, *Z. h. intermedius*, *Z. h. pallidus*, and *Z. h. luteus* as well as a single *Z. princeps* sample. In order to test the monophyly of the King et al. subspecies samples, we combined them with 27 outgroup plus one *Z. h. luteus* sequences provided by R. Ramey and J. Cook. The outgroup samples included 21 *Z. princeps* (ZP), two *Z. trinotatus* (ZT), three *Napaeozapus insignis* (Ntinsig), one *Ratus ratus* (Rratus), and one *Mus musculus* (Mmus). In order to combine these data, the total length of sequence had to be trimmed to 518 bp. The high throughput multiple sequence alignment program MUSCLE (Edgar 2004) was used to align sequences. From the 320 individual sequences 48 distinct haplotypes were found

(Appendix 4). No haplotypes were shared between subspecies groups. Maximum parsimony (MP) trees were generated in PAUP\* (Swofford 1999) by heuristic searches with 100 random additions and using the TBR branch swapping method (see Appendix 5 for PAUP\* haplotype data file). Figure 6 shows the resulting 50% majority rule consensus tree for the 48 MP trees. We also generated Bayesian tree topologies with MRBAYES (Huelsenbeck and Ronquist 2001) using 1,000,000 iterations and a burn-in of 47,000. We used Modeltest (Posada and Crandall 1998) to select the GTR + G + I model as the optimal model of evolution. Because of the short length of the DNA fragment used, separate models for each codon position were not estimated. Tree topologies from the two methods were identical in all major divisions and differed only slightly by levels of resolution, for this reason, only the 50% consensus MP tree is shown and bootstrap and posterior probability values from both analyses combined and placed on the tree (Figure 6).

Figure 6 shows monophyletic groupings with good nodal support for *Z. princeps*, *Z. hudsonius*, and *Napaeozapus insignis*. This is not terribly surprising due to the low numbers of individuals and localities sampled from *N. insignis* and *Z. princeps*. Figure 6 also shows a monophyletic grouping for *Z. h. pallidus* and one for a combined *Z. h. luteus* + *Z. h. pallidus* group. However, given that only a single locality from *Z. h. luteus* was sampled, only limited conclusions can be drawn. All samples from *Z. h. preblei*, *Z. h. campestris*, and *Z. h. intermedius* combined to form a single monophyletic group.



**Figure 6.** Phylogenetic tree based on part of the CytB mtDNA gene. Numbers at taxa represent haplotype numbers listed in Appendix 4.

However, none of these subspecies forms a monophyletic group by themselves and thus fail this particular subspecies test and indeed fail even an evolutionarily significant unit (ESU) test of Moritz (1994). *Z. h. campestris* comes closest to forming a monophyletic group with a single *Z. h. intermedius* haplotype nested within it. As a whole this analysis, although severely restricted by the sampling design as discussed above, provides some preliminary evidence for designation of a two subspecies within the *Z. hudsonius* samples investigated, one including the *Z. h. pallidus* + *Z. h. luteus* samples and another including the *Z. h. preblei* + *Z. h. campestris* + *Z. h. intermedius* samples.

## IV Combined Data Analysis

### A. Combined control region phylogenetics

As mentioned above the only data we were able to combine between the Ramey et al. 2005 and King et al 2006 studies were the sequences generated from the mitochondrial control region. Total sample size for the combined analysis was 520 individuals (including several *Z. princeps* samples) from 14 states. Individuals were pooled by their county and state or origin because exact GPS coordinates were not available for all samples (Table 1). GPS coordinates for all localities were taken from roughly the geographic center of the county. Sequences were aligned in MUSCLE (Edgar 2004) and then trimmed to a total base pair length of 347. Sequences collapsed into 63 distinct haplotypes. Nine of the haplotypes were shared either between our five focal *Z. hudsonius* subspecies and/or *Z. hudsonius* and *Z. princeps* (Table 5).

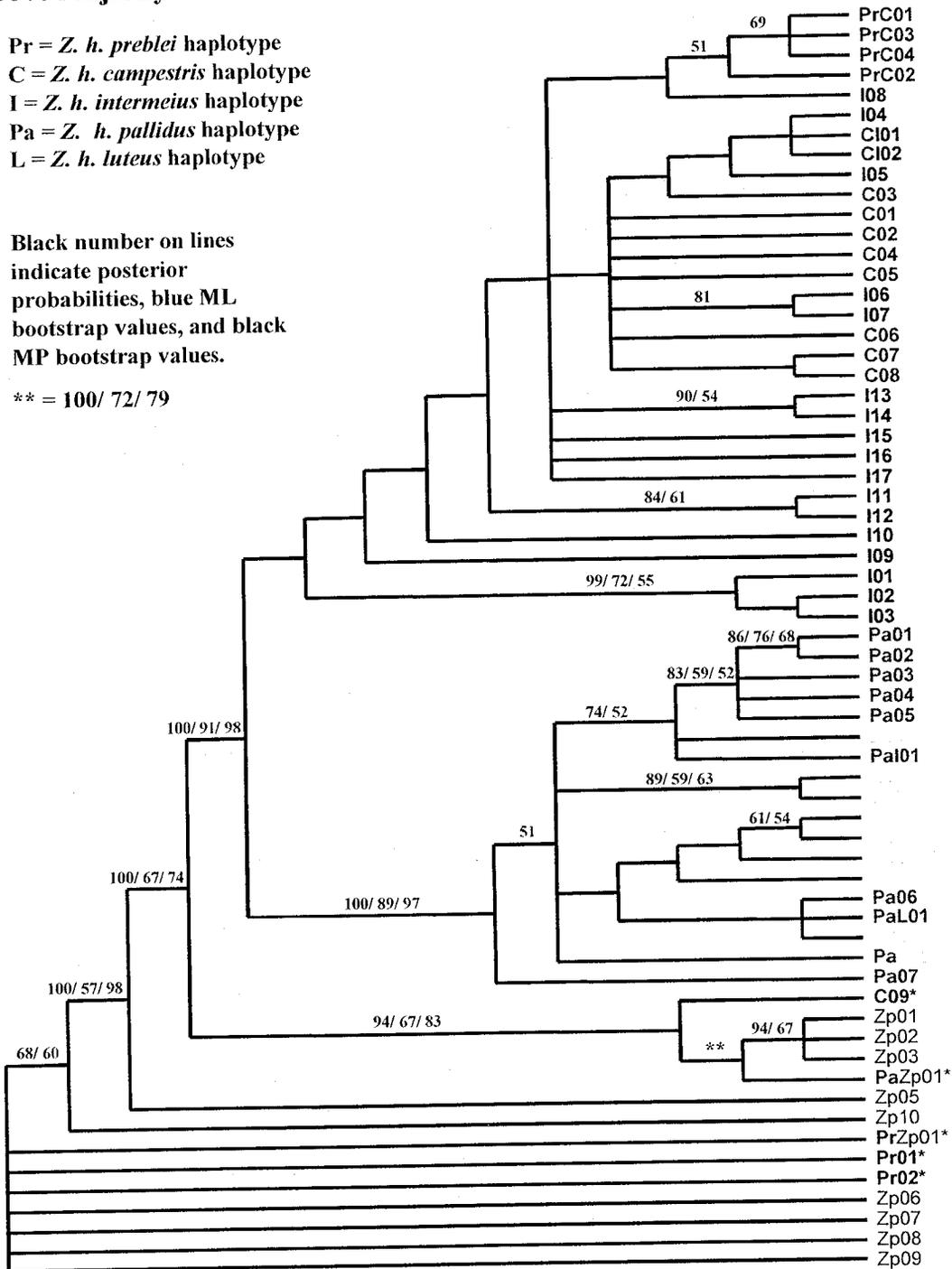
We estimated phylogenetic relationships based on Bayesian, maximum likelihood (ML), and maximum parsimony (MP) criteria. Topologies and well-supported nodes were similar for all three optimality criteria used. Figure 7 shows a 50% majority rule consensus tree of 23,329 most parsimonious trees with a tree length of 146. Trees were

**50% Majority Rule MP Tree**

Pr = *Z. h. preblei* haplotype  
 C = *Z. h. campestris* haplotype  
 I = *Z. h. intermeius* haplotype  
 Pa = *Z. h. pallidus* haplotype  
 L = *Z. h. luteus* haplotype

Black number on lines indicate posterior probabilities, blue ML bootstrap values, and black MP bootstrap values.

\*\* = 100/ 72/ 79



**Figure 7.** Phylogenetic tree based on the combined CR mtDNA data sets from Ramey et al. 2005 and King et al. 2006 studies. Lists of haplotypes represented by each haplotype label are found in Table 5. Colors correspond to different *Z. hudsonius* subspecies. \* = *Z. hudsonius* haplotypes associated with *Z. princeps* haplotypes.

generated using 100 random additions and the TBR branch swapping method. Bootstrap values were based on 1000 replicates where a maximum of  $2 \times 10^7$  rearrangements was set for each replicate. We generated our Bayesian topologies with MRBAYES (Huelsenbeck and Ronquist 2001) using 1,000,000 iterations and a burn-in of 46,000. We used Modeltest (Posada and Crandall 1998) to select the TVM + I + G model as the optimal model of molecular evolution. Maximum likelihood analysis was performed with GARLI v0.94 (Zwickl 2006, <http://www.bio.utexas.edu/grad/zwickl/web/garli.html>) under the TVM + I + G model and 100 replicates used for ML bootstrap values.

Results from the CR phylogenetic analysis are similar to the results from the CytB phylogenetic analysis except with less distinct clustering of subspecies and groups of subspecies. This is not surprising because the current analysis incorporated samples from regions avoided by the King et al. study that were located in area most likely to see gene flow between different subspecies/populations. Noticeably widespread across the tree topology are the *Z. h. preblei* samples. Our analysis included some samples of *Z. h. preblei* dropped from the Ramey et al. study on the basis of their similarity to *Z. princeps* sequences. As mentioned above, gene flow between these species is suspected in southeastern Wyoming and these samples seem equally likely to be a result of interspecific gene flow as a result of misidentification. Some haplotypes found both in *Z. h. pallidus* and *Z. h. campestris* also clustered with *Z. princeps* haplotypes (C09, PaZp01) and together formed a moderately supported (posterior probabilities and bootstrap values = 100/67/74, Figure 7) sister group to most *Z. hudsonius* populations. These results at a minimum merit further study on species boundaries between *Z. hudsonius* and *Z. princeps*. Quantification of levels gene flow between these species could then serve to add a base line level of gene flow between proposed *Z. hudsonius* subspecies and aid in the identification of proper subspecific boundaries if such boundaries exist.

Although many of the nodes in Figure 7 are either unresolved or poorly supported we can draw limited conclusions based on some of the moderately support ones. We see that most *Z. hudsonius* samples cluster into a well-supported (100/91/98) monophyletic group. Within this monophyletic group, we see most *Z. h. luteus* and *Z. h. pallidus* samples clustering into a well-supported (100/89/97) group nested within the larger *Z. hudsonius* clade. However, no support for exclusive clustering of any of the *Z. hudsonius*

subspecies is evident anywhere in the tree. In fact, even combining most *Z. h. campestris*, *Z. h. preblei*, and *Z. h. intermedius* into a single group did not result in a well-supported clade (as opposed to the CytB result in Figure 6). These results could be indicative of different marker resolution, different sampling schemes, or a combination of both. If exclusivity or near-exclusivity of taxa based on mtDNA markers is to be taken as evidence of historical isolation between populations and thus incorporated into subspecific designation then the results in Figure 6 and Figure 7 question subspecific status for any of the individual *Z. hudsonius* subspecies. However the formation of two subspecies by combining *Z. h. pallidus* with *Z. h. luteus*, and *Z. h. campestris* with both *Z. h. preblei* and *Z. h. intermedius* would merit subspecific status under the near-exclusivity criterion.

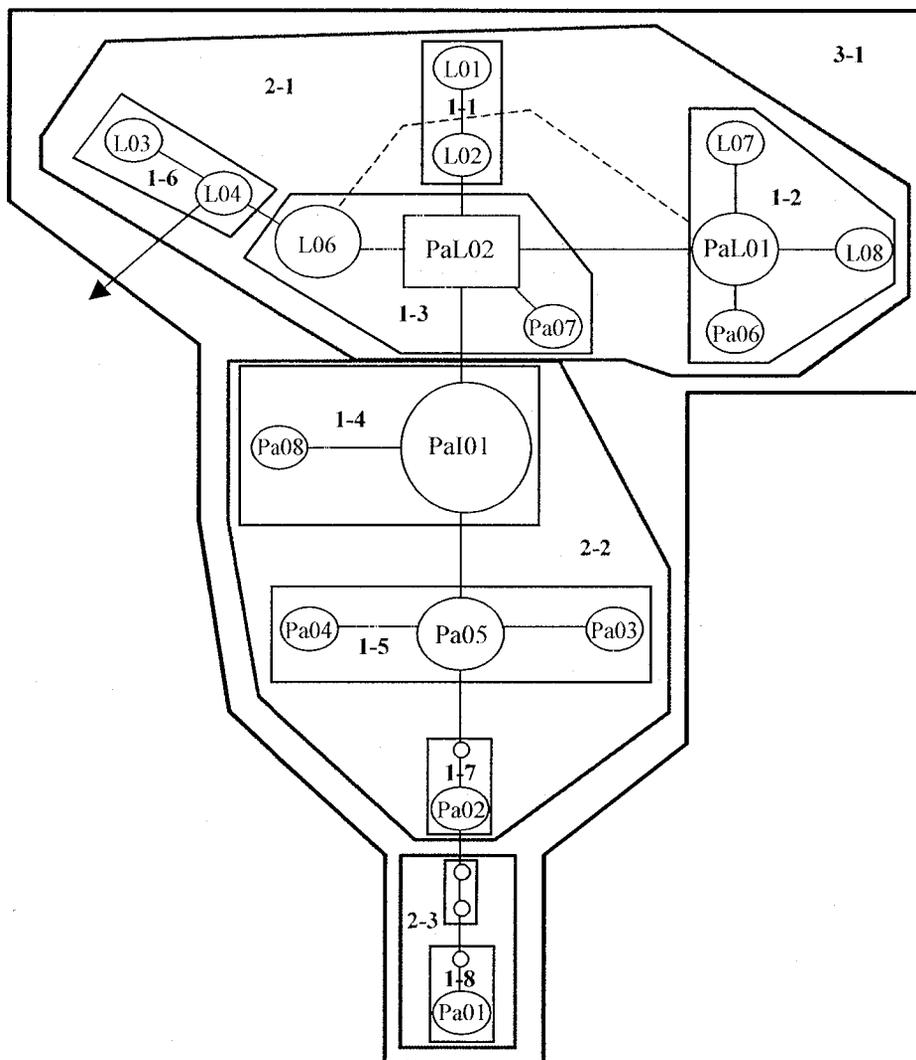
#### **B. Nested clade analysis (NCA) on control region**

When assessing patterns of genetic variation at the intraspecific level, it is often difficult to distinguish current population structure from population history using traditional population genetic estimates such as  $F_{st}$  (Templeton et al. 1995). For instance, two populations sharing similar alleles at similar frequencies could be the product of ongoing gene flow (current population structure) or a past range expansion of the organism (population history). NCA uses haplotype frequencies in conjunction with the genealogical relationships and geographic distribution of the haplotypes in a novel methodology that allows the researcher to distinguish between structure and different historical events (Templeton et al. 1995). Such an analysis was lacking from both the King et al. and the Ramey et al. studies. Thus their studies possibly confound population history and population structure.

To implement the NCA, a parsimony haplotype network was first constructed for the mitochondrial control region sequences using the program TCS (version 1.21, Clement et al. 2000). Haplotypes were connected using a 95% parsimony limit that imposed a maximum of seven mutational steps between connections. Four separate networks plus and unconnected single haplotype (Zp05) resulted (Figures 8-10).

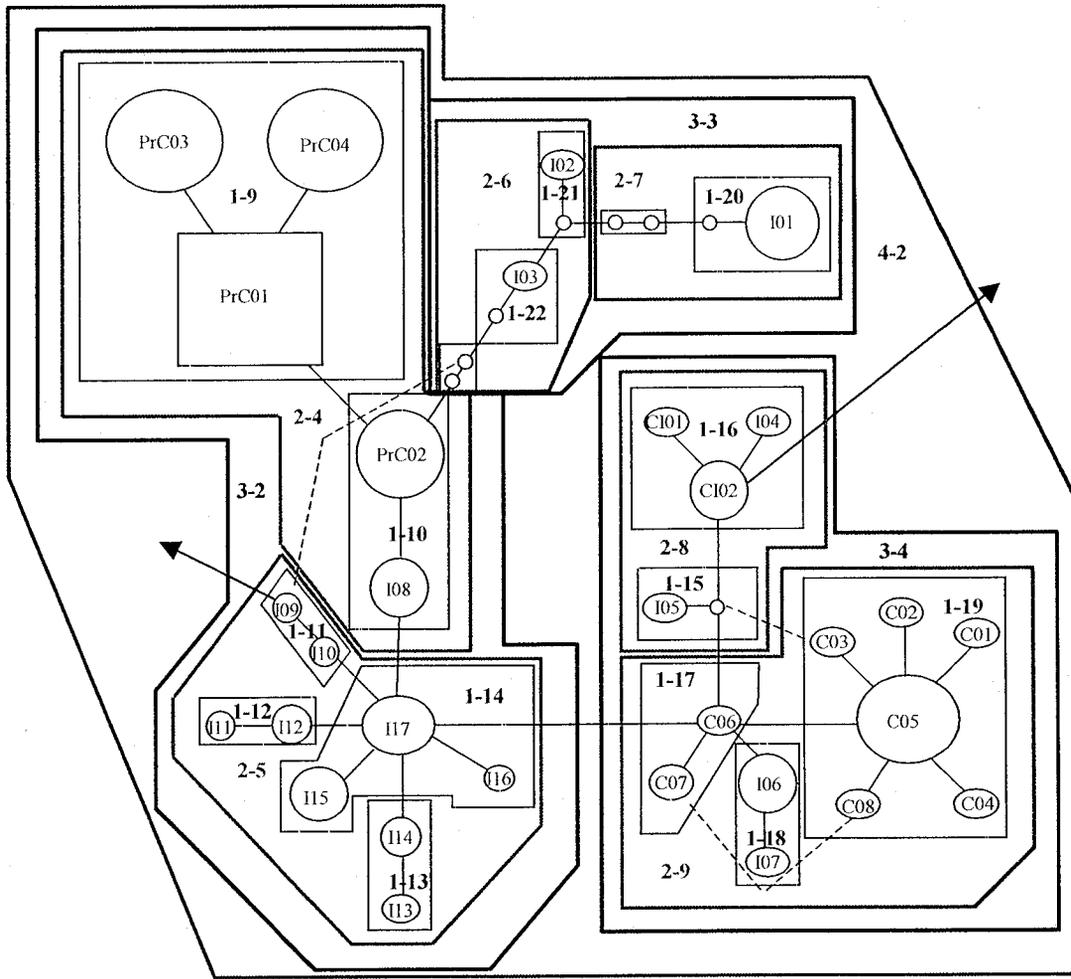
The independent networks were then connected into a total network using TCS by relaxing the 95% parsimony criterion (Figure 10). A number of ambiguous connections or loops in the resulting haplotype networks were resolved using the criteria set forth in Crandall & Templeton (1993). The total network was then nested (Templeton 1998) and input into GEODIS (version 2.4, Posada et al. 2000) together with geographic sampling information (Appendix 7, input file). We then performed permutation tests (1000) to determine association between phylogeny and geographic distribution. Clade distances ( $D_c$ ) and nested clade distances ( $D_n$ ) were measured and interior and tip clade differences estimated. Templeton's revised (2004) inference key (Modified 11 November 2005) was then applied to the clades with significant results from GEODIS to determine the outcome of the NCA.

Appendix 8 provides the extended GEODIS results and Table 9 summarized the general conclusions from the NCA inference key. A total of 33 clades with both geographic and genetic variation from various nesting levels were input into GEODIS; of these clades only 17 resulted in significant results that lent themselves to interpretation. This demonstrates the necessity of the need for a sampling scheme that employs both large numbers of localities (as in Ramey et al. 2005) and large numbers of individuals per locality (as in King et al. 2006) (Sites & Crandall 1997; Morando et al. 2003). However, even with our limited sampling scheme a number of important conclusions can be drawn. Of the 63 distinct haplotypes eleven were shared between species and subspecies. Noting the distribution of these haplotypes on our networks (Figs. 7-9), we see most of the shared haplotypes are interior clades indicating ancestral types.



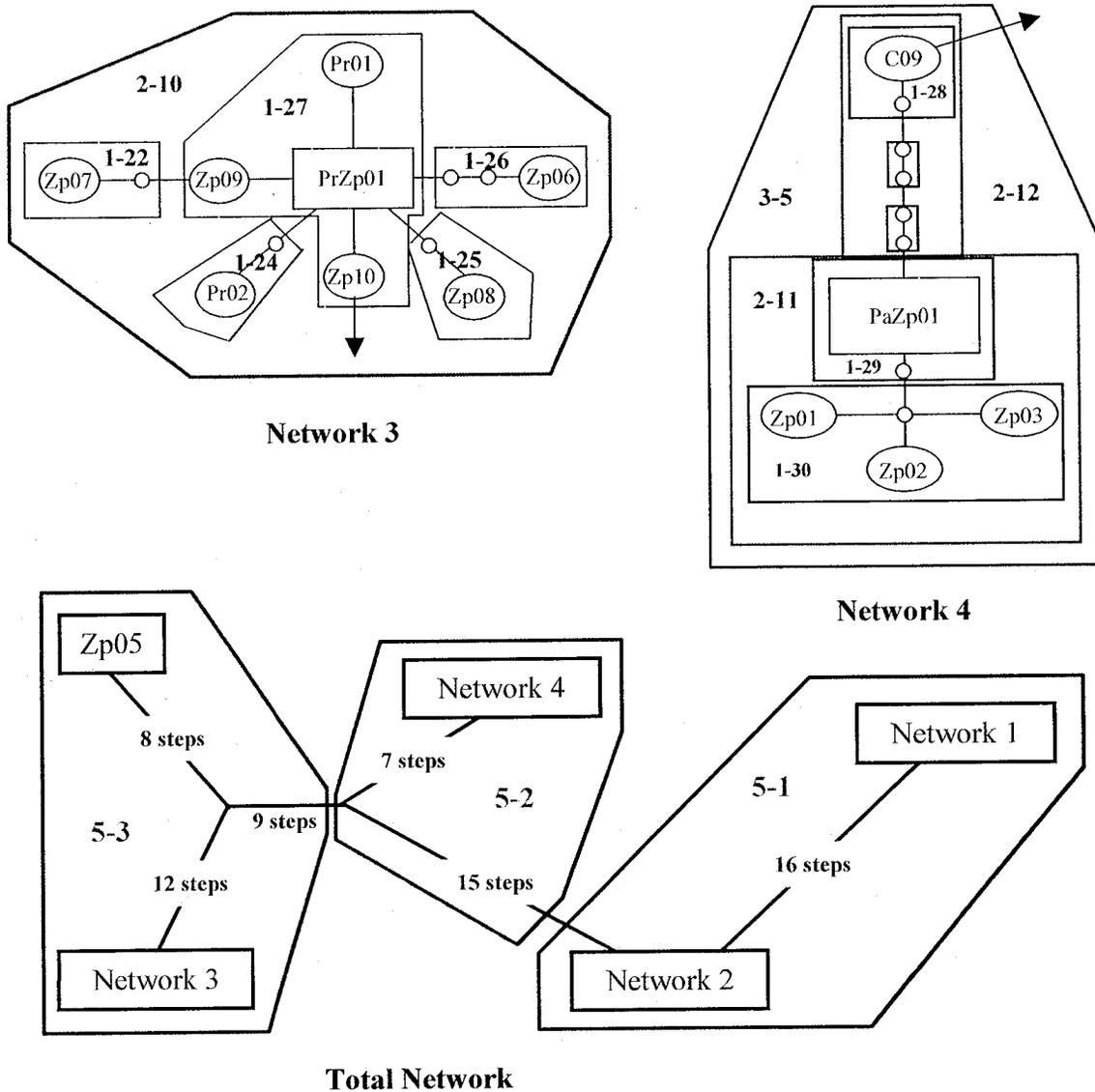
**Figure 8.** Haplotype network 1 estimated in TCS with 1, 2, and 3 step nesting groups shown. Ovals and squares represent haplotypes where labels correspond to labels in Table 5 and size roughly correlated with frequency of haplotype. Lines separating haplotypes and empty circles represent single mutational steps. Arrows indicate connections to other networks. Dashed lines represent broken loops. Colored boxes correspond to different nesting levels. Network consists of haplotypes mostly from *Z. h. pallidus* and *Z. h. luteus* individuals.

Network 1 (Figure 8) consisted of mostly haplotypes from *Z. h. pallidus* and *Z. h. luteus* individuals, with a single haplotype (PAI01) also being found in *Z. h. intermedius*. The distribution of these haplotypes between subspecies showed non-exclusive clustering within the network. Also clades 1-3 and 2-1 both spanned the geographic divide between *Z. h. luteus* and *Z. h. pallidus* populations (Figure 11), thus including non-subspecific populations. However, the NCA inference for clade 1-3 (Table 9) indicated possible allopatric fragmentation across this geographic divide. NCA inferences for clades 2-1



**Figure 9.** Haplotype network 2 estimated in TCS with 1, 2, 3, and 4 step nesting groups shown. Schematics are the same as in Figure 7. Network consists of haplotypes mostly from *Z. h. preblei*, *Z. h. campestris* and *Z. h. intermedius* individuals.

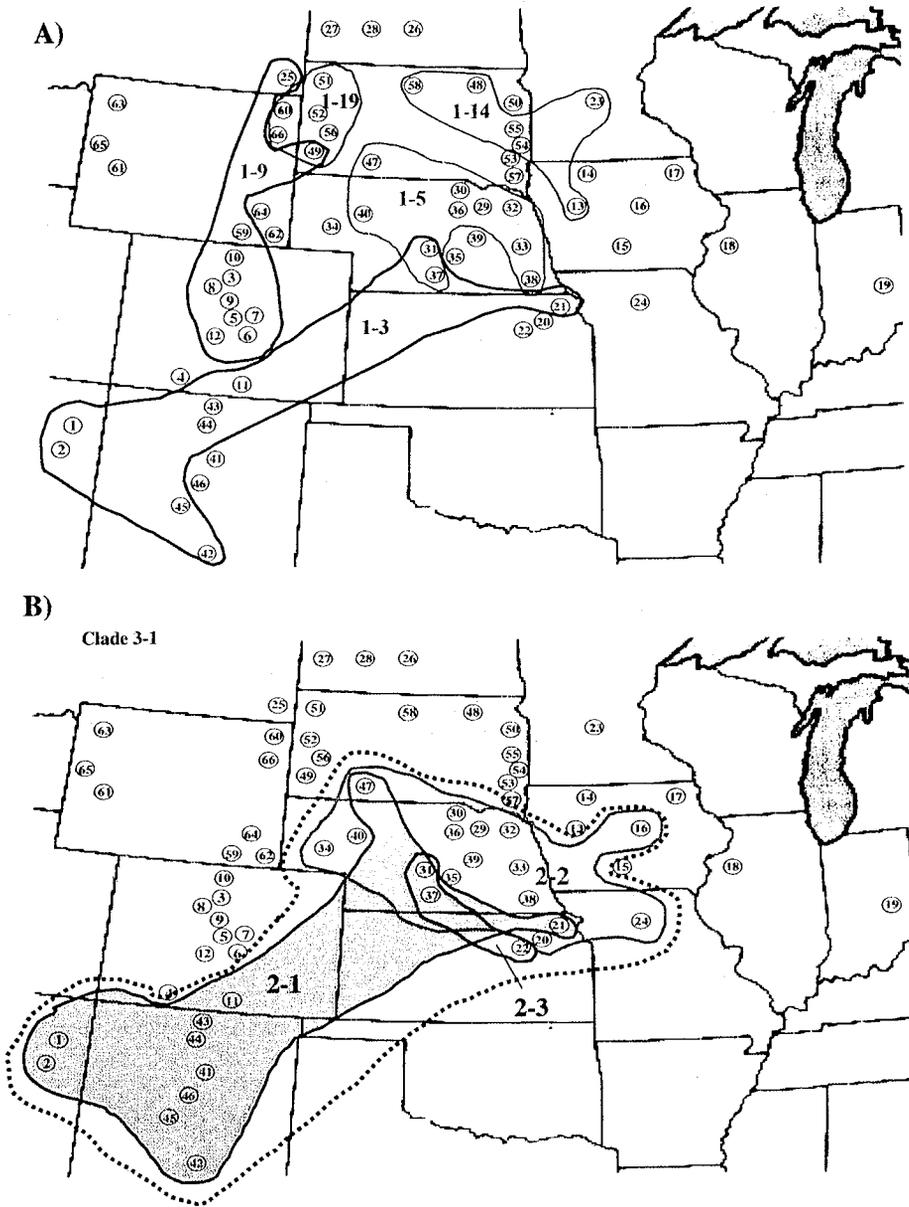
and 3-1 indicate restricted gene flow with isolation by distance and a contiguous range expansion but these results depend on adequate sampling for *Z. hudsonius* populations in eastern Colorado and Kansas. Although further investigation via more dense sampling in New Mexico, Kansas and eastern Colorado is merited to illuminate the extent of separation between the *Z. h. luteus* populations of New Mexico and various *Z. h. pallidus* populations of Kansas and Nebraska, taken as a single unit (*Z. h. pallidus* + *Z. h. luteus*) evidence based on the CR sequence data seems to indicate some separation from the other *Z. hudsonius* populations sampled in these studies. Evidence for this includes, clustering into a single network separated from all other networks by a minimum of 16 mutational steps (Figure 10) and the inference of possible fragmentation within clade 5-1 (Figure 12, Table 5) between clades 4-1 (network 1) and 4-2 (network 2).



**Figure 10.** Haplotype networks 3, 4 and the total network. The total network represent connections above the 95% parsimony cut-off. Schematics are the same as in Figure 7. Networks 3 and 4 consist of haplotypes mostly from *Z. princeps* individuals but contain some individuals from *Z. hudsonius* subspecies indicating possibly low levels of gene flow.

Network 2 (Figure 9) consists of haplotypes primarily derived from *Z. h. preblei*, *Z. h. campestris*, and *Z. h. intermedius* individuals. Like network 1 haplotypes from different subspecies show some clustering but no subspecies form exclusive groups. Further, all the *Z. h. preblei* haplotypes found in this network are shared with *Z. h. campestris* haplotypes (although in every case the group is dominated by *Z. h. preblei* samples). Clades 1-9 (Figure 11) and 3-2 (figure 12) illustrate the geographical

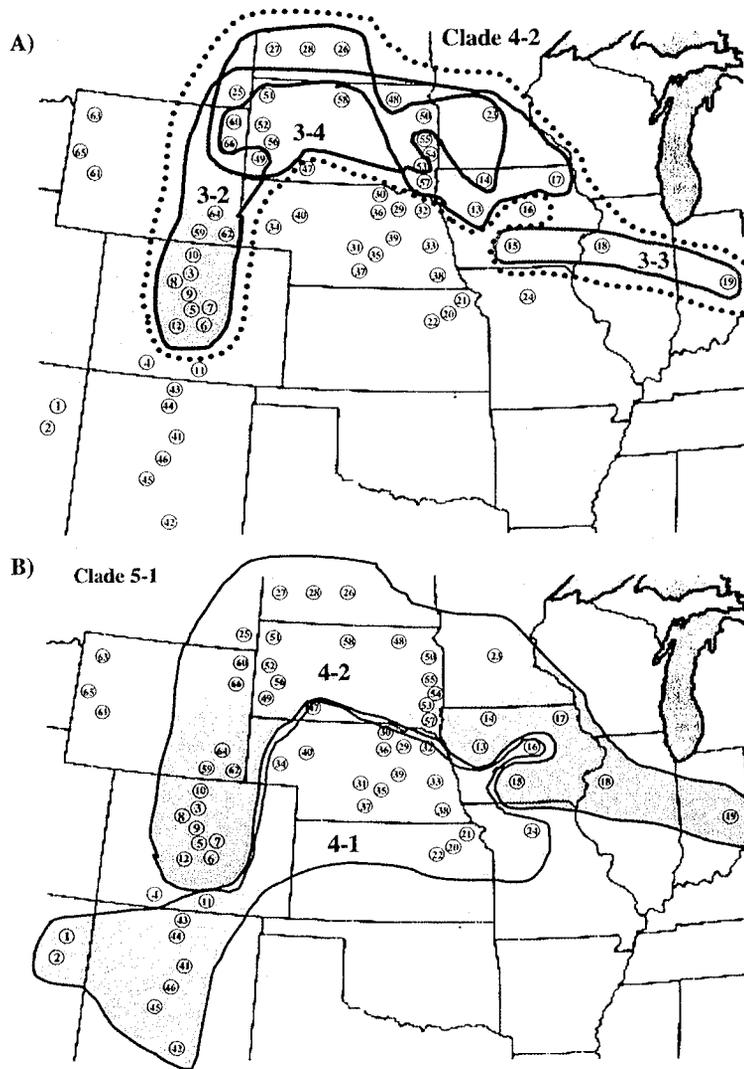
connection between these haplotypes. At no clade level do the *Z. h. preblei* haplotypes separate out. Taking frequency differences into account the NCA inferences for these clades indicate that contiguous range expansion is the best-supported conclusion in both cases (Table 9). At the deeper clade level (Clade 4-2) NCA indicates that restricted gene flow occurs within the *preblei/campestris/intermedius* cluster but is best explained by



**Figure 11.** Geographic spread of selected clades used in NCA. Population numbers correspond to those in Table 1.

long distance dispersal for the populations 15, 18, and 19 (Table 1 and Figure 12) and isolation by distance for the vast majority of all other *preblei*, *campestris*, and *intermedius* populations.

Networks 3 and 4 and haplotype Zp05 were comprised primarily of *Z. princeps* individuals with the exception of a few *Z. hudsonius* individuals. These divergent *Z. hudsonius* samples were discarded by Ramey et al. 2005 under the assumption that they were misclassified. This may very well be the case but as mentioned above the possible introgression of *Z. princeps* haplotypes into *Z. hudsonius* populations should be



**Figure 12.** Geographic spread of selected clades used in NCA. Population numbers correspond to those in Table 1.

considered further. Few *Z. princeps* were sampled and thus few conclusions can be drawn from NCA aside from inadequate geographic sampling in Clade 5-1 (Table 9).

## V. Subspecies designation

Much debate has centered around diagnosing units below the species level (Green 2005). At this time no established universally accepted criteria exist for diagnosing subspecies. Other units such as populations (Waples and Gaggiotti 2006) and evolutionary significant units have received more attention but still a number of competing criteria are used (Moritz 2002, Waples 2005). Crandall et al. 2000 established a methodology for rejecting or accepting evidence of distinctiveness based on genetic, ecological, recent, and historical categories. Crandall et al. established recommended management actions based on the relative strength of evidence for 8 separate cases. A criticism of the King et al. 2006 study is that no criteria are offered. However, Ramey et al. 2005, on the other hand, used the robust criteria from Crandall et al. 2000 to diagnose Distinct Vertebrate Population Segments (DPS) and concluded that ‘our results for *Z. h. preblei* and its neighboring populations [*Z. h. campestris* and *Z. h. intermedius*] do not appear to support the discrete requirement’. Cases such as that of the *Z. h. preblei* illustrate the need for explicit criteria to be established at the species, subspecies, and DPS level (Sites & Crandall 1997) as were detailed and followed in Ramey et al., but not in King et al. The ‘fuzziness’ of boundaries at each of these levels makes this a challenge but a diversity of data from ecologists, taxonomists, population geneticists, and phylogeneticists coupled with explicit criteria evaluated in a robust hypothesis testing framework make it attainable.

## VI. Conclusion

A number of general conclusions can be drawn from our reanalysis of the Ramey et al. 2005 and King et al. 2006 data sets. First, the highest resolution analysis performed in this study was the STRUCTURE analysis using microsatellite data. With both the King

et al. and the Ramey et al. data sets, we saw clusters of subspecies into three groups consisting of *Z. h. pallidus* + *Z. h. luteus*, *Z. h. intermedius* + *Z. h. campestris*, and *Z. h. prebleii*, best accounted for the genetic variation. Although some admixture was evident in the STRUCTURE analysis and moderate levels of migration rates were estimated between these clusters, we still believe relatively good population boundaries exist between these three groups. This is consistent with general population structure within a species and similar structure is found in, for example, human populations (Rosenberg et al. 2005). Such structure does not warrant conservation action and is recent in evolutionary origin.

Analysis of the mtDNA data revealed two more inclusive groupings. Phylogenetic analysis of the CytB and CR data sets fairly consistently revealed two major clades. One consisted of a combined *Z. h. intermedius* + *Z. h. campestris* + *Z. h. prebleii* group and the other a combined *Z. h. pallidus* + *Z. h. luteus* group. In most analysis these groups were monophyletic or nearly so but almost without exception none of the subspecies ever formed monophyletic groups within these distinct clades. The same ‘non-exclusive’ pattern was evident in the haplotype networks. Add to this the NCA results where range expansion or restricted gene flow with isolation by distance were inferred for most clades at most level and preponderance of evidence indicates extensive gene flow within the groups. The most parsimonious conclusion based on the current available data suggests that *Z. h. pallidus* + *Z. h. luteus* may represent a distinct ‘subspecies’ or population segment or ESU and *Z. h. intermedius* + *Z. h. campestris* + *Z. h. prebleii* form another. This conclusion is consistent with that drawn in Ramey et al. 2005.

When taken as a whole, the preponderance of evidence (mtDNA [both control region and cytb] and morphometric data indicate that the taxon *Z. h. prebleii* is not a valid taxonomic unit. The microsatellite data suggest that there may be recent population subdivision consistent with subdivision within, for example, humans (Rosenberg et al. 2005). The data clearly show a grouping of *Z. h. pallidus* + *Z. h. luteus* relative to *Z. h. intermedius* + *Z. h. campestris* + *Z. h. prebleii*. These groupings form what we would consider ESUs and demonstrate the invalidity of the current taxonomy for these subspecies and throws questions on the taxonomy of the species complex as a whole. If

we employ the Crandall et al. (2000) criteria for ESU designation on the “subspecies” *Z. h. preblei*, the subspecies shows, at most, recent genetic inexchangeability, with historical exchangeability and ecological exchangeability both recently and historically. As such, it would fall under Case 8 with a management recommendation to “treat as a single population” (*Z. h. intermedius* + *Z. h. campestris* + *Z. h. preblei*). Note that the “subspecies” *Z. h. preblei* would also not be found to be an ESU under the more stringent criteria of Mortiz (1994), the criterion used by King et al. in their assessment, due to the lack of reciprocal monophyly as required by the Moritz (1994) definition. Thus using an explicit framework for testing taxonomic boundaries (Crandall et al. 2000 & Mortiz 1994), we find that the “subspecies” *Z. h. preblei* does not even warrant ESU status, let alone subspecific status and therefore recommend it be delisted as an endangered entity under the Endangered Species Act.

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**Table 1.** Localities for all samples the King et al. & Ramey et al. studies. Abbr = abbreviations used in combined data set to indicate county and state of sample. Zh1 = *Zapus hudsonius luteus*, Zhpr = *Zapus hudsonius preblei*, Zhi = *Zapus hudsonius intermedius*, Zhpa = *Zapus hudsonius pallidus*, Zhc = *Zapus hudsonius campestris*, ZPp = *Zapus princeps princeps*, ZPid = *Zapus princeps idahoensis*, ZPut = *Zapus princeps utahensis*. Samples were pooled by county and GPS coordinates taken from geographical center of county.

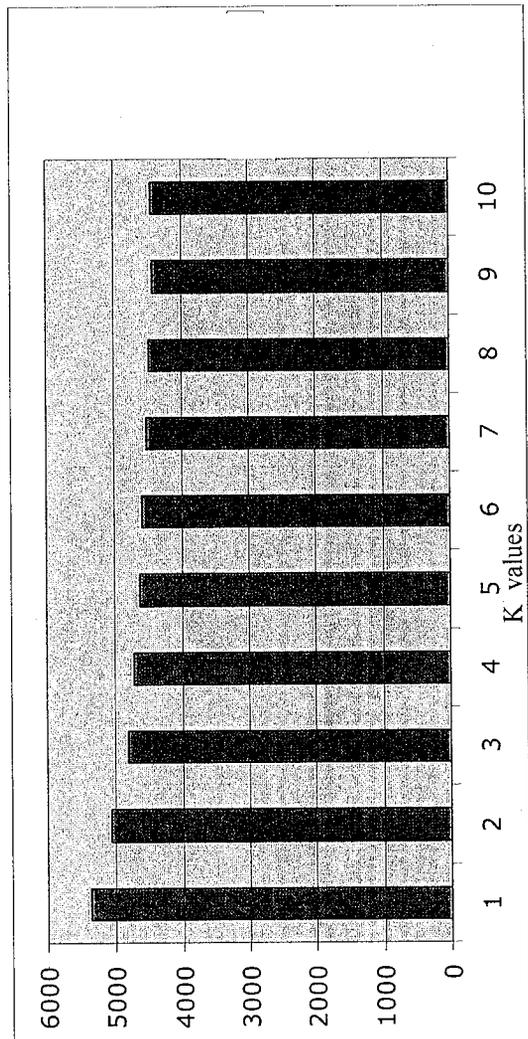
	State	County	Abbr	N	Species	GPS coordinates
1	Arizona	Apache	ApAZ	3	Zh1	N 35 <sup>0</sup> 50' W 109 <sup>0</sup> 32'
2		Navajo	NCAZ	4	Zh1	N 35 <sup>0</sup> 42' W 110 <sup>0</sup> 37'
3	Colorado	Boulder	BCCO	9	Zhpr	N 40 <sup>0</sup> 07' W 105 <sup>0</sup> 29'
4		Conejo	CCCO	1	Zhpr	N 37 <sup>0</sup> 15' W 105 <sup>0</sup> 58'
5		Douglas	DCCO	74	Zhpr	N 39 <sup>0</sup> 18' W 105 <sup>0</sup> 12'
6		El Paso	ECCO	61	Zhpr	N 39 <sup>0</sup> 04' W 104 <sup>0</sup> 15'
7		Elbert	EbCO	1	Zhpr	N 39 <sup>0</sup> 16' W 103 <sup>0</sup> 34'
8		Gilpin	GCCO	1	Zhpr	N 39 <sup>0</sup> 52' W 105 <sup>0</sup> 40'
9		Jefferson	JCCO	1	Zhpr	N 39 <sup>0</sup> 30' W 105 <sup>0</sup> 18'
10		Larimer	LCCO	33	Zhpr	N 40 <sup>0</sup> 40' W 105 <sup>0</sup> 38'
11		Las Animas	LACO	8	Zh1	N 37 <sup>0</sup> 13' W 103 <sup>0</sup> 23'
12		Teller	TCCO	2	Zhpr/ZPp	N 39 <sup>0</sup> 46' W 105 <sup>0</sup> 18'
13	Iowa	Buena Vista	BVIA	1	Zhi	N 42 <sup>0</sup> 38' W 95 <sup>0</sup> 12'
14		Emmet	ECIA	3	Zhi	N 43 <sup>0</sup> 24' W 94 <sup>0</sup> 50'
15		Marion	MCIA	1	Zhi	N 41 <sup>0</sup> 19' W 93 <sup>0</sup> 06'
16		Tama	TCIA	1	Zhi	N 42 <sup>0</sup> 04' W 92 <sup>0</sup> 24'
17		Winneshiek	WCIA	1	Zhi	N 43 <sup>0</sup> 18' W 91 <sup>0</sup> 47'
18	Illinois	Henry	HCIL	1	Zhi	N 41 <sup>0</sup> 06' W 90 <sup>0</sup> 12'
19	Indiana	Wayne	WCIN	1	Zhi	N 39 <sup>0</sup> 36' W 85 <sup>0</sup> 02'
20	Kansas	Douglas	DCKS	2	Zhpa	N 38 <sup>0</sup> 57' W 95 <sup>0</sup> 23'
21		Leavenworth	LCKS	2	Zhpa	N 39 <sup>0</sup> 20' W 94 <sup>0</sup> 59'
22		Osage	OCKS	2	Zhpa	N 38 <sup>0</sup> 38' W 95 <sup>0</sup> 48'
23	Minnesota	Morrison	MCMN	21	Zhi	N 46 <sup>0</sup> 13' W 94 <sup>0</sup> 34'
24	Missouri	Macon	MAMO	2	Zhpa	N 39 <sup>0</sup> 45' W 92 <sup>0</sup> 52'
25	Montana	Carter	CCMT	5	Zhc	N 45 <sup>0</sup> 23' W 104 <sup>0</sup> 42'
26	North Dakota	Burleigh	BCND	6	Zhi	N 47 <sup>0</sup> 14' W 100 <sup>0</sup> 12'
27		Dunn	DCND	5	Zhi	N 47 <sup>0</sup> 23' W 102 <sup>0</sup> 52'
28		Mercer	MCND	1	Zhi	N 47 <sup>0</sup> 20' W 102 <sup>0</sup> 01'
29	Nebraska	Antelope	ACNE	4	Zhpa	N 42 <sup>0</sup> 04' W 97 <sup>0</sup> 58'
30		Boyd	BONE	1	Zhpa	N 42 <sup>0</sup> 52' W 98 <sup>0</sup> 42'
31		Buffalo	BCNE	25	Zhpa	N 40 <sup>0</sup> 47' W 99 <sup>0</sup> 09'
32		Dixon	DCNE	1	Zhpa	N 42 <sup>0</sup> 41' W 97 <sup>0</sup> 02'
33		Dodge	DGNE	1	Zhpa	N 41 <sup>0</sup> 42' W 96 <sup>0</sup> 50'
34		Garden	GCNE	2	Zhpa	N 41 <sup>0</sup> 41' W 102 <sup>0</sup> 20'
35		Hall	HCNE	3	Zhpa	N 40 <sup>0</sup> 55' W 98 <sup>0</sup> 22'
36		Holt	HONE	2	Zhpa	N 42 <sup>0</sup> 27' W 98 <sup>0</sup> 39'
37		Kearney	KCNE	11	Zhpa	N 40 <sup>0</sup> 30' W 98 <sup>0</sup> 57'
38		Lancaster	LCNE	1	Zhpa	N 40 <sup>0</sup> 51' W 96 <sup>0</sup> 43'

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39		Merrick	MCNE	1	Zhpa	N 41 <sup>0</sup> 07' W 97 <sup>0</sup> 60'
40		Thomas	TCNE	1	Zhpa	N 41 <sup>0</sup> 58' W 100 <sup>0</sup> 33'
41	New Mexico	Bernalillo	BCNM	1	Zhl	N 35 <sup>0</sup> 30' W 106 <sup>0</sup> 46'
42		Otero	OCNM	7	Zhl	N 32 <sup>0</sup> 48' W 105 <sup>0</sup> 45'
43		Rio Arriba	RANM	2	Zhl	N 36 <sup>0</sup> 32' W 106 <sup>0</sup> 47'
44		Sandoval	SCNM	26	Zhl	N 35 <sup>0</sup> 52' W 106 <sup>0</sup> 48'
45		Socorro	SONM	1	Zhl	N 33 <sup>0</sup> 50' W 107 <sup>0</sup> 11'
46		Valencia	VCNM	1	Zhl	N 34 <sup>0</sup> 46' W 106 <sup>0</sup> 58'
47	South Dakota	Bennett	BeCSD	18	Zhpa	N 43 <sup>0</sup> 10' W 101 <sup>0</sup> 50'
48		Brown	BrCSD	33	Zhi	N 45 <sup>0</sup> 37' W 98 <sup>0</sup> 26'
49		Custer	CCSD	29	Zhc	N 43 <sup>0</sup> 43' W 103 <sup>0</sup> 01'
50		Deuel	DCSD	3	Zhi	N 44 <sup>0</sup> 40' W 96 <sup>0</sup> 49'
51		Harding	HCSO	3	Zhc	N 45 <sup>0</sup> 27' W 103 <sup>0</sup> 33'
52		Lawrence	LaSD	3	Zhc	N 44 <sup>0</sup> 29' W 103 <sup>0</sup> 44'
53		Lincoln	LCSD	2	Zhi	N 43 <sup>0</sup> 15' W 96 <sup>0</sup> 48'
54		Minnehaha	MCSD	3	Zhi	N 43 <sup>0</sup> 42' W 96 <sup>0</sup> 44'
55		Moody	MOSD	1	Zhi	N 43 <sup>0</sup> 50' W 96 <sup>0</sup> 40'
56		Pennington	PCSD	9	Zhc	N 44 <sup>0</sup> 13' W 102 <sup>0</sup> 30'
57		Union	UCSD	1	Zhi	N 42 <sup>0</sup> 59' W 96 <sup>0</sup> 42'
58		Walworth	WCSD	5	Zhi	N 45 <sup>0</sup> 22' W 100 <sup>0</sup> 03'
59	Wyoming	Albany	AbWY	16	Zhp/ZPp	N 41 <sup>0</sup> 18' W 105 <sup>0</sup> 32'
60		Crook	CCWY	33	Zhc	N 44 <sup>0</sup> 38' W 104 <sup>0</sup> 46'
61		Fremont	FCWY	3	ZPid	N 43 <sup>0</sup> 04' W 108 <sup>0</sup> 14'
62		Larimae	LCWY	2	Zhpr	N 41 <sup>0</sup> 09' W 104 <sup>0</sup> 33'
63		Park	PaWY	3	Zpid	N 44 <sup>0</sup> 31' W 109 <sup>0</sup> 25'
64		Platte	PCWY	1	Zhpr	N 41 <sup>0</sup> 58' W 104 <sup>0</sup> 46'
65		Teton	TCWY	4	ZPut	N 43 <sup>0</sup> 59' W 110 <sup>0</sup> 12'
66		Weston	WCWY	4	Zhc	N 43 <sup>0</sup> 52' W 104 <sup>0</sup> 35'

**Table 2-** A summary of likelihoods scores for each STRUCTURE run for Ramey et al. data, average likelihood scores for each K for all runs, and a visual representation of the absolute values of these scores.

	K									
	1	2	3	4	5	6	7	8	9	10
Run 01	-5366.8	-5057.1	-4802.5	-4695.5	-4590.1	-4582	-4508.1	-4471.6	-4420.6	-4725.8
	-5364.7	-5049.4	-4799.6	-4694.9	-4585	-4530.1	-4517.5	-4507.9	-4463	-4402.4
	-5365.7	-5055.5	-4805.7	-4697.2	-4593.3	-4596.5	-4502.8	-4460.6	-4425	-4409.8
	-5365.9	-5065.1	-4794.3	-4726.5	-4815.9	-4533.3	-4505.8	-4520	-4421.9	-4411.1
	-5360.4	-5075.1	-4801.5	-4717.4	-4586.6	-4527.1	-4509.3	-4461.3	-4413.6	-4452.7
	-5361.2	-5077.3	-4803.9	-4814.8	-4672	-4603.4	-4504	-4445.3	-4421.6	-4434.7
	-5367.2	-5054.1	-4795.4	-4703	-4585	-4802.7	-4508	-4467.9	-4403.9	-4412.6
	-5364.6	-5085.8	-4801.4	-4759.2	-4582.6	-4571.2	-4516.7	-4468.3	-4417.4	-4415.1
	-5365.4	-5058.8	-4801	-4707	-4585.5	-4523.2	-4515.8	-4479.8	-4425.5	-4411
Run 10	-5359.7	-5084.7	-4805.5	-4702.4	-4582.5	-4576.3	-4504.3	-4449.3	-4409.2	-4428.9
Aver.-Ln	5364.16	-5066.29	-4801.08	-4721.79	-4617.85	-4584.58	-4509.23	-4473.2	-4422.17	-4450.41



**Table 3.** Inferred ancestry of all individuals based on the run with the best likelihood score (-4794.3, run 4, Table 2) at K = 3. Probabilities in bold text indicate cluster with highest assignment. Individuals in bold red text indicate individuals with mixed ancestry (no probability > 0.80) and individuals that belong to one subspecies but have highest probability ancestry assigned to a cluster with predominately individuals from a different subspecies are indicated in green. Importantly, individuals indicated in blue text are ones that are *Z.h. campestris* who have a high probability assignment to cluster 3 but have *Z.h. preblei* control region mitochondrial DNA. Cluster 1 consists of predominately *Z.h. preblei* individuals, Cluster 2 consists of predominately *Z.h. luteus* and *Z.h. pallidus* individuals, and, Cluster 3 consists of predominately *Z.h. campestris* and *Z.h. intermedius* individuals.

Inferred ancestry of individuals:

Label	(%Miss)	: Inferred clusters
		: <u>Clstr 1</u> <u>Clstr 2</u> <u>Clstr 3</u>
1 ZhprNM871	(0)	: <b>0.983</b> 0.010 0.008
2 M872Zhpr	(0)	: <b>0.860</b> 0.035 0.105
3 M876Zhpr	(0)	: <b>0.970</b> 0.013 0.018
4 <b>M877Zhpr</b>	(0)	: <b>0.763 0.107 0.130</b>
5 TK86021Zhpr	(0)	: <b>0.840</b> 0.039 0.121
6 TK86034Zhpr	(0)	: <b>0.899</b> 0.026 0.076
7 <b>TK86048Zhpr</b>	(0)	: <b>0.182 0.792</b> 0.027
8 TK86090Zhpr	(0)	: <b>0.873</b> 0.018 0.108
9 TK86105Zhpr	(0)	: <b>0.973</b> 0.013 0.014
10 <b>TK86074Zhpr</b>	(0)	: <b>0.477 0.476</b> 0.047
11 TK86094Zhpr	(0)	: <b>0.895</b> 0.092 0.013
12 9A34Zhpr	(0)	: <b>0.977</b> 0.011 0.012
13 9B89Zhpr	(0)	: <b>0.948</b> 0.024 0.028
14 M874Zhpr	(0)	: <b>0.976</b> 0.010 0.014
15 TK86081Zhpr	(0)	: <b>0.980</b> 0.010 0.011
16 TK86109Zhpr	(0)	: <b>0.962</b> 0.024 0.014
17 TK86117Zhpr	(0)	: <b>0.942</b> 0.023 0.036
18 TK86095Zhpr	(0)	: <b>0.952</b> 0.019 0.029
19 TK86096Zhpr	(0)	: <b>0.970</b> 0.014 0.016
20 TK86097Zhpr	(0)	: <b>0.965</b> 0.028 0.007
21 TK86098Zhpr	(0)	: <b>0.973</b> 0.010 0.017
22 TK86026Zhpr	(0)	: <b>0.986</b> 0.006 0.008
23 TK86029Zhpr	(0)	: <b>0.959</b> 0.030 0.011
24 TK86030Zhpr	(0)	: <b>0.918</b> 0.072 0.009
25 TK86031Zhpr	(0)	: <b>0.979</b> 0.009 0.012
26 TK86032Zhpr	(0)	: <b>0.958</b> 0.020 0.022
27 TK86080Zhpr	(0)	: <b>0.971</b> 0.007 0.022
28 TK86083Zhpr	(0)	: <b>0.978</b> 0.014 0.008
29 TK86115Zhpr	(0)	: <b>0.977</b> 0.010 0.013
30 TK86116Zhpr	(0)	: <b>0.977</b> 0.013 0.011
31 TK86120Zhpr	(0)	: <b>0.981</b> 0.008 0.011

32 TK86121Zhpr	(0)	: <b>0.983</b> 0.008 0.009
33 TK86122Zhpr	(0)	: <b>0.986</b> 0.006 0.008
34 TK86196Zhpr	(0)	: <b>0.946</b> 0.038 0.016
35 TK86163Zhpr	(0)	: <b>0.977</b> 0.009 0.014
36 M875Zhpr	(0)	: <b>0.987</b> 0.007 0.006
37 TK51406Zhpr	(0)	: <b>0.893</b> 0.016 0.090
38 TK86124Zhpr	(0)	: <b>0.980</b> 0.012 0.008
39 TK86088Zhpr	(0)	: <b>0.944</b> 0.045 0.011
40 M879Zhpr	(0)	: <b>0.988</b> 0.006 0.006
41 M1166Zhpr	(0)	: <b>0.951</b> 0.008 0.041
42 TK86093Zhpr	(0)	: <b>0.978</b> 0.009 0.013
43 TK86106Zhpr	(0)	: <b>0.985</b> 0.009 0.006
44 TK86107Zhpr	(0)	: <b>0.974</b> 0.012 0.013
45 TK86118Zhpr	(0)	: <b>0.971</b> 0.009 0.019
46 TK86165Zhpr	(0)	: <b>0.982</b> 0.007 0.011
47 TK86166Zhpr	(0)	: <b>0.981</b> 0.011 0.008
48 TK86167Zhpr	(0)	: <b>0.954</b> 0.035 0.011
49 TK86169Zhpr	(0)	: <b>0.986</b> 0.006 0.008
50 TK86170Zhpr	(0)	: <b>0.983</b> 0.008 0.009
51 TK86173Zhpr	(0)	: <b>0.976</b> 0.010 0.014
52 TK86182Zhpr	(0)	: <b>0.988</b> 0.006 0.006
53 TK86183Zhpr	(0)	: <b>0.982</b> 0.008 0.010
54 TK86185Zhpr	(0)	: <b>0.983</b> 0.008 0.009
55 <b>ZhcTK86190</b>	(0)	: 0.100 <b>0.229 0.671</b>
56 <b>TK86191Zhc</b>	(0)	: <b>0.755</b> 0.015 <b>0.230</b>
57 KU123597Zhc	(0)	: 0.028 0.046 <b>0.926</b>
58 KU123598Zhc	(0)	: 0.014 0.009 <b>0.977</b>
59 <b>KU123599Zhc</b>	(0)	: 0.095 <b>0.535 0.370</b>
60 KU101558Zhc	(0)	: 0.148 0.026 <b>0.827</b>
61 KU109972Zhc	(0)	: 0.023 <b>0.283 0.694</b> --- Zhp mtDNA
62 KU109978Zhc	(0)	: 0.053 0.010 <b>0.937</b> --- Zhp mtDNA
63 <b>KU109984Zhc</b>	(0)	: <b>0.382 0.411</b> 0.207 --- Zhp mtDNA
64 KU109985Zhc	(0)	: 0.014 0.017 <b>0.969</b> --- Zhp mtDNA
65 KU110013Zhc	(0)	: 0.005 0.007 <b>0.988</b> --- Zhp mtDNA
66 <b>KU7Zhc</b>	(0)	: <b>0.466 0.054</b> 0.480
67 KU8Zhc	(0)	: 0.026 0.015 <b>0.959</b>
68 KU13Zhc	(0)	: 0.036 0.017 <b>0.947</b>
69 KU14Zhc	(33)	: 0.016 0.011 <b>0.973</b>
70 KU18Zhc	(0)	: 0.008 0.023 <b>0.969</b>
71 KU19Zhc	(0)	: 0.007 0.012 <b>0.980</b>
72 KU20Zhc	(0)	: 0.007 0.098 <b>0.895</b>
73 KU23Zhc	(16)	: 0.013 0.023 <b>0.964</b>
74 <b>KU24Zhc</b>	(0)	: <b>0.718</b> 0.068 0.215
75 <b>KU25Zhc</b>	(0)	: <b>0.261</b> 0.028 <b>0.711</b>
76 <b>KU26Zhc</b>	(0)	: <b>0.225</b> 0.022 <b>0.753</b>

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77	KU1235Zhc	(0)	:	0.007	0.008	<b>0.985</b>
78	KU123593Zhc	(0)	:	0.006	0.008	<b>0.986</b>
79	KU27Zhc	(16)	:	0.012	0.015	<b>0.972</b>
80	KU28Zhc	(16)	:	0.007	0.010	<b>0.983</b>
81	KU29Zhc	(0)	:	0.067	0.008	<b>0.925</b>
82	<b>KU30Zhc</b>	(0)	:	<b>0.228</b>	0.046	<b>0.726</b>
83	KU34Zhc	(0)	:	0.013	0.008	<b>0.979</b>
188	<b>K127252Zhia</b>	(0)	:	0.008	<b>0.953</b>	0.040
189	<b>K112830Zhi</b>	(0)	:	0.005	<b>0.980</b>	0.014
190	<b>K116263Zhi</b>	(0)	:	<b>0.389</b>	<b>0.315</b>	<b>0.295</b>
191	K116264Zhi	(0)	:	0.027	0.090	<b>0.883</b>
192	<b>K116266Zhi</b>	(0)	:	0.034	<b>0.481</b>	<b>0.485</b>
193	<b>K116269Zhi</b>	(0)	:	0.006	<b>0.958</b>	0.035
194	<b>K104062Zhi</b>	(0)	:	0.165	<b>0.410</b>	<b>0.425</b>
195	<b>K108068Zhi</b>	(0)	:	0.025	<b>0.728</b>	<b>0.247</b>
84	DMNS7764Zhi	(0)	:	0.013	0.008	<b>0.978</b>
85	K115895Zhi	(0)	:	0.008	0.040	<b>0.952</b>
86	K115896Zhi	(0)	:	0.007	0.009	<b>0.984</b>
87	K115897Zhi	(0)	:	0.010	0.031	<b>0.960</b>
88	K123021Zhi	(0)	:	0.013	0.020	<b>0.967</b>
89	K123022Zhi	(0)	:	0.014	0.034	<b>0.952</b>
90	K123031Zhi	(0)	:	0.007	0.133	<b>0.860</b>
91	K123032Zhi	(0)	:	0.023	0.013	<b>0.964</b>
92	K123033Zhi	(0)	:	0.008	0.015	<b>0.976</b>
155	<b>K140721Zhi</b>	(0)	:	<b>0.521</b>	0.132	<b>0.347</b>
156	<b>K140722Zhi</b>	(0)	:	<b>0.199</b>	<b>0.385</b>	<b>0.416</b>
157	<b>K153176Zhi</b>	(0)	:	0.010	<b>0.202</b>	<b>0.788</b>
158	K153177Zhi	(0)	:	0.017	0.030	<b>0.953</b>
159	K153180Zhi	(0)	:	0.039	0.014	<b>0.947</b>
160	K153181Zhi	(0)	:	0.040	0.043	<b>0.917</b>
161	<b>K153190Zhi</b>	(0)	:	<b>0.156</b>	0.059	<b>0.785</b>
162	K153196Zhi	(0)	:	0.013	0.028	<b>0.960</b>
163	K147018Zhi	(0)	:	0.017	0.011	<b>0.972</b>
164	K147020Zhi	(0)	:	0.019	0.017	<b>0.964</b>
165	K153201Zhi	(0)	:	0.119	0.017	<b>0.864</b>
166	K153203Zhi	(8)	:	0.091	0.013	<b>0.897</b>
167	<b>K153205Zhi</b>	(0)	:	0.044	<b>0.919</b>	0.038
168	K153212Zhi	(0)	:	0.021	0.062	<b>0.918</b>
169	K115700Zhi	(0)	:	0.013	0.026	<b>0.962</b>
170	K115702Zhi	(0)	:	0.010	0.040	<b>0.950</b>
171	<b>K115710Zhi</b>	(0)	:	0.026	<b>0.600</b>	<b>0.374</b>
172	<b>K120017Zhi</b>	(0)	:	0.042	<b>0.701</b>	<b>0.256</b>
173	K120018Zhi	(0)	:	0.026	0.012	<b>0.962</b>
174	K120019Zhi	(0)	:	0.018	0.050	<b>0.931</b>
175	<b>K153215Zhi</b>	(0)	:	0.027	<b>0.197</b>	<b>0.775</b>

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176 **K153221Zhi** (0) : 0.088 **0.792 0.120**  
 177 K115730Zhi (0) : 0.008 0.012 **0.980**  
 178 K115731Zhi (0) : 0.006 0.052 **0.942**  
 179 K115732Zhi (0) : 0.023 0.018 **0.958**  
 180 K116265Zhi (0) : 0.025 0.012 **0.963**  
 181 K159190Zhi (0) : 0.094 0.053 **0.853**  
 182 K153230Zhi (0) : 0.099 0.035 **0.865**  
 183 K153229Zhi (0) : 0.016 0.007 **0.977**

93 **ZhpaUNL1UNS** (0) : 0.054 **0.655 0.292**  
 94 ZhpaUNL2UNS (0) : 0.015 **0.916** 0.069  
 95 ZhpaUNL3UNS (0) : 0.017 **0.957** 0.026  
 96 **ZhpaUNL4UNS** (0) : 0.010 **0.372 0.617**  
 97 **ZhpaUNL5UNS** (0) : 0.107 **0.652 0.241**  
 98 ZhpaUNL7UNS (0) : 0.016 **0.971** 0.013  
 99 ZhpaUNL8UNS (0) : 0.010 **0.946** 0.044  
 100 ZhpaUNL9UNS (0) : 0.031 **0.817** 0.152  
 101 ZhpaUNL12UN (0) : 0.068 **0.865** 0.067  
 102 ZhpaUNL16UN (0) : 0.015 **0.945** 0.040  
 103 **ZhpaUNL23UN** (0) : 0.015 **0.355 0.630**  
 104 ZhpaUNL26UN (0) : 0.019 **0.892** 0.090  
 105 ZhpaUNL27UN (0) : 0.077 **0.913** 0.010  
 106 ZhpaUNL28UN (0) : 0.022 **0.953** 0.025  
 107 ZhpaUNL35UN (0) : 0.006 **0.979** 0.015  
 108 ZhpaUNL36UN (0) : 0.030 **0.860** 0.111  
 109 **ZhpaUNL37UN** (0) : 0.009 **0.738 0.253**  
 110 ZhpaUNL41UN (0) : 0.013 **0.962** 0.025  
 111 ZhpaUNL42UN (0) : 0.023 **0.952** 0.025  
 112 ZhpaUNL46UN (0) : 0.023 **0.953** 0.024  
 113 ZhpaUNL51UN (0) : 0.010 **0.961** 0.029  
 114 ZhpaUNL55UN (0) : 0.035 **0.896** 0.070  
 115 ZhpaUNL56UN (0) : 0.023 **0.970** 0.007  
 116 KU40Zhpa (0) : 0.155 **0.801** 0.044  
 117 KU44Zhpa (0) : 0.028 **0.961** 0.011  
 118 KU45Zhpa (0) : 0.080 **0.867** 0.053  
 119 KU47Zhpa (0) : 0.008 **0.970** 0.023  
 120 **KU48Zhpa** (0) : 0.031 **0.716 0.252**  
 121 KU51Zhpa (0) : 0.022 **0.964** 0.014  
 122 KU52Zhpa (0) : 0.014 **0.976** 0.011  
 184 **UNL60Zhpa** (0) : 0.087 0.018 **0.896**  
 185 **UNL61Zhpa** (0) : 0.126 0.040 **0.834**  
 186 KU53Zhpa (0) : 0.018 **0.975** 0.007  
 187 KU54Zhpa (0) : 0.010 **0.982** 0.008

123 DMNH8630Zhl (0) : 0.009 **0.978** 0.013  
 124 DMNH8631Zhl (0) : 0.016 **0.964** 0.019

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125 DMNH8632Zhl	(0)	:	0.010	<b>0.982</b>	0.008
126 DMNH8633Zhl	(0)	:	0.008	<b>0.978</b>	0.014
127 DMNH8634Zhl	(0)	:	0.009	<b>0.975</b>	0.016
128 DMNH8635Zhl	(0)	:	0.008	<b>0.985</b>	0.007
129 NK856Zhl	(0)	:	0.011	<b>0.979</b>	0.010
130 NK871Zhl	(0)	:	0.008	<b>0.986</b>	0.007
131 NK884Zhl	(0)	:	0.034	<b>0.942</b>	0.023
132 NK1584Zhl	(0)	:	0.019	<b>0.951</b>	0.031
133 NK9976Zhl	(0)	:	0.014	<b>0.977</b>	0.009
134 MSB2Zhl	(0)	:	0.021	<b>0.960</b>	0.019
135 MSB4Zhl	(0)	:	0.019	<b>0.964</b>	0.017
136 MSB5Zhl	(0)	:	0.056	<b>0.923</b>	0.021
137 MSB6Zhl	(0)	:	0.014	<b>0.954</b>	0.032
138 MSB7Zhl	(0)	:	0.091	<b>0.882</b>	0.026
139 MSB8Zhl	(0)	:	0.038	<b>0.947</b>	0.015
140 MSB9Zhl	(0)	:	0.022	<b>0.969</b>	0.009
141 MSB11Zhl	(0)	:	0.006	<b>0.985</b>	0.009
142 MSB12Zhl	(0)	:	0.028	<b>0.963</b>	0.009
143 MSB14Zhl	(0)	:	0.010	<b>0.984</b>	0.006
144 MSB16Zhl	(0)	:	0.009	<b>0.953</b>	0.038
145 MSB18Zhl	(0)	:	0.012	<b>0.974</b>	0.014
146 MSB19Zhl	(0)	:	0.022	<b>0.858</b>	0.121
147 MSB20Zhl	(0)	:	0.072	<b>0.914</b>	0.013
148 MSB21Zhl	(0)	:	0.008	<b>0.978</b>	0.014
149 MSB23Zhl	(0)	:	0.013	<b>0.977</b>	0.011
150 MSB24Zhl	(0)	:	0.014	<b>0.975</b>	0.011
151 MSB25Zhl	(0)	:	0.009	<b>0.854</b>	0.137
152 MSB26Zhl	(0)	:	0.011	<b>0.859</b>	0.130
153 MSB27Zhl	(0)	:	0.026	<b>0.959</b>	0.015
154 MSB30Zhl	(0)	:	0.008	<b>0.982</b>	0.009

**Table 4.** Summary of results for MIGRATE analysis of Ramey et al. microsatellite data between three hypothesized populations based on three separate runs. A) Theta ( $\Theta$ ) is equal to the estimated effective population size and Mxy is equal to the relative importance of migration from cluster on ‘x’ axis into cluster on ‘y’ axis relative to mutation rate in introducing new variants into the population. B) Nm estimates based on Migrate results run 2.

**A)**

Runs	Clusters	$\Theta$	Mxy(m/ $\mu$ )			Chains
			Zhpr	Zhc/Zhi	Zhpa/Zhl	
Run 1	Zhpr	1.49165	-----	1.4871	1.1340	<b>Short = 10</b> <b>Long = 3</b>
	Zhc/Zhi	4.75791	1.9401	-----	4.1631	
	Zhpa/Zhl	4.04230	1.3132	5.1773	-----	
Run 2	Zhpr	1.86589	-----	0.9990	1.0143	<b>Short = 10</b> <b>Long = 3</b>
	Zhc/Zhi	3.73727	2.2950	-----	4.4632	
	Zhpa/Zhl	4.60656	1.0230	4.9991	-----	
Run 3	Zhpr	1.14171	-----	1.5501	1.4162	<b>Short = 10</b> <b>Long = 3</b>
	Zhc/Zhi	3.80171	1.7774	-----	5.3583	
	Zhpa/Zhl	6.90139	0.6874	3.2829	-----	

**B)**

	Nm(xy)		
	Zhpr	Zhc/Zhi	Zhpa/Zhl
Zhpr	---	0.46	0.47
Zhc/Zhi	2.14	---	4.17
Zhpa/Zhl	1.18	5.76	---

Number of migrants from ‘x’ axis cluster into ‘y’ axis cluster

**Table 5.** A list of collapsed (identical) control region mitochondrial DNA haplotypes based on a combined data set from Ramey et al. 2005 and King et al. 2006. A total of 63 different haplotypes were found. Haplotypes are named to represent subspecies where haplotype is found. Pr = *Z. h. preblei*, C = *Z. h. campestris*, Pa = *Z. h. pallidus*, I = *Z. h. intermedius*, L = *Z. h. luteus*, and Zp = *Z. princeps*.

List of haplotype names:

**1. PrC01**

DCCOZhprTK86026 [60]  
 AbWYZhprTK86098  
 AbWYZhprTK86124  
 BCCOZhprTK86021  
 BCCOZhprTK86034  
 BCCOZhprTK86048  
 BCCOZhprTK86090  
 BCCOZhprTK86105  
 BCCOZhprXM871  
 BCCOZhprXM872  
 BCCOZhprXM876  
 BCCOZhprXM877  
 DCCOZhprTK86029  
 DCCOZhprTK86030  
 DCCOZhprTK86031  
 DCCOZhprTK86032  
 DCCOZhprTK86080  
 DCCOZhprTK86083  
 DCCOZhprTK86115  
 DCCOZhprTK86116  
 EbCOZhprTK86163  
 GCCOZhprXM874  
 JCCOZhprTK51406  
 LCCOZhprTK86109  
 ZHprDCCOMAY215  
 ZHprDCCOMAY229  
 ZHprDCCOMAY234  
 ZHprDCCOMAY268  
 ZHprDCCOMAY281  
 ZHprDCCOMAY374  
 ZHprDCCOMAY385  
 ZHprDCCOMAY408  
 ZHprDCCOMAY416  
 ZHprDCCOMAY452  
 ZHprDCCOMAY494  
 ZHprDCCOMAY497  
 ZHprDCCOMAY517  
 ZHprDCCOMAY532  
 ZHprDCCOMAY694

ZHprDCCOMAY714  
ZHprDCCOMAY748  
ZHprDCCOMAY798  
ZHprDCCOMAY817  
ZHprDCCOMAY822  
ZHprDCCOMAY880  
ZHprDCCOMAY946  
ZHprDCCOMAY964  
ZHprDCCOMAY9813  
ZHprDCCOMAY9814  
ZHprDCCOMAY940  
ZHprDCCOWH98100  
ZHprDCCOWH98107  
ZHprDCCOWH98109  
ZHprDCCOWH98110  
ZHprDCCOWH98301  
ZHprLCCOSP169  
ZHprLCCOSP223  
ZHprLCCOSP861  
ZHprLCCOYG9803  
CCSDZheaK110013

**2. PrC02**

LCCOZhpr9A43 [35]  
LCCOZhpr9B89  
LCCOZhprTK86081  
LCCOZhprTK86117  
LCWYZhprTK86074  
PCWYZhprTK86094  
ZHprLCCOBG9801  
ZHprLCCOBG9802  
ZHprLCCOCER9801  
ZHprLCCOCER9802  
ZHprLCCOCER9803  
ZHprLCCOCER9804  
ZHprLCCOCER980  
ZHprLCCOHRK981  
ZHprLCCOHRK982  
ZHprLCCOHRK984  
ZHprLCCOMC9801  
ZHprLCCOMC9803  
ZHprLCCONFP9801  
ZHprLCCONFP9802  
ZHprLCCOPGC9801  
ZHprLCCOSP125  
ZHprLCCOSP170  
ZHprLCCOSP243  
ZHprLCCOSP336  
ZHprLCCOSP367  
ZHprLCCOSP375  
ZHprLCCOSP674

ZHprLCCOSP746  
ZHprLCCOYG9801  
AbWYZhprTK86095  
AbWYZhprTK86096  
AbWYZhprTK86097  
CCSDZhcaK109984  
CCSDZhcaK109985

**3. PrC03**

DCCOZhprTK86120 [58]  
DCCOZhprTK86121  
DCCOZhprTK86122  
ECCOZhprTK86093  
ECCOZhprTK86106  
ECCOZhprTK86107  
ECCOZhprTK86118  
ECCOZhprTK86166  
ECCOZhprTK86167  
ECCOZhprXM1166  
ECCOZhprXM875  
ECCOZhprXM879  
ZHprDCCOMAY127  
ZHprDCCOMAY254  
ZHprDCCOMAY368  
ZHprDCCOMAY429  
ZHprDCCOMAY706  
ZHprDCCOMAY785  
ZHprDCCOWH9801  
ZHprDCCOWH9802  
ZHprDCCOWH9803  
ZHprDCCOWH98102  
ZHprDCCOWH98103  
ZHprDCCOWH9810  
ZHprDCCOWH98106  
ZHprDCCOWH98108  
ZHprDCCOWH98120  
ZHprDCCOWH98300  
ZHprDCCOWH98303  
ZHprDCCOWH98304  
ZHprDCCOWH98305  
ZHprDCCOWH98306  
ZHprDCCOWH98309  
ZHprDCCOWH98311  
ZHprDCCOWH98312  
ZHprDCCOWH98313  
ZHprECCO003  
ZHprECCO004  
ZHprECCO005  
ZHprECCO011  
ZHprECCO015  
ZHprECCO016

ZHprECCO020  
ZHprECCO021  
ZHprECCO027  
ZHprECCO080  
ZHprECCO087  
ZHprECCO088  
ZHprECCO091  
ZHprECCO092  
ZHprECCO093  
ZHprECCO095  
ZHprECCO100  
ZHprECCO102  
ZHprECCO103  
ZHprECCO104  
CCMTZheaK123592  
CCSDZheaK109978

**4. PrC04**

DCCOZhprTK86196 [39]  
ECCOZhprTK86165  
ECCOZhprTK86169  
ECCOZhprTK8617  
ECCOZhprTK8613  
ECCOZhprTK86182  
ECCOZhprTK86183  
ECCOZhprTK86185  
TCCOZhprTK86088  
ZHprDCCOWH9805  
ZHprDCCOWH9811  
ZHprDCCOWH98104  
ZHprDCCOWH98121  
ZHprECCO00  
ZHprECCO007  
ZHprECCO008  
ZHprECCO010  
ZHprECCO013  
ZHprECCO018  
ZHprECCO019  
ZHprECCO024  
ZHprECCO025  
ZHprECCO026  
ZHprECCO079  
ZHprECCO081  
ZHprECCO082  
ZHprECCO083  
ZHprECCO084  
ZHprECCO085  
ZHprECCO086  
ZHprECCO089  
ZHprECCO090  
ZHprECCO094

ZHprECCO096  
ZHprECCO097  
ZHprECCO098  
ZHprECCO099  
ZHprECCO101  
CCSDZhcaK109972

**5. PrZp01**

AbWYZhprTK86070 [10]  
AbWYZhprTK86123  
DCCOZPPTK86086  
DCCOZPPTK8608  
TCCOZPPTK8605  
ZpAbWY001  
ZpAbWY004  
ZpAbWY005  
ZpAbWY006  
ZpAbWY007

**6. Pr01**

AbWYZhprTK86202 [1]

**7. Pr02**

AbWYZhprTK86113 [1]

**8. Pa01**

BCNEZhpaUNL9 [10]  
OCKSZhpaKU47  
OCKSZhpaKU48  
ZhpaBCNE030  
ZhpaBCNE032  
ZhpaBCNE040  
ZhpaBCNE047  
ZhpaKCNE021  
ZhpaKCNE024  
ZhpaKCNE025

**9. Pa02**

DCKSZhpaKU40 [4]  
LCKSZhpaKU44  
MAM0ZhpaKU5  
MAM0ZhpaKU52

**10. Pa03**

BONEZhpaUNL7 [1]

**11. Pa 04**

HONEZhpaUNL42 [4]  
TCNEZhpaUNL55  
ZhpaBeCSD008  
ZhpaBeCSD014

**12. Pa05**

ACNEZhpaUNL2 [18]  
ACNEZhpaUNL3  
ACNEZhpaUNL4  
ACNEZhpaUNL5  
DCNEZhpaUNL23  
DGNEZhpaUNL26  
HONEZhpaUNL41  
LCNEZhpaUNL46  
ZhpaBCNE031  
ZhpaBCNE036  
ZhpaBCNE039  
ZhpaBCNE043  
ZhpaBCNE046  
ZhpaKCNE019  
ZhpaKCNE026  
ZhpaKCNE027  
ZhpaKCNE028  
ZhpaBCNE048

**13. L01**

NCAZZhluMSB6 [1]

**14. L02**

ApAZZhluMSB4 [2]  
ApAZZhluMSB40951

**15. L03**

LACOfhluDMNH8631 [1]

**16. L04**

LACOfhluDMNH8632 [2]  
LACOfhluDMNH8634

**17. L05**

BCNEZhpaUNL1 [2]  
BCNEZhpaUNL12

**18. PaI01**

BCNEZhpaUNL16 [32]  
GCNEZhpaUNL27  
GCNEZhpaUNL28  
HCNEZhpaUNL35  
HCNEZhpaUNL36  
HCNEZhpaUNL37  
MCNEZhpaUNL51  
TCIOZhinKU116269  
ZhpaBCNE029  
ZhpaBCNE033  
ZhpaBCNE034  
ZhpaBCNE038  
ZhpaBCNE041  
ZhpaBCNE042  
ZhpaBeCSD004  
ZhpaBeCSD005  
ZhpaBeCSD006  
ZhpaBeCSD007  
ZhpaBeCSD009  
ZhpaBeCSD010  
ZhpaBeCSD011  
ZhpaBeCSD012  
ZhpaBeCSD013  
ZhpaBeCSD015  
ZhpaBeCSD016  
ZhpaBeCSD017  
ZhpaKCNE018  
ZhpaKCNE020  
ZhpaKCNE023  
ZhpaBCNE049  
ZhpaBeCSD002  
ZhpaBeCSD003

**19. L06**

LACOMZhludMNH8630 [10]  
OCNMMSB9  
OCNMZhludMSB61684  
OCNMZhludMSB61690  
OCNMZhludMSB61693  
OCNMZhludMSB61696  
OCNMZhludMSB61712  
OCNMZhludNK871  
RANMZhludMSB58369  
SONMZhludNK884

**20. L07**

BCNMZhludNK9976 [2]

VCNMZhluMSB30

**21. Pa06**

BeSDZhpaKU54 [1]

**22. PaL01**

BeCSDZhpaKU53 [26]  
RANMZhluMSB58370  
SCNMZhluMSB23  
SCNMZhluMSB24  
SCNMZhluMSB25  
SCNMZhluMSB26  
SCNMZhluMSB27  
SCNMZhluMSB56980  
SCNMZhluNK856  
ZhISCNMMSB3826  
ZhISCNMMSB3827  
ZhISCNMMSB3828  
ZhISCNMMSB382  
ZhISCNMMSB3831  
ZhISCNMMSB3832  
ZhISCNMMSB3833  
ZhISCNMMSB3834  
ZhISCNMMSB3835  
ZhISCNMMSB3836  
ZhISCNMMSB3838  
ZhISCNMMSB3840  
ZhISCNMMSB3841  
ZhISCNMMSB3842  
ZhISCNMMSB3843  
ZhISCNMMSB3844  
ZhISCNMMSB3845

**23. L08**

ZhISCNMMSB3837 [2]  
ZhISCNMMSB3839

**24. Pa07**

BCNEZhpaUNL8 [5]  
ZhpaBCNE035  
ZhpaBCNE044  
ZhpaBCNE045  
ZhpaKCNE022

**25. PaL02**

ApAZZhluMSB5 [7]  
ApAZZhluNK1584

LACOHluDMNH8633  
LACOHluDMNH8635  
LCKSZhpaKU45  
NCAZZhluMSB7  
NCAZZhluMSB8

**26. I01**

MCIAZhinKU108068 [1]

**27. I02**

HCILZhinKU127252 [1]

**28. I03**

WCINZhinKU112830 [1]

**29. I04**

DCNDZhinKU123033 [1]

**30. CI01**

LaSDZheaKU112663 [2]  
WCSDZhiKU115730

**31. CI02**

BCNDZhinKU115700 [28]  
BCNDZhinKU115702  
BCNDZhinKU115710  
BCNDZhinKU120018  
BCNDZhinKU120019  
CCMTZheaK123593  
CCMTZheaK123598  
CCMTZheaK123599  
DCNDZhinKU123021  
DCNDZhinKU123022  
DCNDZhinKU123031  
DCNDZhinKU123032  
MCNDZhinDMNS7764  
PCSDZheaK101558  
PCSDZheaKU101564  
WCSDZhiKU115731  
WCSDZhiKU115732  
WCSDZhinKU159190  
WCWYZheaTK86190  
WCWYZheaTK86191  
ZhcCCSD061  
ZhcCCSD066  
ZhcCCSD070  
ZhcPCSD079  
ZhcPCSD080

ZhcPCSD081  
ZhcPCSD082  
ZhcPCSD083

**32. I05**

BCNDZhinKU120017 [1]

**33. C01**

CCWYZhcaKU20839 [1]

**34. C02**

CCWYZhcaKU20843 [2]  
ZhcCCWY054

**35. C03**

WCWYZhcaKU42469 [1]

**36. C04**

ZhcCCWY034 [4]  
ZhcCCWY037  
ZhcCCWY053  
ZhcCCWY088

**37. C05**

CCWYZhcaKU20844 [50]  
HCSDZhcaKU83557  
HCSDZhcaKU87040  
HCSDZhcaKU87042  
LaSDZhcaKU112660  
WCWYZhcaKU42471  
ZhcCCSD056  
ZhcCCSD057  
ZhcCCSD058  
ZhcCCSD059  
ZhcCCSD060  
ZhcCCSD062  
ZhcCCSD063  
ZhcCCSD065  
ZhcCCSD067  
ZhcCCSD068  
ZhcCCSD069  
ZhcCCSD072  
ZhcCCSD073  
ZhcCCSD075  
ZhcCCSD076  
ZhcCCSD077  
ZhcCCSD085  
ZhcCCSD086  
ZhcCCWY028

ZhcCCWY030  
ZhcCCWY031  
ZhcCCWY032  
ZhcCCWY033  
ZhcCCWY035  
ZhcCCWY036  
ZhcCCWY038  
ZhcCCWY039  
ZhcCCWY040  
ZhcCCWY041  
ZhcCCWY042  
ZhcCCWY043  
ZhcCCWY044  
ZhcCCWY045  
ZhcCCWY046  
ZhcCCWY047  
ZhcCCWY048  
ZhcCCWY049  
ZhcCCWY050  
ZhcCCWY051  
ZhcCCWY052  
ZhcCCWY055  
ZhcCCWY087  
ZhcCCWY089  
ZhcPCSD084

**38. I06**

DCSDZhinKU147018 [8]  
DCSDZhinKU153196  
ECIAZhinKU116263  
ECIAZhinKU11626  
LCSDZhinKU153203  
ZhiMCMNMSB41532  
ZhiMCMNMSB80783  
ZhiMCMNMSB80784

**39. I07**

DCSDZhinKU153201 [1]

**40. C06**

ZhcCCSD071 [3]  
ZhcCCSD074  
ZhcCCSD078

**41. C07**

PCSDZhcaKU101552 [1]

**42. C08**

LaSDZhcaKU109970 [1]

**43. I08**

ECIAZhinKU116264 [4]  
UCSDZhinKU153229  
WCIAZhinKU104062  
ZhiMCMNMSB80770

**44. I09**

BrCSDZhinKU140722 [1]

**45. I10**

LCSDZhinKU153205 [2]  
MCSDZhinKU153215

**46. I11**

ZhiMCMNMSB41533 [2]  
ZhiMCMNMSB80767

**47. I12**

ZhiBrCSD003 [5]  
ZhiBrCSD010  
ZhiBrCSD017  
ZhiBrCSD018  
ZhiBrCSD032

**48. I13**

ZhiMCMNMSB80780 [2]  
ZhiMCMNMSB80786

**49. I14**

ZhiMCMNMSB41518 [8]  
ZhiMCMNMSB80766  
ZhiMCMNMSB80768  
ZhiMCMNMSB80771  
ZhiMCMNMSB80773  
ZhiMCMNMSB80774  
ZhiMCMNMSB80779  
ZhiMCMNMSB80782

**50. I15**

BrCSDZhinKU147020 [18]  
BrCSDZhinKU153176  
BrCSDZhinKU153177  
BrCSDZhinKU153180  
BrCSDZhinKU153181  
ZhiBrCSD005  
ZhiBrCSD006  
ZhiBrCSD007  
ZhiBrCSD009  
ZhiBrCSD011

ZhiBrCSD014  
ZhiBrCSD016  
ZhiBrCSD023  
ZhiBrCSD026  
ZhiBrCSD027  
ZhiBrCSD028  
ZhiBrCSD029  
ZhiBrCSD030

**51. I16**

ZhiMCMNMSB80785 [1]

**52. I17**

BVIAZhinKU116266 [18]  
BrCSDZhinKU140721  
MCSDZhinKU153209  
MCSDZhinKU153212  
MOSDZhinKU153221  
WCSDZhinKU15319  
ZhiBrCSD004  
ZhiBrCSD008  
ZhiBrCSD012  
ZhiBrCSD013  
ZhiBrCSD015  
ZhiBrCSD019  
ZhiBrCSD024  
ZhiBrCSD031  
ZhiMCMNMSB80769  
ZhiMCMNMSB80772  
ZhiMCMNMSB80778  
ZhiMCMNMSB80781

**53. C09**

CCMTZhcaK123595 [1]

**54. Zp01**

PaWYZPIdTK86039 [2]  
PaWYZPIdTK86041

**55. Zp02**

TCWYZPUtTK86075 [3]  
TCWYZPUtTK86155  
TCWYZPUtTK86175

**56. Zp03**

TCWYZPUtTK86135 [1]

**57. PaZp01**

DCKSZhpaKU30814 [2]  
PaWYZPIdTK86040

**58. Zp05**

FCWYZPIdTK86028 [3]  
FCWYZPIdTK86037  
FCWYZPIdTK86112

**59. Zp06**

CCCOZPPTK103545 [1]

**60. Zp07**

LACOPPTK103593 [1]

**61. Zp08**

ZpAbWY002 [2]  
ZpAbWY003

**62. Zp09**

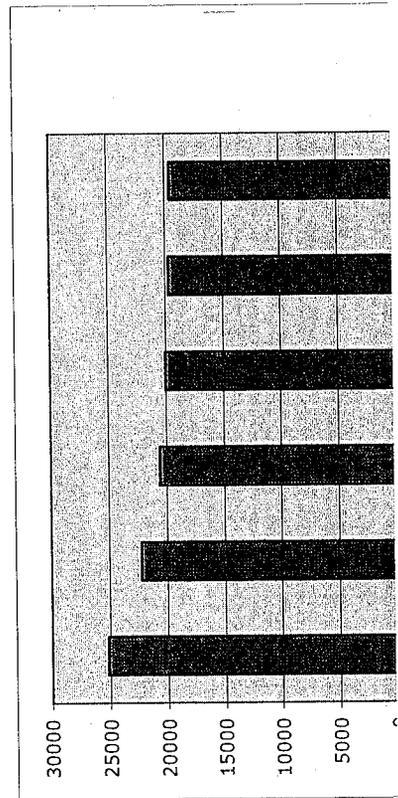
LACOPPTK103589 [1]

**63. Zp10**

LCWYZPPDMNH9316 [1]

**Table 6.** A summary of likelihood scores for each STRUCTURE run for King et al. data, average likelihood scores for each K for all runs, and a visual representation of the absolute values of these scores.

Runs	K									
	1	2	3	4	5	6	7	8	9	10
1	25,162.00	-22,257.80	-20,596.30	-20,067.20	-19,544.60	-20,006.30	-19,721.60	-20,104.20	-77,538.20	
2	25,165.80	-22,257.10	-20,593.60	-19,903.10	-19,378.00	-19,247.30	-19,745.60	-77,544.40	-80,319.90	
3	25,168.50	-22,257.70	-20,590.40	-20,067.00	-19,377.00	-19,994.60	-20,092.90	-19,976.70	-19,629.00	
4	25,157.90	-22,258.40	-20,589.90	-19,904.90	-19,715.10	-20,061.60	-19,708.60	-19,173.80	-19,113.90	
5	25,162.30	-22,266.00	-20,595.00	-20,067.50	-19,918.20	-19,243.90				
6	25,156.70	-22,257.10	-20,593.00	-19,902.30	-19,542.30	-19,020.50				
7	25,160.50	-22,263.30	-20,591.60	-20,069.80	-19,936.20	-19,025.60				
8	25,155.90	-22,258.70	-20,591.50	-20,066.80	-19,717.40	-20,006.30				
9	25,160.60	-22,259.00	-20,593.30	-20,071.40	-19,939.80	-19,020.50				
<u>10</u>	<u>25,157.50</u>	<u>-22,254.20</u>	<u>-20,597.30</u>	<u>-20,069.30</u>	<u>-19,931.20</u>	<u>-20,004.40</u>				
Aver. -Ln	-25,160	-22,258	-20,593	-20,018	-19,699	-19,563				-19,817





**Table 7.** Inferred ancestry of all individuals based on the run with the best likelihood score at  $K = 3$  from the King et al. 2006 microsatellite data. Probabilities in bold text indicate cluster with highest assignment. Individuals in bold red text indicate individuals with mixed ancestry (no probability  $> 0.80$ ) and individuals that belong to one subspecies but have highest probability ancestry assigned to a cluster with predominately individuals from a different subspecies are indicated in green. Individuals 1-94 are *Z. h. preblei*, species for all other samples are given in label. Abbreviations for sample sites are as in Table 1.

Inferred ancestry of individuals:

Label	(%Miss) :	Inferred clusters		
		<u>1</u>	<u>2</u>	<u>3</u>
1 LCC01_CER-9	(0) :	0.002	<b>0.997</b>	0.001
2 LCC01_CER-9	(0) :	0.002	<b>0.997</b>	0.001
3 LCC01_CER-9	(0) :	0.003	<b>0.995</b>	0.001
4 LCC01_CER-9	(0) :	0.002	<b>0.997</b>	0.001
5 LCC01_CER-9	(0) :	0.002	<b>0.996</b>	0.001
6 LCC01_CER-9	(9) :	0.002	<b>0.996</b>	0.001
7 LCC01_HRK-9	(0) :	0.003	<b>0.996</b>	0.001
8 LCC01_HRK-9	(0) :	0.002	<b>0.997</b>	0.001
9 LCC01_HRK-9	(0) :	0.003	<b>0.995</b>	0.002
10 LCC01_HRK-9	(0) :	0.002	<b>0.997</b>	0.001
11 LCC01_MC-98	(0) :	0.002	<b>0.997</b>	0.001
12 LCC01_MC-98	(0) :	0.002	<b>0.997</b>	0.001
13 LCC01_NFP-9	(0) :	0.002	<b>0.997</b>	0.001
14 LCC01_NFP-9	(0) :	0.003	<b>0.996</b>	0.001
15 LCC02_BG-98	(0) :	0.005	<b>0.993</b>	0.001
16 LCC02_BG-98	(0) :	0.017	<b>0.982</b>	0.001
17 LCC02_PGC-9	(0) :	0.002	<b>0.997</b>	0.001
18 LCC02_SP-12	(0) :	0.003	<b>0.995</b>	0.001
19 LCC02_SP-16	(0) :	0.004	<b>0.995</b>	0.001
20 LCC02_SP-17	(0) :	0.003	<b>0.996</b>	0.001
21 LCC02_SP-22	(0) :	0.003	<b>0.996</b>	0.001
22 LCC02_SP-24	(0) :	0.002	<b>0.996</b>	0.001
23 LCC02_SP-33	(0) :	0.003	<b>0.996</b>	0.001
24 LCC02_SP-36	(0) :	0.006	<b>0.993</b>	0.002
25 LCC02_SP-37	(0) :	0.012	<b>0.986</b>	0.002
26 LCC02_SP-67	(0) :	0.003	<b>0.995</b>	0.001
27 LCC02_SP-74	(0) :	0.002	<b>0.996</b>	0.001
28 LCC02_SP-86	(0) :	0.006	<b>0.993</b>	0.001
29 LCC02_YG-98	(0) :	0.003	<b>0.996</b>	0.001
30 LCC02_YG-98	(0) :	0.005	<b>0.994</b>	0.001
31 DCC01_MAY-1	(0) :	0.002	<b>0.997</b>	0.001
32 DCC01_MAY-1	(9) :	0.002	<b>0.997</b>	0.001
33 DCC01_MAY-2	(0) :	0.002	<b>0.997</b>	0.001

34 DCC01_MAY-2	(0) : 0.004 <b>0.995</b> 0.001
35 DCC01_MAY-2	(0) : 0.004 <b>0.995</b> 0.001
36 DCC01_MAY-2	(0) : 0.002 <b>0.996</b> 0.002
37 DCC01_MAY-2	(0) : 0.002 <b>0.997</b> 0.001
38 DCC01_MAY-2	(0) : 0.002 <b>0.997</b> 0.001
39 DCC01_MAY-3	(0) : 0.002 <b>0.996</b> 0.002
40 DCC01_MAY-3	(0) : 0.002 <b>0.995</b> 0.003
41 DCC01_MAY-3	(0) : 0.003 <b>0.996</b> 0.001
42 DCC01_MAY-4	(0) : 0.002 <b>0.997</b> 0.001
43 DCC01_MAY-4	(0) : 0.003 <b>0.996</b> 0.001
44 DCC01_MAY-4	(0) : 0.002 <b>0.997</b> 0.001
45 DCC01_MAY-4	(0) : 0.003 <b>0.996</b> 0.001
46 DCC01_MAY-4	(0) : 0.005 <b>0.994</b> 0.001
47 DCC01_MAY-4	(0) : 0.003 <b>0.996</b> 0.001
48 DCC01_MAY-5	(0) : 0.008 <b>0.987</b> 0.005
49 DCC01_MAY-5	(0) : 0.002 <b>0.997</b> 0.001
50 DCC01_MAY-6	(0) : 0.002 <b>0.997</b> 0.001
51 DCC01_MAY-7	(0) : 0.002 <b>0.996</b> 0.001
52 DCC01_MAY-7	(0) : 0.002 <b>0.998</b> 0.001
53 DCC01_MAY-7	(0) : 0.002 <b>0.996</b> 0.001
54 DCC01_MAY-7	(4) : 0.002 <b>0.996</b> 0.001
55 DCC01_MAY-7	(0) : 0.002 <b>0.997</b> 0.001
56 DCC01_MAY-8	(0) : 0.002 <b>0.997</b> 0.001
57 DCC01_MAY-8	(0) : 0.002 <b>0.997</b> 0.001
58 DCC01_MAY-8	(0) : 0.002 <b>0.997</b> 0.001
59 DCC01_MAY-9	(4) : 0.003 <b>0.995</b> 0.001
60 DCC01_MAY-9	(0) : 0.002 <b>0.997</b> 0.001
61 DCC01_MAY-9	(0) : 0.003 <b>0.996</b> 0.001
62 DCC01_MAY-9	(0) : 0.002 <b>0.997</b> 0.001
63 DCC01_MAY-9	(0) : 0.007 <b>0.990</b> 0.003
64 DCC01_MAY-9	(4) : 0.006 <b>0.988</b> 0.006
65 DCC02_WH-98	(0) : 0.002 <b>0.997</b> 0.001
66 DCC02_WH-98	(0) : 0.005 <b>0.994</b> 0.002
67 DCC02_WH-98	(0) : 0.002 <b>0.997</b> 0.001
68 DCC02_WH-98	(0) : 0.002 <b>0.997</b> 0.001
69 DCC02_WH-98	(0) : 0.002 <b>0.997</b> 0.001
70 DCC02_WH-98	(0) : 0.002 <b>0.983</b> 0.015
71 DCC02_WH-98	(0) : 0.002 <b>0.997</b> 0.001
72 DCC02_WH-98	(0) : 0.001 <b>0.997</b> 0.001
73 DCC02_WH-98	(0) : 0.001 <b>0.998</b> 0.001
74 DCC02_WH-98	(0) : 0.002 <b>0.997</b> 0.001
75 DCC02_WH-98	(0) : 0.002 <b>0.997</b> 0.001
76 DCC02_WH-98	(9) : 0.002 <b>0.996</b> 0.001
77 DCC02_WH-98	(4) : 0.003 <b>0.930</b> 0.067
78 DCC02_WH-98	(0) : 0.002 <b>0.996</b> 0.002
79 DCC02_WH-98	(0) : 0.002 <b>0.997</b> 0.001

80 DCC02_WH-98	(0) : 0.002 <b>0.996</b> 0.001
81 DCC02_WH-98	(4) : 0.002 <b>0.997</b> 0.001
82 DCC02_WH-98	(0) : 0.012 <b>0.986</b> 0.002
83 DCC02_WH-98	(0) : 0.001 <b>0.997</b> 0.001
84 DCC02_WH-98	(0) : 0.002 <b>0.997</b> 0.001
85 DCC02_WH-98	(0) : 0.002 <b>0.997</b> 0.001
86 DCC02_WH-98	(9) : 0.002 <b>0.997</b> 0.001
87 DCC02_WH-98	(0) : 0.002 <b>0.997</b> 0.001
88 DCC02_WH-98	(0) : 0.002 <b>0.996</b> 0.001
89 DCC02_WH-98	(0) : 0.039 <b>0.956</b> 0.005
90 DCC02_WH-98	(0) : 0.002 <b>0.997</b> 0.001
91 DCC02_WH-98	(4) : 0.002 <b>0.996</b> 0.001
92 DCC02_WH-98	(4) : 0.002 <b>0.997</b> 0.001
93 DCC02_WH-98	(4) : 0.002 <b>0.997</b> 0.001
94 DCC02_WH-98	(4) : 0.002 <b>0.997</b> 0.001
95 ECC01_Zhp-0	(0) : 0.002 <b>0.996</b> 0.001
96 ECC01_Zhp-0	(0) : 0.002 <b>0.997</b> 0.001
97 ECC01_Zhp-0	(0) : 0.004 <b>0.995</b> 0.002
98 ECC01_Zhp-0	(0) : 0.003 <b>0.995</b> 0.001
99 ECC01_Zhp-0	(0) : 0.002 <b>0.997</b> 0.002
100 ECC01_Zhp-0	(0) : 0.010 <b>0.988</b> 0.002
101 ECC01_Zhp-0	(0) : 0.002 <b>0.996</b> 0.002
102 ECC01_Zhp-0	(0) : 0.002 <b>0.997</b> 0.001
103 ECC01_Zhp-0	(0) : 0.003 <b>0.996</b> 0.001
104 ECC01_Zhp-0	(0) : 0.002 <b>0.997</b> 0.001
105 ECC01_Zhp-0	(0) : 0.010 <b>0.987</b> 0.003
106 ECC01_Zhp-0	(0) : 0.002 <b>0.997</b> 0.001
107 ECC01_Zhp-0	(0) : 0.002 <b>0.997</b> 0.001
108 ECC01_Zhp-0	(0) : 0.002 <b>0.997</b> 0.001
109 ECC01_Zhp-0	(0) : 0.002 <b>0.996</b> 0.002
110 ECC01_Zhp-0	(0) : 0.003 <b>0.995</b> 0.002
111 ECC01_Zhp-0	(0) : 0.002 <b>0.996</b> 0.001
112 ECC01_Zhp-0	(0) : 0.004 <b>0.995</b> 0.002
113 ECC01_Zhp-0	(0) : 0.006 <b>0.993</b> 0.002
114 ECC01_Zhp-0	(0) : 0.003 <b>0.995</b> 0.002
115 ECC01_Zhp-0	(0) : 0.003 <b>0.996</b> 0.001
116 ECC01_Zhp-0	(0) : 0.002 <b>0.997</b> 0.001
117 ECC02_Zhp-0	(0) : 0.003 <b>0.995</b> 0.002
118 ECC02_Zhp-0	(0) : 0.002 <b>0.997</b> 0.001
119 ECC02_Zhp-0	(0) : 0.006 <b>0.992</b> 0.002
120 ECC02_Zhp-0	(0) : 0.003 <b>0.996</b> 0.001
121 ECC02_Zhp-0	(0) : 0.003 <b>0.996</b> 0.002
122 ECC02_Zhp-0	(0) : 0.004 <b>0.993</b> 0.003
123 ECC02_Zhp-0	(0) : 0.003 <b>0.996</b> 0.001
124 ECC02_Zhp-0	(0) : 0.002 <b>0.997</b> 0.001
125 ECC02_Zhp-0	(0) : 0.002 <b>0.997</b> 0.001

126 ECC02_Zhp-0	(0) : 0.002 <b>0.997</b> 0.001
127 ECC02_Zhp-0	(0) : 0.004 <b>0.994</b> 0.001
128 ECC02_Zhp-0	(0) : 0.002 <b>0.997</b> 0.001
129 ECC02_Zhp-0	(0) : 0.003 <b>0.996</b> 0.001
130 ECC02_Zhp-0	(0) : 0.002 <b>0.997</b> 0.001
131 ECC02_Zhp-0	(0) : 0.002 <b>0.997</b> 0.001
132 ECC02_Zhp-0	(0) : 0.003 <b>0.995</b> 0.001
133 ECC02_Zhp-0	(0) : 0.004 <b>0.994</b> 0.002
134 ECC02_Zhp-0	(0) : 0.002 <b>0.997</b> 0.001
135 ECC02_Zhp-0	(0) : 0.002 <b>0.997</b> 0.001
136 ECC02_Zhp-0	(0) : 0.002 <b>0.996</b> 0.002
137 ECC02_Zhp-0	(0) : 0.002 <b>0.997</b> 0.001
138 ECC02_Zhp-1	(0) : 0.002 <b>0.997</b> 0.001
139 ECC02_Zhp-1	(0) : 0.002 <b>0.996</b> 0.001
140 ECC02_Zhp-1	(0) : 0.014 <b>0.983</b> 0.003
141 ECC02_Zhp-1	(0) : 0.072 <b>0.927</b> 0.002
142 ECC02_Zhp-1	(0) : 0.019 <b>0.979</b> 0.003
143 CCWY_Zhc-02	(0) : <b>0.996</b> 0.003 0.001
144 CCWY_Zhc-03	(0) : <b>0.977</b> 0.022 0.001
145 CCWY_Zhc-03	(0) : <b>0.988</b> 0.011 0.002
146 <b>CCWY_Zhc-03</b>	(4) : <b>0.546 0.452</b> 0.002
147 CCWY_Zhc-03	(0) : <b>0.990</b> 0.008 0.001
148 CCWY_Zhc-03	(0) : <b>0.993</b> 0.006 0.001
149 CCWY_Zhc-03	(0) : <b>0.995</b> 0.003 0.002
150 CCWY_Zhc-03	(0) : <b>0.986</b> 0.004 0.010
151 CCWY_Zhc-03	(0) : <b>0.979</b> 0.019 0.002
152 CCWY_Zhc-03	(0) : <b>0.980</b> 0.019 0.001
153 CCWY_Zhc-03	(0) : <b>0.979</b> 0.017 0.004
154 CCWY_Zhc-04	(0) : <b>0.990</b> 0.007 0.004
155 <b>CCWY_Zhc-04</b>	(0) : <b>0.696 0.303</b> 0.002
156 CCWY_Zhc-04	(0) : <b>0.977</b> 0.021 0.002
157 CCWY_Zhc-04	(0) : <b>0.980</b> 0.018 0.001
158 <b>CCWY_Zhc-04</b>	(0) : <b>0.680 0.318</b> 0.002
159 CCWY_Zhc-04	(0) : <b>0.895</b> 0.103 0.002
160 CCWY_Zhc-04	(0) : <b>0.992</b> 0.006 0.001
161 CCWY_Zhc-04	(0) : <b>0.996</b> 0.002 0.001
162 CCWY_Zhc-04	(0) : <b>0.921</b> 0.077 0.002
163 CCWY_Zhc-04	(0) : <b>0.881</b> 0.118 0.001
164 CCWY_Zhc-05	(0) : <b>0.852</b> 0.146 0.001
165 CCWY_Zhc-05	(0) : <b>0.985</b> 0.012 0.002
166 CCWY_Zhc-05	(0) : <b>0.994</b> 0.005 0.002
167 CCWY_Zhc-05	(0) : <b>0.983</b> 0.016 0.001
168 CCWY_Zhc-05	(0) : <b>0.977</b> 0.021 0.002
169 CCWY_Zhc-05	(0) : <b>0.995</b> 0.003 0.002
170 CCWY_Zhc-08	(0) : <b>0.985</b> 0.012 0.003

171 CCWY_Zhc-08	(0) : <b>0.987</b> 0.011 0.002
172 <b>CCWY_Zhc-08</b>	(0) : <b>0.692 0.306</b> 0.001
173 CCSD_Zhc-05	(0) : <b>0.993</b> 0.005 0.002
174 CCSD_Zhc-05	(0) : <b>0.995</b> 0.003 0.001
175 CCSD_Zhc-05	(0) : <b>0.972</b> 0.026 0.001
176 CCSD_Zhc-05	(0) : <b>0.992</b> 0.007 0.002
177 CCSD_Zhc-06	(0) : <b>0.954</b> 0.043 0.004
178 CCSD_Zhc-06	(14) : <b>0.953</b> 0.045 0.002
179 CCSD_Zhc-06	(4) : <b>0.874</b> 0.122 0.004
180 CCSD_Zhc-06	(0) : <b>0.936</b> 0.063 0.002
181 CCSD_Zhc-06	(0) : <b>0.994</b> 0.005 0.001
182 CCSD_Zhc-06	(0) : <b>0.995</b> 0.003 0.001
183 CCSD_Zhc-06	(0) : <b>0.977</b> 0.019 0.004
184 CCSD_Zhc-06	(0) : <b>0.994</b> 0.004 0.002
185 CCSD_Zhc-06	(0) : <b>0.980</b> 0.019 0.001
186 CCSD_Zhc-06	(0) : <b>0.996</b> 0.002 0.002
187 CCSD_Zhc-07	(0) : <b>0.994</b> 0.004 0.001
188 CCSD_Zhc-07	(0) : <b>0.995</b> 0.003 0.002
189 CCSD_Zhc-07	(0) : <b>0.990</b> 0.008 0.002
190 CCSD_Zhc-07	(0) : <b>0.986</b> 0.012 0.002
191 CCSD_Zhc-07	(0) : <b>0.993</b> 0.006 0.002
192 CCSD_Zhc-07	(0) : <b>0.990</b> 0.009 0.001
193 CCSD_Zhc-07	(0) : <b>0.988</b> 0.008 0.004
194 CCSD_Zhc-07	(0) : <b>0.993</b> 0.006 0.001
195 CCSD_Zhc-07	(0) : <b>0.996</b> 0.003 0.001
196 CCSD_Zhc-07	(4) : <b>0.991</b> 0.007 0.002
197 CCSD_Zhc-08	(0) : <b>0.995</b> 0.003 0.001
198 CCSD_Zhc-08	(0) : <b>0.994</b> 0.004 0.001
199 CCSD_Zhc-08	(0) : <b>0.904</b> 0.093 0.003
200 CCSD_Zhc-08	(0) : <b>0.992</b> 0.006 0.002
201 CCSD_Zhc-08	(0) : <b>0.990</b> 0.008 0.002
202 CCSD_Zhc-08	(0) : <b>0.995</b> 0.004 0.001
203 CCSD_Zhc-08	(0) : <b>0.991</b> 0.007 0.002
204 BRCSD_Zhi-0	(0) : <b>0.991</b> 0.005 0.004
205 BRCSD_Zhi-0	(4) : <b>0.991</b> 0.006 0.002
206 BRCSD_Zhi-0	(0) : <b>0.995</b> 0.003 0.002
207 BRCSD_Zhi-0	(0) : <b>0.997</b> 0.001 0.002
208 BRCSD_Zhi-0	(0) : <b>0.995</b> 0.003 0.002
209 BRCSD_Zhi-0	(0) : <b>0.995</b> 0.002 0.003
210 BRCSD_Zhi-0	(0) : <b>0.996</b> 0.002 0.002
211 BRCSD_Zhi-0	(0) : <b>0.996</b> 0.001 0.002
212 BRCSD_Zhi-0	(0) : <b>0.995</b> 0.003 0.002
213 BRCSD_Zhi-0	(0) : <b>0.996</b> 0.002 0.002
214 BRCSD_Zhi-0	(0) : <b>0.928</b> 0.053 0.019
215 BRCSD_Zhi-0	(0) : <b>0.995</b> 0.003 0.003

216 BRCSD_Zhi-0	(0) : <b>0.991</b> 0.006 0.002
217 BRCSD_Zhi-0	(0) : <b>0.997</b> 0.002 0.002
218 BRCSD_Zhi-0	(0) : <b>0.993</b> 0.004 0.003
219 BRCSD_Zhi-0	(0) : <b>0.996</b> 0.002 0.002
220 BRCSD_Zhi-0	(0) : <b>0.996</b> 0.002 0.002
221 BRCSD_Zhi-0	(0) : <b>0.997</b> 0.001 0.001
222 BRCSD_Zhi-0	(0) : <b>0.996</b> 0.002 0.003
223 BRCSD_Zhi-0	(0) : <b>0.996</b> 0.002 0.002
224 BRCSD_Zhi-0	(0) : <b>0.988</b> 0.010 0.002
225 BRCSD_Zhi-0	(0) : <b>0.992</b> 0.003 0.006
226 BRCSD_Zhi-0	(0) : <b>0.993</b> 0.002 0.005
227 BRCSD_Zhi-0	(0) : <b>0.995</b> 0.003 0.002
228 BRCSD_Zhi-0	(0) : <b>0.990</b> 0.008 0.002
229 BRCSD_Zhi-0	(0) : <b>0.997</b> 0.002 0.001
230 BRCSD_Zhi-0	(0) : <b>0.997</b> 0.002 0.002
231 BRCSD_Zhi-0	(0) : <b>0.976</b> 0.003 0.021
232 MCMN_MSB-41	(0) : <b>0.993</b> 0.004 0.003
233 MCMN_MSB-41	(0) : <b>0.755</b> 0.005 <b>0.240</b>
234 MCMN_MSB-41	(0) : <b>0.961</b> 0.003 0.036
235 <b>MCMN_MSB-80</b>	(0) : <b>0.725</b> 0.003 <b>0.272</b>
236 MCMN_MSB-80	(0) : <b>0.994</b> 0.004 0.002
237 MCMN_MSB-80	(0) : <b>0.990</b> 0.003 0.007
238 MCMN_MSB-80	(0) : <b>0.979</b> 0.014 0.007
239 MCMN_MSB-80	(0) : <b>0.988</b> 0.009 0.003
240 MCMN_MSB-80	(0) : <b>0.977</b> 0.003 0.020
241 MCMN_MSB-80	(0) : <b>0.991</b> 0.004 0.004
242 MCMN_MSB-80	(0) : <b>0.976</b> 0.011 0.013
243 MCMN_MSB-80	(0) : <b>0.974</b> 0.002 0.024
244 MCMN_MSB-80	(0) : <b>0.919</b> 0.003 0.077
245 MCMN_MSB-80	(0) : <b>0.953</b> 0.004 0.043
246 MCMN_MSB-80	(0) : <b>0.981</b> 0.014 0.005
247 MCMN_MSB-80	(0) : <b>0.993</b> 0.004 0.003
248 MCMN_MSB-80	(0) : <b>0.990</b> 0.002 0.008
249 MCMN_MSB-80	(0) : <b>0.910</b> 0.004 0.086
250 MCMN_MSB-80	(0) : <b>0.991</b> 0.005 0.003
251 MCMN_MSB-80	(0) : <b>0.987</b> 0.010 0.003
252 <b>MCMN_MSB-80</b>	(0) : <b>0.736</b> 0.003 <b>0.262</b>
253 BCSD_Zhpa-0	(0) : 0.003 0.001 <b>0.995</b>
254 BCSD_Zhpa-0	(0) : 0.013 0.006 <b>0.981</b>
255 BCSD_Zhpa-0	(0) : 0.002 0.031 <b>0.967</b>
256 BCSD_Zhpa-0	(0) : 0.002 0.002 <b>0.995</b>
257 BCSD_Zhpa-0	(0) : 0.014 0.002 <b>0.984</b>
258 BCSD_Zhpa-0	(0) : 0.007 0.002 <b>0.990</b>
259 BCSD_Zhpa-0	(0) : 0.002 0.002 <b>0.996</b>
260 BCSD_Zhpa-0	(0) : 0.006 0.007 <b>0.987</b>

261 BCSD_Zhpa-0	(0) : 0.005 0.002 <b>0.993</b>
262 BCSD_Zhpa-0	(0) : 0.003 0.002 <b>0.995</b>
263 BCSD_Zhpa-0	(0) : 0.003 0.005 <b>0.993</b>
264 BCSD_Zhpa-0	(0) : 0.002 0.003 <b>0.995</b>
265 BCSD_Zhpa-0	(0) : 0.009 0.014 <b>0.976</b>
266 BCSD_Zhpa-0	(0) : 0.002 0.003 <b>0.995</b>
267 BCSD_Zhpa-0	(0) : 0.002 0.007 <b>0.991</b>
268 BCSD_Zhpa-0	(0) : 0.005 0.001 <b>0.994</b>
269 KBCNE_Zhpa-	(0) : 0.005 0.001 <b>0.994</b>
270 KBCNE_Zhpa-	(0) : 0.008 0.002 <b>0.990</b>
271 KBCNE_Zhpa-	(0) : 0.008 0.002 <b>0.990</b>
272 KBCNE_Zhpa-	(0) : 0.003 0.001 <b>0.996</b>
273 KBCNE_Zhpa-	(4) : 0.002 0.001 <b>0.997</b>
274 KBCNE_Zhpa-	(0) : 0.006 0.005 <b>0.990</b>
275 KBCNE_Zhpa-	(4) : 0.003 0.004 <b>0.992</b>
276 KBCNE_Zhpa-	(0) : 0.002 0.001 <b>0.997</b>
277 KBCNE_Zhpa-	(0) : 0.019 0.032 <b>0.950</b>
278 KBCNE_Zhpa-	(9) : 0.009 0.003 <b>0.988</b>
279 KBCNE_Zhpa-	(0) : 0.005 0.003 <b>0.992</b>
280 KBCNE_Zhpa-	(0) : 0.003 0.005 <b>0.992</b>
281 KBCNE_Zhpa-	(0) : 0.021 0.009 <b>0.970</b>
282 KBCNE_Zhpa-	(0) : 0.003 0.005 <b>0.992</b>
283 KBCNE_Zhpa-	(0) : 0.007 0.007 <b>0.986</b>
284 KBCNE_Zhpa-	(0) : 0.008 0.002 <b>0.991</b>
285 KBCNE_Zhpa-	(0) : 0.003 0.003 <b>0.994</b>
286 KBCNE_Zhpa-	(0) : 0.013 0.083 <b>0.904</b>
287 KBCNE_Zhpa-	(0) : 0.004 0.007 <b>0.990</b>
288 KBCNE_Zhpa-	(0) : 0.004 0.002 <b>0.995</b>
289 KBCNE_Zhpa-	(0) : 0.004 0.004 <b>0.991</b>
290 KBCNE_Zhpa-	(0) : 0.004 0.002 <b>0.995</b>
291 KBCNE_Zhpa-	(0) : 0.003 0.002 <b>0.994</b>
292 KBCNE_Zhpa-	(0) : 0.008 0.007 <b>0.986</b>
293 KBCNE_Zhpa-	(0) : 0.018 0.004 <b>0.978</b>
294 KBCNE_Zhpa-	(0) : 0.009 0.004 <b>0.987</b>
295 KBCNE_Zhpa-	(0) : 0.008 0.024 <b>0.969</b>
296 KBCNE_Zhpa-	(0) : 0.006 0.024 <b>0.970</b>
297 KBCNE_Zhpa-	(0) : 0.005 0.004 <b>0.991</b>
298 KBCNE_Zhpa-	(0) : 0.002 0.002 <b>0.997</b>
299 KBCNE_Zhpa-	(0) : 0.019 0.009 <b>0.972</b>
300 KBCNE_Zhpa-	(0) : 0.027 0.002 <b>0.971</b>
301 SCNM_MSB-38	(0) : 0.001 0.001 <b>0.998</b>
302 SCNM_MSB-38	(0) : 0.001 0.001 <b>0.998</b>
303 SCNM_MSB-38	(0) : 0.001 0.001 <b>0.998</b>
304 SCNM_MSB-38	(0) : 0.001 0.002 <b>0.997</b>
305 SCNM_MSB-38	(0) : 0.002 0.002 <b>0.996</b>

306 SCNM_MSB-38	(0) : 0.002 0.002 <b>0.996</b>
307 SCNM_MSB-38	(0) : 0.001 0.001 <b>0.997</b>
308 SCNM_MSB-38	(0) : 0.001 0.001 <b>0.997</b>
309 SCNM_MSB-38	(0) : 0.002 0.002 <b>0.997</b>
310 SCNM_MSB-38	(0) : 0.001 0.001 <b>0.998</b>
311 SCNM_MSB-38	(0) : 0.002 0.003 <b>0.995</b>
312 SCNM_MSB-38	(0) : 0.001 0.002 <b>0.997</b>
313 SCNM_MSB-38	(0) : 0.001 0.001 <b>0.998</b>
314 SCNM_MSB-38	(0) : 0.001 0.001 <b>0.998</b>
315 SCNM_MSB-38	(0) : 0.001 0.001 <b>0.998</b>
316 SCNM_MSB-38	(0) : 0.002 0.002 <b>0.997</b>
317 SCNM_MSB-38	(0) : 0.001 0.001 <b>0.998</b>
318 SCNM_MSB-38	(0) : 0.001 0.001 <b>0.998</b>
319 SCNM_MSB-38	(0) : 0.001 0.001 <b>0.998</b>
320 SCNM_MSB-38	(0) : 0.001 0.001 <b>0.998</b>

**Table 8.** Summary of results for MIGRATE analysis of King et al. microsatellite data between three hypothesized populations based on four separate runs. Because of slight inconsistencies in the first three runs a fourth was attempted in which length of the run was doubled A) Theta is equal to the estimated effective population size and Mxy is equal to the relative importance of migration from cluster on ‘x’ axis into cluster on ‘y’ axis relative to mutation rate in introducing new variants into the population. B) Nm estimates based on the results of the Migrate run 4.

A)

Runs	Clusters	$\Theta$	Mxy(m/ $\mu$ )			Chains
			Zhpr	Zhc/Zhi	Zhpa/Zhl	
Run 1	Zhpr	1.27503	---	4.5161	1.9411	Short = 10
	Zhc/Zhi	1.27120	7.5431	---	3.5490	Long = 3
	Zhpa/Zhl	1.39109	2.6982	3.1101	---	
Run 2	Zhpr	1.19892	---	4.3438	1.9371	Short = 10
	Zhc/Zhi	1.31505	5.9209	---	3.2387	Long = 3
	Zhpa/Zhl	1.47370	3.1404	3.2982	---	
Run 3	Zhpr	1.20181	---	4.9202	1.8748	Short = 10
	Zhc/Zhi	1.31586	21.2357	---	7.3766	Long = 3
	Zhpa/Zhl	1.41384	2.9735	3.0185	---	
Run 4	Zhpr	1.39925	---	3.4569	1.7511	Short = 20
	Zhc/Zhi	1.39302	5.9200	---	3.1183	Long = 6
	Zhpa/Zhl	2.98891	2.1854	2.2228	---	

B)

Nm(xy)			
	Zhpr	Zhc/Zhi	Zhpa/Zhl
Zhpr	---	1.21	2.45
Zhc/Zhi	2.06	---	1.09
Zhpa/Zhl	1.63	3.32	---

Number of migrants from ‘x’ axis cluster into ‘y’ axis cluster

**Table 9.** A summary of results for the nested clade analysis as per the Templeton (2004) inference key. Clades are labeled as in Figures 7-9. Geographical distribution of important clades (in bold) are shown in Figures 10-13.

<b>Clades</b>	<b>NCA inferences</b>
<b>Clade 1-3</b>	Allopatric fragmentation but the sampling scheme may be inadequate because only 6 samples were taken from Kansas and it is unclear if <i>Z. hudsonius</i> exists here.
Clade 1-5	Contiguous Range Expansion
<b>Clade 1-9</b>	Contiguous Range Expansion
Clade 1-10	Inconclusive
Clade 1-14	Restricted Gene Flow w/IBD
Clade 1-19	Restricted Gene Flow w/IBD
Clade 2-1	Restricted Gene Flow w/IBD
Clade 2-2	Restricted Gene Flow w/IBD
Clade 2-4	Restricted Gene Flow w/IBD
Clade 2-5	Insufficient Genetic Resolution to discriminate between range expansion/colonization and restricted dispersal / gene flow
Clade 2-9	Inconclusive
<b>Clade 3-1</b>	Contiguous range expansion but like with clade 1-3 this depends on adequate sampling in Eastern Colorado and Kansas
<b>Clade 3-2</b>	Contiguous Range Expansion
<b>Clade 3-4</b>	Restricted Gene Flow w/IBD
<b>Clade 4-2</b>	When you compare all nested clades 3-2, 3-3, and 3-4 you get restricted gene flow / dispersal with some long distance dispersal but it depends on the sampling design in the area between 3-2+3-4 and 3-3. If you just compare 3-2 and 3-4 ignoring 3-3 samples then you get Restricted Gene Flow with IBD.
<b>Clade 5-1</b>	Possible fragmentation but may need better sampling between clades 4-1 and 4-2.
Clade 5-3	Inadequate Geographical Sampling

Appendices are not included in this internet posting due to their size. If you wish to obtain copies of these materials, contact Susan Linner, Colorado Field Office Supervisor, at 303-236-4773.

**Should hypothesis testing or selective posthoc interpretation of results guide the allocation of conservation effort: a response to Vignieri *et al.***

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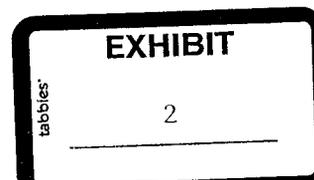
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5/15/06



In their response to Ramey *et al.* (2005), Vignieri *et al.* (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 the Preble's meadow jumping mouse (*Z. h. 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 posthoc 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 3 skulls and comparisons of only 4 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 *Z. hudsonius*. VEA state that our analyses suffered from intercorrelated data because 26 of 36 correlations among the 9 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, and 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 *Z. h. 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 e-mail 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 Whitaker (1972, 1999), Clark & Stromberg (1987) and Cryan (2004) in support our claim that no adaptive differences have been described between *Z. h. preblei* and other subspecies. While 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 Kuchler'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 (Stephan 1998). PNVs are not mutually exclusive categories. For example, each of the PNVs 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, *in press*); 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 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. While this value is low and suggests the possibility of continuing divergence due to 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 *Z. h. 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.

VEA seek to invoke selective posthoc 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

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 that data were never made public (Riggs *et al.* 1997).

VEA raise some important questions with regards 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 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 U. S. 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. preblei* as "threatened", the 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. preblei* was thought to be restricted to fourteen 8<sup>th</sup> 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 9 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 Table 4-5). While 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. preblei* under the ESA. This raises fundamental questions regarding the allocation of conservation effort.

The U.S. 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 (U.S. Government Accountability Office 2006). With 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 and Marzulluff 2001, 2002). In other words, expenditures on local populations of otherwise common species (like *Z. h. preblei*) 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. preblei* ranked 125 out of 1260 listed taxa (USFWS 2006). That put spending for *Z. h. preblei* 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. preblei*, the only verifiable figures on the cost for the 23,632 ha critical habitat designation was conservatively estimated by the USFWS at \$79 to \$183 million from 2005-2015 (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 U.S. 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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Division of  
Open Space and Natural Resources

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MAY 25 2006

May 18, 2006

Ms. Susan Linner  
Field Supervisor  
Colorado Field Office  
Ecological Services  
340 Union Boulevard  
Lakewood, CO 80225

Re: Douglas County's Supplemental Comments on the U.S. Fish and Wildlife Service's 12-Month Finding and Proposed Delisting of the Preble's Meadow Jumping Mouse

Dear Ms. Linner:

The Douglas County Division of Open Space and Natural Resources submits these comments in response to the U.S. Fish and Wildlife Service's (Service) request for comments on two recently published reports regarding data analysis and other available information related to the potential delisting of the Preble's meadow jumping mouse, *Zapus hudsonius preblei* (Preble's). 71 Fed. Reg. 8556 (February 6, 2006), 71 Fed. Reg. 16090 (March 30, 2006). These brief comments are intended to supplement our previous comments provided dated May 3, 2005 regarding the Service's delisting proposal announced on February 2, 2005 (70 Fed. Reg. 5404, 5405).

The Service's 2005 proposal to delist Preble's was initially supported in part by Dr. Rob Roy Ramey's genetic work disputing the taxonomic status of Preble's as a separate subspecies within *Zapus hudsonius*. At that time, Ramey's work was the best available scientific information. However, King, et al. (2006) performed a genetic analysis which brings into question Ramey's work and concludes that Preble's is a distinct subspecies. The differences in methodology and final analysis of these two studies illustrate the ability of science to be used to support divergent conclusions. The result has been uncertainty within the regulated community, unnecessary expenditure of resources and time, and the potential of diverting funds away from those species and subspecies that truly need protection under the ESA.

Therefore, it is our opinion that the time has come for the Service to more clearly define criteria to determine "recognized subspecies" that will be protected under the ESA. A recognized subspecies is a subspecies which after meeting certain criteria would be protected under the ESA. Such a criteria should include: 1) a standard methodology for genetic analysis and identification of subspecies eligible to be protected under the ESA; 2) procedures for analyzing eligible subspecies'

ecological significance or unique ecological niche; and 3) guidelines for determining the conservation status of the eligible species.

King stated that while developing a standard “methodological approach for determining what constitutes species, subspecies and DPSs” is a laudable goal, the practical reality is elusive. Nonetheless, we believe a standard genetic methodology will help the Service avoid dueling genetic conclusions such as is currently the situation with Preble’s and other subspecies throughout the country. This is an issue that will only become more prevalent as time goes on if a standard genetic methodology is not identified. Therefore, the Service should seek a standard that is sensible, consistent, reliable and reproducible, and able to be conducted with an expenditure of a reasonable amount funds within a reasonable amount of time. The standard genetic methodology should not be so broad to allow the interpretation that the slightest divergence in evolutionary lineage represents a recognized subspecies deserving of protection under ESA. Rather, the standard should be reasonably stringent **and** be used in concert with (not exclusive of) other criteria for determining recognized subspecies.

Although genetic analysis is an important tool in defining species and subspecies, it is only one component of what should be considered the “best scientific and commercial data available” under ESA section 4(b). In addition to setting standard practices for genetic analysis for the identification of subspecies eligible for protection under ESA, the Service should also consider if an eligible subspecies has a unique ecological niche or is ecologically significant. This type of analysis is conducted as part of the Service’s determination of discrete population segments, and should also be considered when determining the listing status of subspecies.

Comparing an eligible subspecies’ ecological significance to other similar subspecies and determining whether it has a unique ecological niche should be used in concert with the standard genetic methodology discussed above. For example, the King study identified Preble’s as a unique subspecies and went even further to describe potential evolutionary differences between populations in Larimer County and populations in Douglas County and El Paso County. While King may have identified such evolutionary differences, Preble’s populations as well as other more abundant subspecies of *Z. hudsonius* occupy the same type of habitat, eat the same types of food, and provide the same ecological functions as all *Z. hudsonius* populations throughout North America. As such, Preble’s may not have a unique ecological niche, or be ecologically significant from other *Z. hudsonius* subspecies.

Although King et al. (2006) suggested that mice sampled in Douglas and El Paso counties may be considered “genetically distinct populations worthy of individual management considerations,” we believe other factors should be taken into consideration as the Service considers the listing status of Preble’s and whether certain populations can be designated as distinct population segments (DPS). We previously addressed this issue in our May 27, 2004 comments in response to the Service’s request for public comment on its Status Review for the 12-month finding and 5-year review of Preble’s. In those comments, we specifically discussed: 1) the increase in known distribution, 2) the improvement in habitat conditions, 3) additional conservation measures, 4)

reduction in threats, and 5) the overall conservation status of Preble's as these issues relate to the listing status and consideration of a DPS designation.

All of the points made in our May 27, 2004 comment letter remain true today, and are further bolstered by the fact that Douglas County and the Towns of Parker and Castle Rock have followed through on their commitment to implement a county-wide habitat conservation plan for the benefit of riparian habitat and Preble's. As you know, Douglas County and the Towns were recently issued their incidental take permits for their HCP efforts. The HCP has been designed to conserve habitat supportive of Preble's regardless of the uncertainty related to its listing status. Should the Service determine to delist Preble's and instead seek protection of the species as a DPS, we reserve the right to comment on that issue as part of the required subsequent comment period.

In conclusion, the Service must determine how it will proceed into the future in determining which subspecies will receive ESA protection. Without such a determination, uncertainty, unnecessary expenditure of resources and time, and the diversion of funds away from those species and subspecies that are truly in need of protection under the ESA will continue.

We appreciate the opportunity to provide input concerning the proposed delisting of Preble's. Should you require additional information or clarification regarding our comments, please let me know.

Sincerely,

A handwritten signature in cursive script that reads "Cheryl Matthews".

Cheryl Matthews  
Director