

POSTNATAL ONTOGENY OF MARMOT (RODENTIA, SCIURIDAE) CRANIA: ALLOMETRIC TRAJECTORIES AND SPECIES DIVERGENCE

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Marmots are the largest extant representatives of the squirrel family (Sciuridae). Members of this clade are believed to have conservative skeletal characters and are inclined to convergence in species with similar size and ecology. However, this does not seem to hold for the mandible and cranium of marmots; instead, similarities reflect subgeneric classification or geographic distribution. To understand the pattern of morphological evolution in the genus *Marmota*, the ontogeny of the cranium is investigated in 7 of the 14 living marmot species. In particular, the role of allometry in producing intra- and interspecific differences is analyzed. Sexual dimorphism in allometric trajectories is found to be negligible, whereas shape traits that characterize a specific age are mostly allometric. Allometry accounts for an important proportion, although not for the majority, of shape variation during postnatal ontogeny of the cranium. Interspecific differences in allometric trajectories are generally small and the majority of shape differences in relation to phylogeny appear early in ontogeny. Thus, allometry might have had a limited role in producing the morphological variation of living marmot species or it might even have constrained the range of evolutionary changes in this clade. A very different role of allometry as a source of morphological novelties can be speculated to exist in earlier stages of marmot evolutionary history, when a highly distinctive cranial shape evolved concomitant with a 2-fold increase in size. Three sets of analyses are performed to investigate the ontogeny of cranial form in *Marmota*. Three-dimensional (3D) coordinates of anatomical landmarks are used to describe the whole marmot cranium in the first 3D geometric morphometric analysis of a sciurid taxon. Also, anatomical landmarks that describe the dorsal and lateral sides of the cranium are used for 2-dimensional (2D) analyses complementary to previous studies on the ventral cranium. Despite the complexity of the cranium, which makes it a poor candidate for 2D studies, results of 2D and 3D analyses are generally in good agreement.

Key words: allometry, 2-dimensional and 3 dimensional geometric morphometrics, *Marmota*, ontogeny, *Petromarmota*, species divergence

The genus *Marmota* includes the largest representatives of the ground squirrel tribe (Marmotini), a group of Holarctic rodents related to tree squirrels but specialized for terrestrial life (Hoffmann et al. 1993; Mercer and Roth 2003; Steppan et al. 1999).

Most marmots live in alpine and steppe meadows, where they dig complex systems of underground burrows. Hibernation, sociality, interbirth intervals of 2 or 3 years, and size increase are among the evolutionary responses to the selec-

tive pressures of harsh environments (Armitage 2000; Barash 1974, 1989) such as those of the periglacial zone in which marmots live and are believed to have evolved (Zimina and Gerasimov 1973).

Marmots appeared in North America about 9.5 million years ago and crossed the Bering Strait to colonize Eurasia at the end of the Pliocene or the beginning of the Pleistocene (Mein 1992; Steppan et al. 1999). According to the cytochrome-*b* gene tree (Steppan et al. 1999), 2 main lineages are found. The subgenus *Marmota* includes all Palearctic species plus 2 North American species, *M. broweri* and *M. monax*, whereas the subgenus *Petromarmota* is composed exclusively of Nearctic species. (To avoid confusion between *Marmota* as a genus and *Marmota* as a subgenus, the latter will here be indicated as *Marmota*_{SG}).

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Ecology and ethology of marmots have been deeply investigated (Armitage 1999, 2000; Arnold 1990; Barash 1989; Blumstein and Armitage 1998, 1999). Species of *Marmota* have a variable degree of sociality, which is correlated with environmental variables such as the length of the active season. Thus, they represent an excellent model for the study of the ecological scenarios that might have promoted the evolution of mammal societies.

Compared to ecoethological analyses, marmot phylogeny and morphological evolution have been much less studied. Only recently, marmots have become the subject of detailed interspecific comparisons of genetic (Kruckenhauser et al. 1999; Steppan et al. 1999) and morphological (Cardini 2003, 2004; Cardini et al. 2005; Cardini and O'Higgins 2004; Polly 2003) characters. A multidisciplinary approach to the study of marmot biology is highly desirable for a better understanding of the evolution of this genus. For instance, phylogenetic analyses (Kruckenhauser et al. 1999; Steppan et al. 1999) suggested that complex societies might have evolved twice in the marmot clade, and morphometric studies (Cardini 2003, 2004; Cardini et al. 2005; Cardini and O'Higgins 2004) revealed unexpected levels of morphological divergence in *M. vancouverensis*, the most endangered mammal species of North America.

The study of marmot morphological evolution is of great interest also in the context of studies on the presumed propensity of the sciurid skeleton for convergence induced by ecological or size similarities (Hafner 1984; Roth 1996; Velhagen and Roth 1997). Hafner (1984) found phenetic clusters reflecting either size (small, medium, and large species) or ecological similarities (diggers and climbers, and, among climbers, desert-scrub foragers, terrestrial-arboreal foragers, and adept climbers) in his analysis of cranial and postcranial characters of several sciurid species, and Roth (1996) presented a cladogram based on "qualitative characters of the cranium" that is "what one might have expected had we performed our analysis on just a single character: size." In contrast, recent analyses of mandibular (Cardini 2003, 2004) and cranial (Cardini et al. 2005; Cardini and O'Higgins 2004) form of marmots and other ground squirrel taxa did not produce any clusters clearly reflecting ecological or size similarities. Differences in analytical methods and in the choice of skeletal characters and taxa may help explain differences in results of these various studies.

The study of ontogeny of form can provide useful clues for understanding patterns of morphological evolution. In the late 1980s and early 1990s, the development of Procrustes superimposition methods for Cartesian coordinates of anatomical landmarks (see "Materials and Methods") provided scientists with an effective tool for an efficient separation of size and shape components of form (a basic issue in all quantitative analyses of ontogeny). The newborn field of geometric morphometrics (Adams et al. 2004) has found very fruitful applications, especially in the study of morphology and ontogeny of primates. For instance, new perspectives and insights have been achieved on the role of pedomorphosis in the evolution of *Homo* (Mitteroecker et al. 2004; Penin et al. 2002), the divergence of *Homo sapiens* and *Homo neanderthalensis* (Ponce de León and

Zollikofer 2001), sex and race differences in modern humans (Rosas and Bastir 2002; Strand Viðarsdóttir et al. 2002), and the evolution of homoplastic long faces in baboons and mandrills (Collard and O'Higgins 2001; O'Higgins and Collard 2002).

In contrast, few studies have investigated the ontogeny of skeletal characters in sciurids. The study of allometry, in particular, might provide interesting clues about changes that took place in the evolutionary radiation of the Marmotini (Harrison et al. 2003; Herron et al. 2004), where an increase up to 2-fold in size occurred in 2 distinct lineages, *Marmota* and *Cynomys*. The degree of morphological, ecological, and behavioral divergence is so remarkable that the 2 clades have been considered distinct genera related to *Spermophilus*. Contrary to traditional taxonomy, recent molecular phylogenies (Harrison et al. 2003; Herron et al. 2004) strongly support the evolution of *Marmota* and *Cynomys* as separate branches of the polyphyletic *Spermophilus*, thus suggesting a large increase in the rate of morphological evolution of marmots and prairie dogs compared to other ground squirrels.

Allometry of the sciurid mandible was the subject of studies by Velhagen and Roth (1997) and Swiderski (2003). Velhagen and Roth (1997) focused on evolutionary allometries of adult New World tree squirrels. In these taxa, the scaling of the mandible has both isometric and allometric components. Proportions similar to those predicted for New World tree squirrels are found in Old World tree squirrels of similar size. In contrast, mandibular proportions of ground squirrels are the most divergent in the family. Swiderski (2003) analyzed the mandibular morphology of the eastern fox squirrel (*Sciurus niger*), with special emphasis on the supposed importance of allometry as a constraint on evolutionary changes in shape. The analysis of traditional distance measurements shows that allometry explains almost all ontogenetic variation. However, a geometric morphometric analysis performed on the same sample indicates that less than 50% of shape variance is actually correlated with size. This different outcome is largely attributed to the inability of traditional morphometric analyses to separate size and shape components of form (thus, including both of them in the estimate of the proportion of size-related variation) and warns about possible flaws in the empirical evidence on the role of allometry as an effective evolutionary constraint.

A few studies focus on allometry in the Marmotini. Recently, Cardini and Tongiorgi (2003) showed that, after birth, age-related variation in the shape of the mandible of *M. flaviventris* is mostly allometric, and shape changes are especially pronounced in the 1st months of life, concomitant with the shift from a liquid diet (milk) to solids (plants). Cardini and O'Higgins (2005) compared ontogenetic allometric trajectories of the mandible and ventral cranium of 6 marmot species. Anatomical regions affected by size-related shape variation are similar in different species, but allometric trajectories are divergent. The largest modifications of the mandible and ventral cranium occur in regions directly involved in the mechanics of mastication. Relative to other anatomical regions, the size of areas of muscle insertion increases, whereas the size of sense organs, nerves, and teeth generally decreases. Epigenetic factors, developmental constraints, and size variation

are suggested to be the major contributors in producing the observed allometric patterns. A phylogenetic signal is not evident in the comparison of allometric trajectories, but traits that allow discrimination of the Palearctic marmots from the Nearctic species of *Petromarmota* are present early in development and are conserved during postnatal ontogeny.

Studies of the morphology of the adult mandible and cranium in *Marmota* (Cardini 2003; Cardini et al. 2005; Cardini and O'Higgins 2004) did not find any relevant interspecific allometry that could contribute to an explanation of shape divergence in the genus. However, results of the interspecific comparisons of the dorsal and lateral sides of the cranium (Cardini et al. 2005) were not fully congruent with the outcome of similar analyses on the mandible (Cardini 2003) and ventral cranium (Cardini and O'Higgins 2004). The most relevant differences involved the outgroup species (other sciurid genera). *Spermophilus*, *Cynomys*, *Tamias*, and *Sciurus* grouped together on 1 branch and *Marmota* on the other in the analysis of the dorsal and lateral side of the cranium. This was explained as retention of the ancestral morphology in smaller members of the Marmotini (*Spermophilus*, *Cynomys*, and *Tamias*) and the evolution of a derived morphology in *Marmota*. In contrast, all marmotine genera (*Spermophilus*, *Cynomys*, and *Marmota*) clustered together in studies on the mandible and ventral cranium. Also, the Nearctic *M. monax* was found to have a distinctive cranial morphology, whereas its mandibular shape is similar to that of Palearctic members of *Marmota*_{SG}.

The outcome of studies on marmot cranial morphology may be affected by differences in measurement errors. All studies until now have been performed using 2-dimensional (2D) geometric morphometrics (i.e., landmark coordinates digitized on pictures). The approximation of 3-dimensional (3D) anatomical structures in 2 dimensions may be good for a relatively flat bone such as the sciurid hemimandible, but it is less appropriate for more complex structures such as the cranium. However, to our knowledge, no investigation has yet been performed that uses 3D landmark coordinates for a quantitative description of the sciurid cranium.

This study focuses on the ontogenetic variation of the marmot cranium analyzed with 3D geometric morphometric techniques. Also, pictures of the dorsal and lateral side of the cranium are analyzed to complement previous 2D analyses of the mandible and ventral cranium. Particular emphasis is placed on ontogenetic allometry, and its influence in shaping the marmot cranium. Thus, 5 *Marmota*_{SG} species (*M. caudata* (Geoffroy, 1844), *M. himalayana* (Hodgson, 1841), *M. marmota* (Linnaeus, 1758), *M. monax* (Linnaeus, 1758), and *M. sibirica* (Radde, 1862)) and 2 *Petromarmota* representatives (*M. caligata* (Eschscholtz, 1829) and *M. flaviventris* (Audubon and Bachman, 1841)) are compared in a geometric morphometric analysis of the dorsal and lateral side of the cranium (excluding *M. sibirica*), and in the first 3D analysis of the marmot cranium (excluding *M. caudata*).

The study is aimed at answering the following questions:

1. Does ontogenetic allometry play a significant role in modeling the cranium of marmots?

TABLE 1.—Sample size of marmot (*Marmota*) species examined in 2-dimensional analysis of dorsal and lateral aspects of cranium and in 3-dimensional (3D) analysis of whole cranium. F = females; M = males; total number of specimens includes those of unknown sex.

Species	Abbreviation	Dorsal			Lateral			3D		
		F	M	Total	F	M	Total	F	M	Total
<i>M. caligata</i>	<i>cal</i>	32	26	61	33	25	62	36	30	73
<i>M. caudata</i>	<i>cau</i>	22	18	44	26	22	53			
<i>M. flaviventris</i>	<i>fla</i>	41	34	78	43	36	82	82	61	154
<i>M. himalayana</i>	<i>him</i>	18	24	50	18	26	52	18	22	48
<i>M. marmota</i>	<i>mar</i>	9	11	35	10	10	40	6	10	21
<i>M. monax</i>	<i>mon</i>	28	24	64	33	27	72	39	30	82
<i>M. sibirica</i>	<i>sib</i>							7	16	27
Genus <i>Marmota</i>	<i>tot</i>	150	137	332	163	146	361	188	169	405

2. If allometry is significant in the cranium, do males and females share a common allometric trajectory in all species such that any sexual dimorphism in shape is reached through extension or truncation of a common allometry?
3. Do different marmot species share common allometries that are extended or truncated to generate interspecific differences?
4. If allometry is significant, are ontogenetic changes similar in different species, are patterns of ontogenetic shape variation similar to those found in other structures, or both?
5. What is the congruence between 2D and 3D analyses of the same structure?

MATERIALS AND METHODS

Samples and age classes.—Dorsal (dorsal cranium [DC]) and lateral (lateral cranium [LC]) sides of the cranium of, respectively, 332 and 361 specimens of all available age classes of *M. caligata*, *M. caudata*, *M. flaviventris*, *M. himalayana*, *M. marmota*, and *M. monax* were compared in a 2D geometric morphometric analysis (Table 1). All these species except *M. caudata* are included in the 3D geometric morphometric study of the whole cranium. *M. caudata* was not adequately represented in the 3D sample, but a relatively large sample of another species, *M. sibirica*, was available for the 3D study (Table 1).

Specimens belong to collections of the National Museum of Natural History (Washington, D.C.), American Museum of Natural History (New York), University of Alaska Museum (Fairbanks), British Museum of Natural History (London, United Kingdom), Zoological Institute of the Russian Academy of Sciences (St. Petersburg, Russia), Museo di Storia Naturale di Milano (Milan, Italy), Parco Nazionale del Gran Paradiso (Torino, Italy), Dipartimento di Biologia Animale, Università di Modena e Reggio Emilia (Italy), and private collection of Dino Scaravelli (Forlì, Italy). Specimens analyzed in 2D are the same (with very few exceptions) as in Cardini and O'Higgins (2005), who also provide a list with museum catalog number (p. 201). Detailed information on specimen catalog numbers is available from the authors upon request.

Five age classes were estimated in *M. flaviventris* by using the relationship between age and premolar wear described by Van Vuren and Salsbury (1992). This relies on changes in the distances between the paraconid and protoconid cusps of the lower left premolar as they wear. Measurements were taken with digital calipers and precision was tested as described by Cardini and Tongiorgi (2003). Numbers of specimens of different age classes in *M. flaviventris* were DC:

juveniles, 3; yearlings, 9; 2 years old, 18; 3 years old, 18; and ≥ 4 years old, 26; LC: juveniles, 3; yearlings, 10; 2 years old, 19; 3 years old, 19; and ≥ 4 years old, 26; and 3D: juveniles, 2; yearlings, 21; 2 years old, 44; 3 years old, 38; and ≥ 4 years old, 26. No similar technique was available for evaluating the age of the other marmot species. For this reason our analysis focused on the issue of ontogenetic allometry rather than on differences in shape among age classes (with the exception of *M. flaviventris*). The allometric trajectories do not explicitly incorporate time, but size changes are correlated with age during postnatal ontogeny, as suggested also by the significant correlation between centroid size of the cranium and premolar wear (average $r = 0.727$).

Early stages of postnatal ontogeny were not included in our study. Marmots are altricial—average birth weight for *Marmota monax* is 26–27 g—and museum collections contain almost exclusively postmergent marmots. Completeness of molar eruption, sutures between frontal and parietal bones (visible only in the young), and development of the sagittal crest (absent in the young) were considered for discriminating young from adults. Only recently, Bezuidenhout and Evans (2005) have published a description of the pattern of tooth eruption in *M. monax*. The deciduous premolars erupt when the animals are 35–38 days old and are replaced by permanent premolars when they are 3–4 months old. The molars erupt after the 4th week of life. No information is provided on time that molar eruption is complete. *M. monax* is the only solitary marmot species, the only one that also lives in the forest, and the only one to reach sexual maturity after 1st hibernation. Other species exhibit a medium to high degree of sociality, live almost exclusively in mountain meadows, and reach sexually maturity after the 2nd (*M. flaviventris*) or 3rd (the remaining species) hibernation (Armitage 2000; Blumstein and Armitage 1998, 1999). Generalizing from the developmental data for *M. monax* to other species would seem unwise. However, specimens with visible sutures between frontal and parietal bones, no sagittal crest, and incomplete dentition were present in all samples and were considered juvenile animals that were still growing. The number of individuals with milk teeth or incomplete eruption of 1–3 molars varied from 2 (*M. caudata* and *M. marmota*, for which very few young [see “Discussion”] were available in museums), to 8 (*M. monax*), and 10 or more in the other species.

Geometric morphometrics.—Geometric morphometric techniques allow the comparison of geometric form (size and shape) of organisms or their organs (Bookstein 2000, Encyclopedia of Life Sciences, <http://www.els.net>). Topographically corresponding anatomical points (landmarks—Marcus et al. 2000) are chosen on each structure (Table 2; Figs. 1 and 2) and Cartesian coordinates of a set (configuration) of landmarks are used to compare their form. Differences due to rotation and translation (position) or size are removed by superimposing landmark configurations on their mean (reference or consensus configuration) and standardizing size (unit centroid size, i.e., square root of the sum of square distances between each landmark and the centroid of the landmark configuration). This process is called generalized Procrustes analysis (Rohlf and Slice 1990) or registration (O’Higgins 2000). The matrix of registered landmark coordinates represents the shape information captured by the sampled landmarks. However, landmark configurations have to be projected in a Euclidean space that approximates the curved shape space to aid statistical analyses. This is analogous to approximation of the distance relationship between points of a small region of the earth’s surface on a flat map (Rohlf 1998). This approach is satisfactory when the range of variation is small, as in the present data (virtually identical results were obtained in each data set by regressing the shape distances in the tangent space onto the Procrustes distances: correlation, $r = 1.000$; slope, $b = 0.999$; intercept, $a = 0.000$).

Differences between a reference shape (average of that sample) and a target shape (one of the specimens) can be illustrated with deformation grids (thin plate spline or TPS—Bookstein 1991). The resulting shape variables are linear combinations of the registered Cartesian coordinates and can be analyzed with standard methods of multivariate statistics (Adams et al. 2004).

A useful measure to express shape differences among specimens is the Procrustes distance. The Procrustes distance is the square root of the sum of square differences between positions of the landmarks in 2 registered configurations and it is the metric that defines the shape space (Rohlf 2003). The magnitude of the Procrustes distance is directly proportional to shape differences between 2 landmark configurations.

Strong deviations of young from linearity of size–shape relationships were not evident in scatterplots of the principal components of shape highly correlated to size. A log-transformation of centroid size to correct for nonlinearity (following Cardini and Tongiorgi 2003) only led to a very small increase in overall fit of the model (percentage of size-related shape variation increased an average of 0.5—the maximum increase was in *M. flaviventris*, from 20.8% to 21.5%, and the minimum increase is in *M. marmota*, from 25.8% to 26.0%). Thus, a linear multivariate regression of shape variables onto size (without log-transformation) was used as a test for allometry. The proportion of size-related shape variance (called “degree of allometry”) was computed as 1 minus the ratio between the sum of square distances between each specimen Procrustes superimposed on its predicted configuration based on the regression analysis (lack of fit of the model) and the sum of square Procrustes distances between each specimen and the reference (sample variation).

Statistical significance of differences in allometric trajectories among groups (sexes or species) was tested with tests for common slopes and homogeneity of intercepts (Rohlf 2004) computed in full shape space. A test for common slopes tests the significance of species \times size interaction in a multivariate analysis of covariance with shape as dependent variables, species as grouping variable, and centroid size as covariate. If the interaction term is not significant (i.e., slopes are the same), a 2nd multivariate analysis of covariance is performed after removing the interaction term. Significance of the grouping variable (species) implies that not only do species have parallel allometric trajectories (same slopes) but also similar intercepts (homogeneity of intercepts), and thus share a common allometric trajectory whose extension or truncation may explain size-related shape differences in adults. *M. monax* was 1st included in the comparison of allometric trajectories of species of the subgenus *Marmota*_{SG}. Then, the test was repeated after excluding this species. This was done because *M. monax* was a basal lineage of *Marmota*_{SG} and interspecific comparisons of cranial shape among adults have shown that its cranial shape is the most divergent in the marmot clade. Thus, *M. monax* could have strongly affected results of the comparison of ontogenetic trajectories.

Additionally, angles were computed pairwise between allometric vectors, and the resulting interspecific dissimilarity relationships (angles) between the allometric trajectories were summarized with a phenogram based on unweighted pair-group method using arithmetic averages.

Principal components analyses of shape coordinates after projection in the tangent space (O’Higgins and Jones 1999) were employed to investigate intraspecific and interspecific phenetic relationships during marmot postnatal ontogeny.

Geometric morphometric analyses were performed using computer programs from the TPS series, written by Rohlf (2004, <http://life.bio.sunysb.edu/morph/>), IMP series (Sheets 2004, <http://www.canisius.edu/~sheets/morphsoft.html>), and the programs Morphueus (Slice 1999, <http://life.bio.sunysb.edu/morph/morphueus/>) and

TABLE 2.—Definition and numbering of 3-dimensional (3D landmarks directly digitized on crania), 2-dimensional (2D landmarks digitized on pictures) dorsal and lateral cranial landmarks. The terms “anterior” and “posterior” are used with reference to Figs. 1 and 2. DC = dorsal cranium; LC = lateral cranium.

Landmark number		Definition
3D	2D	
1		Anterior (midsagittal) lower tip of the premaxilla
2		Anterior (midsagittal) upper tip of the premaxilla
3	2 DC, 1 LC	Anterior tip of suture between nasal and premaxilla
4	1 DC	Anterior (midsagittal) tip of the nasal
5	3 DC	Anterior tip of suture between premaxilla and maxilla
6		Meeting point of maxilla, lacrimal, and frontal sutures
7		Meeting point of premaxilla, maxilla, and frontal sutures
8		Meeting point of premaxilla, nasal, and frontal sutures
9	4 DC	Meeting point between nasal and frontal along the midsagittal plane
10	5 DC	Supraorbital notch
11	7 DC	Posterior base of the postorbital process
12		Meeting point of frontal, parietal, and squamosal bones
13	10 DC	Temporal foramen
14	3 LC	Ventralmost meeting point between mastoid process of the occipital bone and the tympanic bulla
15		Meeting point of parietal, squamosal, and occipital bones
16	11 DC, 2 LC	Posteriormost point of the parietal along the midsagittal plane
17		Ventrolateral meeting point of zygomatic process of maxilla and jugal
18		Anteriormost point of region of insertion of the posterior deep masseter on the jugal
19	9 DC	Posterior tip of the zygomatic arch
20		Nasolachrymal foramen
21		Unossified area in maxillary–lacrimal suture
22		Ethmoidal foramen
23		Sphenopalatine foramen
24		Dorsal palatine foramen
25	8 LC	Optic foramen
26		Sphenofrontal foramen
27		Dorsal tip of sphenoidal fissure
28		Masticatory foramen
29		Anterior extremity of suture between alisphenoid and zygomatic process of squamosal
30, 31		Extremities of incisive foramen
32		Dorsal tip of infraorbital foramen
33		Tip of the masseteric tubercle
34		Anteriormost point of the orbit (in ventral view)
35, 36	5 LC, 6 LC	Posterior and anterior end of the maxillary toothrow
37		Posterior maxillary foramen
38		Posterior palatine foramen
39		Suture between maxilla and palatine along the midsagittal plane
40		Point of maximum curvature on posterior edge of the palatine

TABLE 2.—Continued.

Landmark number		Definition
3D	2D	
41		Meeting point between basisphenoid and presphenoid where anterior foramen lacerum typically opens
42		Posterior extremity of foramen ovale
43		Meeting point between basisphenoid, basioccipital, and tympanic bulla
44	4 LC	Ventralmost meeting point between tympanic bulla and alisphenoid
45, 46		Anterior and posterior tip of external auditory meatus
47		Anterior extremity of carotid canal
48		Posteriormost point on ventral region of occipital foramen
49, 50		Lateral tips of the occipital condyle
51		Dorsomedial tip of occipital foramen
52		Mastoid foramen
	6 DC	Tip of postorbital process of frontal
	8 DC	Posteriormost point of orbit along squamosal process of zygomatic arch
	7 LC	Posteriormost ventral point of upper incisor alveolus

Morphologika (O’Higgins and Jones 1999, <http://www.york.ac.uk/res/fme/resources/software.htm>). SPSS for Windows, version 9.0.1 (SPSS Inc. 1999), Statistica for Windows, version 4.5 (StatSoft Inc. 1993), NTSYS-pc, version 2.10z (Rohlf 2002), and TpsRegr 1.28 (Rohlf 2004) were used for these statistical analyses.

Missing landmarks.—One to 3 missing landmarks were present in 12.2% of the specimens in the 3D data set. Centroid size for specimens

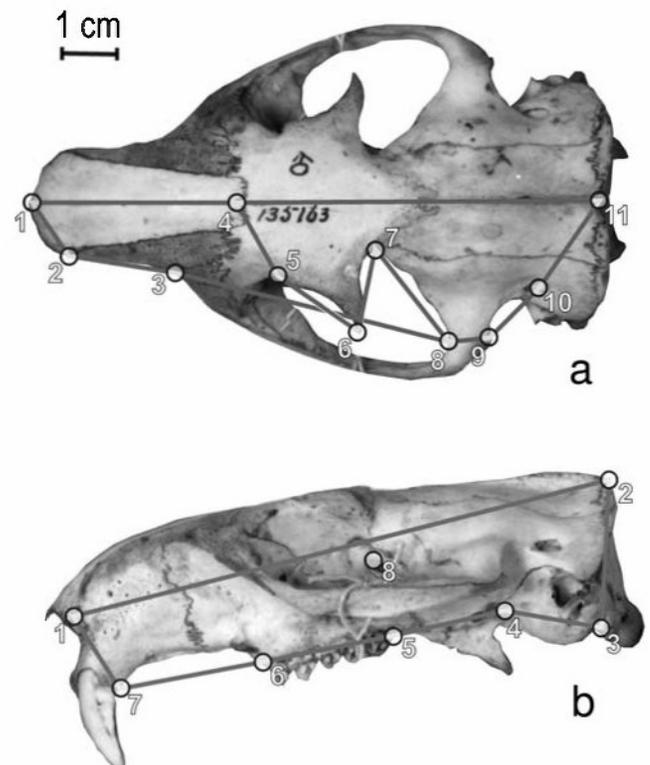


FIG. 1.—Landmark configurations on the a) dorsal and b) lateral marmot cranium.

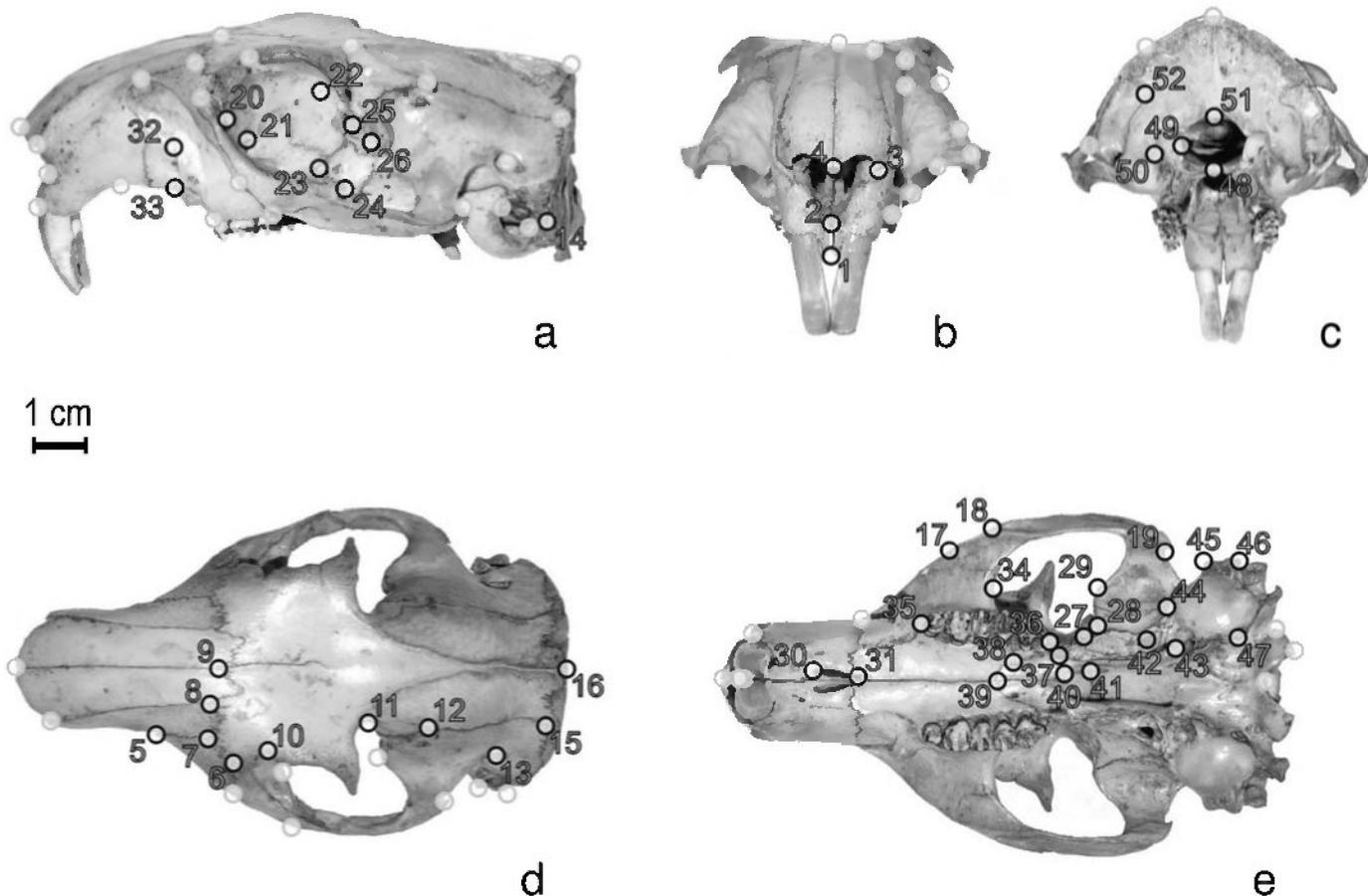


FIG. 2.—Three-dimensional landmark configuration of marmot cranium. Landmarks are shown on a) lateral, b) rostral, c) caudal, d) dorsal, and e) ventral views of the cranium. Landmarks are as described in Table 2.

with missing landmarks was estimated using values predicted by a linear regression of centroid size of specimens with no missing landmarks onto the length of the cranium (interlandmark distance L1–L16). Missing shape variables were estimated using the sample average for the missing coordinate after the registration (Slice 1999).

Including specimens with a few missing landmarks allowed increase of the sample size, and because of the small number of missing landmarks it did not appreciably affect the outcome of the analysis (see “Results”).

Data collection, digitizing error, and 2D versus 3D.—Two-dimensional analyses were performed on pictures taken in standardized conditions and at a distance (1 m) that minimizes photographic distortions (Cardini et al. 2005; Cardini and O’Higgins 2004; Cardini and Tongiorgi 2003). 3D landmark coordinates were directly collected on crania using a 3D digitizer (MicroScribe 3DX, Immersion Corporation, San Jose, California). Landmarks were digitized only on the left side to avoid redundant information in symmetric structures.

In a subsample, landmarks were digitized 5 times on each specimen in each data set. The sum of variances of the registered coordinates was computed for each specimen and then averaged (average digitizing error in the subsample). The ratio between the digitizing error shape variance and the shape variance in the analysis sample (all available specimens) approximates the relative amount of shape variation accounted for by digitizing error. In all 3 data sets (DC, LC, and 3D), the ratio was smaller than 0.1.

Two-dimensional studies of highly 3D structures such as the marmot cranium introduce an error that can be minimized by choosing

almost coplanar landmarks. However, this is especially difficult for highly curved structures such as the lateral side of the cranium. The accuracy of 2D data compared to 3D landmarks was tested in *M. flaviventris*, whose sample was the largest one available. Landmarks that were in common between the 2D data sets (DC and LC) and the 3D configuration were selected. Centroid size was computed and correlation between 2D and 3D measures was calculated. Also, a test for matrix correlation (Rohlf 2002) was performed on the matrices of Procrustes distances computed using 2D and 3D landmarks. In a test for matrix correlation, the correlation between unique off-diagonal elements of 2 distance matrices is computed element by element (i.e., distance between specimens 1 and 2 in the 1st matrix against distance between specimens 1 and 2 in the 2nd matrix, etc.). The significance of the matrix correlation was tested with permutation tests (1,000 random permutations).

RESULTS

The 1st section of the “Results” concerns the predictions for the specimens with missing landmarks. All other sections refer to the 5 questions (numbered from 1 to 5) asked at the end of the “Introduction.”

Missing landmarks.—Centroid size was highly correlated (pooling all species, $r = 0.994$; 1 species at a time, $r = 0.990$ – 0.999) to the length of the cranium (distance L1–L16). The length of the cranium was thus used to predict centroid size for

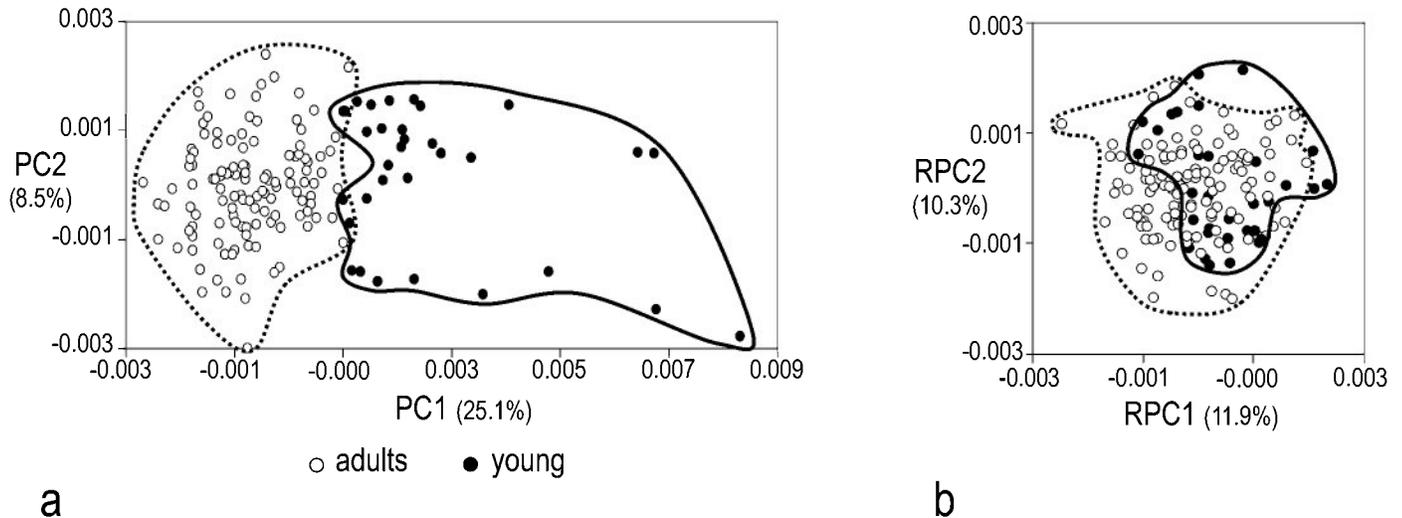


FIG. 3.—Principal component analysis of the 3-dimensional shape variables of *Marmota flaviventris*; percentages of variance are given below titles of each axis. a) All shape information included. b) Only shape variation uncorrelated with size included (RPC, principal component of the matrix of residuals of a multivariate regression of shape onto size).

specimens with missing landmarks. The accuracy of the prediction was tested in the subsample ($n = 361$) that includes only intact specimens without missing landmarks. In this sample, 10% of the specimens were randomly selected and their centroid size was predicted by a linear regression of centroid size of all other specimens onto cranial length. Predictions were compared with measured values of centroid size. The average difference between predicted and observed values of centroid size was 1.9 mm (this corresponds to 1.6% of the size of the smallest cranium in the sample).

Coordinates of missing landmarks were estimated using the sample average after the registration. Overall, there were 74 missing landmarks out of a total of 21,372 landmarks in the sample. As for the size, the accuracy of the prediction was tested in the sample of specimens without missing landmarks ($n = 361$). About 10% of the specimens were randomly selected and the coordinates of 1–3 landmarks of each of these specimens were deleted. “Simulated” missing landmarks approximately mirrored the distribution of missing landmarks in the analysis sample (for instance, if L20 was missing in about 3% of the specimens in the original sample, L20 was deleted in about 3% of the 361 specimens without missing landmarks). “Simulated” missing landmarks were estimated, and Procrustes distances were computed pairwise between all specimens. The matrix of Procrustes distances describes the phenetic relationships among the 361 specimens including those with estimated missing landmarks. These were compared to the relationships described by the matrix of Procrustes distances for the same 361 specimens using the original landmark coordinates (i.e., without any missing landmarks). The correlation between the 2 distance matrices was 0.998, which indicates that the relative positions of the specimens in the shape space are virtually identical in the 2 samples. This observation was supported by a cluster analysis on the matrix of Procrustes distances of all specimens (“simulation” and “real” samples), which produced a phenogram where each

specimen clustered with the corresponding one with estimated missing landmarks (results not shown).

1 and 2. Sexual dimorphism and allometry.—Ability to estimate ages of *M. flaviventris* permitted testing the significance of cranial form differences during postnatal ontogeny. Results of these tests were briefly presented because they are complementary to those of previous studies (Cardini and O’Higgins 2005; Cardini and Tongiorgi 2003), with which they were largely congruent. Sexual dimorphism was small but significant for size and generally negligible for shape. Shape changes during postnatal ontogeny were mainly allometric. Shape differences among age classes became negligible if aspects of shape variation correlated to size increase (growth) are removed by an analysis of covariance with size as covariate. That the shape traits that discriminate young and adults are allometric was supported by results of principal component analyses of the shape variables (3D data set). Young and adults were well separated on the 1st principal component (PC1; Fig. 3a), which explained 25.1% of sample shape variance and it was the only principal component highly correlated to size ($r = -0.87$). PC1 also was the principal component with the highest discriminant function coefficient in a discriminant analysis of age using shape principal components. Age discrimination was no longer visible in the scatterplot (Fig. 3b), if the principal component analysis was performed on the residuals of a multivariate regression of shape onto size (i.e., using nonallometric shape variation). Similar results were found in the 2D data sets.

Differences among age classes were significant even after removing shape changes correlated with size increase only in the 2D analysis on the DC. Sexual dimorphism of the DC shape also was significant, even if smaller than age-related shape differences.

Allometry accounted for a significant amount of postnatal ontogenetic shape variation in both sexes. Allometric trajectories of males and females were similar (Table 3). Slopes and

TABLE 3.—Multivariate regressions of cranial shape (dorsal cranium, lateral cranium, and 3-dimensional [3D] data) of *Marmota* species onto corresponding centroid size, shown as tests for allometry (a = slope; b = intercept; 2D = 2-dimensional).

Species ^a	View	Test for	Sexual dimorphism in allometry				Allometry with pooled sample (males and females)			
			Wilks' λ^b	<i>F</i>	<i>df.</i>	<i>P</i> ^c	Wilks' λ	<i>F</i>	<i>df.</i>	<i>P</i>
<i>M. caligata</i>	Dorsal	a	0.640	1.155	18, 37	0.344	0.056	39.515	18, 42	1×10^{-20}
		b	0.544	1.768	18, 38	0.069				
	Lateral	a	0.778	1.025	12, 43	0.445	0.077	48.673	12, 49	5×10^{-23}
		b	0.852	0.639	12, 44	0.797				
3D ^d	a	0.514	1.041	30, 33	0.453	0.020	66.040	30, 42	8×10^{-27}	
	b	0.337	2.231	30, 34	0.012					
<i>M. caudata</i>	Dorsal					0.097	12.953	18, 25	2×10^{-8}	
	Lateral					0.102	29.485	12, 40	4×10^{-16}	
<i>M. flaviventris</i>	Dorsal	a	0.667	1.495	18, 54	0.128	0.071	43.138	18, 59	3×10^{27}
		b	0.562	2.385	18, 55	0.007				
	Lateral	a	0.716	1.649	12, 50	0.108	0.147	33.399	12, 69	5×10^{-24}
		b	0.827	0.887	12, 51	0.565				
3D	a	0.590	1.250	50, 90	0.178	0.044	44.918	50, 103	1×10^{51}	
	b	0.505	1.781	50, 91	0.009					
<i>M. himalayana</i>	Dorsal	a	0.357	1.801	18, 18	0.111	0.041	40.208	18, 31	1×10^{-16}
		b	0.612	0.668	18, 19	0.802				
	Lateral	a	0.816	0.545	12, 29	0.867	0.085	34.940	12, 39	4×10^{-17}
		b	0.724	0.954	12, 30	0.511				
3D	a	0.331	2.129	18, 19	0.055	0.021	73.741	18, 29	7×10^{-19}	
	b	0.465	1.276	18, 20	0.297					
<i>M. marmota</i>	DC ^d	a	0.672	0.550	8, 9	0.794	0.050	16.888	18, 16	3×10^{-7}
		b	0.834	0.249	8, 10	0.166				
	Lateral	a	0.208	1.584	12, 5	0.320	0.056	38.054	12, 27	9×10^{-14}
		b	0.371	0.847	12, 6	0.621				
3D	a	0.559	0.790	6, 6	0.609	0.070	30.989	6, 14	3×10^{-7}	
	b	0.480	1.264	6, 7	0.379					
<i>M. monax</i>	Dorsal	a	0.516	1.616	18, 31	0.117	0.126	17.394	18, 45	1×10^{-14}
		b	0.712	0.720	18, 32	0.767				
	Lateral	a	0.651	2.010	12, 45	0.046	0.342	9.472	12, 59	7×10^{-10}
		b	0.684	1.769	12, 46	0.083				
3D ^e	a	0.310	2.670	30, 36	0.003	0.060	26.823	30, 51	5×10^{-22}	
	b	0.605	1.212	7, 13	0.362					
<i>M. sibirica</i>	3D	a	0.605	1.212	7, 13	0.362	0.020	131.830	7, 19	1×10^{-16}
		b	0.645	1.101	7, 14	0.414				

^a Tests of sexual dimorphism in allometric trajectories were not performed on *M. caudata*, whose sample of putative juveniles includes only 2 female specimens; *M. caudata* is included only in the 2D analyses and *M. sibirica* only in the 3D study.

^b Wilks' λ statistic is based on a comparison of the residual (deviation from the fitted regression) variance-covariance matrix and total (residuals plus predictions based on the regression) variance-covariance matrix (Rohlf 2004).

^c Highly significant values ($P < 0.01$) are in italics.

^d Tests of 3D data of all species and of dorsal cranium (DC) of *M. marmota* are performed using the 1st q principal components, with q (number of principal components in the analysis) chosen so that the matrix of Euclidean distances is based on those principal components with correlation to the matrix of Procrustes distances ≥ 0.98 computed in the full shape space.

^e Test of homogeneity of intercept was not performed because of high significance of differences in slopes. Excluding the smallest specimen in sample of *M. monax*, results of test of common slopes and homogeneity of intercepts are, respectively, Wilks' $\lambda = 0.349$, $F = 2.173$, $df. = 30, 35$, $P = 0.014$; Wilks' $\lambda = 0.535$, $F = 1.044$, $df. = 30, 36$, $P = 0.447$.

intercepts were not appreciably different ($P > 0.01$) when sexes were compared in each species. Three main exceptions were found to this pattern. *M. monax* seemed to have significantly divergent allometric trajectories of the 3D cranium in males and females. However, in the test of common slopes, P increased from 0.003 to 0.014 when the smallest specimen in the sample was excluded. This specimen is a male that was clearly separated from all other specimens in scatterplots of PC1 and PC2, which together summarize allometric shape, onto centroid size. Thus, it might have strongly influenced the slope of the male trajectory in the comparison with the females for which no specimen of similar size was available. The DC and 3D cranium of *M. flaviventris* showed significant sexual dimorphism in allometric trajectories: the allometric pattern of shape variation was similar in males and females (same slope), but sexual dimorphism was already present in juveniles

(different intercept), thus leading to parallel allometric trajectories. However, if a Bonferroni correction for multiple comparisons was applied, none of the 16 comparisons was significant ($P > 0.003$).

In conclusion, sexual dimorphism in the ontogeny of cranial shape can be considered very modest and does not strongly mitigate against performing all further analyses with pooled sexes. Thus, the proportion of the total shape change that was related to size variation ("degree of allometry"; see definition in "Materials and Methods") in the postnatal ontogeny of the cranium was compared among species. The range of cranial size variation in each species of the 3D data set is shown in Table 4. A significant proportion ($P < 0.001$; Table 3) of shape variation was correlated to size in all species and data sets. However, the degree of allometry was highly variable (Table 5). *M. monax* had the lowest value; *M. caligata*, *M. hima-*

TABLE 4.—Range of cranial size variation for species of *Marmota* in the 3-dimensional data set expressed as condylobasal length (mm). Data are for all ages combined.

Species	Minimum	Maximum	Range
<i>M. caligata</i>	44.0	80.1	36.1
<i>M. flaviventris</i>	35.7	74.8	39.1
<i>M. himalayana</i>	47.6	84.3	36.8
<i>M. marmota</i>	52.7	75.0	22.3
<i>M. monax</i>	44.3	74.0	29.7
<i>M. sibirica</i>	43.6	71.8	28.3

layana, and *M. sibirica* the highest; and *M. caudata* and *M. flaviventris* intermediate values. The relative variation of the degree of allometry of different structures (or sides of the cranium) was relatively constant across species, as indicated by the high correlation between all pairs of data sets ($r \geq 0.90$), although absolute values were generally higher for the DC and smaller for the 3D data set.

3 and 4. Interspecific allometries and pattern of ontogenetic shape change.—Interspecific differences in allometric trajectories were significant (test of common slopes: DC, Wilks' $\lambda = 0.525$, $F = 2.327$, $d.f. = 90$, 1,474.4, $P = 1.6 \times 10^{-10}$; LC, Wilks' $\lambda = 0.629$, $F = 2.748$, $d.f. = 60$, 1,586.5, $P = 5.4 \times 10^{-11}$; 3D, Wilks' $\lambda = 0.430$, $F = 3.450$, $d.f. = 100$, 1,829.2, $P < 0.0001$). The test of common slope for the 3D data set was performed using the first 20 principal components of the shape variables, which accounted for 75.9% of sample variance and include all principal components correlated with size. The correlation of the Euclidean distance matrix computed pairwise using the first 20 principal components to the matrix of Procrustes distances (computed in the full shape space) was 0.993. Thus, the first 20 principal components give a rather good representation of the phenetic relationships in the full shape space. Pairwise comparisons of angles between allometric vectors of 3D crania were summarized in the phenogram of Fig. 4, which suggested some similarity between *M. flaviventris* and *M. himalayana*. Thus, differences in slopes were compared between these 2 species. Again, results were highly significant (first 40 principal components, whose matrix of Euclidean distances had a 0.999 correlation to the matrix of Procrustes distances: Wilks' $\lambda = 0.397$, $F = 6.044$, $d.f. = 40$, 159.0, $P < 0.0001$).

Allometric trajectories also were compared among species of the same subgenus. All tests (results not shown) indicated significant differences in slopes.

Sampling error was large in *M. caudata*. Young specimens were uncommon and the only small juvenile in the sample may have strongly influenced the outcome of the regression of shape onto size. However, the exclusion of this species in the comparisons (all 6 species or only *Marmota*_{SG} species) of the DC and LC trajectories did not substantially change their outcome and differences remain highly significant.

Thus, statistical differences in allometric trajectories were found in the postnatal ontogeny of the cranium of different marmot species. However, large-scale size-related shape changes were quite similar in all species analyzed. This was

TABLE 5.—Percentages of shape variance correlated with size differences (allometric variation) in species of *Marmota*. Results on hemimandible and ventral cranium are from Cardini and O'Higgins (2005). 3D = 3-dimensional.

Species	Hemimandible	Cranium			
		Ventral	Dorsal	Lateral	3D
<i>M. caligata</i>	43.6	37.6	45.9	41.1	32.7
<i>M. caudata</i>	13.2	16.5	31.5	20.5	
<i>M. flaviventris</i>	30.0	31.3	41.1	28.2	20.8
<i>M. himalayana</i>	43.9	40.6	56.2	48.6	42.0
<i>M. marmota</i>	21.9	24.5	40	33.2	25.8
<i>M. monax</i>	7.3	14.5	24.4	15.5	12.5
<i>M. sibirica</i>					53.6

shown in Fig. 4 using surface rendering of the 3D landmark configurations for the specimens at the extremes of size variation in *M. flaviventris*, *M. monax*, *M. himalayana*, and *M. sibirica*. The first 3 species were chosen to illustrate the DC and LC shapes predicted by the allometric trajectories for the smallest and largest specimens of each species using deformation grids (Figs. 5 and 6). Juveniles had a relatively shorter snout, longer cranial vault (frontal and parietal bones), and enlarged braincase and tympanic bulla. Adults had snouts that were not only longer but also deeper (in the dorsoventral axis) than those of the young, the zygomatic arch was larger, the tooththrow as well as the cranial vault were relatively shorter, and the postorbital process was more pronounced and no longer bent backward.

Ontogenetic shape variation in marmot species was summarized in Fig. 7 by scatterplots of the first 3 principal components of the 3D shape variables (46.3% of sample variance). PC1 was highly correlated with size ($r = -0.787$) and thus mainly represented allometric shape variation. Also other principal components (PC2 to PC5) were correlated with size, but correlations ($-0.453 \leq r \leq -0.117$) and percentages of explained variance were smaller. PC2 separated well the Palearctic species of *Marmota*_{SG} and *M. monax*, whose relatively flat skull was the most divergent in the genus, and to a smaller extent discriminated *Petromarmota* from other groups. PC3 also contributed to discriminating these 3 clusters. Divergence of Palearctic species of *Marmota*_{SG}, *Petromarmota*, and *M. monax* seems to begin early in ontogeny (Fig. 7a, right), although young of *M. sibirica* overlapped with those of *Petromarmota* and *M. monax*.

5. Correlation between 2D and 3D data in *M. flaviventris*.—The correlation between measurements of size computed using landmarks in common between the 2D and 3D configurations was very large ($r \geq 0.995$), and the average difference between 2D and 3D measurements of centroid size was 1.74 mm for the DC and 1.26 mm for the LC.

Matrices of Procrustes distances were computed for the DC and LC, and for the 3D data sets, using subsets of common landmarks, and the correlation between the 2 data sets was calculated. Correlations were all highly significant ($r_{3D-DC} = 0.686$, $P_{\text{permutation test}} \leq 0.0010$; $r_{3D-LC} = 0.639$, $P_{\text{permutation test}} \leq 0.0010$).

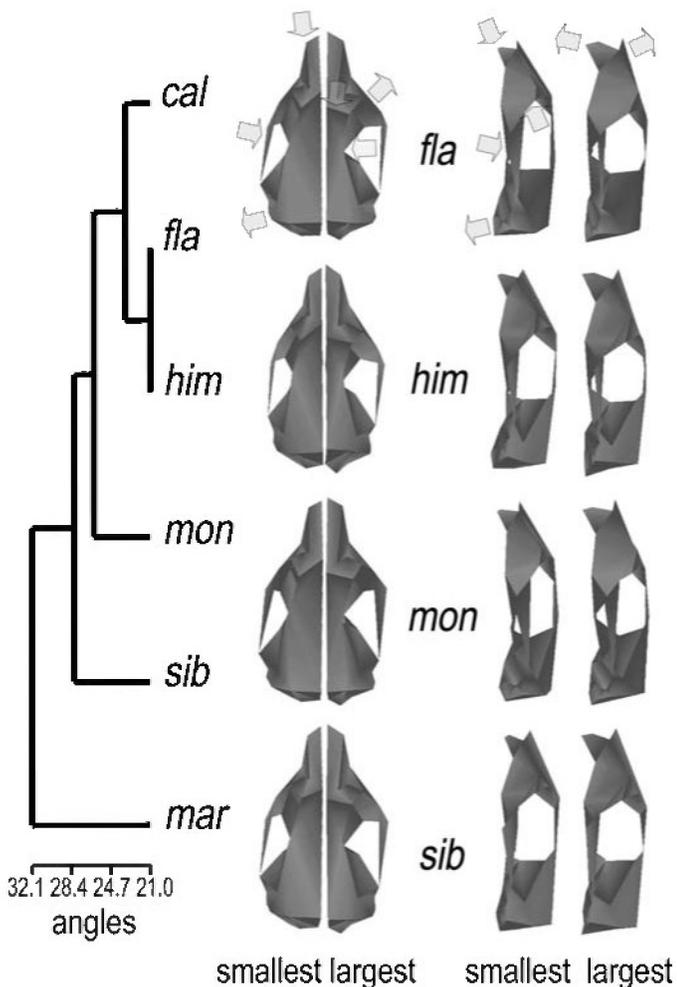


FIG. 4.—Comparison of angles (degrees) between allometric trajectories for the 3-dimensional crania of species of *Marmota* (unweighted pair-group method using arithmetic averages cluster analysis on the matrix of pairwise angles). Dorsal and lateral views of the surface rendering of the landmark configuration describing differences in allometric shape between the smallest (left) and largest (right) specimen of *M. flaviventris* (= fla), *M. himalayana* (= him), *M. monax* (= mon), and *M. sibirica* (= sib). Arrows indicate prominent shape changes at the extremes of size variation in *M. flaviventris*. *M. caligata* = cal; *M. marmota* = mar.

DISCUSSION

Most of the “Discussion” will focus on results of 3D analyses. This is done because 3D data provide a more accurate and comprehensive description of the cranium. Patterns found in the 2D analyses of the DC and the LC are generally the same as in the 3D data set and are briefly discussed. 2D analyses of the DC and LC were performed and included in the present study to complement a previous study (Cardini and O’Higgins 2005), which emphasized areas associated with the teeth and with jaw mechanics, and to compare results obtained with less expensive and faster methods of data collection (photography) with those of more accurate but expensive 3D digitizers.

As in the “Results,” section numbers refer to the 5 questions asked at the end of the “Introduction.”

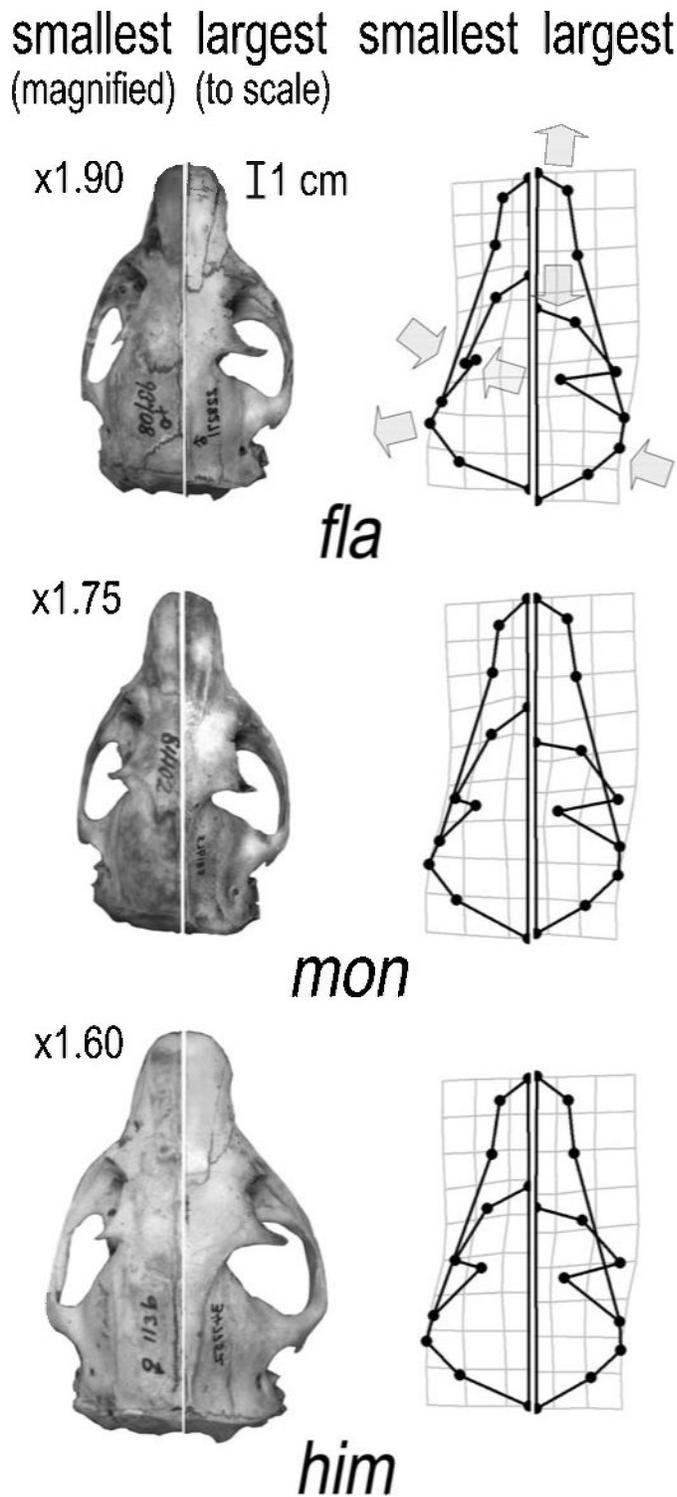


FIG. 5.—Dorsal cranium (DC) of *Marmota flaviventris* (= fla), *M. monax* (= mon), and *M. himalayana* (= him). Left, pictures of the DC for the smallest and largest specimens of each species sample (the largest specimens are drawn to scale, and scale is shown by *M. flaviventris*; the smallest specimens are magnified relative to the scale, according to magnification factors given by each specimen). Right, deformation grids and wireframe for shapes predicted by the multivariate regression of the DC shape onto size for the smallest and largest specimens. Arrows indicate prominent shape changes at the extremes of size variation in *M. flaviventris*.

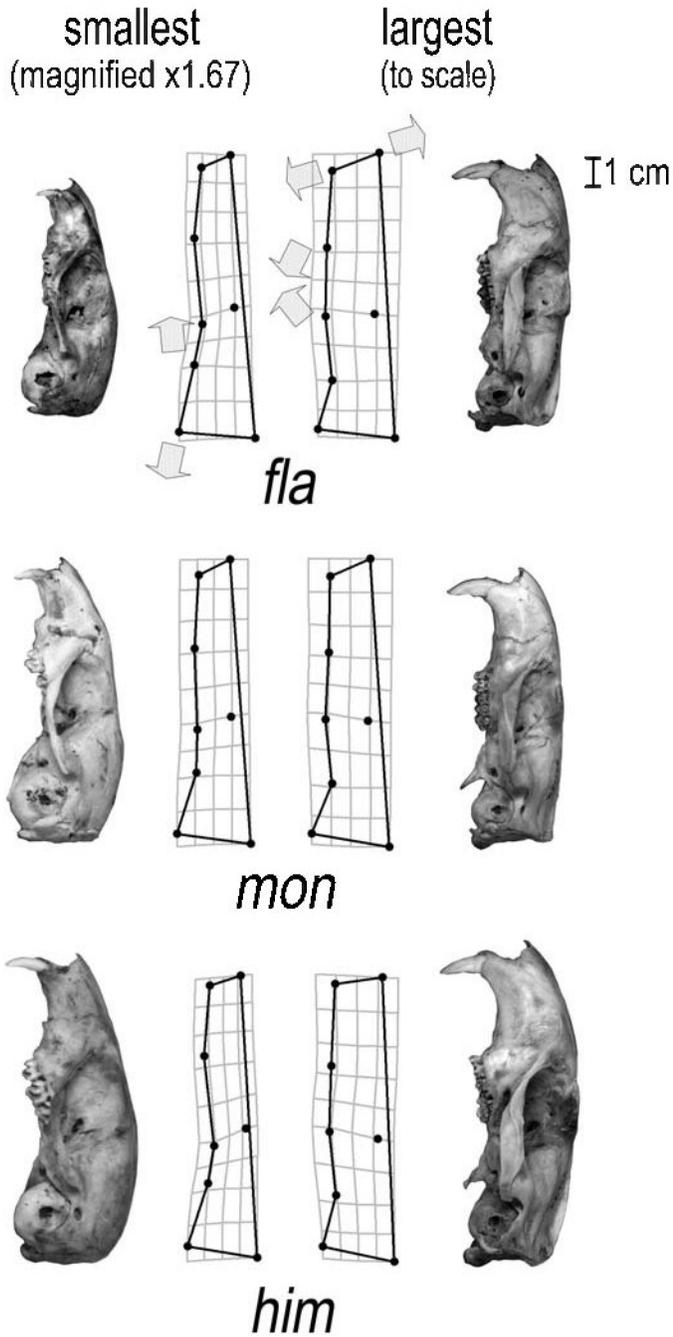


FIG. 6.—Lateral cranium (LC) of *Marmota flaviventris* (= fla), *M. monax* (= mon), and *M. himalayana* (= him). Pictures of the LC for the smallest (left, magnified relative to the scale according to magnification factors given by the specimen) and largest (right) individuals of each species sample, and deformation grids and wireframe (center) for shapes predicted by the multivariate regression of the LC shape onto size for the smallest (left) and largest (right) specimens. Arrows indicate prominent shape changes at the extremes of size variation in *M. flaviventris*.

1 and 2. Sexual dimorphism and allometry.—Allometry accounts for a significant proportion of postnatal shape changes in the marmot cranium. A relative reduction in size during postnatal ontogeny is common in the neurocranium (e.g., cranial vault and tympanic bulla) but also in the toothrow,

consistent with findings by Cardini and O’Higgins (2005), who related these features to the early completion of development of the nervous system and dentition. A relative increase in size is found in the rostrum and zygomatic arch, and it is either part of a trend of early neurocranial and late facial expansion found in most mammals (Laghenbach and Van Eijden 2001) or related to masticatory muscle development.

Males and females share a common allometric trajectory and males are significantly ($P < 0.01$) larger than females. This allometric pattern could lead to shape differences between the 2 sexes because of the extension of the common allometric trajectory in males, but in fact, shape differences in the cranium of male and female marmots are found to be generally negligible. Sexual dimorphism in shape may be difficult to detect in natural population samples, “where nothing is controlled and only coarse toothwear classifications are available to partition ‘age’ effects” (Voss et al. 1990:1578). This is likely to be true for our analysis. On average, crania of males are <3% larger than those of females. Thus, size-related sexual dimorphism must be very subtle. Our findings are consistent with those of all previous analyses on the marmot mandible and ventral cranium (Cardini 2003, 2004; Cardini et al. 2005; Cardini and O’Higgins 2004, 2005; Cardini and Tongiorgi 2003).

The DC of *M. flaviventris* represents the only remarkable exception to the general absence of appreciable sexual dimorphism in the marmot cranium. The pattern of allometric shape change in males and females is similar, but trajectories are parallel. Thus, sexually dimorphic traits that are present in newborn marmots are preserved through postnatal ontogeny into adulthood. Indeed, the observation of deformation grids for the average DC shape of the juveniles (not shown) suggests a relatively shorter and enlarged cranial vault in males. The same dimorphic pattern is seen in deformation grids for the adult average shapes. Although significant, these differences appear to be small compared to interspecific differences in the scatterplot of shape onto size of the DC (not shown), and thus negligible in comparison of allometries of different marmot species.

In conclusion, like all previous studies, this one indicates that sexual dimorphism in marmot skeletal characters is generally small for size and negligible for shape. The slightly larger size of males is related to sexual selection, with males competing for territories and access to females (Armitage 2000; Barash 1989).

3 and 4. Interspecific allometries and pattern of ontogenetic shape change.—The comparison of allometric trajectories in the species of *Marmota* analyzed indicates differences in slopes of the regression lines. Thus, the divergence of allometries might contribute to the development of species-specific traits. Cardini and O’Higgins (2005) found similar results with the mandible and ventral cranium.

In contrast, the observation of deformation grids of shapes predicted by allometric trajectories for specimens at the extremes of size variation of each species suggests similar changes and, thus, a common allometric model despite statistical differences in slopes. Influential cases, which might strongly alter the regression slopes, or high statistical power,

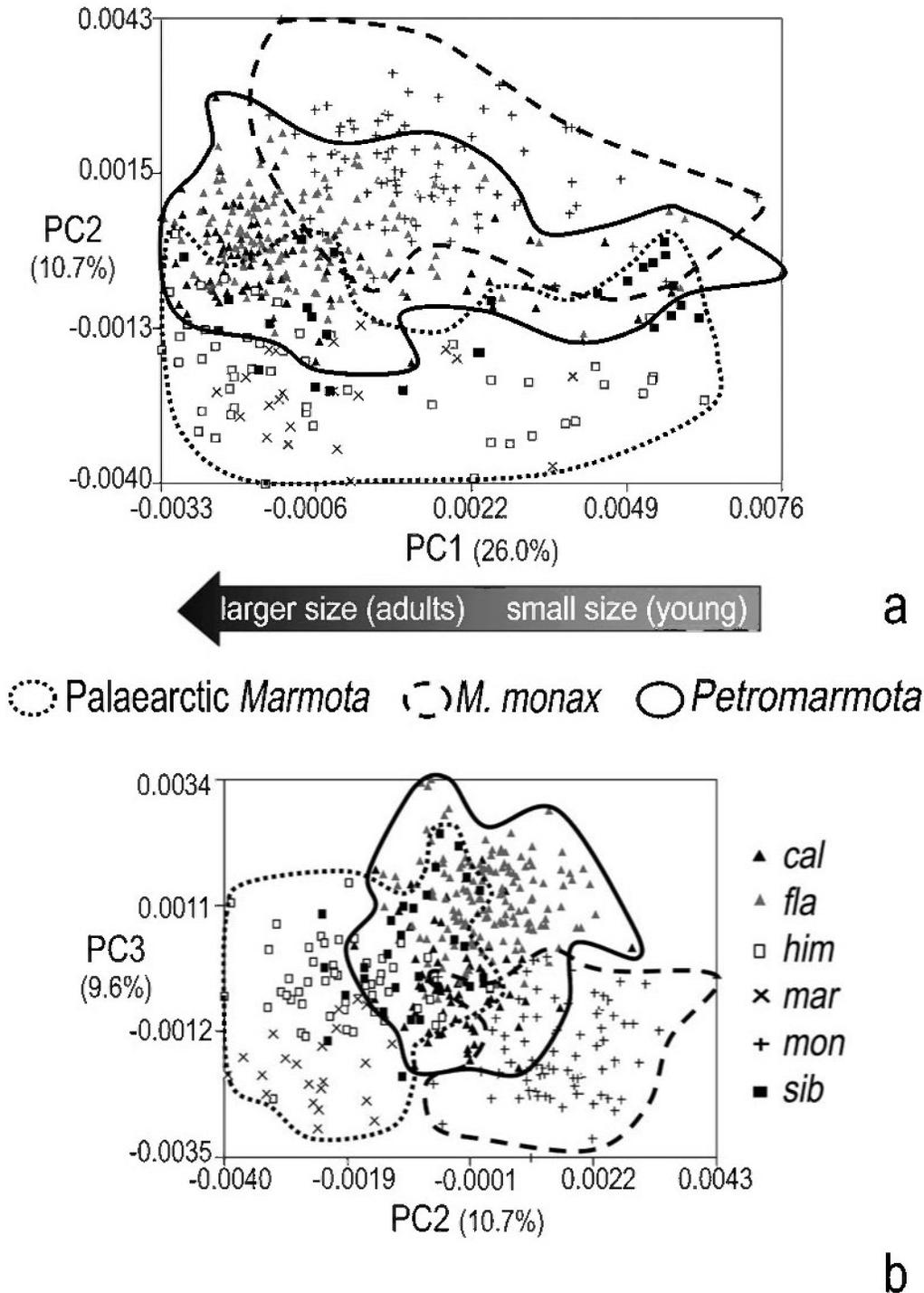


Fig. 7.—Scatterplots of *Marmota* species samples a) projected onto principal component 1 (PC1) and PC2, and b) onto PC2 and PC3 of the 3-dimensional shape variables; percentages of variance given below axis titles; PC1 is negatively correlated with size, and thus smaller and younger specimens tend to be to the right and larger and older ones to the left as indicated by the arrow under PC1. Species abbreviations are as in Table 1.

that allow detection of small but negligible differences, could have influenced the comparison of allometric trajectories. The phenogram of the angles between allometric trajectories of 3D crania indicates a relatively small interspecific divergence. The relatively larger angles of comparisons involving *M. marmota* are likely to be affected by sampling error (see below). The phylogenetic information in the phenogram topology is small.

On the one hand it suggests a modest divergence between the 2 *Petromarmota* species, *M. caligata* and *M. flaviventris*, and on the other hand it shows strong similarities in slopes between the latter and *M. himalayana*, which is not congruent with phylogeny (they belong to different subgenera) nor easily explained by convergence. The 2 species belong to different subgenera, have different degrees of sociality, and are, respec-

tively, the smallest and largest (together with *M. olympus*) marmot species.

Clues about possibly misleading results of statistical tests can be sought by repeating the comparison of allometric trajectories in a subsample of specimens (3D data set; results not shown). Specimens representative of the main range of size variation ($120 \text{ mm} \leq \text{centroid size} \leq 240 \text{ mm}$) in *Marmota* are included, 12 size intervals are created at 10-mm increments (i.e., 120, 130, 140, . . . , 210, 230, and 240 mm), and the analysis sample is built by selecting for each species one specimen having centroid size $\cong 120 \text{ mm}$, one with centroid size $\cong 130 \text{ mm}$, and so on. Thus, allometric trajectories of marmots can be computed using samples of specimens that correspond to comparable increments of size during postnatal ontogeny of each species. The comparison of allometric trajectories now indicates that intercepts are different, but slopes are not. This is explained by a common model of allometric shape change (parallel trajectories), which is shifted to the left in smaller species and to the right in larger ones. If this is the case, most interspecific differences of the cranium are already present in young marmots and are preserved during postnatal ontogeny.

Early separation of *Petromarmota*, Palearctic *Marmota*_{SG}, and *M. monax* is indeed visible, to some extent, in the scatterplot of the first 3 principal components, and it is fairly evident for the latter 2 groups. Also, most of the shape traits that discriminate *M. monax* from Palearctic species of *Marmota*_{SG}, and to a smaller extent these 2 groups from *Petromarmota*, are not strongly allometric, as shown by the good separation of the 3 clusters along PC2 and PC3, whose correlation to size explains only 5.2% and 20.5% of the shape variance summarized by each axis (respectively, 10.7% and 9.6% of total variance). Thus, divergence of allometric trajectories does not seem to be the main factor responsible for the morphological differentiation in *Marmota*.

In conclusion, shape differentiation starts early in marmot ontogeny and seems to account for most interspecific differences. Allometry may contribute to magnifying these differences either by divergence of allometric trajectories (mainly in *M. monax* and *M. sibirica*) or by shifts (lateral transpositions, sensu Klingenberg [1998]) of a common allometric pattern in smaller (paedomorphic *M. flaviventris*) and larger (peramorphic *M. himalayana*) species.

Another interesting aspect of allometric variation of the marmot cranium concerns what Cardini and O'Higgins (2005) called "the degree of allometry," that is, the proportion of size-related shape changes during postnatal ontogeny. In the present study this measurement ranges from about 10% to 50% of shape variance, an outcome congruent with previous studies on marmots (Cardini and O'Higgins 2005; Cardini and Tongiorgi 2003). Our results also are in agreement with Swiderski's (2003) observation that the proportion of allometric shape changes during postnatal ontogeny does not generally account for the majority of shape variation in a sample (at least, when size and shape are efficiently separated using geometric morphometric techniques). However, we argue that most of the age-specific shape traits (i.e., those making a juvenile look like a juvenile, a yearling look like a yearling, etc.) of the cranium

are allometric. Indeed, clear discrimination of age classes is no longer possible if the allometric variation is removed in the sample of *M. flaviventris*.

The degree of allometry is to some extent correlated with social complexity and age at 1st reproduction. This pattern is especially clear if *M. caudata* and *M. marmota* are excluded from the analysis. These 2 samples might not be representative of ontogenetic variation in the wild because of the very low number of putative juveniles available for the study. Thus, after exclusion of *M. caudata* and *M. marmota*, the degree of allometry increases monotonically, and consistently in all data sets, from *M. monax*, a solitary species sexually mature as a yearling, to *M. flaviventris*, which begins reproduction after the 2nd year of life, hibernates solitarily, and is characterized by matrilineal defended by a territorial male, to *M. caligata*, sexually mature at 3 and with group hibernation, and to *M. himalayana* and *M. sibirica*, which start reproduction as 3 year olds, have social hibernation, and often have alloparental care. Cardini and O'Higgins (2005) speculated that, because the degree of allometry correlates with the actual time necessary for an individual to become an adult, and muscles insert on regions that show extensive allometric changes, a prolonged bone remodeling by muscles might be involved in producing allometric modifications whose extent is roughly proportional to the length of preadult life (the period when muscle development is more intense). However, the significance of this correlation between an ontogenetic pattern of the cranium and mandible and life history traits remains unclear, and observations need to be confirmed in other species before an explanation is sought.

5. Correlation between 2D and 3D data in M. flaviventris.—Correlations between 2D and 3D landmarks are very high for size and fairly good for shape. This partly explains why results of 2D analyses of the ventral cranium (Cardini and O'Higgins 2005), DC, and LC are in good agreement with the more accurate and comprehensive description of the cranium obtained in 3D. Nevertheless, congruence in results was higher than expected considering that measurement error increases in 2D analyses of complex 3D structures.

Robustness of results of geometric morphometrics is in relation to the efficiency of this method in providing detailed and accurate quantitative descriptions of form, and increases with the amount of information stored in the landmark configuration. This is suggested by the increase in the coefficient of matrix correlation ($r > 0.7$) between 2D and 3D data when dorsal, lateral (present study), and ventral (Cardini and O'Higgins 2005) 2D data are combined using the separate subset method of Adams (1999).

Thus, after a careful choice of the landmarks, 2D studies can provide fairly accurate descriptions of morphological variation even when applied to complex 3D structures such as the marmot cranium. Further studies that compare 2D and 3D data may help to understand whether this finding has a general applicability or if it depends on the taxon, structure, and landmark configuration (number and type of landmarks, etc.) used for the analysis.

Conclusions.—Our main conclusions support previous findings on the postnatal ontogeny of marmots, which

suggested a modest sexual dimorphism and an important role of allometry in producing the main age-specific traits in the marmot cranium. A relative reduction in size during the postnatal ontogeny of marmots is generally associated with regions whose development is completed early, whereas a relative expansion mainly concerns areas of muscle insertion.

The extent to which allometry contributed to evolutionary divergence in the marmot clade, and especially the different contribution of allometry to ontogenetic shape changes in marmots with different length of preadult life are less clearly understood. The main shape differences are already present in newborn marmots and divergence of allometric trajectories is probably small in most species. Thus, at least in the radiation of the modern species of *Marmota*, allometry is unlikely to have played a major role.

The occurrence of a common allometric pattern in the postnatal ontogeny of the marmot cranium of different marmot species can be interpreted according to the traditional view of allometry as an evolutionary constraint that reduces the range of shape variation susceptible to evolutionary change. This may well be the case in the recent evolutionary history of marmots. However, constraining evolutionary variation to a particular direction can have a positive meaning too: "If any of these phenotypes [those produced by ontogenetic allometries] would benefit the organism at a different size or stage of life, or if any different combination of characters ... might yield increased adaptation, then the existing channel of ordinary ontogeny already holds the raw material in a particularly effective state for evolutionary change" (Gould 2002:1038). Natural selection may be particularly effective on such correlated traits (a possibility also considered by Swiderski [2003]) and allometry may act as a positive accelerator of evolutionary change. Thus, changes that follow the preferential direction set by allometric trajectories might be responsible for important evolutionary changes. Indeed, sciurids might well be conservative and prone to convergence, but an extensive shape divergence took place in the evolution of marmots, which separates this clade from all other ground squirrels (Cardini 2003; Cardini and O'Higgins 2004), despite close phylogenetic relationships with *Spermophilus* (Harrison et al. 2003; Herron et al. 2004). The emergence of marmots as an offshoot of the *Spermophilus* phylogenetic bush also has been characterized by a large increase in size (about 2-fold for the cranium). The concomitance of pronounced shape divergence and large size increase in earlier stages of the marmot evolutionary history might imply that allometry was then an important source of evolutionary novelties, whereas its contribution to later morphological differentiation was small, as suggested by the comparison of allometric trajectories of living species.

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