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Morphological versus chromosomal and molecular divergence in two species of *Eligmodontia*

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Abstract

Karyotypic and mtDNA variation support the presence of at least two distinct species of *Eligmodontia* in the Patagonian region of Argentina. No diagnostic morphological characters are available to identify these species reliably, and few data are available to assess reliably the degree of morphological difference between them. We used univariate and multivariate analyses of external morphological and cranial characters in a sample of *Eligmodontia* collected at 15 localities across northern Patagonia to test the hypothesis that these presumed species (*Eligmodontia typus* and *E. morgani*) also are morphologically distinct. No single diagnostic morphological character was identified based on specimens for which independent identifications were available through mtDNA sequence and species-specific diploid numbers. However, discriminant function analyses were able to assign specimens reliably to correct species as independently determined. Cluster analyses based on various combinations of morphological characters showed some congruence with the other data sets, but specimens of known mtDNA haplotype did not cluster together exclusively. These patterns suggest that although the genetic and karyological differences are substantial and potentially represent a deep divergence, these changes are not mirrored by equivalent morphological divergence.

Introduction

Silky mice of the phyllotine genus *Eligmodontia* occupy arid habitats over a large geographic portion of South America from southern Perú to the southern Patagonian region of Argentina. Hershkovitz (1962) noted that as many as 20 species-group names were commonly used for members of this genus prior to his revision in which he recognized only a single species, *E. typus*, with two subspecies. Hershkovitz's (1962) work was based on morphology, and thus by lumping all previously recognized taxa as a single species he acknowledged the relatively low degree of morphological divergence within the genus. Despite more than 30 years of research on this genus and the fact that several species presently are recognized (Musser and Carleton 1993), there still are no diagnostic morphological characters available to assign specimens of *Eligmodontia* reliably to species. Lack of diagnostic characters hinders all aspects of biological research, as even such fundamental tasks as alpha-level faunal surveys and delineation of species distributions require unequivocal specimen identification. We now have a sample of *Eligmodontia* from northern Patagonia representing two species, *E. typus* and *E. mor-*

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gani, for which unequivocal identification based on karyotypic and mtDNA data are available (see Hillyard et al. 1997). This unique sample makes it possible for us to: 1) assess the degree of morphological divergence between the two species; 2) assess how well previously described morphological characters distinguish between them; and 3) search for diagnostic morphological characters or character combinations that allow reliable species identification.

Cytogenetic studies by Ortells et al. (1989), Kelt et al. (1991), Zambelli et al. (1992), and Spotorno et al. (1994) have reported diploid chromosomal numbers of 50, 43–44, 32–33, and 34 for specimens of *Eligmodontia*, and the names *E. puerulus*, *E. typus*, *E. morgani*, and *E. moreni*, respectively, were associated with these cytotypes by Kelt et al. (1991) and Spotorno et al. (1994). Further evidence of species-level or deeper differences within *Eligmodontia* is provided by Hillyard et al. (1997). They sequenced a 348 base-pair region of the cytochrome b gene of specimens of *Eligmodontia* and found two haplotypes that corresponded exactly to the karyotypically distinct *E. typus* and *E. morgani* and differed from each other by as much as 11.8%. In a recent morphological study Braun (1993) recognized six species of *Eligmodontia* in her treatment of phyllotine rodents and showed discrete separation of each species in distance dendrograms. However, clustering methods such as those used by Braun (1993) will find differences between operational taxonomic units (OTU's) regardless of whether or not there are biologically meaningful differences (Engstrom et al. 1994), and the basis for her a priori recognition of six species was not presented.

Given that phyllotine rodents have been present in southern South America for at least several million years (Patterson and Pascual 1972; Marshall 1979; Reig 1978) and that *Eligmodontia* are geographically widespread, karyotypic and chromosomal divergence within the genus is not surprising. However, these molecular and chromosomal differences seem not to be matched by comparable morphological divergence. The apparent high degree of similarity among some species of *Eligmodontia* could arise through (1) wide-spread introgressive hybridization, (2) relatively recent genetic isolation events that have not allowed sufficient time for species to undergo morphological divergence comparable to that observed in molecular and chromosomal data, or (3) selection favoring a similar phenotype among species that is greater than any selection favoring phenotypic divergence.

Materials and methods

We collected specimens used in these analyses on two expeditions (1992 and 1995) to various localities in the Argentine portion of Patagonia. Voucher specimens presently are deposited in the University of Minnesota Bell Museum of Natural History, St. Paul, MN, and the tissue specimens at Illinois State University, Normal, IL. Tissues used for molecular analyses were removed from fresh animals and immediately frozen in liquid nitrogen (for further details see Hillyard et al. 1997). In 1995 we also prepared chromosome spreads following the technique described by Patton (1967) and modified by Lee and Elder (1980) for a subset of animals captured. These karyotypic data were used to assign mtDNA genotypes to species as defined by Kelt et al. (1991) and Ortells et al. (1989). We measured 18 cranial characters (Fig. 1) to the nearest 0.01 mm with digital calipers from all of our adult specimens (including those with and without independent identifications) and recorded four external body measurements (total length, tail length, hind foot length, and ear length) and sex from specimen labels. We also calculated measurements for 5 characters used by Braun (1993).

Only animals with completely erupted dentition were considered to be adult. These adult specimens were placed into three age groups based on tooth wear following criteria specified by Pearson et al. (1987). Categories used were: N if no wear was evident on M^2, W if wear was evident on M^2 but cusps were still distinct, and O if cusps were no longer distinct on M^2. We ordered individuals based on scores for the discriminant multipliers obtained from the canonical discriminant function analysis and assessed the placement of individuals in this list based on their age category assignment. No pattern of age varia-

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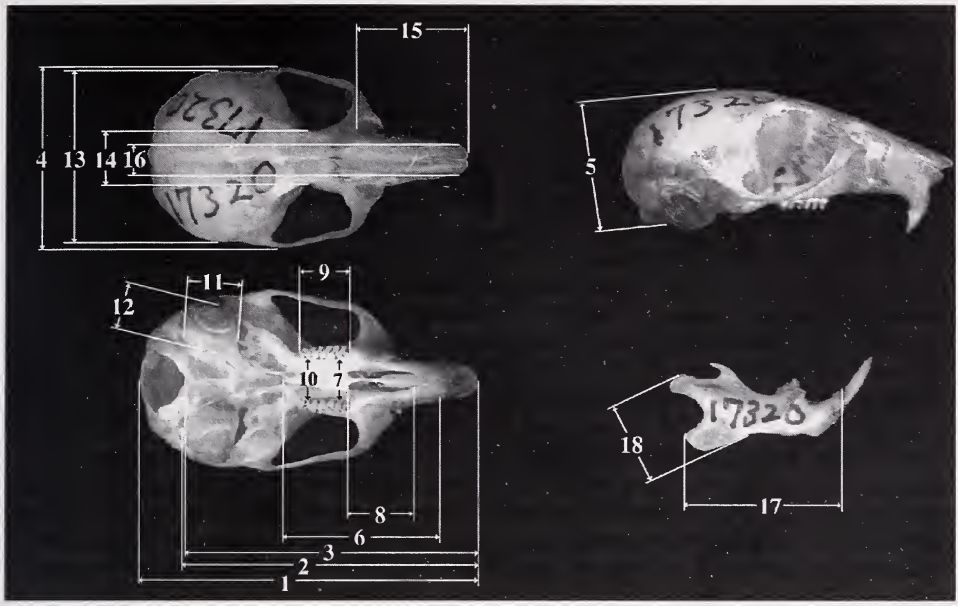

Fig. 1. Digitized image of a skull of *Eligmodontia morgani* illustrating the cranial measurements included in morphological analyses. Numbers represent the following measurements: 1) greatest length of skull; 2) condylobasal length; 3) basal length; 4) zygomatic breadth; 5) greatest depth of skull; 6) length of palate; 7) palatal width at M^1; 8) length of incisive foramen; 9) length of maxillary toothrow; 10) palatal width at M^3; 11) length of auditory bullae; 12) breadth of auditory bullae; 13) breadth of braincase; 14) least interorbital breadth; 15) rostral length; 16) nasal breadth; 17) mandibular length; 18) mandibular height.

tion was revealed, so all individuals classified as adult were included in subsequent analyses and age variation is not discussed beyond. Our data set of adults included 53 animals for which independent identification was available and 124 specimens that lacked independent verification of species assignment. Sample sizes differ slightly among statistical comparisons that follow because only specimens with no missing data for any character were included in multivariate analyses.

Because species identifications in mammals typically are made using cranial or external measurements rather than karyotypes or DNA sequences, our first priority was to perform univariate analyses on morphological characters to try to identify diagnostic characters and to assess variation in individual characters. We used one-way analysis of variance (ANOVA) to test for differences in mean size of each character examined between species and between sexes (excluding the ratios used by Braun 1993). We used Type III Sums of Squares (SS) errors in the General Linear Models (GLM) package of SAS to determine statistical significance (SAS Institute Inc. 1990). Because many one-way comparisons were to be made with each data set, we used a sequential Bonferroni α adjustment to maintain an experiment-wise error rate $\leq 5\%$ (Rice 1989). In these analyses we made 18 one-way comparisons on our morphological characters (including cranial and standard external characters).

We next performed separate cluster analyses on standardized data using all of the cranial and external body measurements with the NT-SYS package (Rohlf et al. 1982) without regard to independent identification based on mtDNA sequences or karyotypes. These cluster analyses were performed to see if specimens could be separated accurately when multiple characters were considered simultaneously. We then performed a similar cluster analysis using only those characters that Braun (1993) found useful in distinguishing between these two species. These characters were: 1) relative tail length (mean tail length divided by mean head-body length); 2) relative ear length (mean ear length divided by mean head-body length); 3) relative hind foot length (mean hind foot length divided by mean head-body length); 4) inflation of tympanic bullae (mean bullar length times mean bullar width divided by mean greatest skull

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length); and 5) relative toothrow length (mean maxillary toothrow length divided by mean greatest skull length). Because one cannot make a priori species identifications to obtain means for the ratios used by Braun (1993), we calculated the ratios using measurements obtained for each individual. We next tested for normality in each of the cranial characters using the Wilks-Shapiro statistic with $\alpha = 0.05$ for significance. These results indicated that four cranial characters: 1) palatal width at M^1; 2) palatal width at M^3; 3) rostral length; and 4) breadth of braincase were not normally distributed and these characters were excluded from further analyses. External body measurements were not tested for normality because some (e. g., hind foot length and ear length) were integer values over only a small range.

We next were interested in determining how well the specimens of known species affiliation could be separated in multivariate character space using each of the data sets considered above. We used discriminant function analysis to assess the utility of morphological data sets (cranial, standard external, and those characters used by Braun 1993) for distinguishing between these two species. Following these discriminant function analyses we used step-wise discriminant function analysis on each data set to identify those characters best able to distinguish between them. Because discriminant function analyses resulted in species separation only in multidimensional space, we next used canonical discriminant function analysis to reduce the dimensionality to the first two canonical dimensions and to compute the raw canonical coefficients that resulted in maximum separation on each axis.

To assess the power of our discriminant function obtained from the previous analyses to assign unidentified specimens to species, we used our sample of animals of known identity as a training set to classify the 124 specimens for which neither mtDNA nor karyotypic data were available. Probability of group membership was calculated based on discriminant scores. We examined a plot of all specimens based on values computed from canonical coefficients that were derived from specimens of known identity (see above) to gain some insight as to the degree to which the specimens clustered into discrete groups.

Because each of the data sets did provide some independent discriminatory power, we next considered all variables in a similar analysis. We again used a stepwise discriminant function to identify the subset of characters best able to classify specimens of known identity correctly. We then performed a discriminant function analysis considering only these characters to classify unknown specimens and computed probability of group membership. We used canonical discriminant function analysis to compute canonical coefficients for the first two eigenvectors derived from those discriminatory variables. Using these canonical coefficients, we computed scores for all specimens and plotted individuals in this two-dimensional space. All analyses except for the cluster programs were performed in SAS (SAS Institute Inc. 1990).

Results

Our karyotypic analyses of a subset of animals (n = 15) collected in 1995 support the conclusions of Ortells et al. (1989), Kelt et al. (1991), and Zambelli et al. (1992) in that we found diploid numbers of 43–44, and 32–33 and clear morphological differences (number and size of metacentrics) for *Eligmodontia typus* and *E. morgani*, respectively. Furthermore, these karyotypes were matched unequivocally to mtDNA haplotypes from tissues taken from the same specimens (Hillyard et al. 1997). By establishing a definite correspondence between mtDNA haplotypes and karyotypes with a subset of individuals, we then were able to assign individuals of known mtDNA haplotypes reliably to species even though corresponding karyotypes were not available. This greatly augmented sample sizes available for morphological comparisons.

Univariate analyses produced no significant differences between the sexes for any character in either species. We detected significant differences between the two species in only 7 of the 18 characters examined using the conservative rejection criterion of the sequential Bonferroni adjustment (Tab. 1). A comparison of means showed that specimens of *E. typus* tended to be larger than those of *E. morgani* for five of these characters, whereas *E. morgani* was larger than *E. typus* for two others. The percent difference between the species for these characters ($\bar{x}$ for *E. typus*/$\bar{x}$ for *E. morgani*) ranged from 19% larger for tail length and 13% larger for ear length in *E. typus* as compared to *E. morgani* and 6% smaller for length of the incisive foramina and 7% smaller for nasal width in

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Table 1. Means, ranges, relative differences (*typus/morgani*), and overlap in the 10 morphological characters that showed the greatest differences between *Eligmodontia typus* and *E. morgani* in univariate analyses. Differences in character means marked with an asterisk were statistically significant with α levels determined by a sequential Bonferroni adjustment to maintain an error rate of 5% across analyses.

Character	n (t, m)	*E. typus*		*E. morgani*		Relative difference	Overlap	P value
		$\bar{x}$	Range	$\bar{x}$	Range			
total length	31,22	175.84	151–205	166.36	146–197	1.06	151–197	0.0112
tail length	31,22	92.71	77–104	78.05	69–90	1.19	77–90	0.0001*
hind foot	31,22	23.13	22–25	22.27	21–25	1.04	22–25	0.0050
ear	31,22	18.05	16–22	16.00	14–19	1.13	16–19	0.0001*
incisive foramen	31,22	4.91	4.34–5.61	5.25	4.72–6.04	0.94	4.72–5.61	0.0007*
maxillary toothrow	31,22	3.74	3.45–3.97	3.60	3.33–3.88	1.04	3.45–3.88	0.0004*
bullar length	31,22	4.05	3.80–4.34	3.61	3.10–4.09	1.12	3.80–4.09	0.0001*
bullar breadth	31,22	4.31	3.91–4.77	4.12	3.75–4.40	1.05	3.91–4.40	0.0003*
nasal breadth	31,21	2.36	2.04–2.61	2.54	2.20–2.88	0.93	2.20–2.61	0.0001*
mandibular height	31,20	5.77	5.22–6.53	5.54	4.92–6.44	1.04	5.22–6.44	0.0304

E. typus. The fact that only 7 characters showed significant differences and that the differences tended to be small illustrate that relatively little morphological difference exists between these species in univariate space. If the α level for rejection were maintained at 0.05 for all comparisons (no Bonferroni correction used to guard against Type I error), 10 characters would have been considered significantly different between the species. However, the small differences in size between these characters further supports our conclusion that morphological differences between these species are subtle at best. In summary, visual inspection of these specimens failed to reveal a diagnostic character that reliably separates specimens of different haplotype lineages and no individual cranial or external measurement was adequate to separate animals of the two haplotypes.

Distance dendrograms based on 18 cranial measurements (Fig. 2) and 4 standard external body measurements (Fig. 3) of 53 independently identified *Eligmodontia* specimens yielded considerable mixing of specimens from different localities and also mixed specimens of known mtDNA haplotype into clusters with no apparent relationship to haplotype. An additional cluster analysis of these animals using only the characters that Braun (1993) regarded as useful in identifying species of *Eligmodontia* showed improved separation of the two species, but still did not match perfectly with the independent identifications (Fig. 4).

A discriminant function analysis based on all cranial measurements was 100% accurate in assigning independently identified individuals to the correct species. A similar analysis based only on the four external body measurements had an error rate of 3%; two *E. morgani* were classified as *E. typus*. The discriminant analysis using only Braun's (1993) characters resulted in an error rate of 6% and misidentified three *E. morgani* as *E. typus*. These analyses demonstrate that given these cranial characters and a sample of individuals of known species identity, the animals could be assigned reliably to the correct species only in multidimensional space.

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Table 2. Specific trapping localities in Argentina and individual specimens of *Eligmodontia* captured at each locality that were identified by mtDNA, karyotype, or both. Individual character identification codes consist of a prefix indicating species (M = *E. morgani*, T = *E. typus*), MMNH specimen number, and suffix for cross-referencing individuals to Figs. 2–5.

Locality	Individuals
~15 km NE Mencué, 40°21.62′S, 69°31.59′W, Río Negro	M16085D, M16086E, M16087F, M16088G, M16089H
S Jose B. Casas, 40°33.25′S, 62°37.49′W, Buenos Aires	T17052x
~18 km SW Viedma, 40°56.41′S, 63°01.25′W, Río Negro	T17053y, T17054z, T17055aa
Arroyo La Fragua, 41°05.11′S, 70°57.26′W, Río Negro	M17419T, M17420U, M17421V
Tembrao, 41°10.19′S, 66°20.16′W, Río Negro	M17097L
Meseta de Somuncurá, 41°21.33′S, 67°55.69′W, Río Negro	T16097v, T16101w, M16098I, M16100J, M16102K
Istmo Ameghino, 42°25.8′S, 64°15.88′W, Chubut	T15963i, T15964j, T15965k, T16006r, T16009s, T16007cc
Caleta Valdés, 42°26.12′S, 67°55.69′W, Chubut	T15863a, T15864b, T15865c, T15871d, T16004p
Puerto Pirámide, 42°33.58′S, 64°15.88′W, Chubut	T159761, T15977m, T15978n, T15979o, T16060ee
Puerto Pirámide, 42°33.94′S, 64°17.27′W, Chubut	T16063u, T15989dd
~100 km W Dolavon, 43°17.14′S, 67°06.25′W, Chubut	T15947h
~30 km NW Pampa de Agnia, 43°28.78′S, 69°49.09′W, Chubut	T16043t
~27 km NW Pampa de Agnia, 43°29.74′S, 69°49.85′W, Chubut	M16011A, M16012B, M16013C
~200 km W Dolavon, 43°32.92′S, 68°07.78′W, Chubut	T15911g
~280 km W Dolavon, 43°45.30′S, 68°57.17′W, Chubut	T15904e, T15905f, T16005q
Ea. La Escondida, 45°19.39′S, 69°50.09′W, Chubut	M17319P, M17320Q, M17321R, M17322S
Meseta El Pedrero, 46°46.55′S, 69°37.59′W, Santa Cruz	T17173bb
Chile Chico, 46°53′S,70°56′W, Santa Cruz	M17112M
Ea. El Rincón, 46°56.35′S, 70°48.57′W, Santa Cruz	M17301O

►

Fig. 2. UPGMA distance dendrogram from cluster analysis based on 14 cranial characters of *Eligmodontia* representing 15 localities. The cophenetic correlation coefficient is 0.704. Identification labels are MMNH catalog numbers with a prefix indicating mtDNA species affiliation ("M" = *E. morgani*, "T" = *E. typus*). Character suffixes are used to cross-reference to individuals in Fig. 5 and Table 2.

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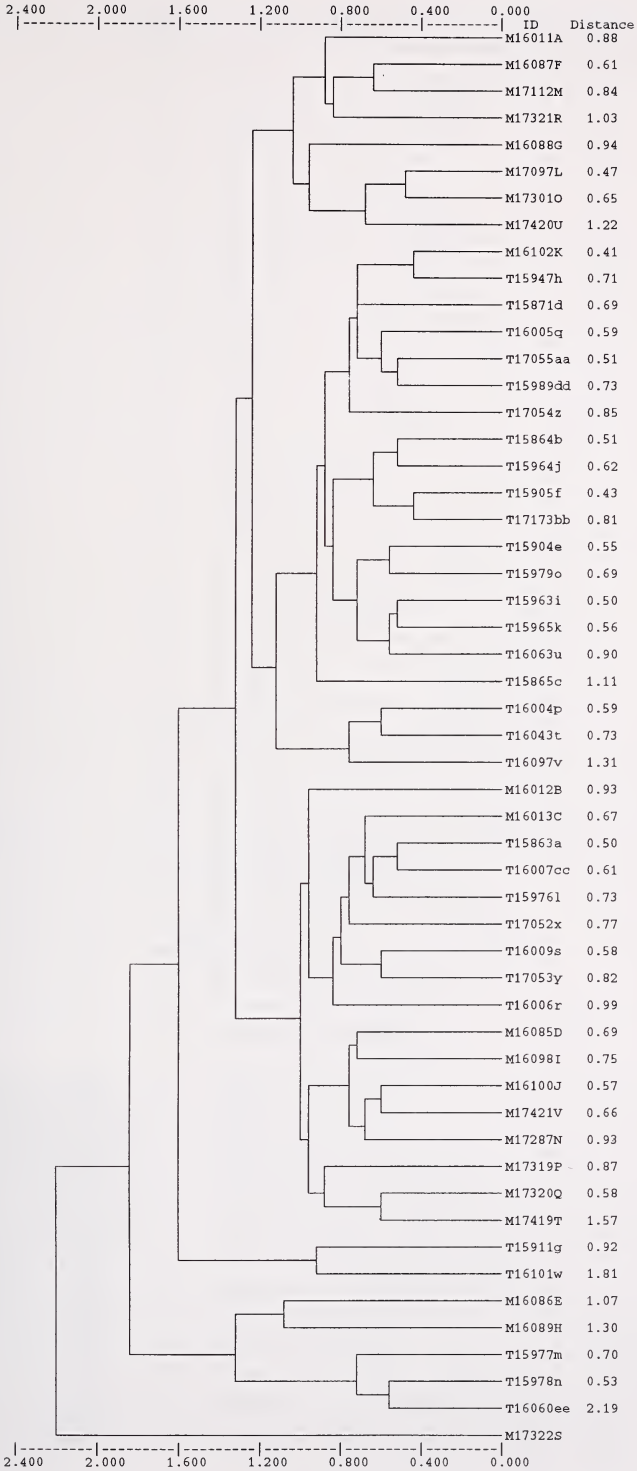

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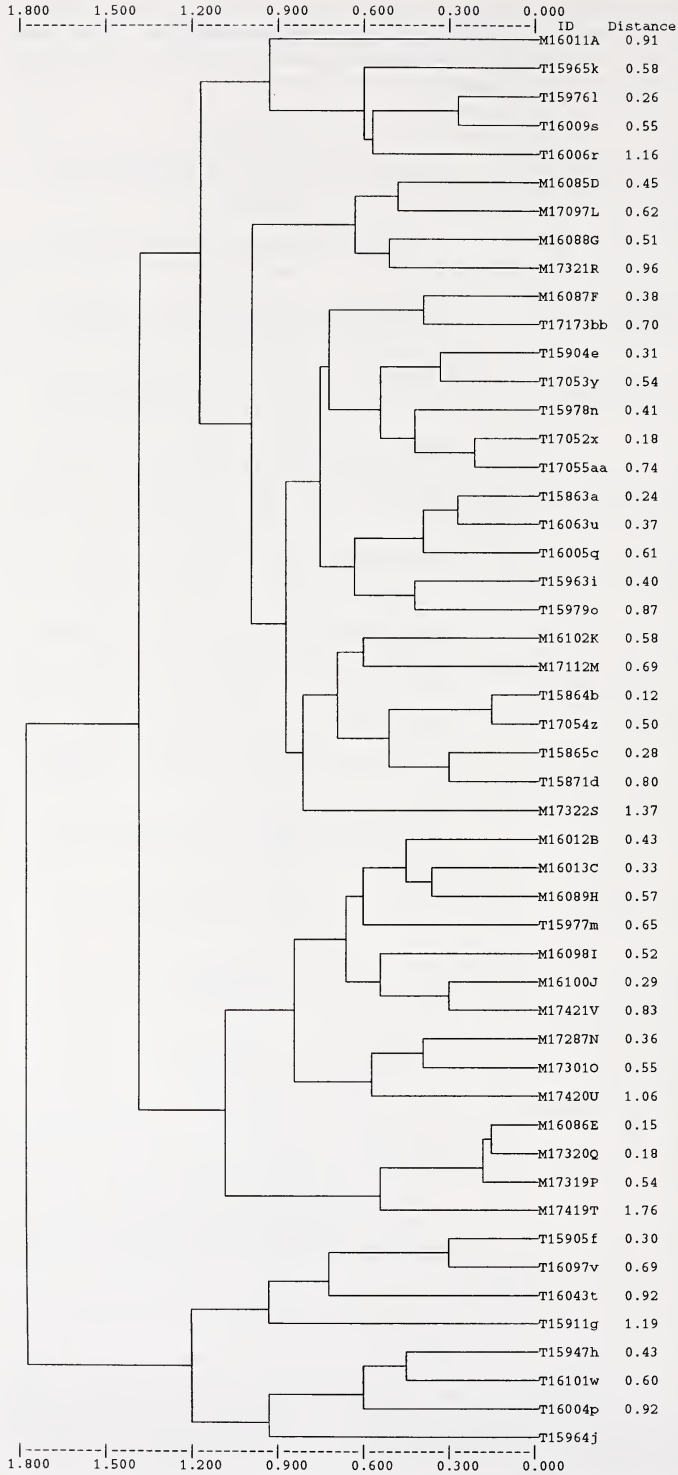

Fig. 3. UPGMA distance dendrogram from cluster analysis based on 4 standard external body measurements of *Eligmodontia* that showed mtDNA separation. The cophenetic correlation coefficient is 0.646. Identification labels are MMNH catalog numbers with a prefix indicating mtDNA species affiliation ("M" = *E. morgani*, "T" = *E. typus*). Character suffixes are used to cross-reference to individuals in Fig. 5 and Table 2.

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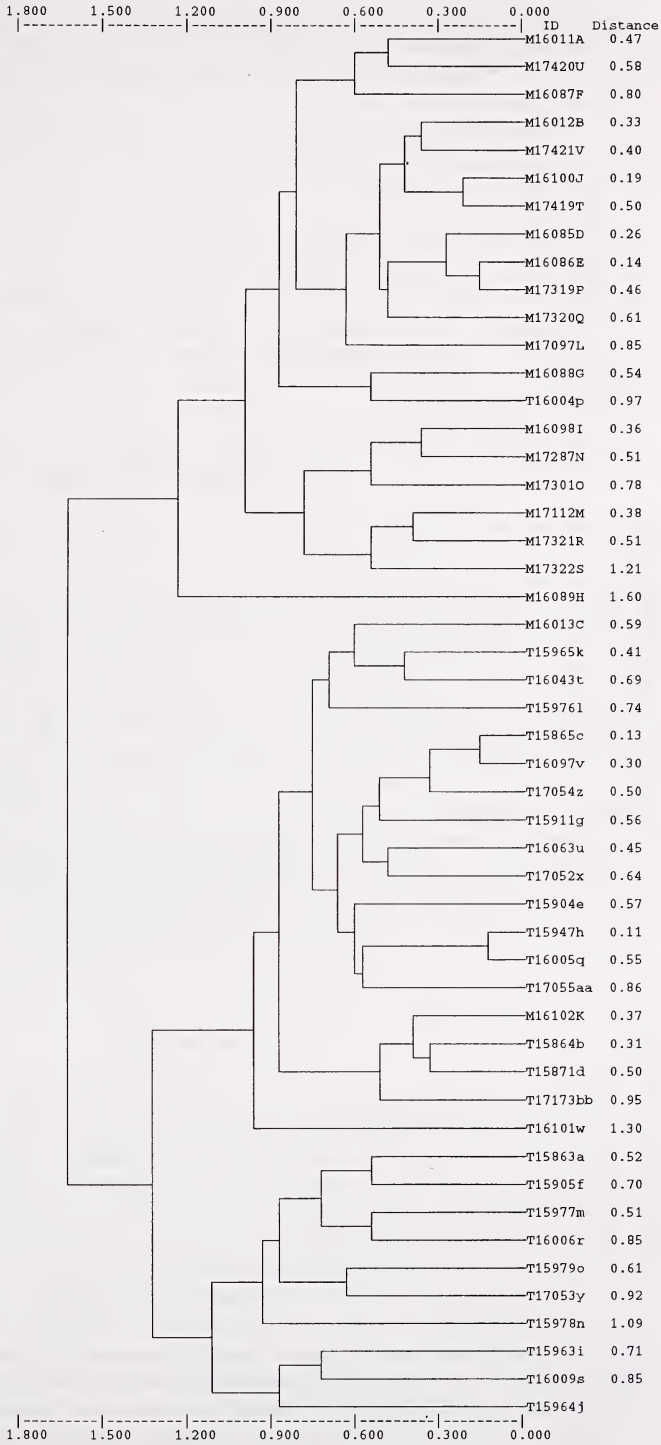

Fig. 4. UPGMA distance dendrogram from cluster analysis based on 5 characters (Braun 1993) of *Eligmodontia* that show mtDNA separation. The cophenetic correlation coefficient is 0.649. Identification labels are MMNH catalog numbers with a prefix indicating mtDNA species affiliation ("M" = *E. morgani*, "T" = *E. typus*). Character suffixes are used to crossreference to individuals in Fig. 5 and Table 2.

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Step-wise discriminant function analyses allowed us to identify the characters with the strongest discriminating ability. Eight cranial characters met the admission criterion of the model. These characters were: 1) length of auditory bullae; 2) zygomatic breadth;

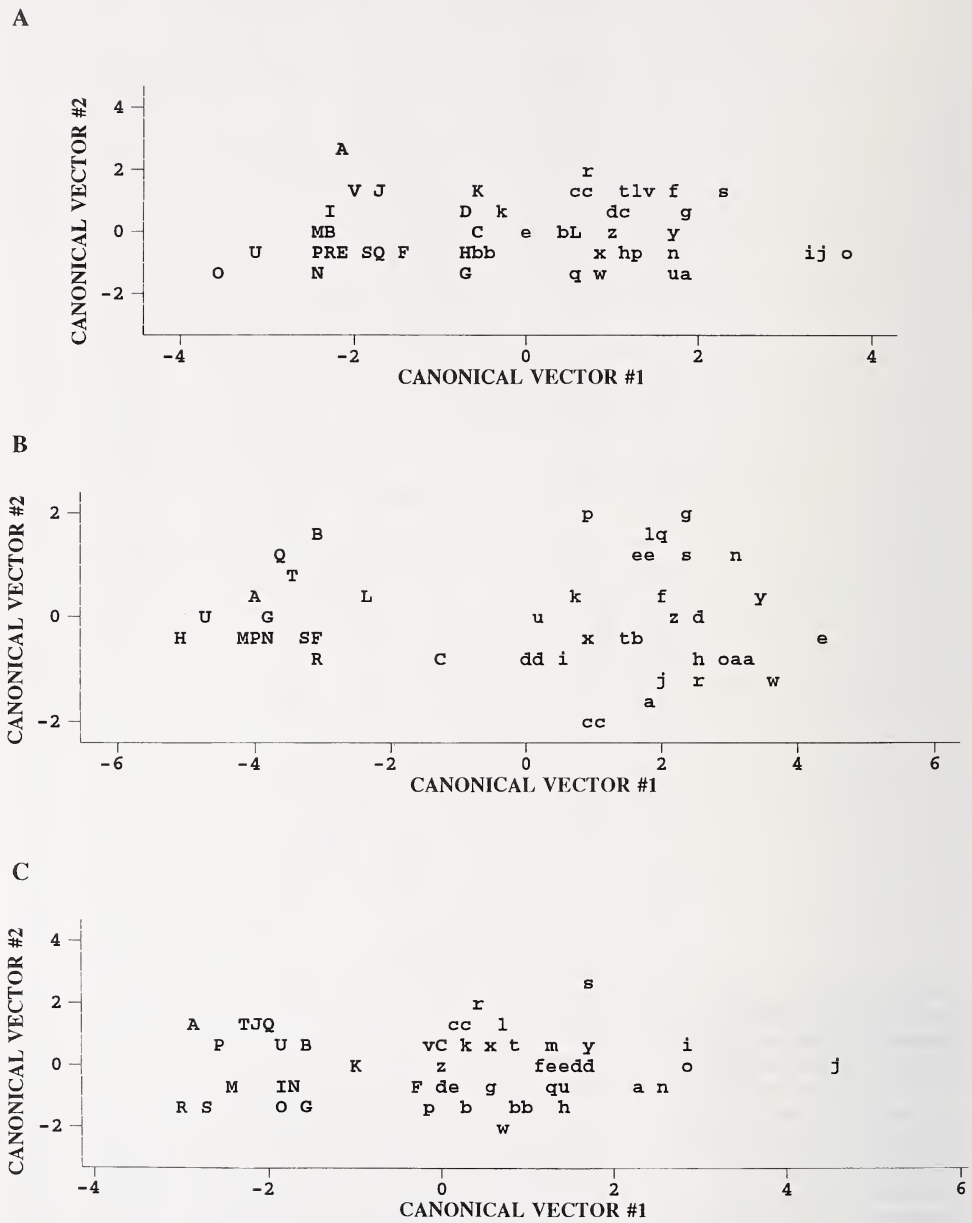

Fig. 5. Plots of canonical scores on first 2 canonical dimensions using those characters found to be significant discriminators by step-wise discriminant function analyses. A) external body measurements, B) cranial measurements, C) Braun's characters. Characters identify individuals with uppercase denoting *E. morgani* and lowercase denoting *E. typus*. Characters can be used to cross-reference individuals in Figs. 2–4 and Table 2.

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3) breadth of auditory bullae; 4) length of palate; 5) length of maxillary toothrow; 6) greatest depth of skull; 7) mandibular length; and 8) length of incisive foramina. The three external characters that best distinguished between the species were: 1) tail length; 2) total length; and 3) length of the ear. Four of Braun's (1993) characters met the criterion for admission to the discriminant model. These characters were: 1) relative tail length; 2) relative length of the hind foot; 3) relative ear length; and 4) relative toothrow length. The step-wise discriminant function analysis eliminated only inflation of the auditory bullae in this last step.

Canonical discriminant function analysis reduced the dimensionality of the multivariate data set created in the step-wise discriminant function analysis (Fig. 5 a–c). When the data sets were considered separately, the combination of cranial characters proved most useful in assigning individuals to species. Results of the canonical discriminant function analysis of cranial characters showed that the first canonical dimension accounted for much of the variation ($R^2 = 0.88$). The species differed most widely in sums of the following linear combination: length of auditory bullae $\times 4.945$ – zygomatic breadth $\times 1.552$ – length of incisive foramen $\times 1.564$ + breadth of auditory bullae $\times 4.080$ + length of maxillary tooth row $\times 5.137$ + mandibular height $\times 1.995$ – length of palate $\times 2.270$ + greatest depth af skull $\times 1.341$.

Comparisons of the ability of the discriminant functions computed from specimens of known identity for each of the character sets to classify specimens of unknown identity showed that the combination of cranial characters was most useful. Ninety-three of 101 specimens (92.1%) were assigned to species with ≥95% probability of correct assignment using the discriminant function for cranial characters. In fact, 91 of 101 (90.1%) were classified with a probability ≥99.5%. However, a plot of specimens based on values computed from these same canonical coefficients did not show completely discrete clusters of individuals. In contrast, only 89 of 117 specimens (76.1%) and 76 of 103 specimens (73.8%), respectively, of unknown identity were assigned to species with ≥95% probability using data from external characters and Braun's (1993) characters. Despite the fact that the characters in each of these three data sets provided some discriminatory power when considered separately, when all of the characters were combined in an attempt to classify the unknowns, the number of individuals assigned to species with a probability ≥95% (82 of 88 specimens = 93.2%) was only slightly greater than results obtained using data on cranial characters alone. As in previous plots, there was no discrete clustering of specimens based in values computed from canonical coefficients.

Discussion

The original description of *Eligmodontia morgani* (Allen 1901) states that it is similar in color to *E. elegans* (now in the synonomy of *E. typus* – see Musser and Carleton 1993), but has a smaller skull, shorter tail, and smaller ears. These characters were insufficient to assign our specimens unequivocally to species because there was overlap between individuals of known mtDNA haplotypes. Furthermore, despite the fact that significant differences were found between species in seven of the 18 morphological characters examined, specimens with independent identifications could not be classified correctly based on any single character. These results underscore the fact that single morphological characters and the ratios used by Braun (1993) are inadequate for reliable species identification. The fact that individuals from different localities and with different mtDNA haplotypes were mixed in our cluster analyses that were performed without regard to independent identifications further emphasizes the minimal degree of morphological divergence between these species. Although it should be noted that these analyses included individuals from 15 localities and included individuals of both sexes, our data indicate that there is lit-

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tle or no sexual dimorphism in the morphological characters that we examined, so sex difference as a confounding factor was nonexistent or minimal. Our data set was insufficient to assess geographic variation within these species, but it is unlikely that "noise" introduced by geographic variation alone could inflate the within species variation to such an extent that between species variation was not detectable. Nevertheless, this is a potential that must be considered in future studies.

Only discriminant function analysis using cranial characters was able to identify correctly 100% of specimens of known mtDNA haplotypes as *E. typus* or *E. morgani*, so complete separation of the species based on morphology was possible only in multivariate character space. When specimens were plotted by canonical discriminant scores using only those characters found useful by the step-wise discriminant procedure, species did produce discrete clusters using eight cranial characters, less discrete clusters using Braun's (1993) ratios, and even less discrete clusters using standard external measurements. The linear combination of cranial characters, although not useful for field identification, should aid interested investigators in verifying the identity of museum specimens. Furthermore, the fact that specimens of unknown identity were assigned to species nearly as reliably based only on a combination of cranial characters as they were with a combination of all available characters suggests that a reduced data set consisting only of those characters found useful with step-wise discriminant function analysis is sufficient to identify most specimens. Despite the fact that we could not assign specimens reliably to species based on external characters, in handling adult animals in the field we sometimes had the general impression that adult individuals later identified as *E. morgani* on the basis of mtDNA haplotype were slightly smaller and more compact in body structure, with shorter ears and tails and softer pelage than those later identified as *E. typus*. This suggests that although external characters cannot be used to identify all specimens, they do provide some discriminatory power. These observations are consistent with the original description of *E. morgani* (Allen 1901).

Given the large difference in diploid chromosome numbers and the high degree of mtDNA divergence between these two groups, the position that they should be included under a single species name (Hershkovitz 1962) is clearly untenable (Ortells et al. 1989; Kelt et al. 1991; Zambelli et al. 1992; Braun 1993; Spotorno et al. 1994). However, the question of why they are so similar morphologically remains. Sympatric species that are reproductively isolated and that occupy similar niches generally are expected to diverge morphologically to minimize competition (Dayan et al. 1989, 1990; Grant and Schluter 1984; Malmquist 1985). We feel that there are at least three possible hypotheses for the lack of morphological divergence that warrant consideration: 1) interspecific hybridization; 2) recent, rapid divergence in mtDNA and karyotypes without differences in selective forces sufficient to cause morphological divergence; and 3) geographically widespread selective forces favoring a single phenotype within Patagonian populations of *Eligmodontia* that are affecting both species.

Introgressive hybrization could account for morphological similarity by the mixing of nuclear DNA. Given that mtDNA is inherited maternally and is not subject to recombination (Avise 1994), such hybridization would not obviate the presence of two distinct mtDNA lineages, which have been reported in mixed populations of *Canis lupus* × *Canis latrans* in North America (Wayne 1996). However, the amount of chromosomal divergence in *Eligmodontia* argues strongly against this hypothesis, suggesting instead that they are reproductively isolated (Kelt et al. 1991). None of the karyotypic studies to date has reported any specimen that was a potential hybrid. Finally, the fact that we were able to obtain discrete separation using discriminant function analysis supports the presence of at least two distinct species and provides no evidence of intermediate forms. On the basis of these considerations we feel that the hybridization hypothesis can be rejected.

Both of the remaining explanations depend on similarity of selective forces to main-

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tain the low degree of morphological divergence between these species. Similarity of at least abiotic selective forces is likely in areas of sympatry, but *E. morgani* supposedly is confined to the western edge of the Argentine Patagonian region, whereas *E. typus* is considered to occur throughout central and eastern Patagonia (Kelt et al. 1991). Nevertheless, sympatry between these two has been documented previously. Zambelli et al. (1992) reported both the 2n = 44 and 2n = 32–33–34 karyotypes (= *E. typus* and *E. morgani*, respectively) at two localities in northern Patagonia–Junín de los Andes (Neuquén Province) and Los Menucos (Río Negro Province). Our data extend the distribution of *E. morgani* further eastward than previously was recorded and document additional localities of sympatry (Hillyard et al. 1997). Furthermore, because identification of many putative distributional records for these species was based on morphology alone without the benefit of a comparison data set to verify identity, or worse, solely on geographic location, the accuracy of most identifications is subject to verification and thus we really do not know distributional limits or degree of sympatry between the two species at this time. Nevertheless, the fact that even specimens from disparate localities show an extremely high degree of morphological similarity suggests either that the chromosomal and molecular divergence has been relatively recent and quite rapid (hypothesis 2) or that the primary selective forces influencing morphology are widespread and uniform (hypothesis 3).

Given the amount of mtDNA divergence reported by Hillyard et al. (1997) and using the most conservative rate of divergence discussed by Smith and Patton (1993), one can estimate that the separation between these two species occurred between 2.5 and 3 million years before the present (Pliocene or early Pleistocene). However, the available fossils of phyllotine rodents such as *Auliscomys*, *Graomys*, and *Reithrodon* dating from 2–3 mybp show little difference in comparison with modern specimens of the same genera (Reig 1978). The chromosomal differences observed in *Eligmodontia* are substantial and appear to have involved much more than simple chromosomal fissions and fusions (Ortells et al. 1989). Furthermore, the magnitude of mtDNA divergence between these two species is greater than typically is seen at the species level (Avise 1994). We can think of no reason why the rate of mtDNA divergence in *Eligmodontia* should be greater than in other rodent species. Collectively, because the fossil data support an early adaptive radiation of the Phyllotini and little morphological change in the intervening time, and because the magnitude of chromosomal and mtDNA differences is substantial, the hypothesis of rapid recent molecular and chromosomal divergence to explain differences between these species, although not fully falsified, seems unlikely.

If *E. morgani* and *E. typus* had non-overlapping distributions and selective forces influencing morphology of these species were consistent across both of their ranges, then extreme morphological similarity would not be surprising. However, in areas of sympatry this degree of morphological similarity would be expected to result in strong interspecific competition favoring character divergence (Dayan et al. 1989, 1990; Grant and Schluter 1984; Malmquist 1985). Although the biogeographic history of the two species is unclear, the genetic data available (Hillyard et al. 1997) and the present (albeit incomplete) distribution maps of these species suggest that they are sympatric in only a small portion of their present ranges. Historically, however, the two species might have interacted ecologically in interdigitating patches of Oriental (*typus*) and Occidental (*morgani*) landscapes as the two waxed and waned with changing temperature and precipitation as envisioned by Hillyard et al. (1997). Even if this hypothesis is correct, however, the total geographic ranges of the two species are largely allopatric and probably have been so for a very long time. Consequently, competition leading to character divergence probably has not been a major factor in their evolutionary history. Nevertheless, the evidence of past and present sympatry raises important ecological questions that now must be addressed concerning mechanisms of coexistence in these areas. How are mice of the two morphologically simi-

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lar species partitioning available resources to minimize competition? To address this question we must first obtain better data concerning the distribution and microhabitat use of both species. By using the morphological data from our specimens as a "training" data set, it is possible to assign specimens of unknown or questionable species affiliation now in systematic collections to species with a high degree of reliability. These data will enable researchers to re-assess identification of existing specimens, better define species ranges, identify environmental features correlated with each species' distribution, and verify areas of presumed allopatry and sympatry. With these types of data in hand we can begin to evaluate species specific habitat requirements and assess how these congeners partition habitat space and resources.

There is no question that the Patagonian region supports at least two species of *Eligmodontia*, but their distribution and habitat requirements are poorly understood. *Eligmodontia* presents an evolutionary and ecological puzzle. Spotorno et al. (1994) suggested that the high degree of chromosomal divergence among the species of this genus might have resulted from isolation by both geographic (extrinsic) and chromosomal (intrinsic) factors. Our data regarding the morphological similarity between these species suggest that if they arose allopatrically the selective regimes affecting them either were too weak or too similar to produce much morphological divergence.

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Zusammenfassung

Morphologische, chromosomale und molekulargenetische Divergenz bei zwei Arten von Eligmodontia

Karyologische Befunde und Untersuchungen der mt-DNA sprechen für das Vorhandensein von mindestens zwei verschiedenen Arten von *Eligmodontia* im argentinischen Patagonien. Die bisher vorhandenen morphologischen Daten reichen jedoch nicht aus, um die Individuen der beiden Arten verläßlich voneinander zu unterscheiden. In der vorliegenden Arbeit wurden an Stichproben von *Eligmodontia* aus 15 Herkünften in Patagonien morphologische Untersuchungen (äußere Körpermerkmale, Schädelmerkmale) durchgeführt. Mittels uni- und multivariater Analysen der Meßwerte wurde die Hypothese getestet, daß die vermuteten Arten *Eligmodontia typus* und *E. morgani* auch morphologisch voneinander verschieden sind. Von den einzelnen morphologischen Merkmalen erwies sich nach einem Vergleich mit chromosomalen und mtDNA-Daten keines als differentialdiagnostisch. Mittels Diskriminanzanalysen konnten die jeweiligen Individuen jedoch zuverlässig der einen oder anderen, aufgrund unabhängiger Daten postulierten Arten zugeordnet werden. Auf verschiedene Kombinationen von morphologischen Merkmalen gestützte Clusteranalysen zeigten einige Übereinstimmung mit den chromosomalen und molekularen Datensätzen. Individuen eines bestimmten mtDNA- Haplotyps bildeten jedoch nicht immer eine einheitliche Gruppe. Obwohl es bei *Eligmodontia* in Patagonien deutliche molekulare und karyologische Unterschiede gibt, werden diese nicht unbedingt von einer entsprechenden morphologischen Divergenz begleitet.

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