doi:10.1111/j.1420-9101.2007.01398.x

Evolutionary acceleration in the most endangered mammal of Canada: speciation and divergence in the Vancouver Island marmot (Rodentia, Sciuridae)

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Keywords:

cytochrome *b*;
endangered species;
evolutionary acceleration;
morphology;
neutral model;
selection.

Abstract

The Vancouver Island marmot is the most endangered mammal of Canada. Factors which have brought this population to the verge of extinction have not yet been fully elucidated, but the effects of deforestation and habitat fragmentation on survival rates, as well as those of variation in rainfall, temperature, snowpack depth and snowmelt strongly suggest that marmots on the island are struggling to keep pace with environmental changes. Genetic analyses, however, seem to indicate that the Vancouver Island marmot may merely represent a melanistic population of its parental species on the mainland. Were it not for its black pelage colour, it is unlikely that it would have attracted much attention as a conservation priority. Our study uses three-dimensional coordinates of cranial landmarks to further assess phenotypic differentiation of the Vancouver Island marmot. A pattern of strong interspecific divergence and low intraspecific variation was found which is consistent with aspects of drift-driven models of speciation. However, the magnitude of shape differences relative to the putatively neutral substitutions in synonymous sites of cytochrome *b* is too large for being compatible with a simple neutral model. A combination of bottlenecks and selective pressures due to natural and human-induced changes in the environment may offer a parsimonious explanation for the large phenotypic differentiation observed in the species. Our study exemplifies the usefulness of a multidisciplinary approach to the study of biological diversity for a better understanding of evolutionary models and to discover aspects of diversity that may be undetected by using only a few genetic markers to characterize population divergence and uniqueness.

Introduction

Marmots are ground-dwelling squirrels with pronounced adaptations to cold climates (Barash, 1989; Armitage, 2003). Fourteen species, subdivided into two subgenera (*Marmota* and *Petromarmota*) are currently recognized (Steppan *et al.*, 1999; Armitage, 2003). Marmots have a Holoarctic distribution and only one of the species, *Marmota vancouverensis*, lives exclusively on an island (see Fig. 1). This species, the Vancouver Island marmot, is the most endangered mammal of Canada and survives in the wild with a population of less than 100 individuals (Bryant, 2002). Concerted efforts have been made to conserve it from extinction and its uncertain fate has been considered an exemplar of the present biodiversity crisis (Wilson, 2002).

Presumably, *M. vancouverensis* represents a population of the continental *Marmota caligata*, which was isolated on Vancouver Island after the sea level rose at the end of the last glaciation. This is consistent with the observation that the genetic divergence between *M. vancouverensis* and *M. caligata* is comparable with that found on average within a marmot species (Steppan *et al.*, 1999). Thus, the

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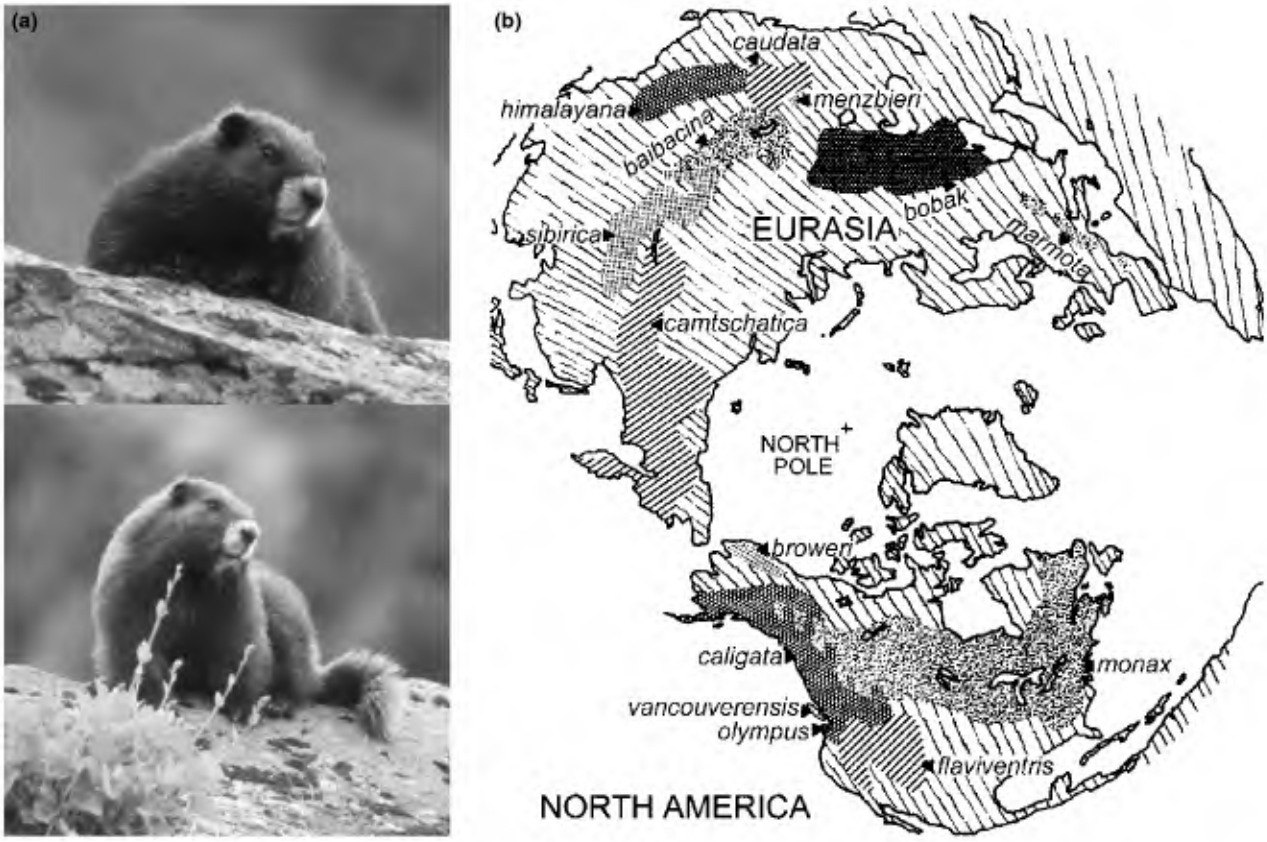

Fig. 1 (a) The Vancouver Island marmot (photos by A. Bryant) and (b) the geographic distribution of living marmot species (from Barash, 1989, modified).

Vancouver Island marmot is the youngest marmot species, and its status as a species comes by virtue of its geographic isolation and its unique black pelage colour. Together with several subspecies of *M. caligata* and with *Marmota olympus*, an endemic of the Olympic Peninsula, it is included in the *M. caligata* species complex.

In the first interspecific morphological comparison of all living marmots (Cardini, 2003), the Vancouver Island marmot was found unexpectedly divergent in mandible shape. Founder effect and genetic drift were suggested as most likely explanations of this rapid differentiation. Studies on teeth and crania also suggested that the species was strongly divergent in dental and skeletal shape (Polly, 2003; Cardini *et al.*, 2005; Cardini & O'Higgins, 2004). The consistency of findings of morphological distinctiveness in the absence of evident genetic differentiation led Cardini & O'Higgins (2004) to suggest that modern quantitative studies of the phenotype may be required to discover aspects of biological uniqueness not revealed by genetic analysis, thus making accurate comparisons of form a highly desirable complement to genetic data in the study of biodiversity. Indeed, the identification of species, subspecies and populations is a fundamental requirement of conservation biology because resources allocated to the recovery of endangered or threatened species are prioritized based on their taxonomic status (Haig, 1998).

The Vancouver Island marmot, however, is not only an example of the usefulness of a multidisciplinary approach to conservation; it is also an opportunity to study population divergence in the context of geographic isolation, climatic and environmental change. The marmots of Vancouver Island faced strong selective pressures after the last glaciation, including human hunting as indicated by cutmarks on bones and Native American artefacts (Nagorsen *et al.*, 1996). The effects of deforestation, habitat fragmentation, variation in rainfall, in temperature, in snowpack depth and in snowmelt, which are all probably affected by global warming and climate change, impact marmot survival rates (Bryant, 2002) strongly suggesting that even today marmots on the island are struggling to keep pace with environmental changes. Thus, the Vancouver Island marmot represents an intriguing example of peripatric speciation, possibly ongoing, in a rapidly changing environment.

The new selective pressures of a newly colonized environment offer a simple explanation for rapid evolution of traits on islands. However, insular populations also go through genetic bottlenecks due to the limited number of individuals in the founding population or to population crashes. Also, island populations are often ecologically similar to populations on the mainland. Thus, Mayr (1963) suggested that genetic drift, and not natural selection, was the main trigger for rapid divergence and speciation of island populations. In Mayr's view, gene flow and coadaptation among sets of genes tend to maintain phenotypic uniformity over large geographic regions so that these populations show little more than gradual clinal variation. This evolutionary inertia or constraint can be overcome by the loss of genetic variation following a founder event. Increased homozygosity and reduced genetic diversity at several loci act as a trigger to produce changes at other loci, causing an 'evolutionary chain reaction' (Mayr's 'genetic revolution') which in turn moves the fitness peak of the island population away from that of the parental population on the mainland. The low fitness of hybrids which fall in the valley between the adaptive peaks of the two populations may be enough to promote reproductive isolation and cladogenesis. Templeton and Carson (Templeton, 1980; Carson & Templeton, 1984), inspired by Mayr, further elaborated models of rapid speciation driven by genetic drift, but severe criticisms were raised by others against the unnecessary emphasis that these models put on drift (see Coyne & Orr, 2004, for a review). Whatever the mechanism is for rapid divergence of insular populations leading to speciation, there is little doubt that morphological evolution on islands is accelerated in mammals (Millien, 2006). Previous studies on the Vancouver Island marmot noted only that its divergence was large, but they did not investigate the tempo and mode of its divergence nor consider how its origin might relate to existing models of speciation. Also, those studies were based on a limited number of two-dimensional morphometric descriptors.

The present study uses precise three-dimensional anatomical landmarks of the cranium to investigate models of evolutionary divergence in *M. vancouverensis*. First, the contribution of the Vancouver Island marmot to diversity in marmot form (i.e. size and shape) is measured. To do this, partial disparities (Foote, 1997; Zelditch *et al.*, 2004) of shape and size are computed, standard errors are estimated by bootstrap, and species are compared to test whether divergence in *M. vancouverensis* is unusually large. Second, phenotypic variance within populations is measured to test whether *M. vancouverensis* has less variation than related species. The significance of the difference variance is tested against populations of the *M. caligata* species complex. A significant reduction in variance in the Vancouver Island marmot would imply a recent population bottleneck. Finally, phenotypic divergence in *M. vancouverensis* was assessed for selection by comparing it with interspecific differences in third codon positions of the mitochondrial cytochrome *b* gene sequence with the assumption that those synonymous sites evolve neutrally by drift (Chamaray *et al.*, 2006). For genetic drift alone to have played a major role in the rapid morphological evolution of *M. vancouverensis*, the distance ratio of *M. vancouverensis*–*M. caligata* would be expected to fall comfortably within the distribution of values observed for other species pairs because both morphological and mtDNA distances are the product of the same neutral drift. If, on the other hand, selection played a role then the amount of morphological divergence should be proportionally large compared with neutral mtDNA divergence.

Materials and methods

Samples and data collection

A total of 380 specimens belonging to all living marmot species were analysed (see Table 1). Sample size and abbreviation of scientific names are shown in Table 1. Only adult specimens were included in the analysis. Completeness of molar eruption, tooth wear, sutures between frontal and parietal bones and development of the sagittal crest were used for separating young from adults.

Specimens belong to the British Museum of Natural History (London, UK), National Museum of Natural History (Washington, DC, USA), American Museum of Natural History (New York, NY, USA), Museum of Vertebrate Zoology (Berkeley, CA, USA), Zoological

Table 1 Adult sample composition.

Species*	$n_{females}$	n_{males}	n_{total}†
Marmotini			
Marmota (MAR)			
Marmota baibacina (*bai*) Kastschenko, 1899	4	6	15
Marmota bobak (*bob*) (Müller, 1776)	0	1	2
Marmota broweri (*bro*) Hall & Gillmore, 1934	6	6	16
Petromarmota caligata (*cal*) (Eschscholtz, 1829)	26	20	52
Marmota camtschatica (*cam*) (Pallas, 1811)	0	1	5
Marmota caudata (*cau*) (Geoffroy, 1844)	14	12	32
Petromarmota flaviventris (*fla*) (Audubon & Bachman, 1841)	59	45	122
Marmota himalayana (*him*) (Hodgson, 1841)	11	9	28
Marmota marmota (*mar*) (Linnaeus, 1758)	4	8	15
Marmota menzbieri (*men*) (Kashkarov, 1925)	1	1	2
Marmota monax (*mon*) (Linnaeus, 1758)	23	20	58
Petromarmota olympus (*oly*) (Merriam, 1898)	5	8	14
Marmota sibirica (*sib*) (Radde, 1862)	5	6	12
Petromarmota vancouverensis (*van*) Swarth, 1911	4	3	7

*Abbreviations for species names used in figures are given in parentheses. †Total sample size may be larger than the sum of females and males of a group due to missing information on sex.

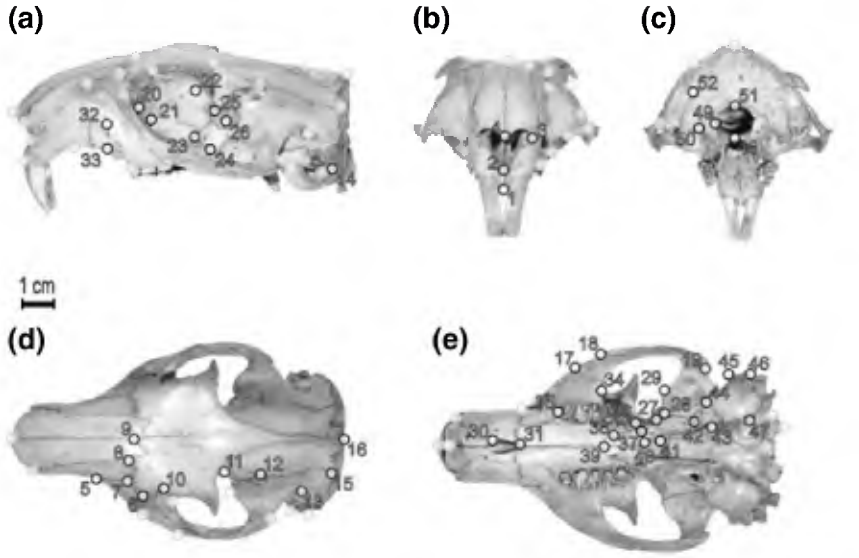

Fig. 2 Three-dimensional landmark configuration of marmot cranium. Landmarks are shown on lateral (a), rostral (b), caudal (c), dorsal (d) and ventral (e) sides of the cranium.

Museum of the University of Montana (Missoula, MT, USA), University of Alaska Museum (Fairbanks, AK, USA), University of Kansas Natural History Museum (Lawrence, KS, USA), Field Museum (Chicago, IL, USA) and R. L. Rausch, private collection.

Three-dimensional coordinates of anatomical landmarks were directly collected by the same person on crania and mandibles using a 3D digitizer (MicroScribe 3DX; Immersion Corporation, San José, CA, USA). Landmarks were digitized only on the left side to avoid redundant information in symmetric structures (see Fig. 2; see Table 2). Measurement error was tested as described in Cardini & Thorington (2006) and found to be negligible. One to three missing landmarks were estimated using means in 9.4% of specimens. The error introduced by this procedure was also found to be negligible (see Cardini & Thorington, 2006).

Geometric morphometrics

Analyses were performed using methods for the comparison of the geometric form of organisms and their organs (Rohlf & Marcus, 1993; Adams *et al.*, 2004; Zelditch *et al.*, 2004). Geometric morphometrics compares forms by using the information captured by Cartesian coordinates of sets (configurations) of topographically corresponding landmarks (Marcus *et al.*, 2000). Differences in coordinates due to rotation and translation of specimens during data collection are removed (Procrustes superimposition, Rohlf & Slice, 1990), and size and shape components of form are separated and analysed with multivariate statistics. Size is measured as centroid size, which is the square root of the sum of squared distances between all landmarks and their centroid. The magnitude of shape differences between two configurations is measured by their Procrustes shape distance, which is the square root of the sum of squared differences between corresponding landmarks of two superimposed landmark configurations.

Geometric morphometric methods have been described in numerous papers and have become standard practice in morphometrics. An extensive introduction to applications of geometric morphometrics in biology is provided by Zelditch *et al.* (2004). Detailed mathematical descriptions of geometric morphometric methods are available in Bookstein (1991) and Dryden & Mardia (1998). Guidelines on how to implement linear statistical models in geometric morphometrics can be found in Rohlf (1998) and Klingenberg & Monteiro (2005).

Geometric morphometric analyses were performed using Morpheus (Slice, 1999), TPSSmall 1.20 (Rohlf, 2003) and NTSYS-pc 2.2f (Rohlf, 2005).

Statistical analyses

Analyses of variance (multivariate for shape) were used to test the significance of sexual dimorphism and species differences in size and shape of the cranium. Results were similar to those of previous studies to which the reader is referred for more information (Cardini, 2003, 2004; Cardini *et al.*, 2005; Cardini & O'Higgins, 2005). Briefly, males were slightly larger than females (on average less than 3%), sexual dimorphism in shape was negligible and interspecific differences in size and shape were highly significant. Thus, all further analyses were performed on species means with pooled sexes.

Principal component analysis (PCA) identifies the axes of the greatest variation in a sample. This method was used to illustrate spatial (similarity) relationships among species mean shapes. A preliminary inspection of scatterplots of the first PCs (not shown), and the observation

Table 2 Anatomical landmark description and numbering.

No.	Definition*
1	Anterior (midsagittal) lower tip of the premaxilla
2	Anterior (midsagittal) upper tip of the premaxilla
3	Anterior tip of suture between nasal and premaxilla
4	Anterior (midsagittal) tip of the nasal
5	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	Meeting point between nasal and frontal along the midsagittal plane
10	Supraorbital notch
11	Posterior base of the post-orbital process
12	Meeting point of frontal, parietal and squamosal bones
13	Temporal foramen
14	Most ventral meeting point between mastoid process of the occipital bone and the tympanic bulla
15	Meeting point of parietal, squamosal and occipital bones
16	Most posterior point of the parietal along the midsagittal plane
17	Ventro-lateral meeting point of zygomatic process of maxilla and jugal
18	Most anterior point of region of insertion of the posterior deep masseter on the jugal
19	Posterior tip of the zygomatic arch
20	Nasolachrymal foramen
21	Unossified area in maxillary-lachrymal suture
22	Ethmoidal foramen
23	Sphenopalatine foramen
24	Dorsal palatine foramen
25	Optic foramen
26	Sphenofrontal foramen
27	Dorsal tip of sphenoidal fissure
28	Masticatory foramen
29	Anterior extremity of the suture between the alisphenoid and the zygomatic process of the squamosal
30–31	Extremities of incisive foramen
32	Dorsal tip of infraorbital foramen
33	Tip of the masseteric tubercle
34	Most anterior point of the orbit (in the ventral view)
35–36	Posterior and anterior end of the 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 the posterior edge of the palatine
41	Meeting point between basisphenoid and presphenoid where the anterior *foramen lacerum* typically opens
42	Posterior extremity of the foramen ovale
43	Meeting point between the basisphenoid, basioccipital and tympanic bulla
44	Most ventral meeting point between tympanic bulla and alisphenoid
45–46	Anterior and posterior tip of the external auditory meatus
47	Anterior extremity of the jugular foramen
48	Most posterior point on the ventral region of the occipital foramen
49–50	Lateral tips of the occipital condyle
51	Dorso-medial tip of the occipital foramen
52	Mastoid foramen

*The terms 'anterior' and 'posterior' are used with reference to Fig. 2.

that *Marmota bobak* has on average the largest shape distances to all other species, suggested highly distinctive cranial traits in this species. However, this observation was at odds with findings of all previous analyses (Cardini, 2003; Cardini *et al.*, 2005; Cardini & O'Higgins, 2005) performed on larger samples of *M. bobak* ($N > 18$). Also, the only two *M. bobak* specimens available to us were unusually large relative to other species (fourth largest average) compared with previous analyses (fifth or sixth smallest average). The unusual divergence of *M. bobak* in the present analysis is, thus, best explained as an artefact of sampling error and led us to exclude this species from all further analyses.

The relative contribution of each species to shape divergence in *Marmota* was measured using partial disparities (Zelditch *et al.*, 2004). Partial disparity (PD) of the *i*th species is given by:

$$\mathrm{PD}_i = (D_i)^2/(\mathrm{N}-1)$$

where D is the shape (Procrustes) distance between the mean of the *i*th species and the grand mean of all species, and N is the sample size. Standard errors of PD were computed by bootstapping original samples, repeating disparity analyses and computing standard deviations (SD) of PD generated by the bootstrap procedure.

Partial disparity of size was measured using the same equation as for shape with D now being equal to the difference between the mean size of the *i*th species and the grand mean of all species.

Shape variance was measured in each sample as the sum of variances of all shape variables. Standard errors of shape variance were computed by bootstrapping original samples, computing the variance for each bootstrap sample and calculating the SD of variances. In general, the number of independent bootstrap samples is given by $(2N-1)!/N!(N-1)$ (Zelditch *et al.*, 2004) and it is three with $N = 2$, 10 with $N = 3$, 70 with $N = 4$, 756 with $N = 5$, etc. Thus, bootstrap standard errors were not computed for *Marmota menzbieri* ($N = 2$) and the number of unique bootstrap means of the smallest samples was smaller than the number of bootstraps (100) for *M. caligata vigilans*. Repeated randomized selection experiments to build progressively smaller samples from an original data set of approximately 400 vervet monkey skulls (Cardini & Elton, in press) indicate that the variation around estimates of parameters such as shape variance increases as sample size decreases. Thus, bootstrap standard errors are expected to become larger in smaller samples.

To test for significance of differences in variance between *M. vancouverensis* and its closely related species/subspecies (*M. caligata* species group), a series of Levene's tests was performed on the Procrustes distances. The Levene's test requires calculating the absolute value of the deviation of each individual from the sample mean (Van Valen, 1978), which is satisfied by using absolute

differences to the mean size or Procrustes distances to the mean shape. These deviations are then compared by ANOVA. Although this test is generally considered relatively robust to departures from normality, we chose to perform a randomization version of the test that compares the observed *F*-statistic with the distribution obtained by randomly reassigning deviations from sample means to the samples.

Variation in size within sample (species or subspecies) was estimated by the SD of centroid size. Resampling statistics (same procedure as for shape) was used to estimate standard errors and to test differences in variance of size among samples.

Phenotypic change in *M. vancouverensis* was tested for selection by determining whether it was greater than expected given the amount of time since divergence. Sequences published in GenBank (accession numbers: gil4769016, gil4769019, gil4769021, gil5565797, gil5565798, gil4769028, gil4769029, gil4769030, gil4769032, gil5565801, gil4769039, gil4769040) were aligned using CLUSTALX 1.81 (Thompson *et al.*, 1997), third codon positions were extracted using BIOEDIT 7.0.5.3 (Hall, 1999) and Kimura 2-parameter genetic distances were calculated using PHYLIP 3.63 (Felsenstein, 2004). Ratios of squared phenotypic shape distances to genetic distances were computed pairwise for all species. Shape distances were squared to linearize phenotypic divergence because the distances increase as a square-root function of time in cases where there is a Brownian motion-like divergence and reversal pattern (Polly, 2003, 2004). The distribution of this ratio among species other than *M. vancouverensis* was used as a null expectation of the amount of phenotypic change per unit time (as measured by neutral substitutions) in marmots against which divergence between *M. vancouverensis* and its sister species *M. caligata* could be tested. Under a model of evolutionary divergence driven exclusively by drift, the shape-to-neutral DNA distance ratio of *M. vancouverensis–M. caligata* should not be a significant outlier in this distribution. *Marmota olympus* was not included in this analysis because the available sequence is incomplete. The same procedure was applied to test whether the divergence in size of *M. vancouverensis–M. caligata* is compatible with a speciation model driven by drift. We chose this procedure over related tests, such as Lynch's (1990) test – where the parameter Δ is estimated as the variance between species over the variance within species multiplied by time since divergence in number of generations – because the only estimate of divergence time available for these marmot species is neutral genetic distance, which is an inherently pairwise measure. It would be possible to convert genetic distances to time since divergence measured in years and then to convert those estimates to time in generations to apply Lynch's equation, but doing so would multiply the uncertainty in the time estimate with each step. Furthermore, the variance in *M. vancouverensis* appears to have decreased substantially compared with other marmot species (see below), which would bias Lynch's Δ by making the denominator smaller and therefore increasing the apparent effect of selection. We do not consider Lynch's test to be inferior, but we prefer our procedure of testing the distance of *M. vancouverensis–M. caligata* against the background distribution of phenotypic change in marmots because: (1) it is directly compatible with pairwise genetic distance measures of divergence time; and (2) it is not dependent on assumptions of equal variance among the species.

Our method of using the distribution of pairwise phenotypic distances to pairwise genetic distances could be biased by sampling error or founder effect in *M. vancouverensis* because either could increase the apparent phenotypic differentiation between *M. vancouverensis* and *M. caligata* with no change in the genetic differentiation. We therefore used a resampling method to determine whether the large difference could be due to chance sampling, either methodological due to our limited sample or biological due to founder effect or other chance processes. We used the species with the largest sample size, *Marmota flaviventris*, as a model for intraspecies variation and drew random subsamples equal in size to our sample of *M. vancouverensis* from it. These were considered to be equivalents of 'founder populations'. The distribution of mean phenotypic differences between random subsamples and the complete *M. flaviventris* sample was calculated and used to test whether the difference between *M. vancouverensis* and *M. caligata* fell outside that range, as would be expected if their divergence were produced by selection rather than drift, founder effect or methodological sampling error.

Statistical analyses were performed using NTSYS-PC 2.2F (Rohlf, 2005) and SPSS 11.5.0 (SPSS for Windows, 2004).

Results

Interspecific relationships are summarized by a PCA of mean shapes (see Fig. 3). Dotted lines connect species means to the grand mean of all species and help visualizing the relative amount of shape divergence. *Marmota vancouverensis*, with its distinctive cranial traits, such as the V-shaped notch at the posterior border of the nasals and the relatively narrow parietal bones, is clearly far from the mean of the genus.

Partial disparity in shape variance was first compared among all species (see Table 3). *Marmota monax*, *M. menzbieri*, *Marmota marmota* and *M. vancouverensis* are strongly divergent with partial disparities more than two standard errors larger than the average (7.7%). Standard errors of partial disparities are negatively correlated with sample size ($r = -0.571$, $P = 0.042$). The average within species shape variance is 0.001536. *Marmota vancouvernsis* has the smallest variance (seven standard errors smaller than the average). Also *M. olympus* has a small shape

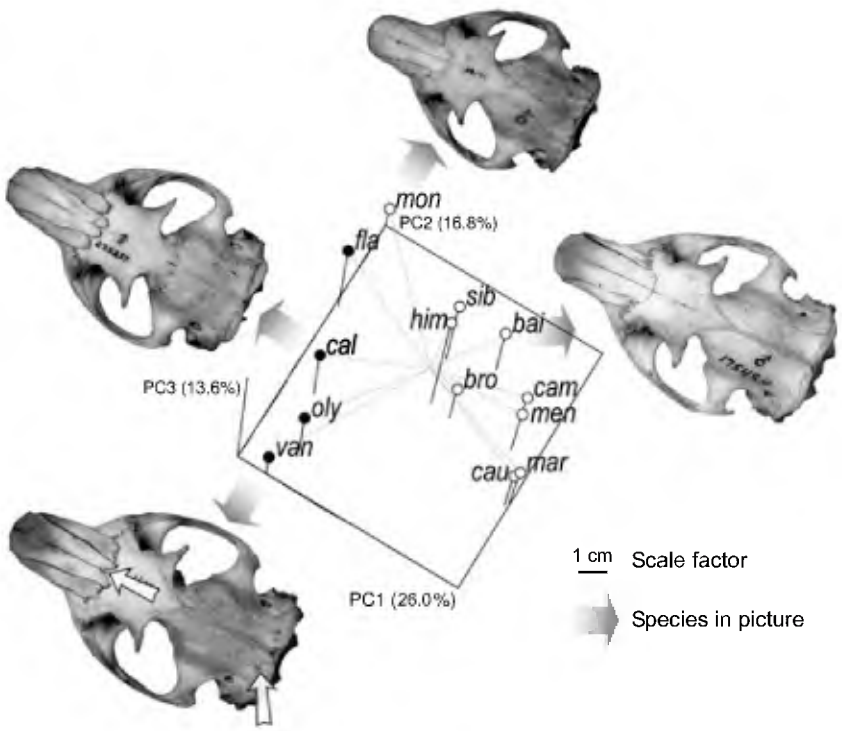

Fig. 3 Scatterplot of first three axes of a PCA of mean shapes. Pictures of *Marmota vancouverensis*, *Marmota caligata*, *Marmota monax* and *Marmota baibacina* crania are shown by their mean shapes. White arrows indicate distinctive traits of *M. vancouverensis*.

variance. As for disparity, standard errors are negatively correlated with sample size but the correlation is not significant ($r = -0.561$, $P = 0.057$).

Results of the analyses of disparity and comparisons of the magnitude of within-species variation in size are also shown in Table 3. *Marmota vancouverensis* contribution to size disparity is very modest (1.7%), whereas *M. flaviventris*, *M. olympus* and *Marmota himalayana* have size partial disparities more than two standard errors larger than the average (7.7%). *Marmota vancouverensis* SD of size (10.3 ± 2.1 mm) is very close to the average (9.9 mm). Measures of size variation have a relatively larger SE compared with shape but none of them is significantly ($P > 0.05$) correlated with sample size.

Neither PD nor SD of size are significantly ($P > 0.05$) correlated with, respectively, PD and variance of shape.

Partial disparities of shape and size also were compared between *M. vancouverensis* and other representatives of the *M. caligata* species complex (see Table 4). Seven of eight subspecies of *M. caligata* described by Hall (1981) are represented in our sample. *Marmota caligata sheldoni* was excluded because only one specimen was available. *Marmota vancouverensis* and *M. olympus* are the most divergent species for shape. Their partial disparities are, respectively, five and three standard errors larger than the average (12.5%). The average within population shape variance is 0.001290. *Marmota vancouverensis* and *M. olympus* have the smallest shape variance (about four standard errors smaller than the average). Standard errors of partial disparities are highly negatively correlated with sample size ($r = -0.955$, $P = 0.0002$), whereas those of variances are not ($r = -0.662$, $P > 0.05$). PD of size is small in most representatives of the *M. caligata* species complex and *M. olympus* and *M. caligata vigilis* with their, respectively, large and small crania, contribute to most of the disparity. The SD of size does not show large differences to the average of all populations (8.9 mm). The SD of size has a significant negative correlation with sample size ($r = -0.735$, $P = 0.038$).

Table 3 Partial disparity (PD) expressed as percentage, shape variance (VAR) in unit of Procrustes distance and standard deviation (SD) of size (in mm) for marmot species.

		Shape		Size	
Species	*N*	PD ± SE	VAR ± SE	PD ± SE	SD ± SE
bai	15	4.4 ± 0.9	0.002251 ± 0.000174	1.3 ± 1.1	8.2 ± 1.5
bro	16	5.4 ± 0.5	0.001466 ± 0.000093	1.7 ± 1.1	9.2 ± 1.1
cal	52	4.6 ± 0.3	0.001567 ± 0.000052	2.9 ± 0.9	9.5 ± 0.9
cam	5	7.4 ± 1.3	0.001932 ± 0.000251	1.6 ± 3.2	17.4 ± 3.6
cau	32	8.1 ± 0.7	0.001646 ± 0.000081	7.2 ± 1.7	10.7 ± 1.1
fla	122	8.1 ± 0.4	0.001543 ± 0.000037	16.9 ± 2.8	10.1 ± 0.8
him	28	7.5 ± 0.6	0.001640 ± 0.000077	14.2 ± 2.3	8.7 ± 1.2
mar	15	9.5 ± 0.7	0.001406 ± 0.000108	0.4 ± 0.4	7.0 ± 1.2
men	2	10.9 ± 1.5	0.001135 ± -	19.2 ± 6.6	8.6 ± -
mon	58	12.7 ± 0.6	0.001813 ± 0.000071	12.2 ± 2.8	12.3 ± 1.2
oly	14	7.0 ± 0.6	0.001027 ± 0.000062	19.3 ± 2.7	7.2 ± 1.0
sib	12	4.7 ± 0.6	0.001618 ± 0.000123	1.3 ± 1.1	10.0 ± 1.9
van	7	9.8 ± 0.8	0.000918 ± 0.000088	1.7 ± 2.1	10.3 ± 2.1

Standard errors were computed with 100 bootstraps.

			Shape		Size	
Species	subspecies	*N*	PD ± SE	VAR ± SE	PD ± SE	SD ± SE
van	–	7	26.5 ± 2.6	0.000918 ± 0.000088	3.7 ± 5.6	10.3 ± 2.1
oly	–	14	16.5 ± 1.3	0.001027 ± 0.000062	43.2 ± 11.0	7.2 ± 1.0
cal	*caligata*	18	6.5 ± 1.0	0.001518 ± 0.000102	6.8 ± 7.1	7.9 ± 1.2
cal	*cascadensis*	7	10.8 ± 2.5	0.001489 ± 0.000133	3.8 ± 5.6	7.0 ± 2.0
cal	*nivaria*	6	8.6 ± 2.7	0.001419 ± 0.000154	1.9 ± 5.2	11.7 ± 4.2
cal	*okanagana*	5	11.2 ± 2.7	0.001173 ± 0.000204	9.9 ± 12.8	11.0 ± 3.2
cal	*oxytona*	10	6.0 ± 1.5	0.001486 ± 0.000126	6.9 ± 6.3	7.6 ± 1.4
cal	*vigilans*	4	13.8 ± 2.8	0.001292 ± 0.000310	23.7 ± 14.1	8.4 ± 2.6

Standard errors were computed with 100 bootstraps.

Table 4 Partial disparity (PD) expressed as percentage, shape variance (VAR) in unit of Procrustes distance and standard deviation (SD) of size (in mm) for the members of *Marmota caligata* species group.

Species	Subspecies	Regression SSQ	Residual SSQ	d.f.	*F*	*P*
cal	–	0.000725	0.001072	1,55	37.172	0.0001
oly	–	0.000159	0.000195	1,19	4.306	0.0518
cal	*caligata*	0.000458	0.000472	1,23	22.295	0.0001
cal	*cascadensis*	0.000204	0.000072	1,12	34.030	0.0009
cal	*nivaria*	0.000130	0.000039	1,11	36.882	0.0008
cal	*okanagana*	0.000016	0.000109	1,10	1.475	0.2709
cal	*oxytona*	0.000291	0.000139	1,15	31.533	0.0002
cal	*vigilans*	0.000022	0.000063	1,9	3.181	0.1132

The shape variances being tested are presented in the first part of Table 4.

Table 5 Levene's test for differences in shape variance of *Marmota vancouverensis* and other members of the *Marmota caligata* species complex (significance of *F*-test tested using 10 000 random permutations).

Results of permutation tests for differences in shape variance between *M. vancouverensis* and other members of the *M. caligata* species complex are shown in Table 5. *Marmota caligata* shape variance is significantly larger than that of *M. vancouverensis*, whereas differences between *M. olympus* and *M. vancouverensis* are not significant. That the significantly larger variance of *M. caligata* does not simply depend on differences in sample size is suggested by significant results of permutation tests on random subsamples of *M. caligata* of the same size as that of *M. vancouverensis* (results not shown). Subspecies of *M. caligata* also tend to have shape variances larger than those of *M. vancouverensis* and only comparisons with the smallest samples are not significant.

Permutation tests for differences in magnitude of size variance in the *M. caligata* species complex are not significant ($P > 0.05$ in all pairwise comparisons).

Ratios of shape and size distances to Kimura 2-parameter genetic distances for the mitochondrial cytochrome *b* third codon positions are computed to put shape differences on the scale of neutral genetic divergence. The distributions of the ratios are shown in Fig. 4 using histograms. *Marmota vancouverensis–M. caligata* size-to-neutral DNA distance ratio is within the range of values observed for other species pairs (see Fig. 4b, black bar). By contrast, *M. vancouverensis–M. caligata* shape-to-neutral DNA distance ratio is a strong outlier (see Fig. 4a, black bar), being about three times larger than the largest ratios observed among other species.

These results indicate that divergence in shape of *M. vancouverensis* relative to *M. caligata* is larger than expected from a subsample of within-species variation, either a methodological subsample or a natural 'founder' subsample (Fig. 5). The Procrustes shape distance between means of *M. vancouverensis* and *M. caligata* is an outlier ($P = 0.001$) in the distribution of mean shape distances between random subsamples of *M. flaviventris* and its species mean. The Procrustes shape distance between means of *M. vancouverensis* and *M. caligata* is also larger than those between means of real subspecies of either *M. caligata* itself or *M. flaviventris* (results not shown).

Discussion

Our first finding, a strong divergence in the cranial shape of *M. vancouverensis*, is consistent with previous studies including all species and using geometric morphometrics (Cardini, 2003; Polly, 2003; Cardini *et al.*, 2005; Cardini & O'Higgins, 2005). This unexpected divergence in a very young species was not discovered by Hoffmann *et al.* (1979) in a traditional morphometric analysis of amphiberigian marmots based on linear size measurements. The large divergence in *M. vancouverensis* shape is not paralleled by a comparable divergence in size, which suggests that size and allometry have not played a significant role in the cranial evolution of this species. This is likely to explain why the morphological distinctiveness of *M. vancouverensis* was not discovered using traditional morphometrics, which do not efficiently

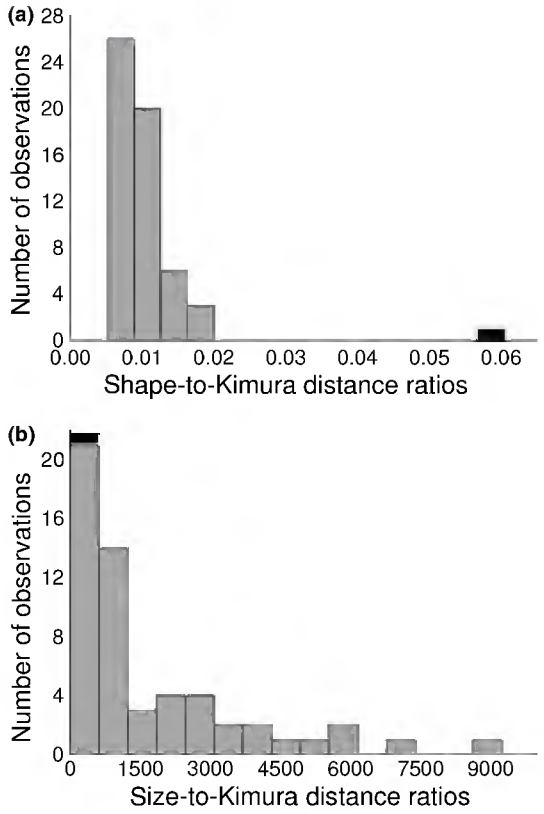

Fig. 4 Histograms showing proportions of shape- (a) or size- (b) to-neutral DNA distance ratios. Distance are computed pairwise among all marmot species except *Marmota vancouverensis*. A black bar shows the interval corresponding to the *M. vancouverensis*–*Marmota caligata* ratio.

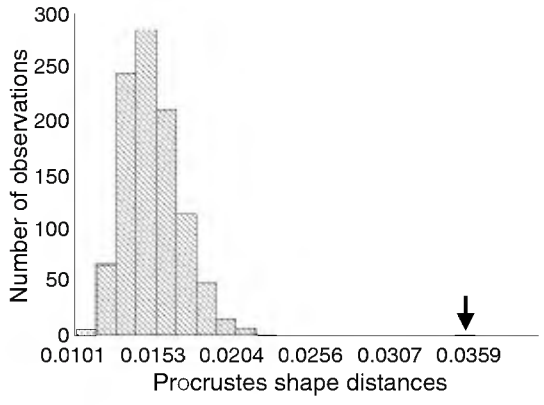

Fig. 5 Histogram showing proportions of Procrustes shape distances of means of *Marmota flaviventris* random subsamples (same size as the sample of *Marmota vancouverensis*) to means of all other *M. flaviventris* specimens. The distance between mean shapes of *M. vancouverensis* and *Marmota caligata* (arrow) is an outlier relative to the range of within-species distances in the random subsamples of *M. flaviventris*.

separate size and shape. A modest divergence in size of an insular vertebrate is, however, in contrast to expectations based on the 'island rule' (Lomolino, 2005), i.e. a graded trend on islands from gigantism in the smaller species to dwarfism in the larger species. Natural selection for changes in size of island species depends on a variety of factors, which often include ecological release (from predation, parasitism and interspecific competition) and resource limitation mediated by relatively high densities of conspecifics and intense intraspecific competition. Cut marks on subfossil bones of *M. vancouverensis* found in caves on the Vancouver Island indicate that this species was hunted by indigenous populations (Nagorsen *et al.*, 1996). Hunting and, more recently, habitat destruction by human activities are likely to have kept the density of marmots low on the island. Thus, none of the main factors thought to be involved in the selection of smaller (or larger) sizes on islands may have had a major role in the evolution of *M. vancouverensis*. Nevertheless, Lomolino (2005) argued that 'measurements of skulls... while... correlated with body size (mass), ...are features that, to different degrees, also reflect differences in shape, in diet, and in other more labile characteristics of insular populations adapting to a diversity of insular environments'. If this holds, it may explain the discrepancy between estimates of size based on crania (present study) and mandibles (Cardini, 2003) and those of body mass and body length (Armitage, 1999), with the former suggesting negligible differences in size between *M. vancouverensis* and *M. caligata*, and the latter showing *M. vancouverensis* as remarkably smaller compared with its sister species on the continent, consistently with the 'island rule'.

For shape, *M. vancouverensis* is about as divergent as *M. menzibieri*, whose mean is based on a very small sample ($N = 2$), and only slightly less divergent than *M. monax*, which has a much longer independent evolutionary history (basal branch of the subgenus *Marmota*; Steppan *et al.*, 1999) and which has an unusual behavioural ecology (it is the only marmot species found also in forests, the only solitary species and the only one which is sexually mature as a yearling; Barash, 1989; Armitage, 2003). *Marmota vancouverensis* is also the most divergent lineage in the *M. caligata* species complex and this cannot be explained by sampling error only, given that subspecies of *M. caligata* with sample size similar to *M. vancouverensis* have partial disparities much smaller (about half) than the latter. That sampling error alone cannot account for the large shape difference between *M. vancouverensis* and *M. caligata* is also suggested by this difference being an outlier in both the distribution of shape distances in the *M. flaviventris* 'founder population' randomization experiment and in that of mean subspecies shape distances within *M. caligata* and *M. flaviventris*. These results are in remarkable contrast with the genetics. Based on molecular markers (Steppan *et al.*, 1999), *M. vancouverensis* could be considered a subspecies of

M. caligata. Karyotypic analyses too (Hoffmann *et al.*, 1979) failed to show any appreciable difference between the two species. Thus, our first conclusion is that the Vancouver Island marmot is a good morphospecies, whose uniqueness does not simply concern the pelage colour and alarm calls, but also hard tissues like the cranium and mandible (Cardini, 2003). Analyses of more genetic markers and morphological studies on larger samples are highly desirable to confirm this apparent discrepancy between rates of phenotypic and genotypic evolution. Faunal remains of Vancouver Island marmots discovered in high elevation cave sites in the Vancouver Island (Nagorsen *et al.*, 1996) may help to determine whether highly divergent traits evolved recently or, by contrast, have a longer history. Radiocarbon dating gives dates of 830–2630 years ago for this material. Several well-preserved skulls found in the caves seem to show at least one of the 'diagnostic traits' of the modern *M. vancouverensis* population, the V-shaped notch at the posterior border of the nasal bones (D.W. Nagorsen, personal communication). If this observation is confirmed by quantitative analyses, it is very likely that at least some of the distinctive characters of *M. vancouverensis* may have evolved at least one millennium ago. Our findings about the magnitude and time of the acceleration in the evolution of form of the Vancouver Island marmot are in very good agreement with a recent study by Millien (2006) showing that rates of morphological evolution over timescales from a few decades up to several thousands of years are up to three times bigger for islands than for mainland mammal populations over timescale.

Our second question concerns the relative amount of form variation in the present population of *M. vancouverensis*. Again, the most interesting outcome came from the analysis of shape. Shape variance is smaller in *M. vancouverensis* than in any other species. Compared with other populations of the *M. caligata* species complex, *M. vancouverensis* shape variance is significantly smaller or comparable with that found within subspecies of *M. caligata*. A potential bias exists that may have led to an underestimate of the shape variance of *M. vancouverensis*. Six of seven specimens available for this study are from the same region (Mount Douglas/King Solomon basin/Golden Eagle Basin) and were collected in the same year (1910). Three considerations, however, suggest that this underestimate may not be very large: (1) the shape distance of the specimen collected 58 years later on Mount Washington to the other six specimens is on average only 5.9% larger than the average shape distance between any of those six; (2) *M. caligata nivaria* has a shape variance much larger (almost 50% larger) than *M. vancouverensis* despite four of six specimens from the same locality and year (Montana, Upper St. Marys Lake, 1895); (3) by contrast, *M. olympus*, a peripheral isolate like *M. vancouverensis*, has a shape variance almost identical to that of the Vancouver Island marmot despite a much larger ($N = 14$) and heterogeneous sample with specimens collected in several localities over at least 30 years. Thus, our second conclusion is that a strong reduction in phenotypic variance has occurred in the history of the Vancouver Island marmot and this is consistent with the small genetic variability found in this population by Kruckenhauser *et al.* (1999) using microsatellites and Frankham's (1997) observation that a highly significant majority of island populations have less allozyme genetic variation than their mainland counterparts with the proportionate reduction in genetic variation greater in island endemic than in nonendemic island populations. The occurrence of at least one recent genetic bottleneck seems thus to be supported by studies of cranial and genetic variation.

Answers to the first two questions in our study are consistent with at least some aspects of speciation models driven by genetic drift. Mayr's (1963) 'genetic revolution', or peripatric speciation, implies: (1) a founding event with a strong reduction in population size; (2) a large drop in the amount of genetic variation due to the initial population bottleneck; (3) the disruption of the old coadapted gene complexes as a consequence of the increased homozygosity. As a result of this 'genetic revolution', the isolated population, free of previous epistatic constraints, can move to a new adaptive peak and evolve new coadapted gene complexes. In Mayr's view, this would explain the morphological divergence between insular species and populations on the mainland in the absence of strong ecological dissimilarities. The first point of Mayr's model fits well the current demographic situation of the Vancouver Island marmot, with little more than 100 individuals surviving in the wild. Genetic bottlenecks might have occurred after the sea level rise separated the island from the mainland either from natural causes or induced by humans. The predominance of Vancouver Island marmot bones in caves with faunal and human remains suggests that aboriginal peoples travelled to the remote mountainous areas inhabited by the marmots to hunt them. Marmots are an easy prey for humans and intensive hunting might have contributed to limit the population size. The second assumption of Mayr's model, a drop in genetic variation, receives some support by our finding of reduced shape variance of the cranium and by Kruckenhauser *et al.* (1999) microsatellite analysis, but neither our results nor those of Kruckenhauser *et al.* say much about the magnitude of this reduction. Whether new coadapted gene complexes have evolved in *M. vancouverensis*, the third assumption, is very hard or impossible to judge. Also the combination of large morphological divergence and minimal ecological differentiation, which inspired Mayr's model, is difficult to ascertain in the Vancouver Island marmot. The divergence in both external and internal morphology is evident and supported by all recent studies, including the present one. However, we cannot make strong claims about the extent of the

ecological differentiation of *M. vancouverensis* and *M. caligata*. The two species have a similar social structure with restricted family groups (Armitage, 2003). However, the habitats of the two species have several differences, with *M. vancouverensis* mostly found in clearcuts or grass-forb alpine meadows at elevations between 1000 and 1400 m, and *M. caligata* typically occupying rock ledges and talus slopes close to subalpine meadows or relatively flat meadows with short mesophytic grassland vegetation above the timberline (Armitage, 2003).

Clues about the prominence of founder effects and genetic drift in the evolution of *M. vancouverensis* are provided by the answer to our third question about the proportion of morphological to neutral genetic differentiation. When shape and size distances are scaled by the amount of neutral genetic divergence (i.e. they are divided by the amount of difference in third codon positions of the cytochrome *b*), the differences in shape between *M. vancouverensis* and *M. caligata* are three times larger than the largest pairwise distances between any other species, making the amount of morphological differentiation much greater than would be expected if it had evolved neutrally. Also, on average, all ratios between shape distances and neutral genetic distances involving *M. vancouverensis* are significantly larger than those of all other species. Size divergence of *M. vancouverensis–M. caligata*, by contrast, is within the range of variation observed for other species pairs. Thus, a model of form divergence of the Vancouver Island marmot driven exclusively by genetic drift cannot be rejected for size, but it is rejected for shape. This implies that, even if genetic bottlenecks occurred which reduced genetic variation and contributed to modify the genetic background on which selection acts, neutral divergence alone cannot explain the magnitude of shape changes in the cranium of *M. vancouverensis*. Selection must be at least partly responsible for the differences in cranial form associated with the origin of *M. vancouverensis*. Again, our findings are consistent with Millien's (2006) observation that the peculiar ecological environment on islands favours faster evolution in mammals, and it is not just the consequence of a founder event immediately after isolation from the mainland.

Before discussing the implications of this finding, two points need to be clarified. First, in the comparison of the shape distance of *M. vancouverensis* to *M. caligata* with all other pairwise interspecific distances, almost all the divergence is implicitly attributed to the Vancouver Island marmot rather than to divergence in *M. caligata*. This assumption is justified by the simple observation that *M. vancouverensis* contribution to the disparity of *Marmota* is more than two times larger than the contribution of *M. caligata*. Thus, there is an asymmetry in their evolutionary divergence and *M. vancouverensis* represents the rapidly evolving lineage. The second point concerns our assumption that third codon positions (synonymous sites) have no effect on the fitness of an organism and become fixed by drift (neutral evolution). This assumption has recently been challenged (Chamaray *et al.*, 2006) based on evidence that indicates that even in mammals some synonymous mutations may be subject to constraint because they affect splicing and/or mRNA stability. The strength of natural selection on synonymous mutations is, however, yet to be clarified, as well as the proportion of third codon positions which do not evolve neutrally. Chamaray *et al.* (2006) present convincing evidence that an assumption that all synonymous mutations are neutral no longer seems safe. However, they also acknowledge that '...it remains highly probable that most mutations are neutral' (p. 103). Thus, the error introduced by using differences in third codon positions to estimate the rate of neutral evolution in a clade of closely related species is likely to be negligible.

It is worth noting that a population bottleneck could affect rates of both phenotypic and genetic divergence associated with the origin of *M. vancouverensis*. Our conclusion that phenotypic selection played a part in the origin of *M. vancouverensis* is based on the ratio of these two variables. If a bottleneck affected either type of divergence, its effect would have been to increase the rate of phenotypic change, selective or neutral, and to increase the rate at which mutations are fixed in the population, both due to smaller population size (Kimura, 1983). We have shown that the phenotypic change in *M. vancouverensis* is larger than expected given reduced population and/or sample size with our bootstrap analysis. We have not tested whether genetic distance between *M. vancouverensis* and *M. caligata* is greater due to higher fixation, but our conclusions are conservative with respect to this possibility: if fixation was increased in *M. vancouverensis* due to bottlenecking then our measure of genetic differentiation is an overestimate of the time since divergence, which would make the ratio of phenotypic to genetic divergence smaller than it really was. Because we found that phenotypic divergence was larger than expected due to chance sampling and because it was also larger than expected given even a potentially inflated genetic distance, our conclusion that selection must have played a role in the origin of *M. vancouverensis* remains justifiable in the face of bottlenecking effects.

It is also worth noting that our measure of phenotypic evolution does not explicitly differentiate between genetic and nongenetic components. The phenotype can be partitioned into $\boldsymbol{P} = \boldsymbol{G} + \boldsymbol{E}$, where P symbolizes phenotypic variance, G symbolizes heritable genetic variance and E symbolizes nonheritable variance attributable to various environmental effects (Falconer & Mackay, 1996). Evolutionary change due to selection and drift is associated only with the genetic portion, but phenotypic differences between two species can also be due to systematic differences in their environments. The differences found in *M. vancouverensis* are unlikely to be due exclusively to differences in the environment of Vancouver Island. Our null measure of phenotypic variance

in other species is based on samples taken from environments across the West of North America that vary much more than does the environment of Vancouver Island from the rest of the coastal British Columbia, Washington or Oregon. Thus, environmental variance would inflate our null distribution of phenotypic variance among marmots than it would the specific difference between *M. vancouverensis* and *M. caligata*, both of which live in these same coastal environments. Our conclusion that selection played a role in the origin of *M. vancouverensis* is also conservative in light of the above equation. Change in phenotype can be expressed as $\Delta\bar{z} = \beta G$, where β is a vector of selection coefficients and G is the genetic component of variance (Lande, 1979). Our study measured the change in phenotype with the goal of determining whether selection was important without knowing what portion of P was genetic. Examination of Lande's equation shows that for any measured change in phenotype, selection must be higher to achieve that change when G is a small proportion of P than when it is a high proportion. Conversely, selection would have been smaller for any measured change in phenotype if the proportion of G were higher. Our conclusion that selection contributed to the origin of *M. vancouverensis* would still be justified even if the genetic component of variance in marmot skull shape were 100% of the phenotypic variance, which it almost certainly is not. For any lower proportion of G, selection would have been even more intense than we have assumed (see Wójcik *et al.*, 2006 for quantitative assessments of the effects of different heritabilities on studies that measure only the phenotype).

Then, what does an observation of large differences in shape relative to neutral genetic differences tell us about the evolutionary divergence of *M. vancouverensis*? This finding rules out a model of speciation/divergence which is simply neutral. However, it does not provide conclusive evidence against peak shift models of speciation by drift (Mayr's 'genetic revolutions' and its 'variants'), which assume that drift overcomes selection and acts as a trigger to accelerate divergence thus promoting a shift to a new adaptive peak. This is because speciation models which explain divergence as a consequence of either a drop in genetic variation which breaks the evolutionary inertia or new strong selective pressures on the founder populations are both compatible with accelerated evolution.

Peak shift models of speciation have been severely criticized (Coyne & Orr, 2004). The main criticisms are that founder effects are unnecessary to explain radiations on islands, which may be simply be the result of strong natural selection on allopatric populations in a novel habitat and that the reduction in genetic variation after a founder event is actually modest; if peak shifts do occur, according to these authors, they tend to be too small to cause reproductive isolation. In the light of these criticisms, a parsimonious explanation for the observed pattern of evolutionary divergence in the Vancouver Island marmot could be that, even if a strong founder effect is unlikely, the reduction in genetic variance following one or more population bottlenecks might has significantly contributed to modify the frequency of the alleles which were the target of natural selection. Environmental changes after the glaciation and more recently because of anthropic activities (for instance, hunting and forest industry) might have put strong selective pressures on the small population of marmots inhabiting the Vancouver Island. Thus, a strong natural selection, in a new or rapidly changing habitat, acting on genetic variation reduced by population bottlenecks might explain the observed pattern. Berry (1996, p. 753) suggested that 'Studies of voles on Orkney, long-tailed field mice on the Hebrides and Shetland, and house mice on the Faroe archipelago the main factor in differentiating island races from their mainland ancestors is the chance genetic composition of the founding animals. Subsequent change has necessarily to be based on the genes and frequencies carried by this colonizing group. Probably most post-colonization changes are adaptive, although possibly limited in extent both by the initial paucity of variation and by the conservative effect of intragenomic interactions'.

Beside the implications for the study of models of population divergence and speciation, the story of the Vancouver Island marmot can be read also from a rather different perspective. Species with a large phenotypic divergence but small genetic differentiation (measured using a common genetic marker like the mitochondrial cytochrome *b*) can be seen as the 'other side of coin' of the cryptic species concept. Cryptic, or sibling, species are reproductively isolated natural populations that are morphologically similar or identical (Mayr, 1942, 1963) but genetically distinct, whereas the Vancouver marmot is genetically similar to its sibling species, but morphologically distinct. Although this definition of cryptic species oversimplifies the issue (for instance, not mentioning competing species definitions), it helps to emphasize the general perception that DNA sequences can tell us more about the evolutionary divergence of organisms. DNA barcoding, for instance, has been shown to be capable of identifying cryptic species (Hebert *et al.*, 2004) and it has even been suggested that 'A COI [mitochondrial cytochrome *c* oxidase] based identification system will undoubtedly provide taxonomic resolution that exceeds that which can be achieved through morphological studies' (Hebert *et al.*, 2003). DNA barcoding and, more generally, the comparison of DNA sequences can indeed provide powerful tools to discriminate species and discover hidden taxonomic diversity in need of protection (Haig, 1998; Savolainen *et al.*, 2005).

The usefulness of genetic analyses in this context is indisputable, but problems may also arise if a limited amount of knowledge from a single disciplinary sector is used as the only taxonomic criterion for setting priorities.

For instance, Rubinoff (2006) emphasized that adopting the 'bar code' as a universal mechanism of identifying species concept may fail to recognize subsets of diversity because of gene lineage sorting problems, thus erroneously focussing conservation efforts on populations with minor genetic divergences. Also, despite his enthusiasm on the multiplicity of applications of DNA barcodes, Marshall (2005) warned that very young species may not be easily distinguished by bar coding. Orangutans, for instance, may be hardly identified using COI bar code. Thus, the discrepancy observed in *M. vancouverensis* between morphology and genetics may well represent a similar instance of failure to recognize a very distinctive population in a different group of mammals using a common genetic marker (cytochrome *b*). Had it not been melanistic, the Vancouver Island marmot would have probably been considered an isolated population of *M. caligata* with minor behavioural differences. Studies of bone and tooth morphology using modern quantitative methods strongly suggest that the evolutionary divergence of the Vancouver Island marmot actually concerns numerous and complex traits besides fur colour and a component of its alarm calls. Thus, detailed morphological studies as well as behavioural and genetic analyses may be needed to fully appreciate biological diversity.

Acknowledgments

We are extremely grateful to all museum curators and collection managers who allowed and helped us to study their collections. Special thanks also to K.B. Armitage, for his always helpful advice on aspects of marmot behaviour and ecology, to D.W. Nagorsen for sharing with us his knowledge on the Vancouver Island marmot subfossils, to A. Bryant for lending us his very beautiful pictures of *M. vancouverensis*. Finally, we would like to thank two anonymous referees for their helpful comments and suggestions, which greatly contributed to improve the manuscript.

Part of this work was done when A. Cardini was a Smithsonian Fellow at the National Museum of Natural History, Washington, DC.

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Received 27 February 2007; revised 23 April 2007; accepted 24 April 2007