Accepted on 29 June 2008
J Zool Syst Evol Res doi: 10.1111/j.1439-0469.2008.00503.x

[1]*Mammalia Biological Consulting, Victoria, BC, Canada;* [2]*Dipartimento del Museo di Paleobiologia e dell'Orto Botanico, Universitá di Modena e Reggio Emilia, Modena, Italy*

Tempo and mode of evolutionary divergence in modern and Holocene Vancouver Island marmots (*Marmota vancouverensis*) (Mammalia, Rodentia)

D. W. Nagorsen[1] and A. Cardini[2]

Abstract

Marmota vancouverensis is the only insular species among the 14 species of marmots. The evolutionary history of this species is unresolved. Although *M. vancouverensis* is strongly differentiated in osteological and other morphological characters, its low genetic divergence suggests recent evolution from an ancestral continental species. We used geometric morphometric techniques to assess the morphology of hemimandibles from 239 modern *M. vancouverensis*, *Marmota caligata*, *Marmota flaviventris*, *Marmota olympus* and 30 Holocene (9435–735 cal. yr bp) subfossil *M. vancouverensis*. Our results confirm that the mandible of *M. vancouverensis* is strongly differentiated in shape from continental marmot species, but similar in size to its mainland sister species *M. caligata*. Temporal variation in size and shape over the past 2500 years among allochronic samples of *M. vancouverensis* was minimal suggesting that the morphological divergence of this species occurred in a period of rapid change following its isolation from mainland populations in the late Pleistocene. Selection pressures associated with environmental changes and founder effects and genetic drift resulting from population bottlenecks created by population declines and habitat fragmentation are hypothesized as factors contributing to the morphological divergence of this species.

Key words: Marmot evolution – mandible – geometric morphometrics – island evolution – climate changes

Introduction

Insular mammals have long intrigued evolutionary biologists because they often undergo rapid evolutionary changes in body size and other morphologies (Millien 2006). Endemic to Vancouver Island off the northwest coast of North America, *Marmota vancouverensis* is the only insular species among the 14 species recognized in the genus *Marmota*. Phylogenetic studies (Kruckenhauser et al. 1999a; Steppan et al. 1999) based on mtDNA revealed that *M. vancouverensis* is a member of the Petromarmota group with only minor divergence in DNA sequences from the mainland species *Marmota olympus* and *Marmota caligata*. In contrast to its low genetic divergence, this species differs in pelage colour, vocalizations and behaviour from continental species (Hoffmann et al. 1979; Nagorsen 1987; Barash 1989). The *M. vancouverensis* skull and mandible is also peculiar with some morphological traits atypical of the genus (Cardini 2003; Cardini and O'Higgins 2004; Cardini et al. 2005, 2007).

The evolutionary history of *M. vancouverensis* is unresolved. It is generally considered to be a sister species of *M. caligata* (Hoffmann et al. 1979; Steppan et al. 1999). Hoffmann et al. (1979) and Hoffmann (1981) interpreted *M. vancouverensis* as a Pleistocene isolate that evolved in a coastal refugium. The oldest marmot fossils from Vancouver Island are 20 685–19 250 cal. yr bp post-cranial remains (species indeterminate, compare with *M. caligata*, *M. vancouverensis* or *M. olympus*) excavated from a sea cave (Al-Suwaidi et al. 2006). They date from a time when the Cordilleran ice sheet was advancing over the island and the cave was subsequently covered by glacial ice ~16 000 years ago. Although McCabe and Cowan (1945) speculated that *M. vancouverensis* persisted in the mountains of Vancouver Island during the last glaciation, there are no fossils to support their hypothesis and the scenario seems inconsistent with the glacial history of the island. During the last glacial maximum ~15 000 years ago, ice reached >1300 m thick with ice-free areas limited to nunataks in the Vancouver Island Ranges and a 7 km^2 ridge on the Brooks Peninsula (Clague 1981; Howes 1981, 1997). It is unlikely that marmots could have persisted in these small and highly fragmented refugial areas for 1500 years. A more plausible scenario (Nagorsen 2005) is that *M. vancouverensis* is a more recent form that evolved during the past 13 000–12 000 years after colonizing Vancouver Island following the last glaciation. If the ancestral populations of this marmot were only isolated only during the brief post-glacial period, then the morphological evolution of this insular species appears to have been more rapid than that of continental *Marmota* species.

Three evolutionary scenarios could account for the rapid morphological divergence of *M. vancouverensis* (Fig. 1). Differentiation was initially slow after isolation from continental populations followed by rapid changes in the recent past. With this scenario, ancient and modern *M. vancouverensis* would show major differences and ancient *M. vancouverensis* should resemble the mainland ancestral form. Alternatively, evolution was rapid after the initial isolation followed by a slow rate of change in the recent past. Thus, ancient populations would be strongly divergent from continental marmots but with only minor differences from modern *M. vancouverensis*. A third scenario is that there was a rapid and constant rate of morphological evolution with differences between modern and ancient populations of *M. vancouverensis* and other marmot species proportional to divergence time.

Genetic drift resulting from founder effects and population bottlenecks (Cardini 2003; Cardini and O'Higgins 2004; Cardini et al. 2005) or strong selective pressures (Cardini et al. 2007) have contributed to the morphological differentiation of *M. vancouverensis*. A range collapse that began in the late Pleistocene or early Holocene (Nagorsen et al. 1996) and major population declines in the past 50 years (Bryant and Janz 1996) would have created bottlenecks and the potential for genetic drift (see Table S1). However, selection pressures associated with long-term climatic changes (Hebda 1995) or

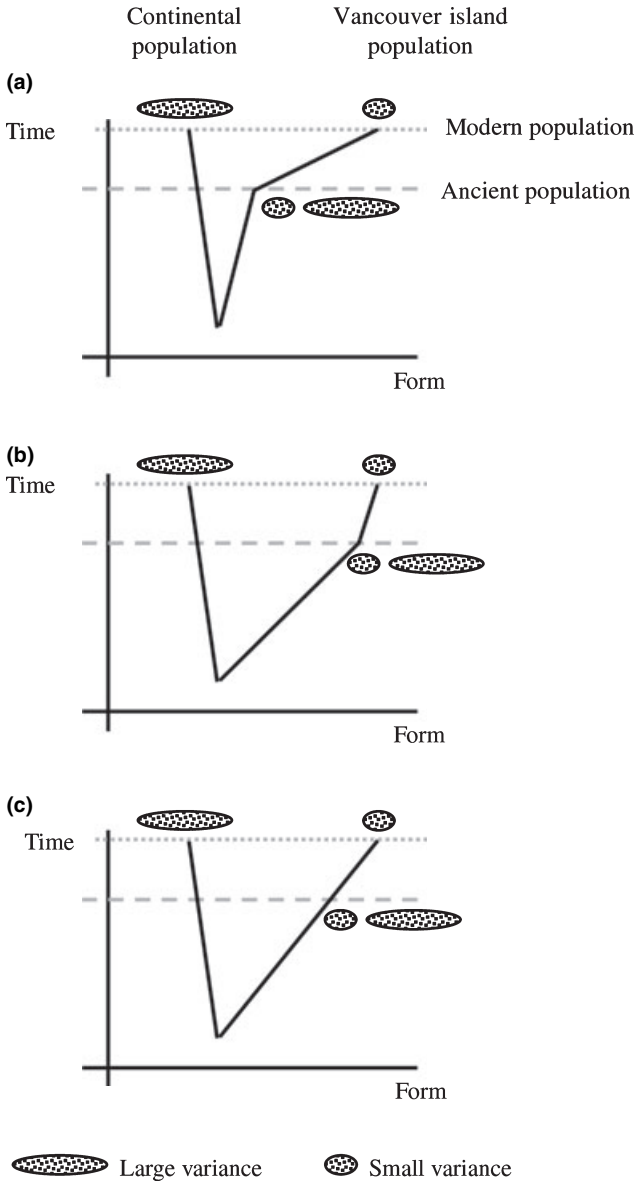

Fig. 1. Three evolutionary scenarios for the morphological divergence of *Marmota vancouverensis*. (a) slow morphological evolution after isolation from continental populations followed by rapid evolution in the recent past; (b) rapid evolution after isolation followed by little change in the recent past; (c) rapid and constant rate of morphological evolution. Plots assume that modern *M. vancouverensis* populations have a highly distinctive morphology and show reduced morphological variance relative to mainland marmots

rapid faunal turnover in the early post-glacial (Nagorsen and Keddie 2000) may have also contributed to its evolution. Because previous morphological studies of *M. vancouverensis* (Hoffmann et al. 1979; Cardini 2003; Cardini and O'Higgins 2004; Cardini et al. 2005) were based on small samples sizes, we cannot rule out sampling error as an explanation for the distinctive morphology of this species.

The availability of additional modern *M. vancouverensis* mandibles increasing the sample size by three fold from previous analyses and samples of Holocene subfossil mandibles of *M. vancouverensis* recovered from caves prompted us to re-examine the morphology of this species. Our study addresses four questions: (1) do modern and ancient populations of *M. vancouverensis* share the same morphology including the atypical mandibular traits described by Cardini (2003), (2) do Holocene and modern populations of *M. vancouverensis* differ from modern continental populations of *Marmota*, (3) what are the similarity relationships between allochronic populations of *M. vancouverensis* and modern marmot species on the mainland and (4) are the patterns of variation among modern and Holocene *M. vancouverensis* consistent with population bottlenecks?

Materials and Methods

Samples and data collection

The samples comprised 239 mandible specimens of adult modern marmots and 30 subfossil Holocene specimens of *M. vancouverensis* (Table S2; Appendix S1; Fig. 2). Modern samples included museum specimens of four species: *M. vancouverensis* and the continental species *M. caligata*, *M. olympus* and *Marmota flaviventris* the closest living relatives of *M. vancouverensis* (Steppan et al. 1999). Modern specimens of *M. vancouverensis* dated from 1910 to 1992. We selected subspecies of *M. caligata* and *M. flaviventris* with a large number of specimens.

Subfossil mandibles were from marmot faunal remains recovered from five caves on Vancouver Island (Table S2). Limestone Cave is within the modern range of *M. vancouverensis* only 3 km from the type locality (Swarth 1911), the location of eight of our modern specimens. The other cave sites are peripheral to the modern range. Clayoquot, Limestone and Mariner caves were archaeological sites where marmot remains were the result of aboriginal hunters; Mausoleum and Pellucidar caves were natural trap sites (see Nagorsen et al. 1996). Radiocarbon dates obtained from marmot bones were: Clayoquot Cave, 2757–2630 cal.yr bp ($n = 2$); Limestone Cave, 960–935 cal. yr bp ($n = 2$); Mariner Cave, 923–739 cal. yr bp ($n = 6$); Mausolelum Cave, 1240 cal. yr bp ($n = 1$); Pellucidar Cave, 9435 cal. yr bp ($n = 1$).

Only specimens with permanent dentition and fully erupted teeth were included in the analyses. No information is available on tooth eruption in species of the Petromarmota group. However, Mashkin and Kolesnikov (1990) reported that the deciduous premolars of the Old World species *Marmota bobac*, *Marmota babacina* and *Marmota menziberi* were replaced by permanent dentition before animals entered their first hibernation. Mandibles with permanent dentition but immature shape features (Cardini and Tongiorgi 2003; Cardini and O'Higgins 2005), small size and no dental wear were excluded.

Images were captured using a flatbed scanner at a resolution of 300 dpi. Mandibles were laid horizontally on the labial side. Nine anatomical landmarks used by Cardini (2003), Cardini and Tongiorgi (2003) and Cardini and O'Higgins (2005) were digitized on the hemimandible labial side (Fig. 3) using TPSDIG (Rohlf 2006a): (1) upper extreme anterior part of the incisor alveolus, (2) anterior top of the mandibular symphysis, (3) anterior extremity of the maxillary toothrow (premolar alveolus), (4) intersection of the dental ridge with the dorsal portion of the masseteric ridge (base of the coronoid process), (5) tip of the coronoid process, (6) and (7) anterior and posterior tip of the condyle (8) posterior extremity of the angular process and (9) mental foramen.

Digitizing error of landmarks used in this study was determined by Cardini and Tongiorgi (2003) to be negligible. A two dimensional approach to the study of three-dimensional structures allows an effective visualization of shape differences using transformation grids. Any loss of information by this approach is minimal for a nearly flat object, as a marmot mandible (Velhagen and Roth 1997; Cardini and Tongiorgi 2003).

Geometric morphometrics

Analyses were performed using methods for the comparison of the geometric form of organisms and their organs (Rohlf and Marcus 1993; Adams et al. 2004; Zelditch et al. 2004). Specimens were described using Cartesian coordinates of anatomical landmarks. Configurations were optimally superimposed using generalized Procrustes analysis (Rohlf and Slice 1990) to obtain a matrix of shape

 doi: 10.1111/j.1439-0469.2008.00503.x

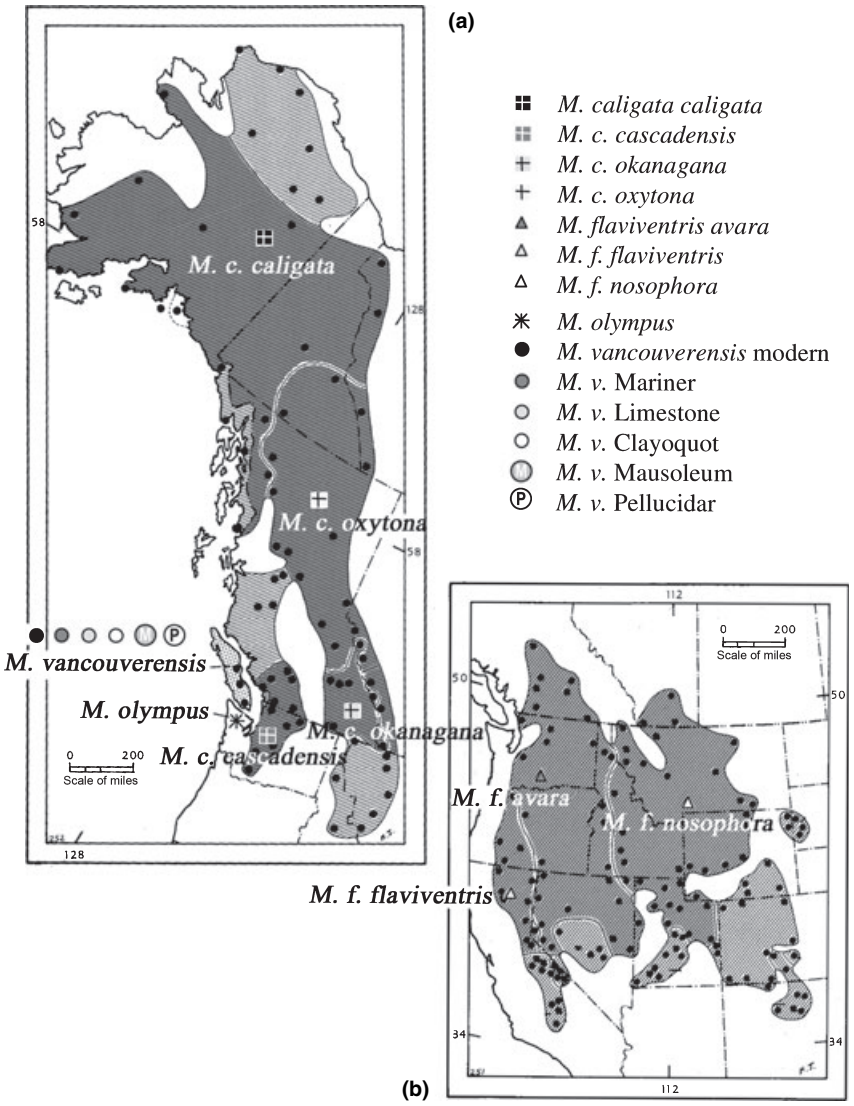

Fig. 2. Geographic distribution of *Petromarmota* species (modified from Hall 1981) with taxa used in the study. Ranges of subspecies of *Marmota caligata* (dark gray) and *Marmota flaviventris* (light gray) used in analysis are emphasized with a shaded background

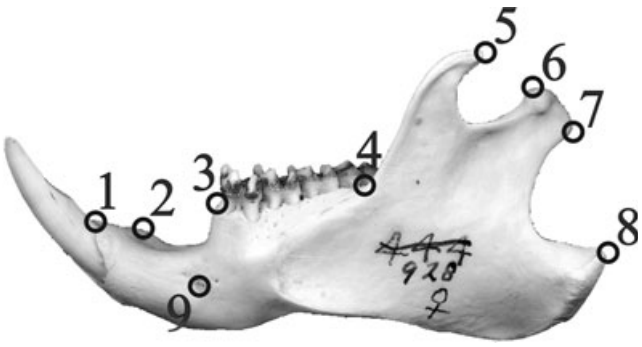

Fig. 3. Landmark configuration on the labial side of the mandible

coordinates. Size was measured as centroid size, the square root of the sum of squared distances between all landmarks and their centroid. The magnitude of shape differences between two configurations was measured by their Procrustes shape distance, the square root of the sum of squared differences between corresponding landmarks of two superimposed landmark configurations. Morphological features were displayed by deformation grids (Thompson 1917; Bookstein 1991) computed using the THIN-PLATE SPLINE (TPS) algorithm that expresses a shape change as the smoothest possible deformation from a reference configuration of landmarks to a target configuration. Geometric morphometric analyses were performed using MORPHEUS (Slice 1999), TPSSMALL 1.20 (Rohlf 2006a), NTSYS-PC 2.2L (Rohlf 2006b) and MORPHOLOGIKA (O'Higgins and Jones 2006).

Statistical analyses

Specimens from Mausoleum and Pellucidar caves were excluded from statistical comparisons of means and variances because they were represented by only one individual. For the other 12 groups, analyses were based left hemimandibles as in previous studies (Cardini 2003; Cardini 2004; Cardini and Tongiorgi 2003; Cardini and O'Higgins 2005). An exception was the three subfossil samples where a few right hemimandbiles were included to increase sample size. Because subfossil mandibles were disarticulated when found in cave deposits, associated left and right dentaries of an individual could not be matched. We tested if left and right hemimandibles belonged to the same individual. We first assessed differences in size and shape between left and right hemimandibles in modern marmot species (Table S3). Permutation tests were performed by randomly swapping left and right configurations for each individual (Klingenberg et al. 2002) and computing differences in means of the corresponding pseudosamples. The p-value of the observed left-right difference was the proportion of differences in means of pseudosamples larger than or equal to the observed. Differences in mean shapes were measured by the Procrustes shape distance between sample means.

To determine if any of the left and right hemimandibles from a cave sample belonged to the same individual, we constructed an empirical distribution of Procrustes shape distances and size differences among left and right hemimandibles with a modern species sample. For both shape and size, 95th percentiles of empirical distributions were computed for the four *Petromarmota* species samples used for the test of left-right differences (sample sizes in Table S3). Matrices of Procrustes shape distances and of absolute differences in centroid size were then computed pairwise between all possible combinations of left and right hemimandibles from a deposit of subfossil *M. vancouverensis*. Finally, we included in the analysis only right hemimandibles which, compared with any left hemimandible from the same deposit, had both a Procrustes shape distance and an absolute difference in size larger than the 95th percentiles from step 1. The largest 95th percentile of shape (0.03140 units of Procrustes shape distance) and size (1.92 mm) differences were used among those from the four empirical distributions of modern species samples. This rather conservative approach was chosen to minimize chances of erroneously including left and right hemimandibles from the same individual.

Differences among sample means were tested with permutation tests (Zelditch et al. 2004). Unlike standard parametric statistics, permutation tests are unaffected by the large number of shape variables relative to sample size. Because type I error rates are inflated if a large number of pairwise tests is performed, a sequential Bonferroni correction for multiple comparisons using Holm's method (Howell 2002) was used. Significant values emphasized in tables were those at the 0.05 level (in italics) and those (undescored italics) which were significant also after correcting for the total number of pairwise comparisons. We used a principal component analysis (PCA) of shape variables to illustrate spatial (similarity) relationships among all individuals in the shape space. Phenetic relationships were summarized also with a cluster analysis on the matrix of mean shape Procrustes distances. Six clustering algorithms were applied to the matrix of Procrustes shape distances (UPGMA, unweighted pair-group method using arithmetic average; UPGMC, unweighted pair-group method using centroid average; WPGMA, weighted pair-group method using arithmetic average; WPGMC, weighted pair-group method using centroid average). The goodness of fit was measured by the coefficient of cophenetic correlation (Rohlf 1970). The UPGMA algorithm was used because it had the largest cophenetic correlation ($r = 0.857$) to the matrix of Procrustes shape distances.

The repeatability of the topologies was estimated by bootstraps (Cole et al. 2002; Caumul and Polly 2005). Thus, we randomly selected with replacement k individuals from each of the original samples (where k is the number of specimens in the sample). Each bootstrapped pseudosample was Procrustes superimposed and a new mean calculated. The resulting bootstrap sample mean shapes were themselves superimposed and the corresponding matrix of Procrustes distances computed. This was repeated 1000 times, thus obtaining 1000 Procrustes distance matrices of bootstrap mean shapes. Eventually, matrices of Procrustes shape distances of bootstrap pseudosamples were used for building trees. The percentage of trees in which each observed node grouping appeared was reported. Nodes with low percentages were strongly affected by sampling. A PCA of sample mean shapes was also performed, and scatterplots of means with 95% bootstrap confidence ellipses (based on bootstrapped mean shapes computed as for the cluster analysis) summarize similarity relationships.

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 1000 times the original samples, computing the variance for each bootstrap pseudosample and calculating the SD of variances. We applied the same bootstrap procedure to estimate standard errors of variance in centroid size. We tested for differences in variance between samples with a series of Levene's tests on the Procrustes distances. Levene's test requires calculating the absolute value of the deviation of each individual from the sample mean (Van Valen 1978). In geometric morphometrics, this is satisfied for shape by using the Procrustes distances to the mean shape and for size by computing the absolute differences to the mean centroid size. These deviations were then compared by an ANOVA. Although this test is generally considered relatively robust to departures from normality, we chose to perform a randomization version that compared the observed F-statistic with the distribution obtained by randomly reassigning deviations from sample means to the samples (Manly 1997). To test mean differences, a sequential Bonferroni correction for multiple comparisons was used for controlling type I error rates. All statistical analyses were performed using NTSYS-PC 2.2L (Rohlf 2006b).

Results

Sexual dimorphism and left-right differences

Sexual dimorphism was shown to be negligible in all previous studies (Cardini 2003; Cardini 2004; Cardini and Tongiorgi 2003; Cardini and O'Higgins 2005). This observation was confirmed in the present study by permutation tests for mean differences which never produced significant results (not shown). Therefore, we pooled sexes in all analyses.

Shapes of left and right hemimandibles differed only for *M. flaviventris* and *M. olympus* (Table S3). The percentage of variance explained by these differences was small for *M. flaviventris* (0.4%) but large (4.0%) for *M. olympus*. This was about three to nine times smaller than the variance explained by interspecific differences between this species and any of the others (13.2–36.3%). The significance of left-right differences of *M. olympus* was mostly related to a single specimen (USNM 67611). Excluding this individual, the permutation test was not significant ($p = 0.053$). Thus, small and generally negligible differences between left and right hemimandibles of modern species did not strongly mitigate against including right hemimandibles in the sample of subfossil *M. vancouverensis*.

For nine out of the available 19 right hemimandibles both shape and size differences to any left hemimandible from the same deposit were larger the 95th percentiles of left-right differences measured in modern species. Therefore, four right hemimandibles from Mariner Cave (catalogue nos: MA 104, MA 121, MA 136 and MA 144), four from Limestone Cave (catalogue nos: LI 40, LI 42, LI 46 and LI 47) and one from Clayoquot Cave (catalogue no.: CL 20) were estimated not to belong to individuals for which a left hemimandible was also available and were included in the analysis.

Differences among modern and Holocene *M. vancouverensis*

Although a sequential Bonferroni correction using all possible pairwise comparisons was used in Table S4 and S5, for the six pairs of comparisons between the three Holocene and one modern *M. vancouverensis* samples a sequential Bonferroni correction for six simultaneous tests was more appropriate. It revealed shape differences between modern and Holocene *M. vancouverensis* populations were significant or very close ($P_{\text{modern/Limestone}} = 0.0133 \approx P_{\text{sequential Bonferroni}} = 0.0125$) to significance, but the three Holocene populations showed no differences (Table S4). Size differences, in contrast, did not differ among the four *M. vancouverensis* samples (Table S5).

Differences among *M. vancouverensis* and continental marmots

To address this issue, only comparisons between populations of *M. vancouverensis* and modern continental marmot populations must be considered. However, significant differences were so large that even with a very conservative Bonferroni corrections for all (66) pairwise comparisons results did not change. Shape differed between *M. vancouverensis* and all mainland samples of *M. caligata*, *M. flaviventris* and

 doi: 10.1111/j.1439-0469.2008.00503.x

M. olympus (Table S4). *Marmota vancouverensis* samples differed in size from *M. flaviventris* and *M. olympus* but not *M. caligata* (Table S5).

Similarity relationships

Scatterplots derived from the PCA of all individuals (Fig. 4) showed large overlap among different marmot samples. But, *M. vancouverensis* specimens generally separated from other species on PC1 and PC3. Also, *M. vancouverensis*, *M. olympus* and *M. caligata* tended to have lower scores on PC2 than *M. flaviventris*. The single specimens from Mausoleum and Pellucidar caves fell within the range of variation of other *M. vancouverensis* samples. Deformation grids for shape changes along PCs revealed that the pronounced differences between *M. vancouverensis* and other marmots mainly involve the coronoid and condylar processes which bend respectively backward and forward in *M. vancouverensis* (Fig. 4a), and the relatively inconspicuous mandibular symphysis 'ridge' in *M. vancouverensis* (Fig. 4c).

The phenogram derived from mean shape correctly clustered population means according to species and these clusters had high repeatability (Fig. 5). The same topology was obtained either including or excluding the single specimens from Mausoleum and Pellucidar caves. The phenogram that included these two specimens (Fig. 5) depicts the branches of Mausoleum and Pellucidar subfossils with dotted lines to emphasize that they were based on real specimens not population means as with the other samples. The Pellucidar mandible grouped with means of *M. vancouverensis* populations, but the Mausoleum specimen was 'sister' to all other *M. vancouverensis* populations and *M. olympus* despite being only slightly older than the Mariner and Limestone specimens. This paraphyletic relationship is likely an artefact of sampling error when a single individual or a mean based on a small sample is used to represent the shape of a population as shown by Cardini and Elton's (2007) rarefaction analysis and by Cardini et al. (2007) for the atypical shape of *Marmota bobak* based on a sample of $n = 2$. Clusters suggested by the scatterplot of the first two PCA axes (Fig. 6) were congruent with those from the cluster analysis; 95% bootstrap confidence intervals of population means largely overlapped within species. This observation was congruent with the low repeatability of within-species clusters of mean shapes in the

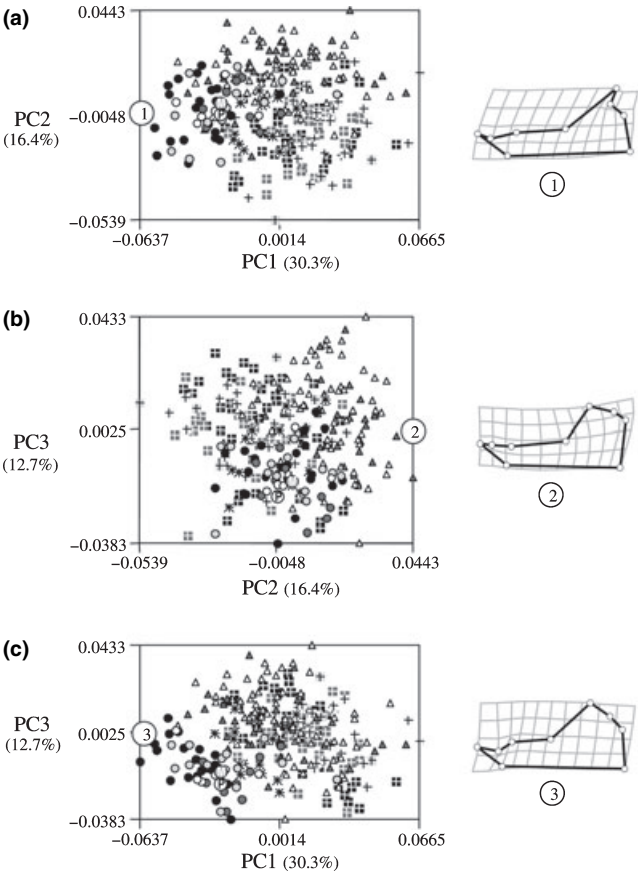

Fig. 4. Scatterplots of the first principal components (PCs) of shape variables based on all specimens (percentages of explained variance in parentheses). Deformation grids depict shape changes (magnified ×2) at extremes of the first three PCs. Arabic numbers indicate the position of specimens in the plots illustrated with deformation grids

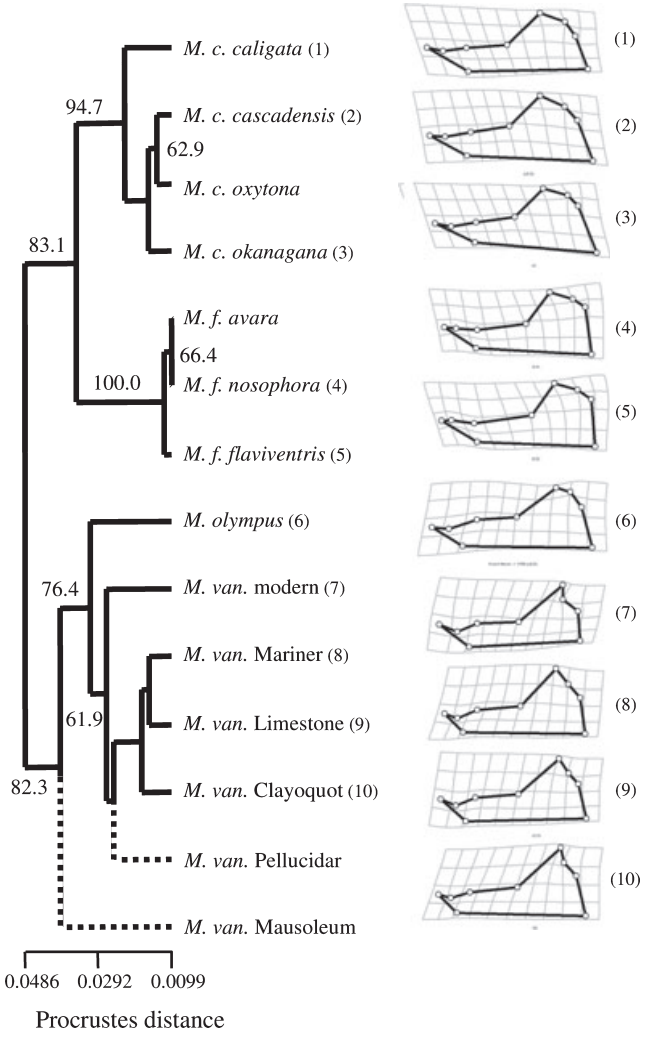

Fig. 5. Unweighted pair-group method using arithmetic average (UPGMA) phenogram of mean shapes for 14 samples. Bootstrap percentages of node repeatability are shown when larger than 50%. Subfossils from Mausoleum and Pellucidar caves were included (dotted lines), but real specimen configurations were used because $n = 1$. Deformation grids (magnified ×3) depict differences between population means of 10 samples and the grand mean based on all populations. Arabic numbers match sample and its associated deformation grids

 doi: 10.1111/j.1439-0469.2008.00503.x

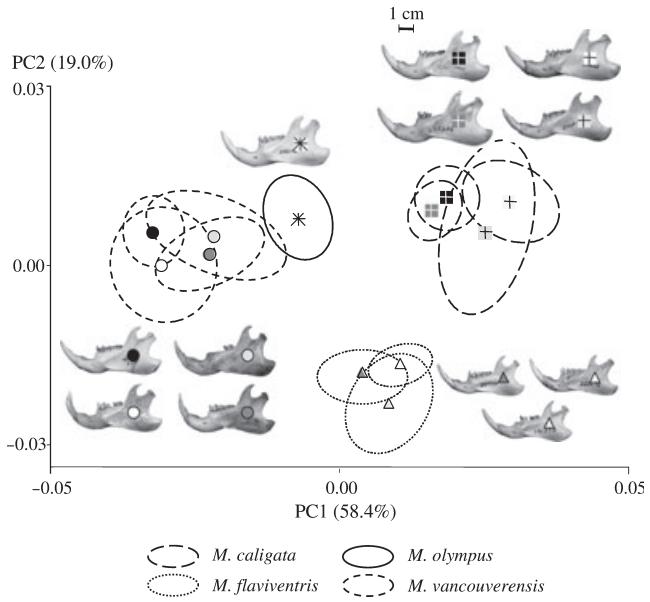

Fig. 6. Scatterplots of the first two principal components of mean shapes for 12 samples (symbols defined in Fig. 2); percentages of explained variance in parentheses. Variation around the centroid is illustrated with 95% confidence ellipses of bootstrap mean shapes. Images show the specimen closest to the sample's mean shape

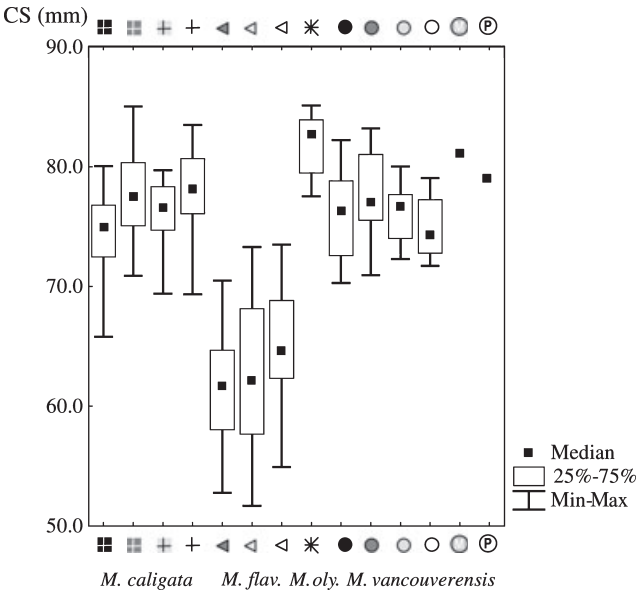

Fig. 7. Box-plots of the mandible centroid size for 12 samples; symbols are defined in Fig. 2

phenogram. The PCA and phenogram both suggest some similarities between *M. vancouverensis* and *M. olympus*, and their clear separation from *M. caligata* and *M. flaviventris*.

Deformation grids of species mean shapes (Fig. 5) supported the observation that *M. vancouverensis* distinctive shape traits mostly involve the coronoid, condyle and mandibular symphysis. The bending in opposite direction of the coronoid and condyle was more pronounced in the modern population but visible also in Holocene populations even the early Holocene specimen from Pellucidar Cave (not shown). The relatively inconspicuous symphysis 'ridge' also characterized all our samples of *M. vancouverensis*. This combination of traits was not found in any of the continental marmot populations.

Mandibular size (Fig. 7) of *M. vancouverensis* samples largely overlapped that of *M. caligata*. *Marmota flaviventris* and *M. olympus* were at opposite extremes for size, representing the smallest and largest marmots. Size ranges were most pronounced for *M. flaviventris* samples but rarely overlapped with those of other species. *Marmota olympus*, in contrast, demonstrated a small size range that showed some overlap with *M. caligata* and *M. vancouverensis* samples. Both the Mausoleum and Pellucidar specimens were larger than the mean size of other *M. vancouverensis* samples and the Mausoleum specimen approached *M. olympus* in size.

Variation in morphology

Shape variances did not differ ($p > 0.05$) among the four *M. vancouverensis* samples (Table S6 and S7). They exhibited relatively small shape variances except the Limestone Cave sample. Except for *M. olympus*, shape variances within continental samples exceeded those of the *M. vancouverensis* populations. However, only 15–10% of tests (depending on whether we considered all 66 pairwise comparisons or only the 32 between *M. vancouverensis* populations and any other one) were significant after a sequential Bonferroni correction. Our hypothesis of a reduction of shape variance in *M. vancouverensis* over past 2500 years was not supported by the data. In contrast, when we tested a hypothesis of reduction of shape variance in a specific *M. vancouverensis* population by comparing a single sample to any of the eight continental populations, half of the tests of modern *M. vancouverensis* and all those with the Mariner and Clayoquot caves were significant even after a sequential Bonferroni correction.

Size variances were greatest for the *M. flaviventris* samples (Table S6)- more than twice the values of other species. *Marmota olympus* and the Limestone and Clayoquot cave samples of *M. vancouverensis* demonstrated the smallest size variances in size. However, few comparisons of size variances were significant at the 0.05 level (Table S8). Size variances did not differ among the four *M. vancouverensis* samples or between *M. vancouverensis* and *M. olympus* and *M. caligata* samples.

Discussion

That *M. vancouverensis* has a highly distinctive shaped mandible is confirmed by our analysis with a sample of modern specimens about three times larger than those of previous studies that reported this finding (Cardini 2003; Cardini and O'Higgins 2004; Cardini et al. 2005). Moreover, the distinctive shape traits that characterize modern *M. vancouverensis* are evident in Holocene marmots from the island. Although shape differences between modern and Holocene populations were significant, percentages of variance explained by groups and the low support for branches among *M. vancouverensis* samples in the phenogram of mean shapes suggests minor divergence among modern and Late Holocene populations. In contrast, *M. vancouverensis* demonstrates marked differences from continental marmot species.

Our results are congruent with the second scenario of Fig. 1, a pronounced acceleration of morphological evolution that mainly predates our subfossil samples. Traits similar to those

of modern and late Holocene specimens were also evident in the early Holocene mandible from Pellucidar Cave. This observation, if confirmed by future fossil finds, suggests that most of the morphological differentiation of *M. vancouverensis* occurred soon after the separation of Vancouver Island from the mainland in the late Pleistocene and implies that the morphological differentiation between *M. vancouverensis* and its sister species *M. caligata* occurred in a briefer time-period than previously proposed. Although Caumul and Polly (2005) found that mandible shape of marmots evolved more rapidly than other osteological structures, the distinct cranial shape (Cardini et al. 2005, 2007) and molar shape (Polly 2003) of *M. vancouverensis* is evidence that this rapid evolution occurred in other morphological structures. This pattern of divergence is consistent with the model proposed by Millien (2006) where insular species undergo more rapid evolution over a shorter time-period compared with mainland species with most of the change occurring in a brief period following isolation.

Clearly, the morphological differentiation of *M. vancouverensis* predates recent climate change and anthropogenic impacts such as hunting and habitat changes from forest harvesting (Bryant and Janz 1996; Nagorsen et al. 1996; Laroque et al. 2001). The most pronounced environmental changes on Vancouver Island following the last glaciation occurred in late Pleistocene early Holocene transition when a tundra parkland landscape associated with a cool moist environment changed to a more forested landscape in response to an early Holocene warming trend (Hebda 1995). Alpine mammals such as the mountain goat (*Oreamnos americanus*) and marmots likely were widely distributed in the early post-glacial period even living at sea level (Nagorsen and Keddie 2000; Al-Suwaidi et al. 2006). With early Holocene warming and associated forest expansion, marmots retreated to alpine areas on the island a pattern that has been well documented in Europe for *M. marmota* (Preleuthner et al. 1995). The tree line quickly advanced fragmenting alpine habitats into isolated habitat islands. According to Rochefort et al. (1994) the treeline in southwestern British Columbia in the early Holocene was 60–130 m higher than today. Selective pressures on small isolated populations during this early post-glacial period may have contributed to accelerate the rate of morphological change in *M. vancouverensis*.

To what extent population bottlenecks played a central role in the morphological evolution of *M. vancouverensis* is unclear. Reduced variance in shape relative to mainland marmots is evident in the modern sample and two of three samples of Holocene *M. vancouverensis*. But, the hypothesis of reduced variance over the past 2500 years among *M. vancouverensis* populations is not supported by our data. Nevertheless, population bottlenecks almost certainly occurred during the evolution of a peripheral isolate that evolved on an island where major environmental changes impacting population size and connectivity occurred during its evolutionary history. The potential for a role of genetic drift in the evolution of the Vancouver Island marmot is also supported by the small genetic variability found in this population by Kruckenhauser et al. (1999b) using microsatellites. Population bottlenecks may also account for the small shape variance shown by *M. olympus*, which, although on a peninsula, shares with *M. vancouverensis* a similar history of geographic isolation and a restricted range (Hoffmann 1981). Despite bottlenecks, sufficient variation may have been retained in the gene pool for populations to recover to higher levels of morphological variation after a population crash. If this speculation, somewhat supported by observed changes in shape variance of allochronic populations of *M. vancouverensis*, holds true, bottlenecks may have increased the rate of fixation of alleles by reducing population size without strongly reducing genetic variance and allowing sufficient variation for natural selection to promote evolutionary divergence.

In contrast to its shape, size of the *M. vancouverensis* mandible has changed little over the last few thousands years and it shows similar levels of variance as the continental marmots. Size is generally considered more labile than shape. Interspecific differences in marmot body mass, however, are influenced by selective pressures related to the length of hibernation and growing season (Armitage 1999). A heavier body mass is required to survive the longer hibernation period associated with harsher environments; therefore, marmots that occupy high altitude habitats would be expected to be larger than species that inhabit lower altitudes and latitudes. Moreover, a species that inhabits a wide range of environments should demonstrate a greater size range than species restricted to a narrow habitat type. These predictions are congruent with our findings on marmot mandibular size. The alpine species (*M. caligata*, *M. olympus* and *M. vancouverensis*) with restricted habitats have larger mandibles but demonstrate less variation in size than *M. flaviventris* a species that ranges from low elevation grasslands to montane meadows in western North America (Frase and Hoffmann 1980). The so-called island 'rule' (Lomolino 1985) predicts a tendency towards gigantism of smaller species and dwarfism of larger species of mammals that inhabit islands. The size trends for insular rodents described by Lomolino (2005) suggest that large rodents (~5 kg) such as marmots should be smaller on islands. The smaller body mass and body length of *M. vancouverensis* compared with its continental sister species *M. caligata* (Armitage 1999) is consistent with this prediction. However, to what extent the body size of modern *M. vancouverensis* is the result of various selective pressures associated with island effects such as competition and food limitation or selection for an optimum size for hibernation is not clear. Bryant and Page (2005) observed that most mortality of *M. vancouverensis* occurs in late summer rather than over winter during hibernation suggesting that this marmot is near optimum body size for winter survival. In contrast to body size, mandible size and cranial size (Cardini et al. 2007) of modern *M. vancouverensis* differ little from continental *M. caligata* populations. Body mass or body length may better measures of size. Selection pressures associated with diet and feeding or ecophenotypic effects may obscure the correlation of mandible size with body size.

We found no appreciable differences in mandible size among four allochronic populations *M. vancouverensis* that span the last 2500 years. Climate and environmental harshness varied during this time-period and some size fluctuations would be expected. Hadly (1997) found significant temporal changes in the diastemal length (a trait correlated with body size) of pocket gopher (*Thomomys talpoides*) mandibles from the past 3200 years with pocket gophers large during the Little Ice Age and small during warm dry periods. Possibly mandible size of marmots is more constrained and less sensitive to ecophenotypic effects than the pocket gopher mandible. Body and mandible size in pocket gophers are highly plastic traits that reflect nutritional quality (Patton and Brylski 1987). Alternatively, the similarity in size among our allochronic populations

 doi: 10.1111/j.1439-0469.2008.00503.x

may be an artefact of the time-periods sampled (Table S1). Two Holocene samples (Mariner Cave and Limestone Cave) date from 960–739 cal. yr bp when the climate on Vancouver Island resembled modern conditions according to Hebda (1995). The Clayoquot Cave sample is from an earlier period (2757–2630 cal. yr bp) with slightly cooler moister conditions, but these small climatic differences may have been insufficient to result in appreciable size changes. Most of our modern specimens are from the first half of the 20th century (60% are from 1910 to 1931), when the effects of global warming and climate change were less pronounced than in the last few decades. No specimens are available from the Little Ice Age when significant cooling and increased moisture resulted in several glacial advances in the mountains on Vancouver Island in the early 1700s and late 1800s (Lewis and Smith 2004). Based on a study of evolutionary rates since the last glaciation, Millien and Damuth (2004) attributed insular size trends to two opposing selection pressures – a trend for decreasing size over time in response to climate warming countered by selective pressures that operate to maintain a large body size. According to their hypothesis, insular mammals were larger than modern populations in the early post-glacial in response to a cool harsh climate and their rate of evolutionary change to a smaller size was determined by the strength of island effects. Evidently, early post-glacial *O. americanus* and American black bears (*Ursus americanus*) from Vancouver Island were larger than modern forms (Nagorsen et al. 1995; Nagorsen and Keddie 2000), but no late Pleistocene fossils of *M. vancouverensis* are available to test the hypothesis of Millien and Damuth (2004).

Based on a substantially larger sample size than previous studies, our study is the first to provide strong confirmation of the distinctiveness of *M. vancouverensis* hard tissue morphology despite its very young evolutionary age. We can rule out the hypothesis that previous findings of unexpectedly distinctive mandibular shape (Cardini 2003) were an artefact of sampling error. Our study also underscores the value of modern and fossil museum specimens in systematic studies. For the first time, Holocene fossil specimens were included in an accurate quantitative study of form and evolution in this endangered endemic mammal. More fossils are required to clarify the evolutionary history of *M. vancouverensis*. A late Pleistocene sample, for example, would permit a more rigorous test of our hypothesis of rapid evolutionary change in both morphological shape and size during the early post-glacial. If most of the evolutionary change in form rapidly occurred in the period of most pronounced environmental alteration on the island since the last glaciation, it suggests that marmots may indeed be very susceptible to climate change (Davis 2005).

Acknowledgements

We are indebted to museum curators and staff of the Cowan Vertebrate Museum, University of British Columbia; Field Museum of Natural History; Museum of Vertebrate Zoology, University of California; National Museum of Natural History; University of Kansas Natural History Museum; Royal British Columbia Museum; and Zoological Museum, University of Montana for access to collections and assistance with data collection. We thank F. J. Rohlf (State University of New York, Stony Brook) for his invaluable aid on statistical software, and S. Elton (University of Hull) and P. O'Higgins (University of York) for allowing A. Cardini to use for this study facilities and equipment of the Functional Morphology and Evolution Unit of the Hull York Medical School. We thank A. Bryant for information on modern *Marmota vancouverensis*. Radiocarbon dates were funded by the Royal British Columbia Museum and the British Columbia Ministry of Environment. Finally, we acknowledge various cavers and other individuals who discovered the Holocene cave bones and assisted with their recovery.

Riassunto

Tempi e modi della divergenza evolutiva in popolazioni moderne e oloceniche della marmotta dell'Isola di Vancouver (Marmota vancouverensis)

Marmota vancouverensis è la sola specie insulare tra le 14 specie attuali di marmotta. La sua storia evolutiva, tuttavia, è ancora da chiarire. Sebbene si differenzi in maniera maracata per caratteristiche osteologiche ed altri tratti morfologici, presenta un basso grado di divergenza genetica, e questo suggerisce una recente origine a partire da una popolazione ancestrale continentale. Abbiamo utilizzato metodi di morfometria geometrica per studiare la morfologia dell'emimandibola di 239 esemplari di specie contemporanee (*M. vancouverensis*, *M. caligata*, *M. flaviventris*, *M. olympus*) insieme con 30 subfossili (9435–735 cal. yr bp) di *M. vancouverensis*. I risultati dell'indagine confermano che la forma della mandibola di *M. vancouverensis* è spiccatamente diversa dal quella delle specie che vivono sul continente, mentre le dimensioni differiscono poco da quelle della specie sorella *M. caligata*. La variazione morfologica nel corso degli ultimi 2500 anni è minima nei campioni allocronici da noi studiati. Questo suggerisce che la differenziazione morfologica della specie si sia verificata in un periodo di rapido cambiamento successivo all'isolamento geografico dal continente al termine del Pleistocene. Pressioni selettive associate ai cambiamenti ambientali e fenomeni di deriva genetica da effetto del fondatore e per colli di bottiglia demografici causati dalla frammentazione dell'habitat hanno contribuito a produrre il modello osservato di variazione morfologica.

References

Adams DC, Slice DE, Rohlf FJ (2004) Geometric morphometrics: ten years of progress following the 'revolution'. Ital J Zool **71:**5–16.

Al-Suwaidi M, Ward BC, Wilson MC, Hebda RJ, Nagorsen DW, Marshall D, Ghaleb B, Wigen RJ, Enkin RJ (2006) Late Wisconsin Port Eliza cave deposits and their implications for human coastal migration, Vancouver Island, Canada. Geoarchaeology **21:**307–332.

Armitage KB (1999) Evolution of sociality in marmots. J Mammal **80:**1–10.

Barash DP (1989) Marmots: Social Behavior and Ecology. Stanford University Press, Stanford, CA.

Bookstein FL (1991) Morphometric Tools for Landmark Data. Cambridge University Press, Cambridge, MA.

Bryant AA, Janz DW (1996) Distribution and abundance of Vancouver Island marmots (*Marmota vancouverensis*). Can J Zool **74:**667–677.

Bryant A, Page RE (2005) Timing and causes of mortality in the endangered Vancouver Island marmot (*Marmota vancouverensis*). Can J Zool **83:**674–682.

Cardini A (2003) The geometry of the marmot (Rodentia: Sciuridae) mandible: phylogeny and patterns of morphological evolution. Syst Biol **52:**186–205.

Cardini A (2004) Evolution of marmots (Rodentia, Scuridae): combining information on labial and lingual sides of the mandible. Acta Theriol **49:**301–318.

Cardini A, Elton S (2007) Sample size and sampling error in geometric studies of morphometric studies of size and shape. Zoomorphology **126:**121–134.

Cardini A, O'Higgins P (2004) Patterns of morphological evolution in *Marmota* (Rodentia: Sciuridae): geometric morphometrics of the cranium in the context of marmot phylogeny, ecology and conservation. Biol J Linn Soc **82:**385–407.

Cardini A, O'Higgins P (2005) Post-natal ontogeny of the mandible and ventral cranium in *Marmota* species (Rodentia, Sciuridae): allometry and phylogeny. Zoomorphology **124:**189–203.

 doi: 10.1111/j.1439-0469.2008.00503.x

Cardini A, Tongiorgi P (2003) Yellow-bellied marmots (*Marmota flaviventris*) 'in the shape space' (Rodentia, Sciuridae): sexual dimorphism, growth and allometry of the mandible. Zoomorphology **122:**11–23.

Cardini A, Hoffmann RS, Thorington RW (2005) Morphological evolution in marmots (Rodentia, Sciuridae): size and shape of the dorsal and lateral surfaces of the cranium. J Zool Syst Evol Res **43:**258–268.

Cardini A, Thorington RW, Polly PD (2007) Evolutionary acceleration in the most endangered mammal of Canada: phylogenetic signal and cranial divergence in the Vancouver Island marmot (Rodentia, Sciuridae). J Evol Biol **20:**1833–1846.

Caumul R, Polly PD (2005) Phylogenetic and environmental components of morphological variation: skull, mandible and molar shape in marmots (*Marmota*, Rodentia). Evolution **59:**2460–2472.

Clague JJ (1981) Late Quaternary geology and geochronology of British Columbia. Part 2. Summary and discussion of radiocarbon-dated Quaternary history. Geol Surv Canada (Ottawa), Paper 80 **35:**1–41.

Cole TM III, Lele S, Richtsmeier JT (2002) A parametric bootstrap approach to the detection of phylogenetic signals in landmark data. In: MacLeod N, Forey P (eds), Morphology, Shape and Phylogeny. Taylor and Francis, NY, pp 194–219.

Davis EB (2005) Comparison of climate space and phylogeny of *Marmota* (Mammalia: Rodentia) indicates a connection between evolutionary history and climate preference. Proc Royal Soc B **272:**519–526.

Frase BA, Hoffmann RS (1980) *Marmota flaviventris*. Mamm Spec **135:**1–8.

Hadly EA (1997) Evolutionary and ecological response of pocket gophers to late-Holocene climatic change. Biol J Linn Soc **60:**277–296.

Hall ER (1981) The Mammals of North America. John Wiley and Sons, New York.

Hebda RJ (1995) British Columbia vegetation and climate history with focus on 6 KA BP. Geog Phys Quatern **49:**55–79.

Hoffmann RS, Koeppl JW, Nadler CF (1979) The relationships of the amphiberingian marmots (Mammalia: Sciuridae). Univ Kansas, Mus Nat Hist **83:**1–56. Occas. Papers.

Hoffmann RS (1981) Different holes for different voles: environmenatl restrictions on refugial survival of mammals. In: Scudder GGE, Reveal J (eds), Evolution Today. Proccedings of the Second International Congress of Systematic and Evolutionary Biology, Vancouver, pp 25–45.

Howell DC (2002) Statistical Methods for Psychology, 5th edn. Duxbury Thompson Learning, Pacific Grove, CA.

Howes DE (1981) Late Quaternary sediments and geomorphic history of north central Vancouver Island. Can J Earth Sci **18:**1–12.

Howes DE (1997) Quaternary geology of Brooks Peninsula. In: Hebda RJ, Haggarty JC (eds), Brooks Peninsula: An Ice Age Refugium on Vancouver Island. Occas. Paper 5. Ministry of Environment, Lands and Parks, BC Parks, Victoria, pp 3.1–3.19.

Klingenberg CP, Barluenga M, Meyer A (2002) Shape analysis of symmetric structures: quantifying variation among individuals and asymmetry. Evolution **56:**1909–1920.

Kruckenhauser L, Pinsker W, Haring E, Arnold W (1999a) Marmot phylogeny revisited: molecular evidence for a diphyletic origin of sociality. J Zool Syst Evol Res **37:**49–56.

Kruckenhauser L, Haring E, Pinsker W (1999b) Microsatellite variation in two species of the genus *Marmota* (Rodentia, Sciuridae). Folia Zool **48:**29–36.

Laroque C, Lewis D, Smith D (2001) Treeline dynamics on southern Vancouver Island, British Columbia. Western Geography **10/11:**43–63.

Lewis D, Smith D (2004) Little Ice Age glacial activity in Strathcona Provincial Park, Vancouver Island, British Columbia. Can J Earth Sci **41:**285–297.

Lomolino MV (1985) Body size of mammals on islands: the rule reexamined. Am Nat **125:**310–316.

Lomolino MV (2005) Body size of evolution in insular vertebrates: generality of the island rule. J. Biogeog **32:**1683–1699.

Manly B (1997) Randomization, Bootstrap, and Monte Carlo Methods in Biology, 2nd edn. Chapman and Hall, London.

Mashkin VI, Kolesnikov VV (1990) Age determination in marmots (*Marmota*, Scuiridae) by the patterns of wear of chewing surface of teeth. Zoolog Zh **69:**124–131 [In Russian].

McCabe TT, Cowan M (1945) *Peromyscus maniculatus macrorhinus* and the problem of insularity. Trans Royal Can Inst **25:**117–216.

Millien V (2006) Morphological evolution is accelerated among island mammals. PLoS Biol **4:**1863–1868.

Millien V, Damuth J (2004) Climate change and size evolution in an island rodent species: new perspectives on the island rule. Evolution **58:**1353–1360.

Nagorsen DW (1987) *Marmota vancouverensis*. Mammal Spec **270:**1–5.

Nagorsen DW (2005) Rodents and Lagomorphs of British Columbia. Royal British Columbia Museum, Victoria.

Nagorsen DW, Keddie G (2000) Late Pleistocene mountain goats (*Oreamnos americanus*) from Vancouver Island: Biogeographic implications. J Mammal **81:**666–675.

Nagorsen DW, Keddie G, Hebda RJ (1995) Early Holocene black bears, *Ursus americanus*, from Vancouver Island. Can Field-Nat **109:**11–18.

Nagorsen DW, Keddie G, Luszcz T (1996) Vancouver Island Marmot Bones in Subalpine Caves: Archaeological and Biological Implications. Ministry of Environment, Lands and Parks, BC Parks, Victoria. Occas. Paper 4.

O'Higgins P, Jones N (2006) Tools for Statistical Shape Analysis. Hull York Medical School. URL http://hyms.fme.googlepages.com/resources [accessed on 21 October 2008].

Patton JL, Brylski PV (1987) Pocket gophers in alfalfa fields: causes and consequences of habitat-related body size variation. Am Nat **130:**493–506.

Polly PD (2003) Paleophylogeography: the tempo of geographic differentiation in marmots (*Marmota*). J Mamm **84:**369–384.

Preleuthner M, Pinsker W, Kruckenhauser L, Miller WJ, Prosl H (1995) Alpine marmots in Austria. The present population structure as a result of postglacial distribution history. Acta Theriol Suppl **3:**87–100.

Rochefort RM, Little RL, Woodward A, Peterson DL (1994) Changes in sub-alpine tree distribution in western North America: a review of climatic and other casual factors. Holocene **4:**89–100.

Rohlf FJ (1970) Adaptive hierarchial clustering schemes. Sys Zool **19:**58–82.

Rohlf FJ (2006a) Tps Series. Department of Ecology and Evolution, State University of New York, Stony Brook, New York. http://life.bio.sunysb.edu/morph/ [accessed on 21 October 2008].

Rohlf FJ (2006b) NTSYSpc, Version 2.20L. Exeter Software, Setauket, NY.

Rohlf FJ, Marcus LF (1993) A revolution in morphometrics. Trends Ecol Evol **8:**129–132.

Rohlf FJ, Slice DE (1990) Extensions of the Procrustes method for the optimal superimposition of landmarks. Syst Zool **39:**40–59.

Slice DE (1999) Morpheus (Beta Version). Department of Ecology and Evolution, State University of New York, Stony Brook, NY.

Steppan SJ, Akhverdyan MR, Lyapunova EA, Fraser DG, Vorontsov N, Hoffmann RS, Braun MJ (1999) Molecular phylogeny of the marmots (Rodentia: sciuridae): tests of evolutionary and biogeographic hypotheses. Syst Biol **48:**715–734.

Swarth HS (1911) Two new species of marmots from northwestern North America. Univ Calif Pub Zool **7:**201–204.

Thompson D'AW (1917) On Growth and Form. Cambridge University Press, London.

Van Valen L (1978) The statistics of variation. Evol Theory **4:**33–43.

Velhagen WA, Roth VL (1997) Scaling the mandible in squirrels. J Morphol **232:**107–132.

Zelditch ML, Swiderski DL, Sheets HD, Fink WL (2004) Geometric Morphometrics for Biologists: A Primer. Elsevier Academic Press, NY.

Authors' addresses: David W. Nagorsen (for correspondence), Mammalia Biological Consulting, 4268 Metchosin Road, Victoria, BC V9C 3Z4, Canada. E-mail: mammalia@shaw.ca; A. Cardini, Dipartimento del Museo di Paleobiologia e dell'Orto Botanico, Universitá di Modena e Reggio Emilia, via Università 4, 41100, Modena, Italy and Hull York Medical School, The University of Hull, Cottingham Road, Hull HU6 7RX, UK. E-mail: alcardini@interfree.it, cardini@unimo.it, andrea.cardini@hyms.ac.uk

 doi: 10.1111/j.1439-0469.2008.00503.x

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Summary of environmental changes on Vancouver Island during the past 15 000 years and associated *Marmota vancouverensis* samples used in study.

Table S2. Sample sizes of the Petromarmota group (*Marmota caligata*, *Marmota flaviventris*, *Marmota olympus* and *Marmota vancouverensis*) used in analyses.

Table S3. 1000 permutation tests for differences in size and shape between left and right hemimandibles of *Petromarmota* species.

Table S4. 10 000 permutation tests for pairwise differences in shape of 12 *Petromarmota* samples.

Table S5. 10 000 permutation tests for pairwise differences in size of 12 *Petromarmota* samples.

Table S6. Variance of shape and centroid size with bootstrap standard errors for 12 *Petromarmota* samples.

Table S7. Results of Levene's test for pairwise differences in shape variance of 12 *Petromarmota* samples (10 000 random permutations).

Table S8. Results of Levene's test for pairwise differences in size variance of 12 *Petromarmota* samples (10 000 random permutations).

Appendix S1. Specimens examined

Please note: Wiley-Blackwell are not responsible for the content or functionality of any supporting materials supplied by the authors. Any queries (other than missing material) should be directed to the corresponding author for the article.

 doi: 10.1111/j.1439-0469.2008.00503.x

Appendix 1. Specimens examined

Field Museum of Natural History (FMNH, Chicago, USA), University of Kansas Natural History Museum (KUNH, Lawrence, USA), Museum of Vertebrate Zoology (MVZ, Berkeley, USA), Royal British Columbia Museum (RBCM, Victoria, Canada), University of British Columbia (UBC, Vancouver, Canada), Zoological Museum of the University of Montana (UMZ, Missoula, USA), National Museum of Natural History (USNM, Washington, D.C., USA).

Modern species samples:

FMNH 6322, 6324, 6328; KUNH 120012; MVZ 12090, 12091, 12092, 12093, 12095, 12098, 12099, 12100; RBCM 1206, 2437, 5192, 9078, 10709, 11210, 11393, 16749, 16968, 18546, 18549, 20253; UBC 158, 928, 2979, 5850, 5853, 5861, 5863, 5864, 5865, 5866, 5870, 8149, 8150, 8152, 8153, 8154, UMZ 13521, USNM, 3674, 3676, 4206, 6861, 6871, 12406, 12407, 23951, 30141, 42606, 42608, 42792, 42793, 53594, 53595, 55239, 55374, 55376, 55378, 55765, 55766, 55830, 66696, 66697, 66709, 66944, 66950, 67073, 67074, 67075, 67076, 67077, 67080, 67612, 67861, 74370, 74996, 78351, 78352, 79390, 80360, 80489, 80770, 81913, 88005, 88006, 89311, 89312, 89814, 89815, 90131, 90132, 90133, 90516, 92768, 94343, 96205, 96206, 96207, 96533, 98153, 98154, 99759, 101299, 101300, 108783, 127762, 127763, 128067, 128069, 128070, 128072, 128073, 128082, 128084, 128665, 128666, 131437, 131438, 131440, 131441, 131442, 131443, 131444, 135161, 135162, 135163, 141948, 146449, 146450, 146451, 147183, 148580, 150433, 156923, 156924, 168472, 168473, 168475, 168476, 168477, 168493, 168799, 169027, 169246, 169248, 170683, 170741, 174502, 175566, 176732, 176735, 178842, 191350, 191355, 191357, 191358, 191363, 202403, 202787, 202788, 202789, 202790, 202791, 204992, 208107, 208108, 209035, 209401, 211232, 212433, 212469, 212470, 212471, 221894, 221895, 221896, 221898, 222995, 225144, 225582, 225583, 225639, 225801, 225802, 225803, 225804, 225805, 225806, 226055, 226057, 226147, 226148, 226471, 226472, 226473, 228672, 228673, 228674, 228675, 228677, 229840, 230653, 230654, 231981, 233212, 233213, 233382, 233386, 234960, 235432, 235433, 236593, 241657, 241658, 241659, 241947, 241948, 242102, 242645, 243663, 243664, 247774, 249317, 249318, 256659, 257448, 257449, 264225, 271698, 271699, 271701, 274340, 274342, 291191, 291192, 291194, 575170.

M. vancouverensis subfossils:

RBCM collections. Clayoquot Cave CL20, CL29, CL32, CL33, CL34, CL35; Limestone Cave LI1, LI2, LI3, LI4, LI6, LI7, ;I11, LI14, LI30, LI40, LI42, LI46, LI47; Mariner Cave MA44, MA60, MA62, MA74, MA85, MA104, MA121, MA136, MA144; Mausoleum Cave 1; Pellucidar Cave1.

Table 1.

Years BP	Significant changes and events	Marmot samples
0-100	Forest harvesting in subalpine	Modern specimens
	Climate change, tree invasion of alpine	
	M. vancouverensis population declines	
100-500	Glacial fluctuations associated with Little Ice Age	-
800-2000	Modern climate	Mariner, Limestone, Mausoleum Cave bones
2000-4000	Slightly cooler and wetter	Clayoquot Cave bones
4000-7500	Slightly warmer, modern precipitation	-
7500-10000	Drier and warmer by 2°C	Pellucidar Cave bones
	Forest expansion	
	First evidence of human occupation ~8000 BP	
13500-10000	Glacial retreat; island isolated from mainland	-
	Pleistocene-Holocene transition vegetation	
	Earliest postglacial mammal remains 12300 BP	
15000-13500	Last glacial maximum	-
	Island covered by Cordilleran ice sheet	

Table 2.

Samples	$n_{females}$	n_{males}	n_u[1]	n_{total}
Modern specimens				
M. c. caligata (Eschscholtz, 1829)	9	11	20	40
M. c. cascadensis Howell, 1914)	13	12	5	30
M. c. okanagana (King, 1836)	5	3	-	8
M. c. oxytona Hollister, 1912)	10	9	2	21
M. f. avara (Bangs, 1899)	18	16	2	36
M. f. flaviventris (Audubon and Bachman, 1841)	10	5	-	15
M. f. nosophora Howell (1914)	20	24	8	52
M. olympus (Merriam, 1898)	7	7	-	14
M. vancouverensis Swarth,1911	9	11	3	23
Subfossil specimens				
M. vancouverensis Mariner Cave	-	-	9	9
M. vancouverensis Limestone Cave	-	-	13	13
M. vancouverensis Mausoleum Cave	-	-	1	1
M. vancouverensis Clayoquot Cave	-	-	6	6
M. vancouverensis Pellucidar Cave	-	-	1	1

[1]specimens of unknown sex.

Table 3.

species	N	size		shape	
		P[1]	% VAR[2]	P[1]	% VAR
M. caligata	42	*0.041*	<0.1	0.415	0.2
M. flaviventris	60	0.402	<0.1	*0.002*	0.4
M. olympus	9	0.057	2.1	*0.002*	4.0
M. vancouverensis	8	0.953	<0.1	0.161	1.9

[1]Significant P in this and other tables are in italics ($P < 0.05$); underscored P are significant after a sequential Bonferroni correction for multiple comparisons. [2]Percentage of variance explained by differences between left or right hemimandibles.

Table 4.

population		(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)
M. caligata caligata	(1)	-	5.8%	5.5%	6.6%	15.2%	13.8%	13.5%	14.0%	29.9%	18.8%	16.7%	18.8%
M. c. cascadensis	(2)	*0.0006*	-	2.5%	3.1%	16.4%	17.0%	11.7%	14.9%	37.1%	20.7%	21.0%	22.0%
M. c. okanagana	(3)	*0.0179*	0.5022	-	3.7%	10.5%	16.4%	5.6%	22.5%	38.3%	36.5%	28.7%	40.0%
M. c. oxytona	(4)	*0.0017*	0.1331	0.3997	-	18.6%	21.1%	13.1%	23.5%	45.6%	31.8%	30.6%	33.5%
M. flaviventris avara	(5)	*0.0001*	*0.0001*	*0.0004*	*0.0001*	-	1.7%	1.4%	13.6%	27.2%	12.8%	13.8%	12.8%
M. f. flaviventris	(6)	*0.0001*	*0.0001*	*0.0018*	*0.0001*	0.5007	-	1.6%	23.5%	34.3%	26.1%	23.5%	26.6%
M. f. nosophora	(7)	*0.0001*	*0.0001*	*0.0036*	*0.0001*	0.2582	0.3927	-	11.8%	28.0%	11.7%	13.7%	11.2%
M. olympus	(8)	*0.0001*	*0.0001*	*0.0001*	*0.0001*	*0.0001*	*0.0001*	*0.0001*	-	22.6%	17.6%	11.2%	21.4%
M. vancouverensis modern	(9)	*0.0001*	*0.0001*	*0.0001*	*0.0001*	*0.0001*	*0.0001*	*0.0001*	*0.0001*	-	15.8%	7.1%	11.6%
M. vancouverensis Mariner Cave	(10)	*0.0001*	*0.0001*	*0.0001*	*0.0001*	*0.0001*	*0.0001*	*0.0001*	*0.0001*	*0.0001*	-	5.3%	8.3%
M. vancouverensis Limestone Cave	(11)	*0.0001*	*0.0001*	*0.0001*	*0.0001*	*0.0005*	*0.0001*	*0.0001*	*0.0051*	*0.0133*	0.3387	-	6.4%
M. vancouverensis Clayoquot Cave	(12)	*0.0001*	*0.0001*	*0.0002*	*0.0001*	*0.0001*	*0.0001*	*0.0001*	*0.0001*	*0.0011*	0.3141	0.3089	-

Table 5.

population		(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)
M. caligata caligata	(1)	-	17.7%	2.7%	19.0%	74.5%	63.6%	58.4%	53.2%	3.9%	11.4%	3.8%	0.1%
M. c. cascadensis	(2)	*0.0003*	-	4.0%	0.0%	79.3%	71.4%	67.7%	22.9%	6.9%	0.1%	5.9%	8.5%
M. c. okanagana	(3)	0.2615	0.2298	-	6.3%	64.3%	62.8%	42.9%	53.8%	0.01%	5.6%	0.0%	3.9%
M. c. oxytona	(4)	*0.0005*	0.9421	0.1860	-	79.2%	73.8%	66.0%	28.7%	8.6%	0.2%	8.8%	13.3%
M. flaviventris avara	(5)	*0.0001*	*0.0001*	*0.0001*	*0.0001*	-	0.9%	14.1%	84.5%	76.5%	69.9%	72.5%	55.6%
M. f. flaviventris	(6)	*0.0001*	*0.0001*	*0.0002*	*0.0001*	0.5129	-	5.0%	82.8%	69.5%	68.0%	69.4%	55.2%
M. f. nosophora	(7)	*0.0001*	*0.0001*	*0.0001*	*0.0001*	*0.0005*	0.0689	-	74.1%	59.9%	51.4%	53.1%	32.4%
M. olympus	(8)	*0.0001*	*0.0009*	*0.0002*	*0.0014*	*0.0001*	*0.0001*	*0.0001*	-	50.8%	34.0%	61.8%	63.5%
M. vancouverensis modern	(9)	0.1185	0.0593	0.9535	0.0550	*0.0001*	*0.0001*	*0.0001*	*0.0001*	-	5.2%	0.01%	2.0%
M. vancouverensis Mariner Cave	(10)	*0.0175*	0.1294	0.3622	0.8261	*0.0001*	*0.0001*	*0.0001*	*0.0034*	0.2140	-	7.3%	15.0%
M. vancouverensis Limestone Cave	(11)	0.1540	0.1166	0.9892	0.0866	*0.0001*	*0.0001*	*0.0001*	*0.0001*	0.9496	0.2226	-	4.7%
M. vancouverensis Clayoquot Cave	(12)	0.8468	0.0877	0.4981	0.0599	*0.0001*	*0.0003*	*0.0001*	*0.0001*	0.4662	0.1542	0.3726	-

Table 6.

population	shape		size	
	VAR	SE_{VAR}	VAR	SE_{VAR}
M. c. caligata	0.001594	0.000142	10.7	2.3
M. c. cascadensis	0.001479	0.000118	15.8	3.2
M. c. okanagana	0.001794	0.000323	10.7	5.4
M. c. oxytona	0.001585	0.000177	12.8	3.7
M. f. avara	0.001662	0.000144	19.7	3.8
M. f. flaviventris	0.001677	0.000196	34.7	9.7
M. f. nosophora	0.001666	0.000107	19.9	3.2
M. Olympus	0.000905	0.000091	6.3	1.5
M. vancouverensis modern	0.001055	0.000124	10.4	2.3
M. vancouverensis Mariner Cave	0.000856	0.000111	14.8	5.4
M. vancouverensis Limestone Cave	0.001414	0.000225	5.7	1.5
M. vancouverensis Clayoquot Cave	0.000868	0.000180	8.2	2.8

Table 7.

population		(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)
M. caligata caligata	(1)	-	-	-	-	-	-	-	-	-	-	-
M. c. cascadensis	(2)	0.6258	-	-	-	-	-	-	-	-	-	-
M. c. okanagana	(3)	0.9431	0.6875	-	-	-	-	-	-	-	-	-
M. c. oxytona	(4)	0.8472	0.8239	0.8456	-	-	-	-	-	-	-	-
M. flaviventris avara	(5)	0.7770	0.4485	0.9258	0.6774	-	-	-	-	-	-	-
M. f. flaviventris	(6)	0.8756	0.5616	0.9658	0.7567	0.9496	-	-	-	-	-	-
M. f. nosophora	(7)	0.5012	0.2162	0.7653	0.4479	0.7676	0.7542	-	-	-	-	-
M. olympus	(8)	*0.0015*	*0.0016*	*0.0090*	*0.0043*	*0.0014*	*0.0016*	*0.0004*	-	-	-	-
M. vancouverensis modern	(9)	*0.0054*	*0.0122*	0.0534	*0.0218*	*0.0037*	*0.0111*	*0.0003*	0.4270	-	-	-
M. vancouverensis. Mariner Cave	(10)	*0.0047*	*0.0020*	*0.0099*	*0.0084*	*0.0032*	*0.0027*	*0.0002*	0.6114	0.2992	-	-
M. vancouverensis Limestone Cave	(11)	0.3114	0.4664	0.4496	0.4411	0.2439	0.2988	0.1026	0.0803	0.2245	0.0659	-
M. vancouverensis Clayoquot Cave	(12)	*0.0099*	*0.0067*	*0.0341*	*0.0176*	*0.0088*	*0.0080*	*0.0011*	0.4464	0.2610	0.7053	0.0987

Table 8.

population		(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)
M. caligata caligata	(1)	-	-	-	-	-	-	-	-	-	-	-
M. c. cascadensis	(2)	0.1455	-	-	-	-	-	-	-	-	-	-
M. c. okanagana	(3)	0.7883	0.2737	-	-	-	-	-	-	-	-	-
M. c. oxytona	(4)	0.5455	0.5087	0.5346	-	-	-	-	-	-	-	-
M. flaviventris avara	(5)	0.0753	0.7001	0.2410	0.3527	-	-	-	-	-	-	-
M. f. flaviventris	(6)	*0.0016*	0.0536	0.0517	*0.0269*	0.1246	-	-	-	-	-	-
M. f. nosophora	(7)	*0.0329*	0.5449	0.1841	0.2370	0.8474	0.1242	-	-	-	-	-
M. olympus	(8)	0.4447	0.0697	0.7472	0.2172	0.0652	*0.0034*	*0.0334*	-	-	-	-
M. vancouverensis modern	(9)	0.8684	0.2407	0.7100	0.6760	0.1616	*0.0063*	0.0940	0.3338	-	-	-
M. vancouverensis. Mariner Cave	(10)	0.6331	0.6542	0.5950	0.9698	0.5384	0.1194	0.4431	0.2863	0.7091	-	-
M. vancouverensis Limestone Cave	(11)	0.3773	0.0563	0.6646	0.1774	0.0576	*0.0030*	0.0305	0.8613	0.2714	0.2449	-
M. vancouverensis Clayoquot Cave	(12)	0.8364	0.3225	0.9734	0.5770	0.3051	0.0688	0.2423	0.6643	0.7324	0.6018	0.5584

Table Legend

Table 1. Summary of environmental changes on Vancouver Island during the past 15,000 years and associated *Marmota vancouverensis* samples used in study. Sources: Claque (1981), Hebda (1995), Bryant (1996), Nagorsen et al. (1996), Nagorsen and Keddie (2000), Laroque et al. (2001), Lewis and Smith (2004), Al_Suwaidi et al. (2006).

Table 2. Sample sizes of the Petromarmota group (*Marmota caligata*, *Marmota flaviventris, Marmota olympus,* and *Marmota vancouverensis*) used in analyses.

Table 3. 1000 permutation tests for differences in size and shape between left and right hemimandibles of *Petromarmota* species.

Table 4. 10000 permutation tests for pairwise differences in shape of 12 *Petromarmota* samples. P values and variances explained by interspecific differences are shown respectively below and above main diagonal.

Table 5. 10000 permutation tests for pairwise differences in size of 12 *Petromarmota* samples. P values and variances explained by interspecific differences are shown respectively below and above main diagonal.

Table 6. Variance of shape and centroid size with bootstrap standard errors for 12 *Petromarmota* samples.

Table 7. Results of Levene's test for pairwise differences in shape variance of 12 *Petromarmota* samples (10000 random permutations).

Table 8. Results of Levene's test for pairwise differences in size variance of 12 *Petromarmota* samples (10000 random permutations).