

PHYLOGENETIC AND ENVIRONMENTAL COMPONENTS OF MORPHOLOGICAL VARIATION: SKULL, MANDIBLE, AND MOLAR SHAPE IN MARMOTS (*MARMOTA*, RODENTIA)

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Abstract.—The phenotype is a product of its phylogenetic history and its recent adaptation to local environments, but the relative importance of the two factors is controversial. We assessed the effects of diet, habitat, elevation, temperature, precipitation, body size, and mtDNA genetic divergence on shape variation in skulls, mandibles, and molars, structures that differ in their genetic and functional control. We asked whether these structures have adapted to environment to the same extent and whether they retain the same amount of phylogenetic signal. We studied these traits in intra- and interspecific populations of Eurasian marmots whose last common ancestor lived 2–5 million years ago. Path Analysis revealed that body size explained 10% of variation in skulls, 7% in mandibles, and 15% in molars. Local vegetation explained 7% of variation in skulls, 11% in mandibles, and 12% in molars. Dietary category explained 25% of variation in skulls, 11% in mandibles, and 9% in molars. Cyt *b* mtDNA divergence (phylogeny) explained 15% of variation in skulls, 7% in mandibles, and 5% in molars. Despite the percentages of phylogenetic variance, maximum-likelihood trees based on molar and skull shape recovered most phylogenetic groupings correctly, but mandible shape did not. The good performance of molars and skulls was probably due to different factors. Skulls are genetically and functionally more complicated than teeth, and they had more mathematically independent components of variation (5–6 in skulls compared to 3 in molars). The high proportion of diet-related variance was not enough to mask the phylogenetic signal. Molars had fewer independent components, but they also have less ecophenotypic variation and evolve more slowly, giving each component a proportionally stronger phylogenetic signal. Molars require larger samples for each operational taxonomic unit than the other structures because the proportion of within-taxon to between-taxon variation was higher. Good phylogenetic signal in quantitative skeletal morphology is likely to be found only when the taxa have a common ancestry no older than hundreds of thousands or millions of years (1% to 10% mtDNA divergence)—under these conditions skulls and molars provide stronger signal than mandibles.

Key words.—Geometric morphometrics, *Marmota*, maximum-likelihood, morphology, phylogeny.

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The phenotype is the product both of phylogenetic history and recent adaptation to local environments, but the relative importance of the two factors in explaining morphometric variation is contested (MacLeod and Forey 2002). Some studies have concluded that morphology is an unreliable indicator of phylogenetic relationships because of capricious, adaptation-driven homoplasy (e.g., Graur et al. 1997; Collard and Wood 2001; Stone and Cook 2002) or because of lack of differentiation among genetically distinct populations (e.g., Barratt et al. 1997; Mayer and von Helversen 2001; Roca et al. 2004). But other studies have found significant phylogeographic structuring in morphometric data among closely related taxa (Thorpe 1976; Straney and Patton 1980; Hausser 1984; Malhotra and Thorpe 1997; Polly 2003a,b). Although not thoroughly assessed, the difference in results appears partly due to recency of common ancestry. In the latter studies, which displayed a significant phylogenetic signal, the taxa were populations of the same species or members of recently diverged species groups whose mtDNA sequence divergence was no more than 5–10%; however, in the earlier studies, in which little phylogenetic signal was apparent, the species divergences were much deeper. This suggests that morphological traits probably have a finite optimum range for phylogeny reconstruction. The recent end of the range will be constrained by the minimum time required for taxa to evolve measurable differences in population means; the deeper end will be constrained by the time required for sta-

bilizing or adaptive selection to erase deeper phylogenetic history through convergence. The principle is the same as in genes that evolve at different rates depending on their functional role; the range of phylogenetic usefulness of a particular gene is determined by the rate of mutation, the sequence length, and selection on nonsynonymous sites (Lemos et al. 2005). For morphological traits, the range will be influenced by the trait's adaptive response, which is a function of its genetic control and the strength of selection. Despite the long history of morphological analysis, there have been few comparisons of morphological traits in this context, partly because complicated structures have been difficult to quantify.

In this paper, we systematically compared the environmental and phylogenetic components of variance in three morphological structures—skulls, mandibles, and teeth—belonging to Eurasian marmots. These skeletal structures were chosen because they are common in the fossil record, where morphology remains crucial for phylogenetic reconstruction, and because their genetics, development, and function differ in ways that could influence their adaptive responses. Marmots were chosen because they have mtDNA sequence divergences that fall within the 5–10% range, and because previous studies found phylogenetic patterning in all three skeletal structures (Cardini 2003; Polly 2003a; Cardini and O'Higgins 2004). We specifically focused on quantitative, geometric morphometric representations of morphology because of current interest in such traits. We assessed the effects of diet, habitat, elevation, temperature, precipitation, body size, and mtDNA genetic divergence on variance among pop-

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ulation means. Our goal was to determine whether the three structures respond to environmental factors in the same way, and to determine the extent to which each structure carries a phylogenetic signal given its degree of environmental adaptation. Our specific objectives were as follows: (1) to measure within- and between-population variance in the shape of the ventral cranium, the lateral mandible, and the occlusal surface of the lower molars; (2) to partition the variance among environmental and genetic (i.e., phylogenetic) factors; (3) to assess how well trees based on the morphological data replicate those based on cytochrome *b* mtDNA sequences; and (4) to evaluate how the genetic, developmental, and functional differences relate to usefulness of each trait for phylogeny reconstruction. Previous morphometric studies have compared skeletal structures (Monteiro et al. 2003; Nicola et al. 2003; Cardini and O'Higgins 2004), but they did not comparatively assess the contribution of phylogenetic and environmental factors.

We expected that the three morphological structures might respond individually because their genetic, developmental, and functional controls differ. The precise number of individual genes that control skull and mandible shape is unknown, but more than 50 are known to be expressed in developing teeth (Thesleff 2003). Regardless of gene number, genetic effects are likely to be combined pleiotropically into a smaller number of independent factors. Previous quantitative trait locus analysis identified more than 26 independent loci in the skull vault and face (Leamy et al. 1999), more than 25 in mandible shape (Atchley and Hall 1991; Klingenberg and Leamy 2001; Klingenberg et al. 2001, 2003), and 18 in the shape of the lower molar row (Workman et al. 2002). The number of independent factors affecting individual molars may be fewer (Salazar-Ciudad and Jernvall 2002, 2004; Hlusko et al. 2004). Developmentally, the skull is built around the brain and sensory placodes, with the braincase, auditory region, orbits, nasal cavity, and jaws developing semi-independently (Hanken and Hall 1993; Rowe et al. 2005). Mandible development is closely related to the facial region of the skull, and the mandible has fewer developmental modules than does the skull as a whole (Atchley and Hall 1991). Tooth development is partly influenced by regionalization of the face (Coburne and Sharpe 2003), but each tooth undergoes morphogenesis inside a follicle buried in the tissue of the jaw (Jernvall 1995; Polly 2000; Thesleff 2003). An important difference among the structures is the duration of their ontogenetic growth. Skulls and mandibles grow through an individual's entire lifetime, partly in response to physical and metabolic stresses (Grüneberg 1963; Moore and Lavelle 1974). On the other hand, bunodont tooth crowns like those in marmots do not grow after mineralization because the ameloblast cells that deposit enamel do not persist after eruption (Hillson 1986; Schroeder 1987). Ontogenetic change in tooth shape thus stops before the tooth is first directly exposed to the external environment. Skulls and mandibles consequently have more ecophenotypic variance than molars.

Ecophenotypic variance is nongenetic variance that results from environmental stimuli during the life of an individual. In quantitative genetics, ecophenotypy is known as the environmental component of phenotypic variation, and is distinct from evolutionary adaptation to environment, which is

a heritable, genetic process. In this study, it was not possible to distinguish between ecophenotypic and adaptive variation, but the two are discussed again below. Functionally, mandibles and the facial part of skull shape are related to one another through jaw mechanics, brain size, and olfaction (Atchley and Hall 1991; Hanken and Hall 1993; Monteiro et al. 2003; Rowe et al. 2005). Molar shape reflects the interlocking of occluding teeth during mastication (Evans and Sanson 2003; Polly et al. 2005). In short, the genetic and control of skulls and mandibles is more complicated than teeth, the development of mandibles overlaps with that of the facial region of the skull, but is mostly independent of teeth, and the morphology of skulls and mandibles should have greater ecophenotypic plasticity than teeth.

The ecology and phylogeny of marmots are comparatively well studied. Eurasia has eight species (Barash 1989; Wilson and Reeder 1993), which occupy a wide range of cold-climate terrestrial habitats. *Marmota himalayana*, *M. caudata*, and *M. marmota* live in montane habitats, whereas *M. bobak*, *M. sibirica*, and *M. baibacina* inhabit lower hills and steppe. In the field, the species are distinguished by their size, coat color, tail length, and alarm calls. The animals are mainly herbivores, consuming a wide diversity of plants, supplemented by occasional meat, insects, and eggs during the intense feeding before hibernation (Nowak 1999). Marmots most likely originated in North America during the early Miocene (Black 1963; Thomas and Martin 1993; Steppan et al. 1999; Armitage 2000). The fossil record documents the arrival of marmots in Asia no later than 2.0 million years ago (mya) (Fang et al. 2003; Qiu et al. 2002) and both the *M. marmota* and *M. bobak* groups were present as far west as Europe by the middle Pleistocene (Kalthoff 1999). Phylogenetic analysis using cytochrome *b* mtDNA has been performed at species level (Kruckenhauser et al. 1999; Steppan et al. 1999), but only size, pelage, and geographic differences have been used in the recognition of subspecies (Ognev 1947; Gromov et al. 1965; Roberts 1977), although morphometric and microsatellite data have been used to study intraspecific variation in a few species (Hoffmann et al. 1979; Polly 2003a; Kruckenhauser et al. 1997).

MATERIALS AND METHODS

We examined 101 museum specimens (see Acknowledgments for a list of institutions) of Eurasian marmots from 51 localities, representing seven subspecies and five species: *M. baibacina* (subspecies *M. b. baibacina*), *M. caudata* (subspecies *M. c. caudata* and *M. c. aurea*), *M. himalayana* (subspecies *M. h. himalayana* and *M. h. robusta*), *M. marmota*, and *M. sibirica* (subspecies *M. s. sibirica*). Species nomenclature followed Wilson and Reeder (1993). Juveniles and aged individuals were excluded to minimize the effects of ontogenetic growth. We grouped localities into 12 localized analytical samples (Fig. 1) because morphometric means based on fewer than five individuals can be wildly inaccurate for intraspecific comparisons (Polly 2003a,b, 2005). Localities populated by the same subspecies were agglomerated with their nearest geographic neighbors if the localities were extremely near one another (e.g., analytical samples b–d, k, and l in Fig. 1) or if their individual sample size was only

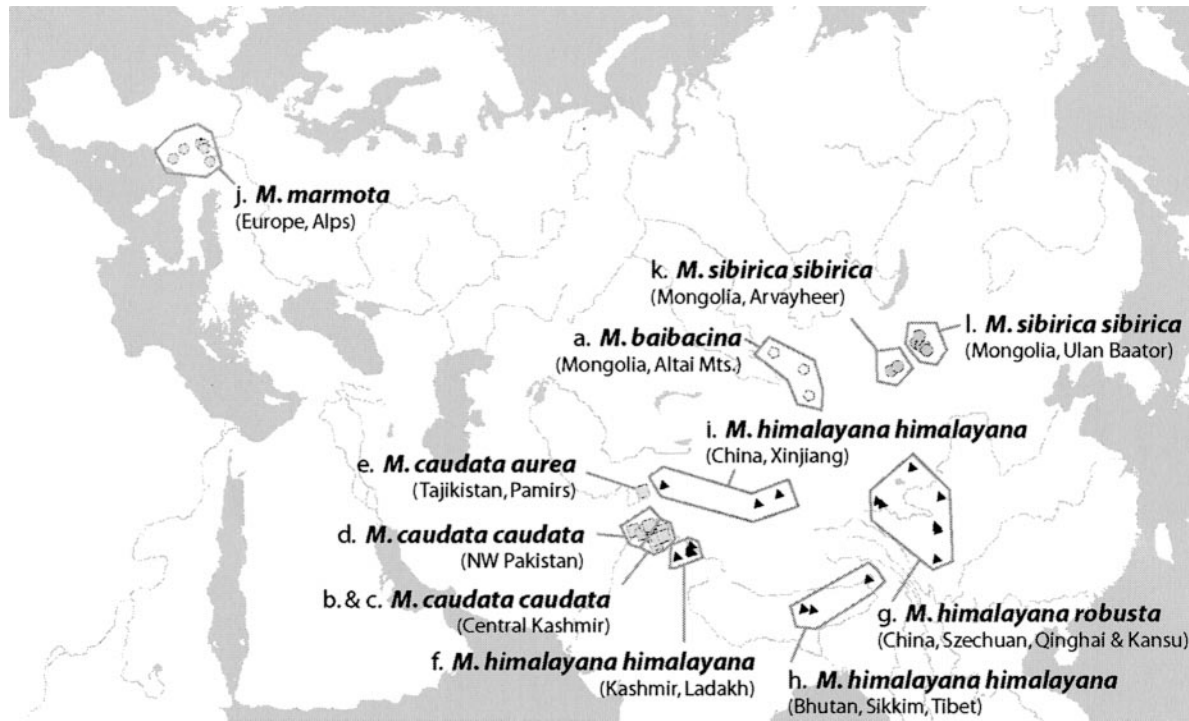


FIG. 1. Location of the samples used in this study. Specimens came from fifty one different locations, which were grouped into twelve analytical samples (A–L).

one or two individuals (e.g., analytical samples a, e, g, h, and j). The size of individual analytical samples differed slightly from one structure to another because the occasional skull, mandible, or tooth was broken. In three instances this unavoidably caused samples to have fewer than five individuals, the effect of which is discussed below. Analytical sample sizes are reported in the Results.

Ventral skull shape was represented with 31 two-dimensional landmarks and the lateral side of the mandible was represented with 13 such landmarks (Fig. 2). Skull landmarks were modified from Polly and Head (2004). Mandible landmarks were similar to those used by Monteiro et al. (2003). Both skull and mandible shape were analyzed using principal components analysis (PCA; Rohlf and Slice 1990; Bookstein 1991; Dryden and Mardia 1998; Polly 2003b). The mean shape of each analytical sample was calculated after Procrustes superimposition of the several individuals in the sample. The 12 sample means were then superimposed and mean centered. Principal components (PCs) were calculated from the covariance matrix of the residuals using singular-value decomposition (SVD). Scores were produced by projecting the sample mean shapes onto the resulting PCs.

Lower third molar shape was represented as the outline around the margin of the crown. We chose outlines because marmot molars do not have many distinct features, such as cusp tips or notches, and those features that do exist are readily obliterated by wear. We did not include excessively worn specimens in our analysis. Standard eigenshape analysis (Lohmann 1983; MacLeod 1999; MacLeod and Rose 1993), a form of principal components analysis, was used. Outlines were obtained from digital images as a series of 100 equally spaced two-dimensional coordinates (Fig. 2). Coordinates

were converted to unstandardized ϕ shape functions and the sample mean calculated. The covariance matrix of the mean centered sample ϕ functions was decomposed into its PCs using SVD, and the shapes were projected onto the PC axes to obtain a matrix of scores for further analysis.

Skull length was measured from the posterior nuchal crest to the base of the incisors with digital calipers. The arithmetic mean each of the 12 samples was used as a size variable. Body mass was estimated as the average of published pre- and posthibernation body masses (Armitage and Blumstein 2002). Body mass was only available at species level. See Table 1.

Latitude and longitude of each locality were taken from museum records. Coordinates from the original collector were available for about 25% of the samples. The remainder had only a site description, which could usually be determined within 15 km or less. Three localities had only a regional descriptor (“Altai,” “Ladakh,” and “NW Mongolia”), and for those we chose an arbitrary position within the current range of the species that lay along the route of the collecting expedition. The effects of error in geographic placement will be a reduction in the amount of shape variance explained by related variables. Elevations for each locality were taken from the Terrain Base of Global Land Elevation (Dater 2003), with coverage at the 5-min level. Temperature and precipitation were taken from Legates and Willmott’s (1990) interpolated data set of global monthly and annual mean air temperature and precipitation, with coverage at the 30-min level. Local vegetation data were taken from Matthews’ (1983) global vegetation database, with coverage at the 1 degree level (see Table 1).

Diet was categorized for each species as one of three types:

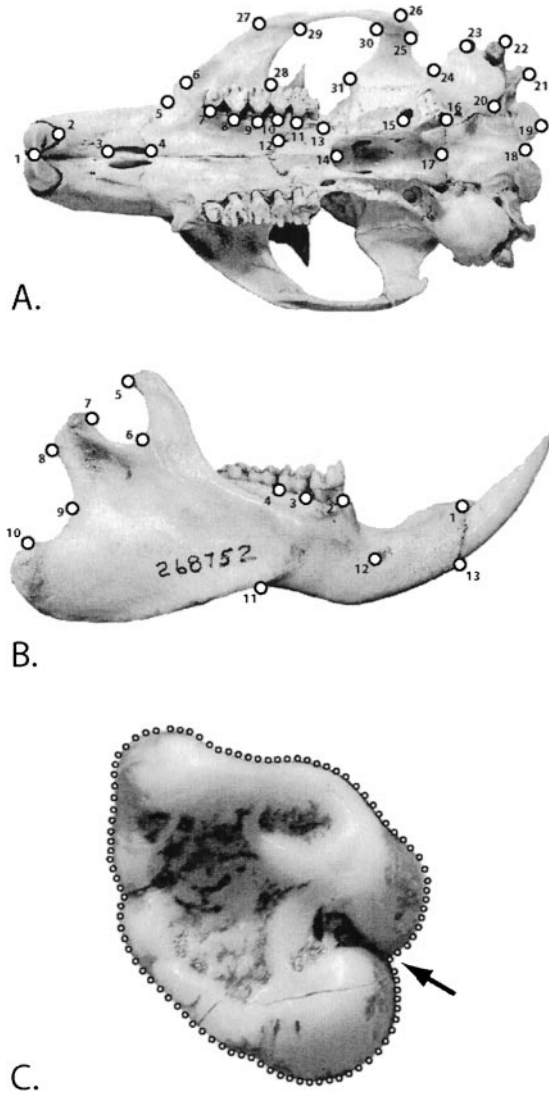


FIG. 2. (A) The positions of 31 landmarks used to characterize the shape of the ventral skull. (B) The positions of 13 landmarks used to characterize the shape of the lateral mandible. (C) One-hundred outline points used to characterize the shape in occlusal view of the crown of the lower third molar. The arrow indicates the first point in the sequence.

(1) diet of woody, dry plants with few fruits; (2) softer, thin plants, often with flowers; and (3) watery plants, bulbs, and fruits (Table 1). Categorization was based on published sources (Allen 1940; Ognev 1947; Gromov et al. 1965; Davis 1976; Barash 1989; Mann et al. 1993; Le Berre and Ramousse 1994; Massemin et al. 1996; Nowak 1999; Armitage and Blumstein 2002), and information was better for some species than others.

Sexual dimorphism was tested using a nested multivariate analysis of variance (MANOVA) with sex nested inside analytical sample. This analysis was restricted to those samples with individuals of both sexes (groups B, D through G, and I in Figure 1). Statistical significance was assessed using Wilk's λ test.

Maximum-likelihood (ML) trees were estimated for each dataset by applying the continuous quantitative traits algo-

TABLE 1. Size, diet, climatic, and vegetation data associated with each analytical sample.

Group	Taxon	Skull length (mm)	Mass (kg)	Diet	Lat.	Long.	Elev. (m)	Total precip. (mm)	Min. monthly avg. precip. (mm)	Max. monthly avg. precip. (mm)	Mean ann. temp. (°C)	Min. monthly avg. temp. (°C)	Max. monthly avg. temp. (°C)	Vegetation
A	<i>M. baibacina</i>	96.6	4.8	A	48.4	90.45	1729	163.25	4.83	30	-1.48	-24.78	18.1	desert
B	<i>M. c. caudata</i>	100.3	3.3	C	34.2	74.37	1964	1045.1	17.11	180.02	14.39	2.34	24.61	grassland with 10%–40% trees
C	<i>M. c. caudata</i>	101.6	3.3	C	34.6	75.39	3688	607.03	14.13	106.27	8.73	-6.17	21.9	short grassland, no woody cover
D	<i>M. c. caudata</i>	99.6	3.3	C	34.8	73.6	3082	1425.5	14.28	209.03	16.14	4.64	26.48	short grassland, no woody cover
E	<i>M. c. aurea</i>	89.6	3.3	C	38	73	3960	146.6	3.7	21.7	5.2	-10.6	19.2	short grassland, no woody cover
F	<i>M. h. himalayana</i>	99.2	4.9	B	33.2	77.88	5120	853.3	7.78	219.62	9.95	-2.06	20.03	cold deciduous forest, without evergreens
G	<i>M. h. robusta</i>	99.4	6.4	B	33.9	100.5	3462	545.73	2.59	110.11	1.53	-5.67	16.7	temperate evergreen forest
H	<i>M. h. himalayana</i>	89.3	4.9	B	28.3	90.38	4390	767.18	3.15	166.5	14.05	6.63	19.4	temperate evergreen forest
I	<i>M. h. himalayana</i>	92.8	4.9	B	37.6	83.59	3699	39.28	0.64	14.54	9.76	-8.52	24.06	grassland with 10%–40% trees
J	<i>M. marmota</i>	88.1	3.4	A	46.4	7.93	1241	1148.4	66.46	134.22	5.78	-2.93	14.39	evergreen broadleaved forest
K	<i>M. sibirica</i>	91.8	3.3	A	46.4	103.2	1851	230.8	0	78.17	-2.3	-23.56	16.38	grassland with shrub cover
L	<i>M. sibirica</i>	89.6	3.3	A	47.4	107.4	1505	222.73	0.09	77.18	-2.09	-23.63	16.86	xeromorphic shrubland

rithm (Felsenstein 1973, 1988, 1993) to the matrix of PCA scores from the ordination of the twelve sample mean shapes. Principal component analysis scores satisfy the requirements for ML because they are uncorrelated and continuously distributed. Scores were standardized to a mean of zero and a variance equal to the proportion of the variance explained by the corresponding principal component (Polly 2003a,b). The usual procedure of standardizing to unit variance is not appropriate for PCA scores because it heavily weights less important PCs, which contain little information about shape. Trees were rooted using *M. marmota* (the Alpine marmot) as the outgroup (Kruckenhauser et al. 1999; Steppan et al. 1999).

Support for the trees was evaluated using a bootstrap procedure (Felsenstein 1985a; Manly 1991). For ordinary phylogenetic data, the characters are sampled with replacement to generate a new dataset. For morphometric data, the characters are scores whose values depend on the ordination of the mean shapes (Cole et al. 2002). We randomly selected with replacement k individuals from each of the original twelve samples (where k is the number of specimens in the sample). Each bootstrapped sample was Procrustes superimposed and a new mean calculated. The resulting 12 sample means were themselves superimposed and submitted to PCA to yield a bootstrapped set of scores from which a tree was constructed. The alignment steps were unnecessary for molar data because the ϕ functions do not require superimposition. The bootstrap was repeated 1000 times, and support values reported as the percentage of trees in which each node grouping appeared. The trees shown in our figures are fully resolved majority-rule consensus of the 1000 replicate trees (Margush and McMorris 1981; Felsenstein 1985a, 1993).

For evaluation of the morphometric trees, a molecular phylogenetic tree and Kimura two-parameter genetic distances were estimated from the cytochrome *b* mtDNA sequences from Steppan et al. (1999). A maximum-likelihood tree with bootstrap support values was estimated using the parameters described by Steppan et al. We matched the sequences to our analytical samples by choosing the sequence from the population from Steppan et al. that was taxonomically and geographically closest to ours.

Path analysis (Wright 1968; Sokal and Rohlf 1995) was used to partition shape variation among phylogenetic, size, dietary, and environmental factors. Path analysis allows variance in the dependant variables, morphological shape in this case, to be partitioned among predictor variables that are not independent themselves. Previous studies used regression to remove the effects of either phylogeny or environment to study the other as residual variation, an approach which treats the two as independent; however, phylogeny can introduce morphological covariation indirectly through factors like diet which themselves may have phylogenetic structuring (Felsenstein 1985b; Harvey and Pagel 1991; Martins and Hansen 1997). Path analysis allows phylogenetic history, measured here as mtDNA divergence, to interact directly with shape and indirectly via vegetation, diet, and size. Path analysis has some similarities to multiple regression or to two-block partial least squares (Rohlf and Corti 2000), but differs in that it allows particular interactions to be specified. Predictor variables were diet (categorical), habitat (categorical), local vegetation type (categorical), elevation (continuous), latitude

(continuous), temperature (continuous), precipitation (continuous), body mass (continuous), and skull length (continuous). Diet, local vegetation type, and size were presumed to have direct effects on morphological shape, whereas the other variables were presumed to have indirect effects via these three factors. Latitude, elevation, precipitation, and temperature were combined into a single "climate" factor consisting of the first principal component of the correlation matrix of these variables (Crespi and Bookstein, 1989). The "climate" factor accounted for about 60% of the variation in its constituent data. Skull length and body mass were likewise combined into a "size" factor. Shape, the dependent variable, was represented by PC scores as described above. Kimura distances from *cyt b* mtDNA were used as a measure of phylogenetic relationship, which was allowed to have both a direct influence on shape and indirect influences via size, vegetation, and diet. The full path diagram is presented below. Correlation coefficients were calculated for each path using the product-moment coefficient for continuous variables and as the square-root of the multiple coefficient of determination from ANOVA for categorical variables. Path coefficients were estimated as the partial regression coefficients by solving the set of simultaneous equations from the path diagram. The coefficients for paths leading to morphological shape were calculated as the square root of the sum of the squared path coefficients for each PC weighted by the proportion of shape variance explained by each axis. The unexplained variance (U) was estimated as one minus the sum of the squared values over the four paths connected to shape.

Variation in morphological shape within and between samples was explored using squared Mahalanobis distances (D^2). Within-sample distances were measured as the distance between an individual and its sample mean. Between-sample distances were measured as the distance between the means of two samples. Specimens were ordinated using PCA and the appropriate squared Euclidean distances calculated as the sum across all axes of the squared differences between score and mean. For the two structures represented by landmarks, D^2 was the same as squared Procrustes distances (Rohlf and Slice 1990). Note that the units for landmarks and outlines are not directly comparable.

RESULTS

Sexual Dimorphism

After controlling for group membership, there was no significant difference between males and females in skull shape (MANOVA: $F_{174,14} = 1.32$, $P = 0.29$), mandible shape (MANOVA: $F_{78,116} = 0.98$, $P = 0.53$), or molar shape (MANOVA: $F_{108,99} = 0.1.03$, $P = 0.44$). Sexes were pooled for further analyses.

Maximum-Likelihood Trees

The molecular tree of Eurasian marmot species (Fig. 3a) was, unsurprisingly, congruent with the tree of all marmot species published by Steppan et al. (1999). The two *M. caudata* subspecies formed a clade that was the sister taxon to the remaining Asian taxa. Within the latter, *M. himalayana*

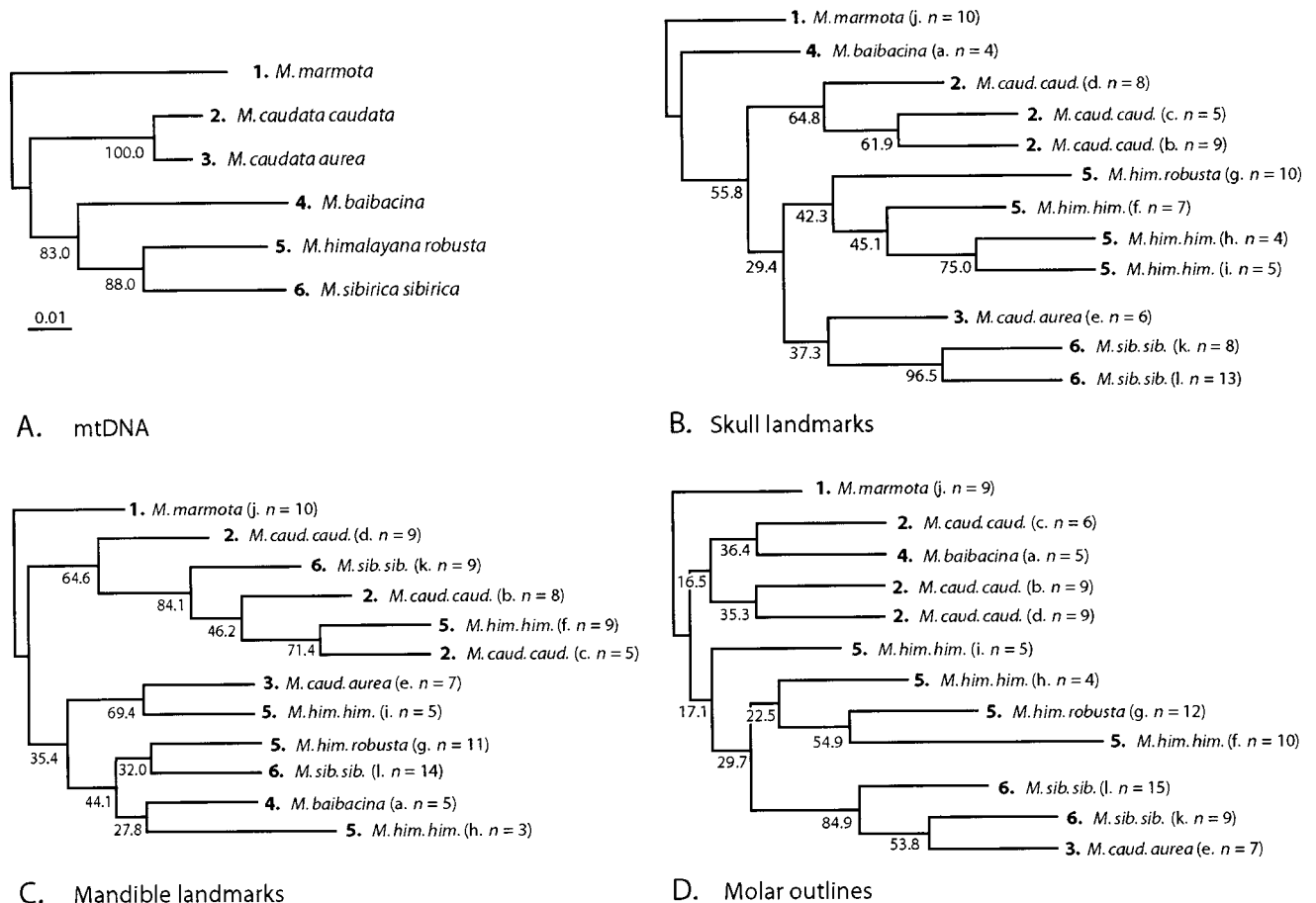


FIG. 3. (A) Maximum-likelihood tree based on aligned *cyt b* mtDNA sequences. Numbers at the nodes are bootstrap values indicating the percentage of trees in which the node was replicated. (B) Continuous trait maximum-likelihood tree based on PC scores of ventral skull landmark shape. Bootstrap values are shown as percentages at the nodes. Bold-face numbers in the OTU labels correspond to the taxa in tree A. Parentheses contain sample size and a letter identifying the analytical sample from Figure 1. (C) Continuous trait maximum-likelihood tree based on PC scores of lateral mandible shape. Notation is the same as in B. (D) Continuous trait maximum-likelihood tree based on PC scores of lower third molar outlines. Notation is the same as in B.

and *M. sibirica* were closely related. Bootstrap values greater than 75% at all nodes suggest that the tree is well supported. The mtDNA tree was used to evaluate the following morphological tree topologies.

The tree based on skull shape had the best congruence with the mtDNA tree (Fig. 3b). Three of the *Marmota caudata* samples formed a clade that was the sister-group to *M. himalayana* and *M. sibirica*. *M. himalayana* taxa formed a clade, with *M. h. himalayana* monophyletic within it. *Marmota sibirica* was also monophyletic. The positions of *M. baibacina* and *M. caudata aurea* were not congruent with the mtDNA tree. The former lay outside the *M. caudata-sibirica-himalayana* clade and the latter was clustered with *M. sibirica*. Despite the phylogenetic congruence, bootstrap values were lower than those normally expected for well-supported nodes in molecular or discrete character trees.

The tree based on mandible shape had the worst congruence with the mtDNA tree (Fig. 3c). *Marmota caudata*, *M. sibirica*, and *M. himalayana* taxa were mixed and no multi-operational taxonomic unit (OTU) species was monophyletic. Interestingly, bootstrap values for the mandible tree were often higher than for the skull tree.

The tree based on molar shape was generally congruent with the mtDNA tree, but not with the fidelity found in the skull tree (Fig. 3d). The *Marmota caudata caudata* taxa were monophyletic, but had *M. baibacina* grouped within them. *Marmota sibirica* was also monophyletic (but with *M. caudata aurea* grouped within) and grouped with *M. himalayana* (*M. himalayana* was paraphyletic with respect to *M. sibirica*). The bootstrap values were generally the lowest of any of the four trees.

Path Analysis

Path analysis indicated that mtDNA distance, size, vegetation, and diet explained a total of 57% of skull shape variation, 35% of mandible shape, and 42% of molar shape (Table 2, Fig. 4). Of the three morphological structures, skulls had the greatest direct interaction with mtDNA distance, with 15% of the variance explained. Mandibles and molars had 7% and 5% explained, respectively. Indirect interactions between genetic distance and morphological shape via size, vegetation, and diet added to the proportion of shape variance that could be attributed to phylogeny. Indirect influence of

TABLE 2. Percent of morphological variance (R^2) explained by each of the four paths leading to morphological shape in Figure 4. R^2 is the square of the path coefficients multiplied by 100.

Path	Skull shape	Mandible shape	M ₃ shape
mtDNA	15%	7%	5%
Size	10%	7%	15%
Vegetation	7%	11%	12%
Diet	25%	11%	9%
Total explained	67%	36%	42%
Unexplained	43%	64%	58%

mtDNA via the paths stemming from its connection to size was 1% for all three structures. Thus, the total shape variance explained by genetic distance was 16% in skulls, 8% in mandibles, and 6% in molars.

Size had its largest direct influence on molar shape (15% of the total variance), and least on mandible shape (7%). Local vegetation type explained 12% of molar shape, 11% of mandible shape, and 7% of skull shape. Dietary category explained 25% of skull shape (the largest single component of variation in any of the structures), 11% of mandible shape, and 9% of molar shape.

Variation within and between Groups

The ratio of variation in within samples to between samples was highest in molar shape and lowest in mandible shape (Fig. 5). For skulls, the median D^2 between individuals and their sample mean was 0.001 and the median D^2 between sample means and the grand mean was 0.0007, yielding a ratio of 1.43. The highest within-sample variation in skull

shape was in *Marmota himalayana himalayana* from Bhutan, Sikkim, and Tibet (Sample H, $n = 4$) and the lowest was in *M. h. robusta* (Sample G, $n = 10$). In mandibles, the median within-sample D^2 was 0.0020 and between-sample was 0.0015, for a ratio of 1.33. The highest within-sample variation in mandible shape was in *M. caudata aurea* (Sample E, $n = 7$) and the lowest was in *M. h. himalayana* (Sample H, $n = 3$). For molars, the median within-sample D^2 was 91.6 and between-sample was 37.6, for a ratio of 2.44. The highest within-sample variation in molar shape was in *M. baibacina* (Sample A, $n = 5$) and the lowest in *M sibirica sibirica* from Arvayheer (Sample K, $n = 9$). Our data give no indication that variation within a particular species is consistently higher than another.

Differentiation within groups (white bars) was notably greater than variation between groups (black bars) for all three structures, especially for molars (Fig. 5). Note that the tails of the distributions, especially the higher end, are likely to be elongated because of poorly estimated variances in the smallest samples.

DISCUSSION

Phylogenetic Components of Shape Variance

Ventral skull shape had stronger phylogenetic signal than molars or mandibles. The skull-shape tree had the greatest congruence with the mtDNA tree and the largest variance explained by mtDNA distance (15%), more than twice as much as in the other two. Even so, it is remarkable that the skull tree was so accurate given that only 15% of the variation could be directly explained by phylogenetic distance and giv-

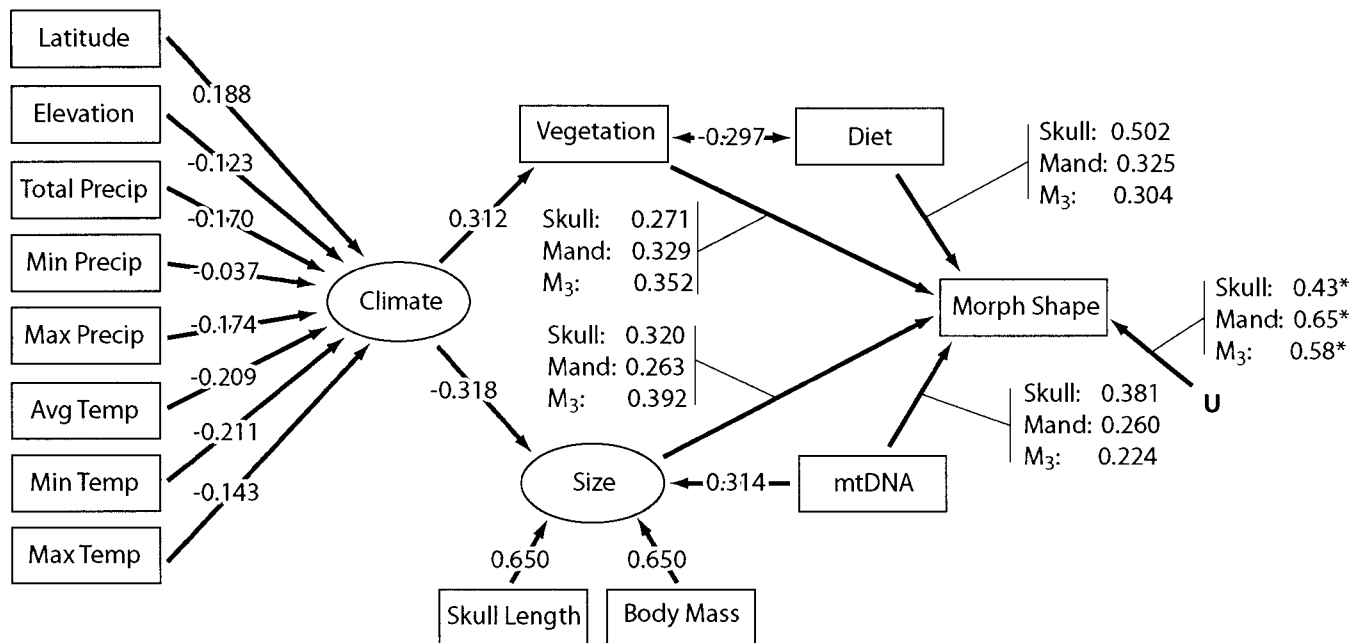


FIG. 4. Results of the path analysis of the contributions of phylogenetic and environmental factors to morphological shape variance. Measured variables are shown in boxes and composite factors in circles. Factors were estimated as the first PC of the contributing variables. Single-headed arrows indicate relationships that are presumed to be causal and double-headed arrows indicate correlation relationships. Values on paths are the partial correlation path coefficients. Coefficients of paths leading to morphological shape are summed across all the PCs of the morphological structure. U is the unexplained variance as a proportion out of 1.

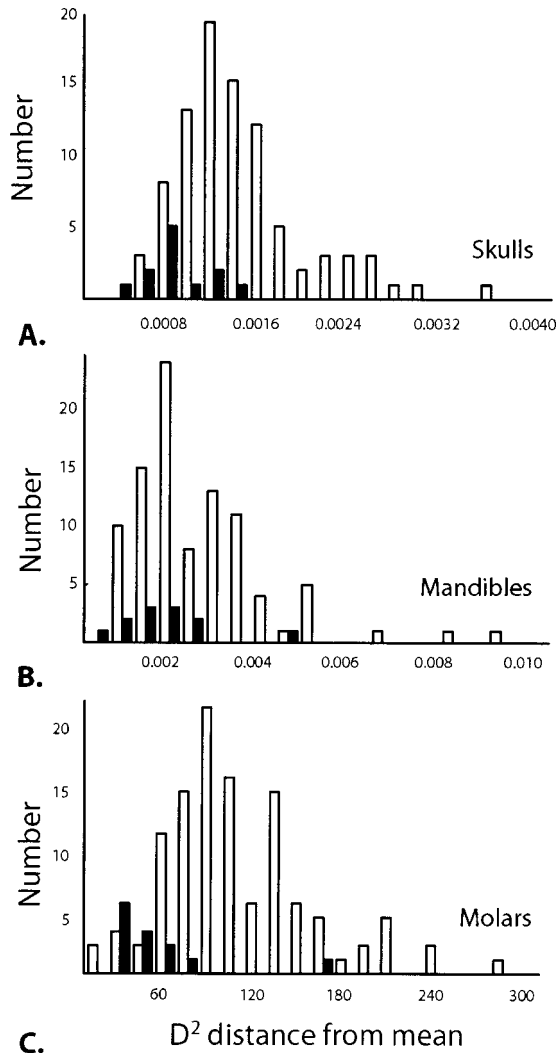


FIG. 5. Comparison of within- and between-sample variation in (A) skulls, (B) mandibles, and (C) molars. Each panel shows a histogram of squared Mahalanobis distances (D^2) between individuals and sample means (within-groups; white bars) and between sample means and the grand mean (between-groups; black bars).

en that 25% was explained by diet. This indicates that phylogenetic signal is still recoverable even when diluted by presumably adaptive trends. The good phylogenetic performance of skulls is probably best explained by its structural complexity of its several functional and developmental modules: the teeth, palate, and pharynx; the auditory region; the paths of major blood vessels; and origination points of masticatory muscles. Each module is likely to have had a semi-independent evolutionary history, collectively providing several independent lines of evidence for phylogenetic relationship. Skull shape is also controlled by a greater number of genetic loci and has more varied functions, including mastication, support of the brain, support of the head, and hearing. Neither mandibles nor molar crowns are as rich in anatomical or genetic complexity.

Despite the phylogenetic signal in the tree, node bootstrap values were low. Normally nodes with values below 70% are considered to be unsupported. Our two highest values were

above that threshold, but other phylogenetically correct nodes had values as low as 29.4%. Such low values can be expected when within-sample variation is high relative to between-sample variation and when sample sizes are low. The topology of the tree is highly dependent on accurate estimation of the mean shape of the OTU. Small sample size combined with high within-sample variation increases the error in estimation of the mean, which in turn increases the probability that its relation to other OTUs will be distorted. When within-OTU variation is high compared to between-OTU, the bootstrap replicates of OTU means with small sample sizes may be more different from one another than they are from the other OTUs. These taxa are likely to jump all over the tree, lowering all of the node values. The ratio of within-sample to between-sample variance was highest in molars and lowest in mandibles, the same rank order as the average bootstrap values on the trees. Therefore, it is likely that small sample size explains the poor placement of *Marmota baibacina* (Sample A; $n = 4$ or 5).

The bootstrap procedure coupled with majority-rule consensus doubtlessly improves the accuracy with which the tree topology describes the morphometric relations among the taxa. Each bootstrap iteration provides a new estimate of each sample mean. Poor estimates jump around the tree, whereas good estimates remain comparatively stable; majority-rule consensus prunes the poor estimates if they do not appear predominantly in any one place, leaving the stable ones. Thus, bootstrapped consensus trees will have filtered out weakly supported nodes that might be present in trees generated directly from the original data. Bootstrapping and majority rule consensus may be a strategy for improving the structure of morphometric trees when within-taxon variation is large compared to between-taxon variation. Because of this, the issues with majority-rule and morphometric data are different from those associated with majority-rule and discrete-character cladistic trees, which were criticized by Sharkey and Leathers (2001).

Why was mandible shape so bad at recovering phylogeny when skulls and molars were so good? The mandible tree was worst in terms of phylogeny, even though a greater amount of mandible shape variance was explained directly by phylogenetic distance than was molar shape. Interestingly, the high bootstrap values indicate that the mandible tree is better supported by its data than the skull or molar trees, something that can be explained by the low within- to between-sample variation in mandibles (ratio of 1.33 compared to 1.43 and 2.44), but the signal in the mandible data is clearly not phylogenetic. Unfortunately, our analysis does not explain the nature of the signal. Mandible shape variance was not strongly associated with size, local vegetation, or diet, and a large proportion (65%) remained unexplained. Of the structures in our study, mandibles are the most likely to be affected by life history and ecophenotypic effects. Conversely, molar crowns do not change shape after eruption (except through wear, and worn teeth were excluded) and the basicranial aspect of the skull is relatively immune to postadult growth (juveniles were also excluded from our study). If the unexplained variance is ecophenotypic, then geographically close but unrelated taxa might be more similar. Our results found some evidence for this in the close groupings of Sam-

ples F and C and Samples E and I. Kryštufek and Griffiths (2000), working on water shrews, also found that mandible shape was more unreliable for discriminant function classification than was ventral skull shape.

Few studies exist with which to compare our results. Hausser (1984) found that the environmental component of mandibular variation in shrews was much greater than the phylogenetic component (26.3% and 2.9%, respectively), although he was able to retrieve the latter by regressing out environmental variance. Straney and Patton (1980) found an environmental component of 28.7% and a phylogenetic component of 36.2% in a variety of external and cranial measurements from pocket mice. Both studies differed from ours methodologically, but the percentages of morphometric variation explained by phylogenetic and environmental factors were broadly similar to those that we found.

Environmental Components of Shape Variance

Skull, mandible, and molar shape differed in their associations with size, local vegetation, and diet (Table 2, Fig. 4). Perhaps surprisingly, diet had its strongest association with the skull shape, explaining 25% of its variance. Molar crowns have a more intimate association with food materials, but diet explained comparatively little of their shape variance (9%). The association of diet and skull shape was strong because the majority of structures are related to masticatory function, notably the position of the teeth, the shape of the palate, and the placement of the zygomatic and palatine originations of the muscles of mastication. But why was the dietary contribution to tooth shape so low? Two reasons may be the lack of a dietary ecophenotypic component to molar shape and the relatively amorphous occlusion of marmot molars. Tooth shape does not have ecophenotypic response to diet because enamel (an acellular tissue) cannot grow or be reworked after it is deposited prior to the eruption of the tooth. Because some of the dietary component of each morphological structure are likely to be adaptive (genetic) and some ecophenotypic, teeth may have a smaller proportion of variance explained by diet because the ecophenotypic component is missing. Furthermore, marmots have teeth that are low, rounded, and without interlocking features, and variation within populations is much greater than the differentiation between them. This suggests that a range of diets may be functionally compatible with any tooth shape. If so, then the power of selection to change molar form in response to diet would be lowered.

Recovery of Phylogenetic Signal

To recover hierarchical structure from morphometric data, the use of an appropriate method is imperative. One aspect of method is tree building; UPGMA trees are the most common in the morphometric literature, yet obtain the poorest results. Huelsenbeck (1995) demonstrated with simulations that UPGMA performed more poorly than other methods and was especially sensitive to unequal branch lengths or variable rates of evolution. Maximum-likelihood and related Bayesian methods are based on assumptions that are more consistent with the evolution of quantitative shape. When divergences are less than a few million years, multivariate skeletal shape

appears to evolve in a manner consistent with multivariate Brownian motion, a mode suited for likelihood methods (Polly 2003a,b, 2004). Least-squares trees, which do not assume equal rates and were developed for a Brownian motion model, are sometimes used in morphometrics (Polly 2001; Viguier 2002) and therefore perform better than UPGMA (Huelsenbeck 1995); however, they are still limited to pairwise distances. The likelihood trees produced in our study use the shape coordinates as data and treat each separate shape component as an independent piece of phylogenetic evidence, optimizing the tree across them all (Felsenstein 1973, 1981, 2002). Evolving multivariate shape diffuses through a large, multidimensional space and the probability of two lineages converging on exactly the same point are vanishing small when dimensionality is high (Polly 2004; Pie and Weitz 2005). Another aspect of method is how the data are treated. Coding continuous data into discrete characters will reduce phylogenetic recoverability. Collard and Wood (2001) found no phylogenetic signal after first arbitrarily dividing continuous morphometric variables into discrete characters and constructing a tree with a parsimony algorithm. Not only did their procedure violate assumptions about homology inherent in parsimony, but lowered the information content of their data, producing a matrix whose variance is explained by fewer principal components than the original mensural data.

Marmota caudata aurea from the High Pamirs

The position of *Marmota caudata aurea* from the High Pamirs of Tajikistan in the morphometric trees was strange because it did not cluster with other *M. caudata*, grouping instead with *M. sibirica sibirica* in the skull and molar trees, which otherwise had strong phylogenetic congruence. While the sample size was relatively small ($n = 6$), it was no lower than in taxa that were seemingly accurately placed. The sample is not taxonomically mixed, having been collected in 1894 by W. L. Abbott from a single locality in the Tagdumbash range. Several explanations exist. *Marmota caudata aurea* may have diverged from other *M. caudata* at a rapid rate, obscuring the phylogenetic association. The sample may be misidentified: *M. caudata* overlaps the range of *M. baibacina* (Wilson and Reeder 1993), but the two are unlikely to be confused because they are distinctive externally and anatomically; *M. menzbieri* also occurs nearby in the Tien Shan Mountains (Wilson and Reeder 1993), but is closely related to *M. caudata* and so would be expected to be similar in morphology. The sample could also belong to an as yet unrecognized species unrelated to *M. caudata*. Field studies in the Pamirs have shown two forms with distinctive alarm calls (Davydov 1991; Nikol'skii et al. 1999). One form lives on the south side of the Amu Darya river at its headwaters in Tajikistan and the other on the northern side, with some evidence of intermediates between the two. Unfortunately, our Tagdumbash sample lies just southeast of the area where this distribution has been studied. The distinctiveness of our sample supports the hypothesis of previously unrecognized variation in these high-altitude populations, but resolution of the identity of the Pamir marmots awaits further study, including molecular sequence analysis.

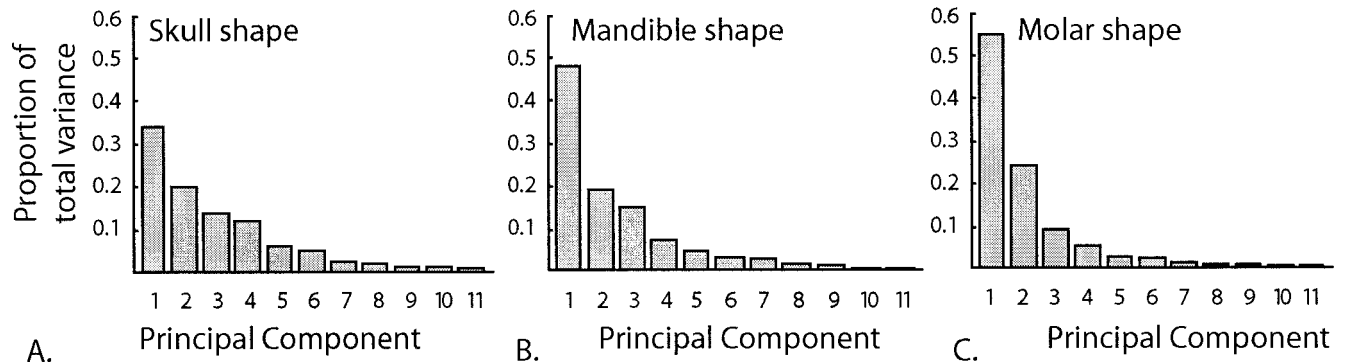


FIG. 6. Proportion of the total variance in each data set explained by its 11 principal components for (A) skull shape, (B) mandible shape, and (C) molar shape.

CONCLUSIONS

When do morphometric data exhibit strong phylogenetic structuring? The role of quantitative data in phylogenetics continues to provoke debate, even over basic issues such as whether they contain any phylogenetic signal or whether they only reflect environmental adaptation. Our results and those of others before us, indeed logic itself, suggests that because morphological structures have evolved, whether in response to selection or due to genetic drift, quantitative data must have phylogenetic structuring (Felsenstein 1988, 2002). The hypothesis that recoverability of phylogenetic hierarchy will vary from trait to trait and from rate to rate, is also supported by our results.

We found that ventral skull shape was the best morphological feature for recovering phylogenetic relationships and lateral mandible shape was the poorest. The ventral skull is genetically and functionally the most diverse of the three structures, and so contains a larger number of evolutionarily independent components of variation. The difference in independent factors is seen in the proportion of the total variance of each dataset explained by the several PCs (Fig. 6). Skull shape variation was spread more evenly across more components than either mandible or molar shape. Skull variance was concentrated on the first five or six components, whereas mandible variance was concentrated on the first three or four, and molar variance on only the first two or three. Although the number of independent factors is difficult to compare mathematically when the shape spaces are defined by different variables as they are in our study, the fact that our phylogenetic analysis is based on the scores weighted by the proportion of variance explained by their respective components makes this an important practical consideration.

Molars had good phylogenetic recoverability, despite having a low association with mtDNA structuring, a small number of meaningful PCs, and high within-sample variation. This success indicates that valid results can be obtained even when phylogenetic signal is weak, a possibility suggested by Hausser (1984). Molars are expected to have fewer effects from ecophenotypic plasticity than skulls and mandibles, and their developmental and genetic covariances may be less constrained than in the other structures. The skulls and mandibles of all mammals share certain developmental patterns, like ontogenetic facial elongation, that are likely to constrain their

variance (Moore and Lavelle 1974; Zelditch et al. 1992; Colvard and O'Higgins, 2001). Even though these structures have a greater number of independent PCs than teeth, their between taxon variation may be more conservative and susceptible to greater homoplasy. Teeth may not be so constrained (Salazar-Ciudad and Jernvall 2004; Polly 2005).

Mandibles behaved poorly for reasons that remain unclear. They had a better association with mtDNA divergence than molars and a lower association with diet than skulls, associations that should have been advantages for phylogeny reconstruction. However, much of mandibular variance remained unexplained by our analysis, possibly because a higher proportion may be nongenetic ecophenotypic variance associated with the life histories of the individual animals than in the other structures. Mandible shape may also evolve more rapidly than molar or skull shape, as indicated by greater between sample divergence relative to within sample variance, which may make it more prone to homoplasy. By the same criterion, molar shape diverged most slowly, perhaps offering an additional explanation for the phylogenetic accuracy of its tree because it may be evolving a rate appropriate for the level of divergence among the marmots.

Good phylogenetic results can be obtained from geometric shape of fossilizable traits even when environmental adaptation is strong, but they will depend on the trait used, its developmental and genetic properties, and the rate of its evolution relative to the divergences being studied. Our results suggest that for mammals whose last common ancestry can be measured in hundreds of thousands or millions of year (roughly equivalent to 1%–10% divergence in mtDNA sequence), skulls contain the best data because of their complex nature and high phylogenetic correlation. Complete skulls are rare in the fossil record because they are easily broken. Molar teeth, perhaps the most common type of fossil specimen, are next best choice for paleophylogeographic reconstruction.

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