Review of Ramey et al.: Testing the taxonomic validity of Preble's Meadow Jumping Mouse (*Zapus hudsonius preblei*); and Testing the uniqueness of *Z. h. intermedius* relative to *Z. h. campestris*

David J. Hafner, Ph.D
New Mexico Museum of Natural History
1801 Mountain Road NW
Albuquerque, NM 87104
(505) 841-2825
Fax (505) 841-2866
dhafner@nmmnh.state.nm.us

20 May 2005

In my original review of the first of Ramey et al.'s reports, I generally agreed with the methods and taxonomic conclusions made by the authors, but disagreed strongly with their view of conservation implications of those taxonomic conclusions. I was also critical of the condescending and ponderous, "preachy" tone of the report.

Subsequent to filing my review, I have been increasingly offended by Ramey's attempts to portray himself as some sort of champion of truth and right opposed to poor scientists entrenched in dogma. Ramey has also charged, in newspaper articles, that only two of the eight reviews of his work were "independent," and did not include my name among those "independent" reviews. I take personal exception to his implication that my review was in some fashion biased. Such a comment is unprofessional and uncalled for, and serves to underscore his grandstanding, soapbox approach to science.

More to the point, the addition of samples of *Z. h. intermedius* samples to this study and the resulting addition of geographic and taxonomic perspective has revealed to me some basic errors in my initial review and, importantly, in the taxonomic as well as conservation conclusions of Ramey et al.'s reports. I now interpret the pattern of mtDNA haplotypes among these four western subspecies of *Z. hudsonius* to support retention of all four subspecies, including *Z. h. preblei* and *Z. h. campestris.*

The most important and overriding consideration of both of these reports is that this is the application of mtDNA sequence data to *subspecies*, not to taxa of a higher level that have been reproductively isolated. Even in *species* that have been recently isolated reproductively, one would expect to find common, ancestral haplotypes during the time necessary to sort mtDNA haplotypes (referred to as "sorting errors"). Thus, the ancestral haplotypes shared among multiple subspecies of *Z. hudsonius* mean nothing, and one cannot simply dismiss subspecific differentiation based on the existence of shared mtDNA haplotypes between neighboring subspecies. Such sharing is *expected*.

Based on mtDNA sequence data alone, recognition of subspecies (to my mind) becomes rather "squishy" and subjective. Furthermore, while Ramey et al. may preach against it, the fact is that it has always been more difficult to synonymize subspecies than to recognize them: *any* difference becomes a measure of differentiation, however slight. The challenge, then, is to establish a "benchmark" for comparision within the broader geographic group: if one recognizes a particular form as a valid subspecies, then any geographic group *more* differentiated must also be recognized, and those *less* differentiated may be considered as candidates for synonymy. It's not an exact science, no matter how much Ramey et al. rail against reality.

The only thing that the bootstrap-supported branches on Fig. 2 (second report) tell you is that there are two major mtDNA groups: *luteus* and *pallidus* on the one hand, *intermedius*, *campestris*, and *hudsonius* on the other. If you want to go out on a limb (sorry about that pun) and interpret branching patterns that are not supported by bootstrap values, I would interpret each branch in this manner: the

luteus-pallidus branches are all mixed up, while within the *intermedius-campestris-preblei* clade we first have the geographically distant, eastern *intermedius*, then a *preblei* clade, then a mixture of *intermedius-campestris*, with most of the *campestris* in one clade. Looks like sorting of ancestrally shared haplotypes is going on, just as one would expect for subspecies in the process of differentiation.

No one, not even Ramey et al., has questioned the subspecific recognition of *Z. h. luteus*. It is geographically quite isolated from *pallidus*, and is clearly differentiated from *preblei* based on mtDNA (again, simply ignore those haplotypes shared by multiple taxa between the two major branches of the mtDNA tree). Yet look at the degree of mtDNA differentiation between *luteus* and *pallidus*: there's nothing there! Populations of *luteus* are found basal to that branch and at the very tips. If you accept *luteus* as a valid subspecies, then that means you have to accept *preblei* and *campestris* as well: they're at least as differentiated, if not more so, from *intermedius* than is *luteus* from *pallidus*.

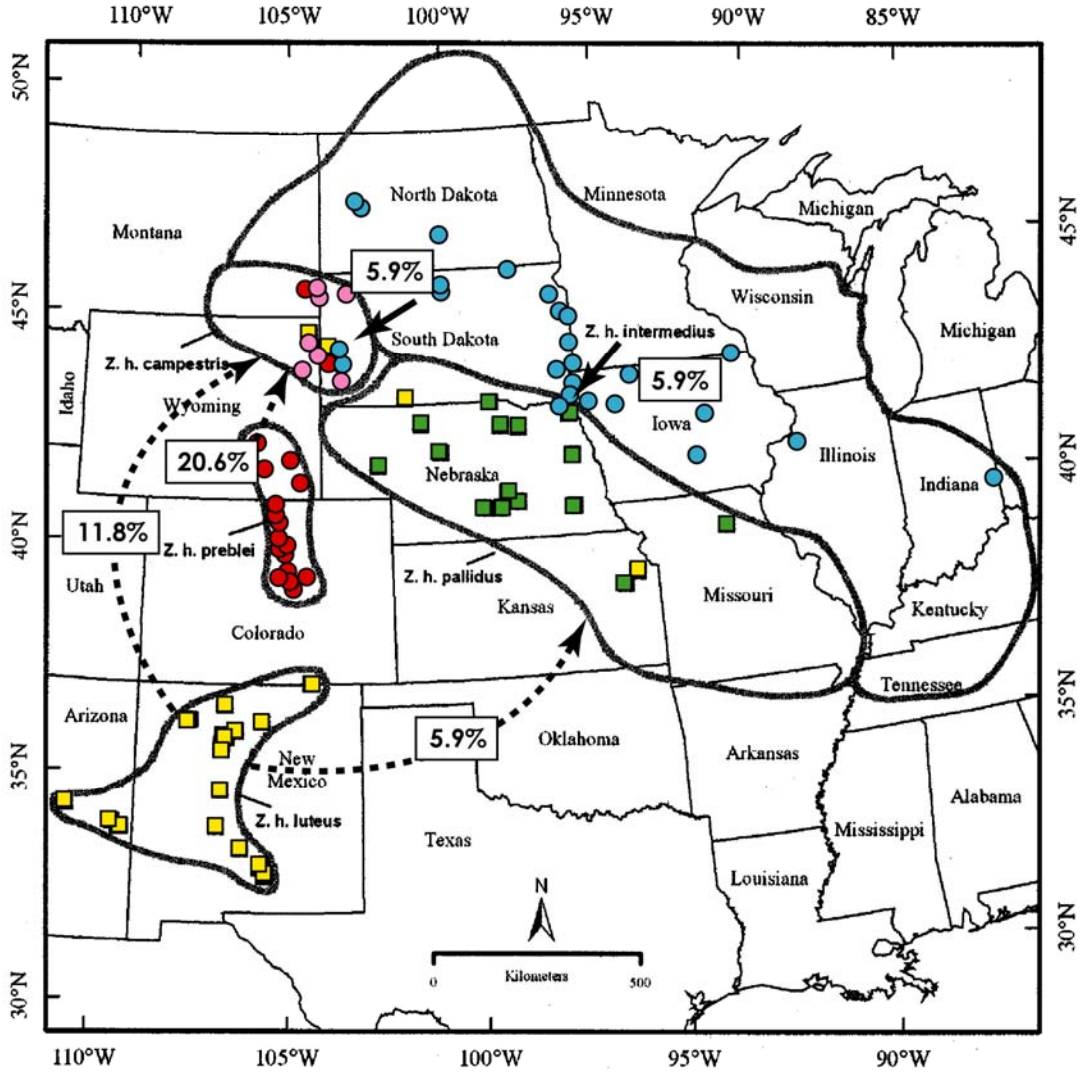
By the way, there are a number of omissions in the reports that would certainly have been picked up if this were reviewed for publication. For example, Ramey et al. report the number of variable sites within each species, but never report the number of *phyletically informative* sites (i.e., those that indicate shared relationships among taxa). With the many inconsistencies among the reports, tables, and figures, it's nearly impossible to determine the sample sizes of each group. I have listed errors or disagreements between text, Table 1, and/or Figure 2 (in the second report) in Appendix I.

Another way to look at the data, with the idea of sorting in mind, is to see just how "mixed" those haplotypes still are. Using the assumed sample sizes, I've assigned the haplotypes to one of the five subspecies based on which subspecies has the highest percentage of that haplotype, then indicated the percentages of each subspecies sample that has that subspecies haplotype (the "correct" haplotype) vs haplotypes assigned to other subspecies ("shared" haplotypes; Table 1). (I've admittedly wiggled the data in one case: one individual each of the *intermedius* and *campestris* samples shared the C9/INT7 haplotype. Because the *intermedius* sample size is higher, its percentage was slightly lower (2.3 vs 2.9%). Because *campestris* has retained many other haplotypes, and this would be the only case of *intermedius* sharing a haplotype, I've assigned C9/INT7 to *intermedius* instead of *campestris*.) Three of the subspecies (*luteus*, *intermedius*, and *preblei*) have only the "correct" haplotype. In contrast, the subspecies *pallidus* and *campestris* share haplotypes assigned to other subspecies: *pallidus* has 5.9% each of *luteus* and *intermedius* haplotypes, while *campestris* has 11.8% *luteus* haplotypes, 5.9% *intermedius* haplotypes, and 20.6% *preblei* haplotypes. The *intermedius* haplotype is found in *pallidus* only at the subspecific boundary, and could represent either misidentification or migration. There are a number of possible explanations why *pallidus* and *campestris* should retain such diversity. The most simple explanation is that *luteus*, *intermedius*, and *preblei* have experienced population bottlenecks, thus speeding the sorting process, whereas *pallidus* and *campestris* historically have enjoyed larger and more continuous populations. The great amount of mixing in *campestris* could also be due to immigration from neighboring *intermedius* (5.9%) and *preblei* (20.6%). I think it most likely that the *luteus* haplotypes retained by *campestris* and *pallidus*, and the *preblei* haplotypes retained by *campestris* represent incomplete sorting, while the *intermedius* haplotypes found in *campestris* and *pallidus* probably represent migration and/or misidentification (respectively). I've indicated this in Figure 1 (revised from Ramey et al.).

TABLE 1: Distribution of haplotypes (assigned to subspecies) among subspecies.

Subspecies (*n*)	Haplotype	***luteus*** (32)	***pallidus*** (34)	***intermedius*** (44)	***campestris*** (34)	***preblei*** (54)
luteus	L1	3.1				
(32)	L2	6.3				
	L3	3.1				
	L4	31.3				
	L5	3.1				
	L6	6.2				
	L/PAL/C1	28.1	2.9		5.9	
	L/PAL/C2	18.8	2.9		5.9	
Σ		**100.0**	5.9		11.8	
pallidus	PAL2		2.9			
(34)	PAL3		11.8			
	PAL4		8.8			
	PAL5		2.9			
	PAL6		5.9			
	PAL7		23.5			
	PAL8		23.5			
	PAL9		5.9			
	PAL10		2.9			
Σ			**88.2**			
intermedius	INT1			2.3		
(44)	INT2			2.3		
	INT3			2.3		
	INT4			2.3		
	INT5			2.3		
	C8/INT-6			29.5	2.9	
	C9/INT7			2.3	2.9	
	INT8			9.1		
	INT9			2.3		
	INT10			2.3		
	INT11			4.5		
	INT12			11.4		
	INT14			11.4		
	PAL1/INT15		5.9	13.6		
	INT16			2.3		
Σ			5.9	**100.0**	5.9	
campestris	C1				2.9	
(34)	C2				2.9	
	C3				2.9	
	C4				2.9	
	C5/INT13				26.5	
	C6				2.9	
	C7				2.9	
	C10				17.6	
Σ					**61.8**	
preblei	C/P1				5.9	16.7
(54)	C/P2				5.9	22.2
	C/P3				5.9	44.4
	C/P4				2.9	16.7
Σ					20.6	**100.0**

Figure 1. Distribution of haplotypes among subspecies of *Zapus hudsonius*.



Shared-primitive (incomplete sorting):

***luteus* haplotypes in *pallidus:* 2 haplotypes (2.9%, 2.9%)**

***luteus* haplotypes in *campestris:* 2 haplotypes (5.9%, 5.9%)**

***preblei* haplotypes in *campestris:* 4 haplotypes (2.9%, 5.9%, 5.9%, 5.9%)**

Migration or mistaken assignment to subspecies:

***intermedius* haplotypes in *campestris:* 2 haplotypes (2.9%, 2.9%)**

***intermedius* haplotype in *pallidus:* 1 haplotype (5.9%)**

Figure 2. Summary of distribution of haplotypes among subspecies and clades of *Zapus hudsonius*.

	Clade I		Clade II		
	luteus	*pallidus*	*intermedius*	*campestris*	*preblei*
luteus	100.0				
pallidus	* 5.9	88.1	** 5.9		
intermedius			100.0		
campestris	* 11.8	** 5.9		61.8	* 20.6
preblei					100.0

* Shared-primitive (incomplete sorting)

** Migration or mistaken assignment to subspecies

Diagonal: % of samples in subspecies with that subspecies' haplotype

I've summarized the distribution of haplotypes among the subspecies and two major clades in Figu`re 2. Note that two of the three cases of incomplete sorting are within clades, and of course the two probable cases of migration or misidentification are between neighboring subspecies. That leaves only one somewhat unexpected case of incomplete sorting: the *luteus* haplotypes found in *campestris*. This to me argues that the current restricted distribution of *campestris* is a recent event, and that the subspecies historically enjoyed a far more extensive and continuous distribution.

In summary, I find no justification for supporting synonymy of *preblei*, *campestris*, and *intermedius*. Instead, the mtDNA data underscore the restricted distribution of *Z. h. preblei* and provide interesting clues to the past biogeographic history of these western subspecies of *Z. hudsonius*. In terms of identifying phyletic groups, it would be more informative to examine an appropriate nuclear marker. It appears that cranial morphology may not provide sufficient discrimination, and most nuclear DNA sequences would likely be too slow-evolving to capture adequate information. Perhaps old-fashioned allozymes might be a more appropriate marker. At any rate, the evident degree of subspecies-specific mtDNA haplotypes supports, rather than refutes, recognition of *Z. h. preblei*.

APPENDIX I: Errors or disagreements between text, Table 1, and/or Figure 2.

1. Fig. 2, C9/INT7: SD(1) should be in pink [*campestris*] rather than light green [*pallidus*]).

2. The branch labeled "INT-VII" towards the bottom of Fig. 2 should instead read "INT-VIII."

3. Sample sizes per subspecies (after correcting for [1] and [2] above):

	luteus	*pallidus*	*intermedius*	*campestris*	*preblei*
Text (initial report)	32	34	N/A	31	54
Table 1 (2nd report)	32	34	47	31	54
Fig. 2 (2nd report)	32	33	51	36	54

a. 3 specimens from "SD: Harding Co." are mis-assigned to *intermedius* in Table 1 (C5/INT13), and are double listed as *campestris* and *intermedius* in Fig. 2. Also, this haplotype is now found only in *campestris*.

b. Table 1 (INT16) lists 1 specimen of *intermedius* (no locality); Fig. 2 indicates 6 specimens. If this locality is near the distribution of *pallidus* (e.g., Clay Co.), it could simply represent mis-assigned specimens (i.e., they are *pallidus*, as they are grouped in Fig. 2, not *intermedius*).

c. Table 1 (PAL3) lists 2 each from KS and MO; Fig. 2 lists 4 from KS.

d. Table 1 (PAL1/INT15) lists 2 *pallidus* from SD; Fig. 2 lists only 1.

e. Table 1 (PAL1/INT15) lists 6 *intermedius* from SD; Fig. 2 lists only 5.

f. Table 1 combines 20 specimens into C8/C10/INT6: 7 *campestris* (WY[2], SD[2], MT[3] and 13 *intermedius* (SD[3], ND[10]); Fig. 2 divides these into C10 (8 *campestris*, WY[4], SD[1], MT[3]) and C8/INT6 (1 *campestris*, SD[1], and 13 *intermedius*, SD[3], ND[10]). I used the lower number of specimens from the Table (20) and the separation indicated in Fig. 2, for the following allocation: C10 (6 *campestris*, WY[2], SD[1], MT[3]) and C8/INT6 (1 *campestris*, SD[1], and 13 *intermedius*, SD[3], ND[10]). Haplotype C10 is found only in *campestris*.

Assuming the Table is correct (exceptions noted in [a] and [f] above):

	luteus	*pallidus*	*intermedius*	*campestris*	*preblei*
Sample Size	32	34	44	34	54