



The influence of environmental factors on the morphology of red-backed voles *Myodes gapperi* (Rodentia: Arvicolinae) in Québec and western Labrador

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Received 9 October 2013; revised 8 January 2014; accepted for publication 8 January 2014

Geographical patterns of morphological variation in small mammals are often associated with environmental factors. The southern red-backed vole *Myodes gapperi* is a widespread and abundant small mammal in Canada, occurring in environments as diverse as mixed-wood forests and taiga. First upper molars and skulls from nine populations of southern red-backed voles distributed across three ecozones and approximately 10° of latitude were analysed by means of geometric morphometric techniques, and their relationships with environmental variables were examined. A weak, non-linear trend of size increase towards higher latitudes was observed in voles' skulls. Environmental variables appeared to be important drivers of shape differentiation among populations from the three distinct ecozones analysed. © 2014 The Linnean Society of London, *Biological Journal of the Linnean Society*, 2014, **112**, 204–218.

ADDITIONAL KEYWORDS: Ecozones – environmental gradient – geometric morphometrics.

INTRODUCTION

The relationship between intraspecific morphological variation and environmental factors has been widely documented in mammals (e.g. Renaud & Millien, 2001; Monteiro, Duarte & Reis, 2003; Piras *et al.*, 2010). Gradients of morphological variation often parallel physiogeographical gradients, which led to empirical generalizations in the form of ecogeographical rules. Of these, Bergmann's rule, the negative relationship between body size and temperature in endotherms (Mayr, 1956), is a classical example.

Climate is hypothesized to influence the morphology of endotherms either directly, through thermoregulatory mechanisms (Scholander, 1955; Mayr, 1956; Brown & Lee, 1969), or indirectly, through its influence on food type and abundance in the environment

(McNab, 1971, 2010; Geist, 1987; Renaud & Michaux, 2003; Cardini, Jansson & Elton, 2007; Piras *et al.*, 2012). Climate-related morphological variation in endotherms has been observed both at the spatial and at the temporal scales (Millien *et al.*, 2006; Teplitsky & Millien, 2013). Examples of temporal variation studies include investigations of variation in external measurements and body mass of heteromyid rodents over 8 years (Wolf, Friggens & Salazar-Bravo, 2009), size and shape of skulls and mandibles of shrews over 27 years (Poroshin, Polly & Wójcik, 2010), external measurements and body mass of shrews over 54 years (Yom-Tov & Yom-Tov, 2005), and size and shape of teeth of the related fossil rodents *Apodemus* and *Stephanomys* over 9 Myr (Renaud *et al.*, 2005). Morphological variation in space is commonly described in terms of more or less gradual changes in the size and shape of some anatomical structure, such as skulls (Cardini *et al.*, 2007, Martínez & Di Cola, 2011), teeth (McGuire, 2010; Ledevin *et al.*, 2010a) or mandibles (Renaud & Millien, 2001), across a species' geographical distribution. Typically, a relationship is then

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described between the spatial morphological variation and a set of environmental variables.

The pattern of geographical variation in a trait of a given species can result from past migration history of this species, which can then be inferred through a phylogeographical study. The variation in morphology can also be a response to the environment and be driven by several mechanisms, including evolutionary adaptation (e.g. Endler, 1995; Ims, 1997), or phenotypic plasticity (e.g. Meyer, 1987; Peres-Neto & Magnan, 2004). Genetic drift can also influence phenotypic variation, especially in the context of changing environmental conditions. These processes are non-exclusive, and the morphology of individuals in a population may reflect an interaction between them (Renaud & Michaux, 2003; Ackermann & Cheverud, 2004; Ledevin & Millien, 2013). Identifying the environmental factors that are correlated with variation in size and shape of a structure may help better understand how these factors influence the spatial patterns of morphological variation.

Given its wide geographical distribution, its abundance and ability to thrive in various environments, the southern red-backed vole (*Myodes gapperi*, Vigors 1830) provides a good model to investigate the effects of environmental factors on size and shape variation in small mammals. The southern red-backed vole is a small arvicoline rodent widespread in North America, living in environments as diverse as deciduous forests and tundra, although showing a clear preference for forested habitats (Merritt, 1981). In Québec, these voles are distributed throughout the province, except for the north of the Ungava Peninsula and the north-eastern tip of the Labrador Peninsula (Merritt, 1981; Desrosiers, Morin & Jutras, 2002), where they are replaced by lemmings (*Dicrostonyx hudsonius*) (Desrosiers *et al.*, 2002). The range of red-backed voles in Québec displays marked gradients of temperature and precipitation (Gérardin & McKenney, 2001), a marked gradient of net primary productivity (Liu *et al.*, 2002) and a succession of ecozones (Ecological Stratification Working Group, 1995). Southern red-backed voles are opportunistic omnivores; although the bulk of their diet is composed of fungi and vegetative plant parts (Orrock & Pagels, 2002), their feeding habits change considerably according to the spatial and seasonal availability of food items (Schloyer, 1977; Martell, 1981; Merritt, 1981). These animals remain active throughout the winter, moving mostly in the subnivean space between the ground and the snow sheet (Merritt & Merritt, 1978).

In this study we investigated the relationships between morphological variation in red-backed vole populations and climate, productivity and ecozones along a latitudinal gradient in Québec. We first

assessed if the size of skulls and teeth, as proxies for body size, would conform to Bergmann's rule. We also tested the hypothesis that skulls and teeth of voles from distinct ecozones would have significantly different shapes and identified the environmental factors that best correlated with shape variation in these structures. We expected to detect contrasting patterns of morphological variation in the tooth – a structure mostly related to food acquisition, and the skull – the shape of which is also probably constrained by sensory functions such as olfaction or vision, in addition to food acquisition.

MATERIAL AND METHODS

SPECIMENS

A total of 260 voles were collected from 2006 to 2011 in Québec and western Labrador. Most of the populations were sampled in summer (July–August), except for Plée Bleue and Lake Champlain, which were sampled in autumn (end of September). The nine populations sampled were distributed across approximately 10° in latitude, or roughly 1000 km along a south–north gradient (Fig. 1), spanning three ecozones. Ecozone classification is a broad generalization of biotic and abiotic characteristics of large tracts of land (Wiken, 1986), which is based on the known conditions of climate, landform, biota and soils (Rowe & Sheard, 1981). The ecozones in which our study populations were included are the Mixedwood Plains, the Boreal Shield and the Taiga Shield (Ecological Stratification Working Group, 1995).

Only adult specimens (third upper molar fully erupted) were considered for the analyses. We used a subsample of 212 specimens for the analysis of skulls, as some specimens had damaged skulls. For the tooth analyses, some specimens were excluded due either to excessive tooth wear or to abnormal morphology (e.g. the absence of dental triangle). These outliers were identified as specimens outside the quartiles of boxplots for univariate data (size analysis); multivariate outliers were identified as specimens with Mahalanobis distances larger than the critical value from a chi-squared distribution with $P < 0.001$ (shape analysis). We thus used 257 specimens for tooth size and 244 specimens for tooth shape analyses (Table 1).

ENVIRONMENTAL VARIABLES

Ten climate variables were considered to characterize the local environment of vole populations (Table 2). Climate variables were extracted from WorldClim (Hijmans *et al.*, 2005) and followed the BIOCLIM arrangement originally proposed by Nix (1986). Net primary productivity (NPP, total annual net primary

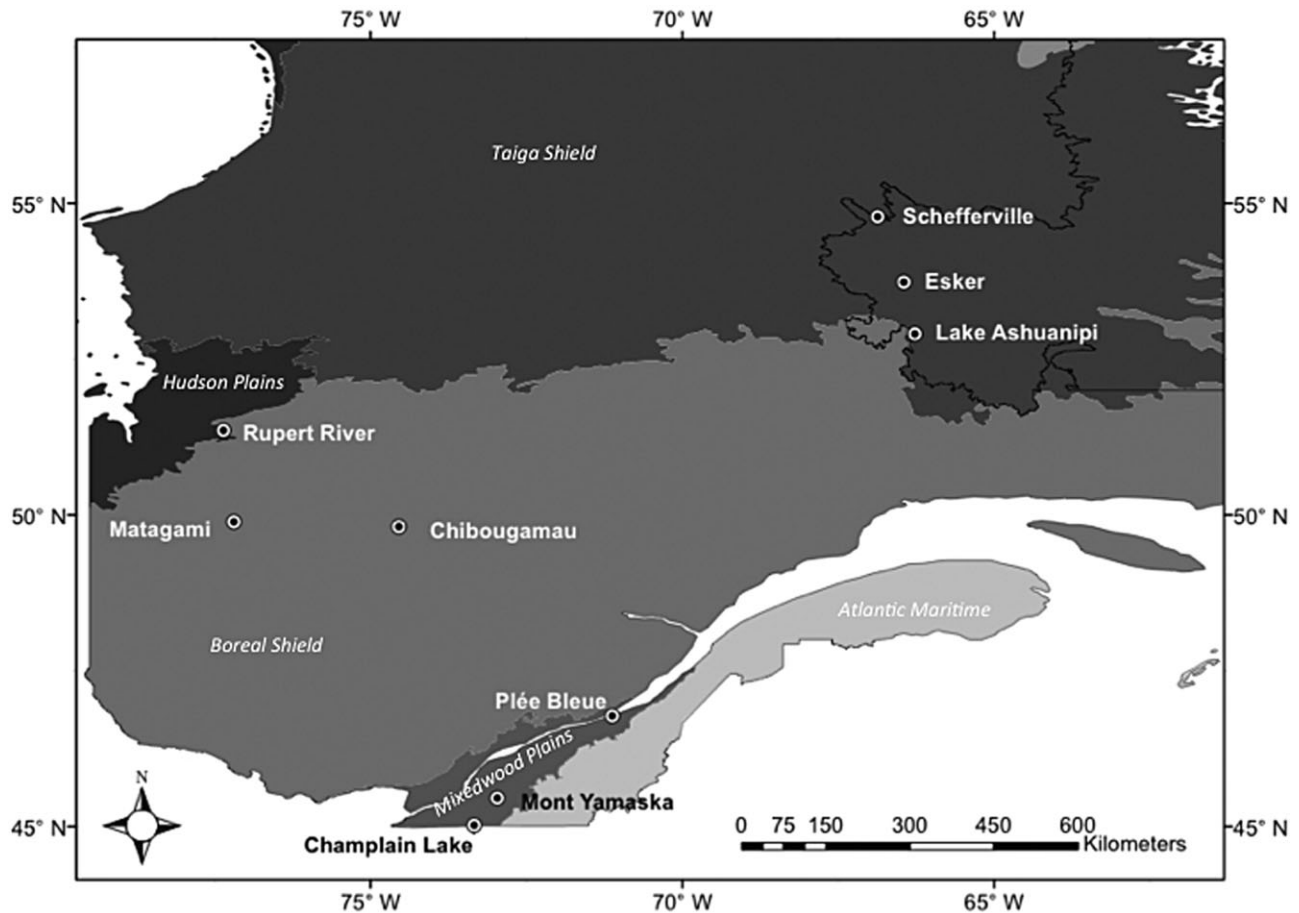


Figure 1. The nine vole populations sampled in Québec and western Labrador. Ecozones are represented in different shades of grey. From south to north, the ecozones sampled are the Mixedwood Plains, Boreal Shield and Taiga Shield.

Table 1. Populations sampled, total number of adults analysed and sample size for each analysis

| Population | Latitude (°N) | Longitude (°W) | Sample size | | | | |
|--------------------------|---------------|----------------|-------------|---------|----------|------------|-------------|
| | | | Adults | M1 size | M1 shape | Skull size | Skull shape |
| Lake Champlain | 45.02 | -73.25 | 23 | 22 | 22 | 13 | 13 |
| Monts Yamaska/St Hilaire | 45.46 | -72.88 | 14 | 14 | 13 | 13 | 13 |
| Grande Plée Bleue | 46.77 | -71.05 | 34 | 34 | 34 | 21 | 21 |
| Chibougamau | 49.81 | -74.46 | 36 | 36 | 34 | 33 | 33 |
| Matagami | 49.89 | -77.11 | 18 | 18 | 17 | 17 | 17 |
| Rupert River | 51.36 | -77.40 | 9 | 9 | 8 | 9 | 9 |
| Lake Ashuanipi | 52.91 | -66.19 | 27 | 25 | 24 | 27 | 27 |
| Esker | 53.73 | -66.36 | 32 | 32 | 31 | 32 | 32 |
| Schefferville | 54.78 | -66.78 | 67 | 67 | 61 | 47 | 47 |
| Total | | | 260 | 257 | 244 | 212 | 212 |

productivity in $\text{gC m}^{-2} \text{ year}^{-1}$) was also included in the analyses as an environmental variable. NPP was calculated with the EALCO model (Wang, Trishchenko & Sun, 2007; Wang, 2008; Wang *et al.*, 2009), and the

mean NPP values from 1999 to 2008 were used as proxies for local productivity. These climatic and productivity variables were henceforth collectively referred to as the environment.

Table 2. BIOCLIM variables used to describe the climate of sampling sites

| Bioclimatic variable | Description |
|----------------------|--------------------------------------|
| BIOCLIM1 | Annual Mean Temperature |
| BIOCLIM4 | Temperature Seasonality |
| BIOCLIM5 | Maximum Temperature of Warmest Month |
| BIOCLIM6 | Minimum Temperature of Coldest Month |
| BIOCLIM10 | Mean Temperature of Warmest Quarter |
| BIOCLIM11 | Mean Temperature of Coldest Quarter |
| BIOCLIM12 | Annual Precipitation |
| BIOCLIM15 | Precipitation Seasonality |
| BIOCLIM18 | Precipitation of Warmest Quarter |
| BIOCLIM19 | Precipitation of Coldest Quarter |

Temperature variables are given in °C, precipitation variables are given in millimetres. Temperature seasonality is the standard deviation of weekly mean temperatures, and precipitation seasonality is the standard deviation of the weekly precipitation estimates expressed as a percentage of the mean of those estimates.

IMAGE ACQUISITION

Skulls were photographed with a Lumenera Infinity 1 digital camera mounted on a Leica MS5 stereomicroscope. Skulls were placed in a container filled with beads with the ventral surface facing up, and were partially submerged in the beads so that the occlusal surface of the molars was parallel to the lens of the stereomicroscope (Fig. 2). A Leica $\times 0.32$ Achromat lens was used for the acquisition of skull images, and a Leica $\times 1.0$ Plan lens was used for the acquisition of teeth images. For each specimen the first upper right molar was preferentially used; when it was damaged or absent, the first upper left molar was photographed and the flipped image was used instead.

OUTLINE ANALYSIS OF TEETH

An outline analysis was performed on first upper molars (M1, Fig. 2). Two methods have been used in the past to quantify the shape of arvicoline rodent teeth, both relying on points recorded along the outline of the tooth: the sliding semi-landmarks method (e.g. Piras *et al.*, 2010) and the Fourier analysis of the outline (e.g. Ledevin, Quéré & Renaud, 2010b). Here, we used the latter method and outlines were manually traced around the external edge of the occlusal surface along the enamel crest starting at the innermost point of the most anterior lingual re-entrant angle (Fig. 2) using TPS Dig 2.16 (Rohlf, 2010). The outlines were resampled as 64 equally spaced points. Outline area was calculated and an elliptic Fourier transform was performed. The

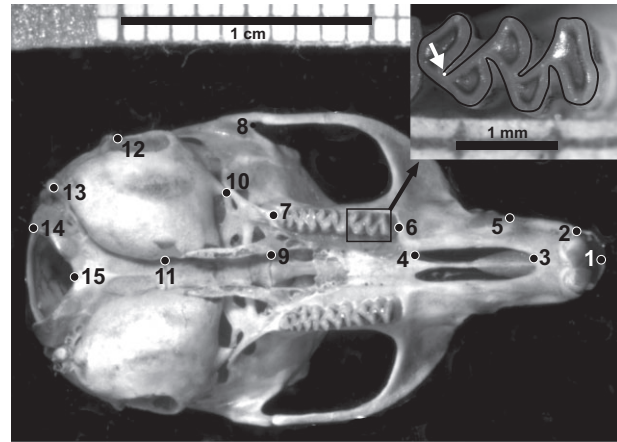


Figure 2. Skull of a red-back vole in ventral view. A total of 15 landmarks were used for the analyses of skull shape: 1, anterior extremity of the suture between nasals; 2, lateralmost point of incisive alveolus; 3, anterior margin of the incisive foramina; 4, posterior margin of the incisive foramina; 5, suture between premaxilla and maxilla where it intercepts the skull outline on the plan of the photo; 6, anterior extremity of first upper molar where it intercepts the maxillary; 7, posterior extremity of third upper molar where it intercepts the maxillary; 8, posterior point of maximum curvature of the zygomatic arch; 9, lateralmost point of the suture between presphenoid and basisphenoid; 10, tip of Eustachian tube; 11, suture between basisphenoid and basioccipital where it contacts the tympanic bulla; 12, mastoid apophysis where it intercepts the superior edge of the auditory meatus on the plan of the photo; 13, tip of paraoccipital process; 14, most posterior point of occipital condyle; 15, anterior extremity of foramen magnum (basion). Inset: outline of the first upper molar (M1) in occlusal view; arrow indicates the starting point of the outline.

square root of the outline area was used as an estimate of tooth size. For shape analysis, outlines were standardized for size and orientation using the area and orientation of the ellipse described by the first harmonic, which is the best-fitting ellipse of the outline.

To determine the number of harmonics to use in our analyses, two factors were considered: the cumulative shape information and the measurement error introduced by an increasing number of harmonics (e.g. Renaud & Millien, 2001; Ledevin *et al.*, 2010a). Measurement error and cumulative shape information were estimated by ten repetitions of outline acquisition on one specimen. Measurement error on each harmonic was calculated as the percentage of the mean amplitude represented by the standard deviation of repetitions. The cumulative information content contributed by each harmonic (an estimate of

shape information) was calculated by summing the mean amplitudes of all harmonics and then computing the cumulative per cent of the total amplitude represented by each harmonic added. We retained the first ten harmonics that represented 92.6% of shape information, while displaying low measurement error (between 0.06% for the first harmonic and 2.93% for the tenth harmonic). We thus obtained 37 coefficients (40 minus the first three coefficients of the first harmonic, which represent residuals after normalization) that we used as shape variables.

Tooth wear has been recognized as a major source of morphological variation in arvicoline teeth (Fominykh *et al.*, 2010; Guérécheau *et al.*, 2010; Ledevin *et al.*, 2010b), acting as a confounding factor when analysing covariates of tooth morphology. Therefore, we developed a tooth wear classification method based on the number of closed dentinal space isthmuses (Abe, 1973), continuity of the enamel crest and presence of dentin in the re-entrant angles (Appendix 1). Teeth were classified in five wear stages. The shape variables obtained with the elliptic Fourier method were regressed on wear stages to control for the effect of wear on tooth shape, and the residuals from this regression were the shape variables used in all statistical analyses. Similarly, we used the residuals from a regression of tooth size on wear stages as our size variables to control for the effect of wear on size. Lastly, we found that skull size was positively correlated with tooth wear, and we took into account the effect of size on skull shape to control for age (or tooth wear).

GEOMETRIC MORPHOMETRIC ANALYSIS OF SKULLS

Fifteen landmarks were digitized on the ventral surface of skulls (Fig. 2) using TPS Dig 2.16 (Rohlf, 2010). Landmarks were only digitized on the right side of the skull to reduce the number of shape variables in comparison with sample sizes. Landmark coordinates were submitted to a Procrustes superimposition and projected on the tangent space. The obtained Procrustes coordinates were regressed on log-transformed centroid size (our measure of size) to control for allometry. Residuals from this regression were used as shape variables in all multivariate statistical analyses.

To estimate the magnitude of digitizing errors present in the landmark data, we randomly selected one specimen from each of nine populations, reproduced their images nine times, randomly ordered the resulting 81 images and digitized landmarks on all of them. These 81 configurations were then superimposed and submitted to a Procrustes analysis of variance (ANOVA) using specimen as a factor. The proportion of the total sum of squares represented by the factor and the residual were then used to estimate

the percentage of variation due to digitization error. Digitization error represented 0.04% of skull size variation and 2.77% of skull shape variation.

PRELIMINARY ANALYSES

Sexual dimorphism and differences between populations were investigated using ANOVAs of size and a permutational (non-parametric) multivariate ANOVA (MANOVA) (Anderson, 2001) of shape performed on dissimilarity matrices, with sex and population as factors, as well as the interaction factor between sex and population.

We then used a subset of 182 specimens to assess whether the square root of M1 occlusal area and skull centroid size were good proxies for body size. We calculated the Pearson correlation coefficients and associated *P* values between these two log-transformed size estimators, the total body length (distance from the tip of the nose to the tip of the tail) and body mass measured on the specimens.

SIZE AND SHAPE VARIATION

To detect a possible latitudinal trend in size variation we first conducted a linear regression using tooth or skull size as the response variable and latitude as the explanatory variable.

A second set of analyses was then performed using ecozones as a factor. ANOVAs on size were performed using the R package stats (R Development Core Team, 2012), while permutational MANOVAs were performed on shape variables using the R package vegan. Size differences among ecozones identified in the ANOVAs were further investigated with Tukey's HSD post-hoc mean comparison tests. Shape differences among ecozones identified in the permutational MANOVAs were further investigated with Hotelling's T^2 mean comparison post-hoc tests using the R package Hotelling. A Bonferroni correction was applied to multiple comparison tests. To further investigate the relationship between ecozones and tooth and skull shape variation in our samples, we performed a between-group principal components analysis (gPCA) (Mitteroecker & Bookstein, 2011) using the R package Morpho.

We then used a hierarchical partition analysis (Chevan & Sutherland, 1991) using the R package hier.part to identify the environmental factors that best predicted tooth and skull size. Hierarchical partition analysis alleviates multicollinearity problems as it averages the importance of each variable in all multiple regression models possible with a given set of variables (Mac Nally, 2002). Finally, we used a two-block partial least squares analysis (PLS) to characterize the relationship between shape variation and

environmental variation, using the ten climate variables and NPP as the environment block (Rohlf & Corti, 2000). This analysis is also well suited for accounting for multicollinearity in the data. Teeth PLS was performed using the software PAST version 2.15 (Hammer, Harper & Ryan, 2001) and skulls PLS was performed using the software MorphoJ version 1.05a (Klingenberg, 2011).

RESULTS

SEXUAL DIMORPHISM

No sexual dimorphism was detected in size or shape of skulls and teeth (all $P > 0.05$). Interaction factors of sex*population were not significant either (all $P > 0.05$). Sexes were thus pooled together for further analyses.

EFFECT OF WEAR STAGE ON TOOTH SIZE AND SHAPE

There was a strong effect of wear stage on the size of the first upper molar ($F = 116$, $R^2 = 0.31$, $P < 0.001$). More worn teeth were larger, and pairwise differences in size among teeth in different wear stages were all significant except for adjacent wear stages (i.e. all $P < 0.01$, except for 0–1, 1–2, 2–3, 3–4). Subsequent analyses were thus conducted on ‘wear-controlled’ tooth size, using the residuals from a regression of tooth size on wear stages. We also found a significant effect of wear stage on the shape of the molar (Wilk’s lambda = 0.19, $P < 0.001$). All subsequent analyses were thus conducted on ‘wear-controlled’ shape variables, using the residuals of the regression of the original shape variables on wear stages.

RELATIONSHIP OF TOOTH SIZE AND SKULL SIZE WITH OTHER SIZE MEASUREMENTS

The square root of M1 area increased with total body length ($R^2 = 0.16$, $P < 0.001$) and body mass ($R^2 = 0.33$, $P < 0.001$), although these trends were weaker when considering the M1 size corrected for wear ($R^2 = 0.02$, $P = 0.08$ for body length and $R^2 = 0.06$, $P < 0.001$ for body mass). The skull centroid size appeared to be a better size proxy than the first upper molar for both total body length ($R^2 = 0.41$, $P < 0.001$) and body mass ($R^2 = 0.62$, $P < 0.001$).

SIZE VARIATION WITH LATITUDE

The size of the first upper molar decreased with latitude (slope = -0.002 , $t = -2.27$, $R^2 = 0.02$, $P = 0.024$), while skull size increased towards higher latitudes (slope = 0.004 , $t = 5.57$, $R^2 = 0.13$, $P < 0.001$). While significant, both these size trends appeared to be weak.

SIZE AND SHAPE VARIATION ACROSS ECOZONES

An ANOVA on M1 size revealed significant differences between ecozones ($F = 7.43$, $R^2 = 0.06$, $P < 0.001$). Voles from the Mixedwood Plains had significantly larger teeth than those from voles in the Boreal Shield and Taiga Shield (Table 3, Fig. 3A). An ANOVA on log centroid size revealed significant differences in skull size between ecozones ($F = 24.63$, $R^2 = 0.19$, $P < 0.001$). Post-hoc tests showed that voles from the Taiga Shield had skulls significantly larger than those from the Mixedwood Plains and the Boreal Shield (both $P < 0.001$, Fig. 3B). Skull size was not significantly different between voles from the Mixedwood Plains and the Boreal Shield ($P = 0.51$).

A permutational MANOVA identified significant differences in tooth shape among ecozones ($F = 8.07$, $P < 0.001$), and post-hoc tests revealed significant differences in tooth shape between all three ecozones (all $P < 0.001$, Table 3). In the between-group PCA analysis, voles from the Taiga Shield tended to be more on the positive side of gPC1 (Fig. 4A). Higher scores on gPC1 represented teeth in which the labial side presented less prominent dental triangles and anterior loop, and deeper and more posteriorly oriented re-entrant angles (Fig. 4C). Specimens from the Boreal Shield were located mostly on the positive side of the second axis gPC2, and those from the Mixedwood Plains mostly on the negative side of gPC2 (Fig. 4B). Increasing scores on gPC2 represented mostly a decrease in relative size of the anterior loop.

A permutational MANOVA revealed significant differences in skull shape among ecozones ($F = 5.55$, $P < 0.001$), and post-hoc tests indicated significant pairwise differences between all three ecozones (all $P < 0.001$; Table 3). The between group PCA showed a segregation of skulls of voles from the Mixedwood Plains on the first principal axis gPC1 (Fig. 5A). Specimens from the Mixedwood Plains were mostly on the negative side of gPC1, while those from the two other ecozones tended to be on the positive side of this

Table 3. Pairwise differences in morphology between ecozones

| | Skull | | Molar | |
|--------------------|-------------------|-------------------|---------------|-------------------|
| | Size | Shape | Size | Shape |
| Mixedwood – Boreal | 0.3557 | <0.0001 | 0.0011 | <0.0001 |
| Mixedwood – Taiga | <0.0001 | <0.0001 | 0.0035 | <0.0001 |
| Boreal – Taiga | <0.0001 | <0.0001 | 0.6498 | <0.0001 |

Numbers are P values of Tukey’s HSD tests for size and permutation P values of Hotelling’s T^2 tests for shape. Values in bold are significant after Bonferroni correction.

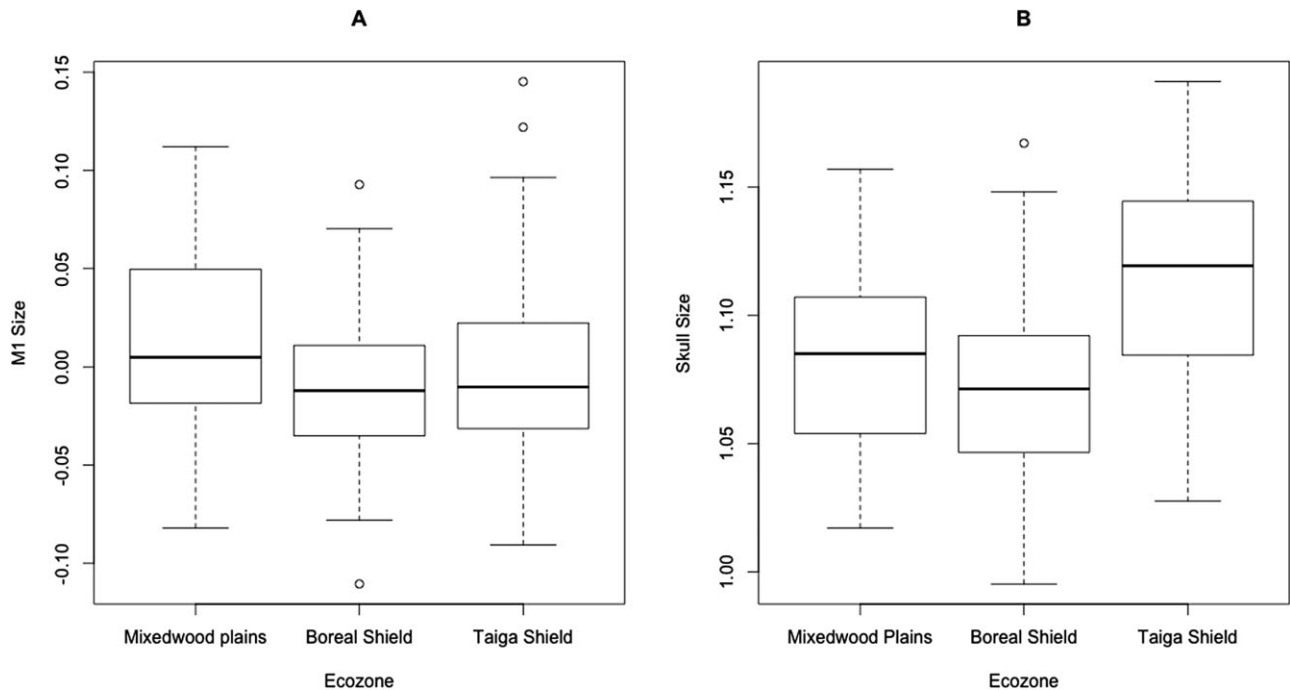


Figure 3. Variation in the first upper molar (A) and skull (B) size across the three ecozones in our study area. The boxes delimitate the 25th and 75th quartiles, the horizontal bar indicates the median, the whiskers extend to the most extreme data points (outliers not considered), and outliers are displayed with an open circle. Redbacked voles were larger in M1 size in the Mixedwood Plains than in the Taiga Shields or Boreal Forest. The size of the skull was smallest in the Boreal Shield, resulting in a U-shaped pattern of size variation with latitude.

axis. Lower scores on gPC1 represented skulls with narrower temporal fossae and tooth rows positioned more laterally (Fig. 5C). The second principal component, gPC2, segregated Boreal Shield specimens, mostly on the negative side of the axis, from Taiga Shield specimens, which tended to be positioned more towards the positive side of gPC2. Lower scores on gPC2 represented generally wider skulls, with larger tympanic bulla and wider zygomatic arches (Fig. 5B).

SIGNIFICANT ENVIRONMENTAL VARIABLES

A hierarchical partition analysis indicated that four environmental variables equally contributed to M1 size variation, all significant (all $P < 0.05$) and with an explanatory power ranging from 10.79 to 12.75%. In order of importance, they were mean temperature of the coldest month, temperature seasonality, mean temperature of the coldest quarter and precipitation seasonality. For skull size, the environmental variables with the highest explanatory power were precipitation of the warmest quarter and total annual precipitation, as well as mean temperature of the warmest quarter, representing respectively 18.44, 14.82 and 10.10% of the independent explanatory power of all environmental variables (all $P < 0.05$).

In the two-block PLS analysis, the first pair of PLS axes explained 92.35% of the total covariation between M1 shape and the environment, and M1 shape was significantly associated with the environment on the first PLS axis ($r = 0.44$, $P < 0.0001$, Fig. 6A). The environmental variables with the highest absolute loadings on the first environment axis were net primary productivity, maximum temperature of the warmest month, annual mean temperature, mean temperature of the warmest quarter and annual precipitation (Table 4). These variables had negative loadings, meaning that higher scores on the shape block were mostly associated with less productive and drier areas presenting colder summers. Temperature and precipitation seasonality presented positive loadings on the first environment axis. Overall, there was an anterior displacement of the labial components and a posterior displacement of the lingual components of the tooth as the environment became colder, drier, more seasonal and less productive. More posteriorly orientated re-entrant angles on the labial side were also evident along the same environmental gradient, and the entire pattern could be summarized as a rotation of the labial and lingual triangles along the antero-posterior axis of the tooth in occlusal view (Fig. 6B).

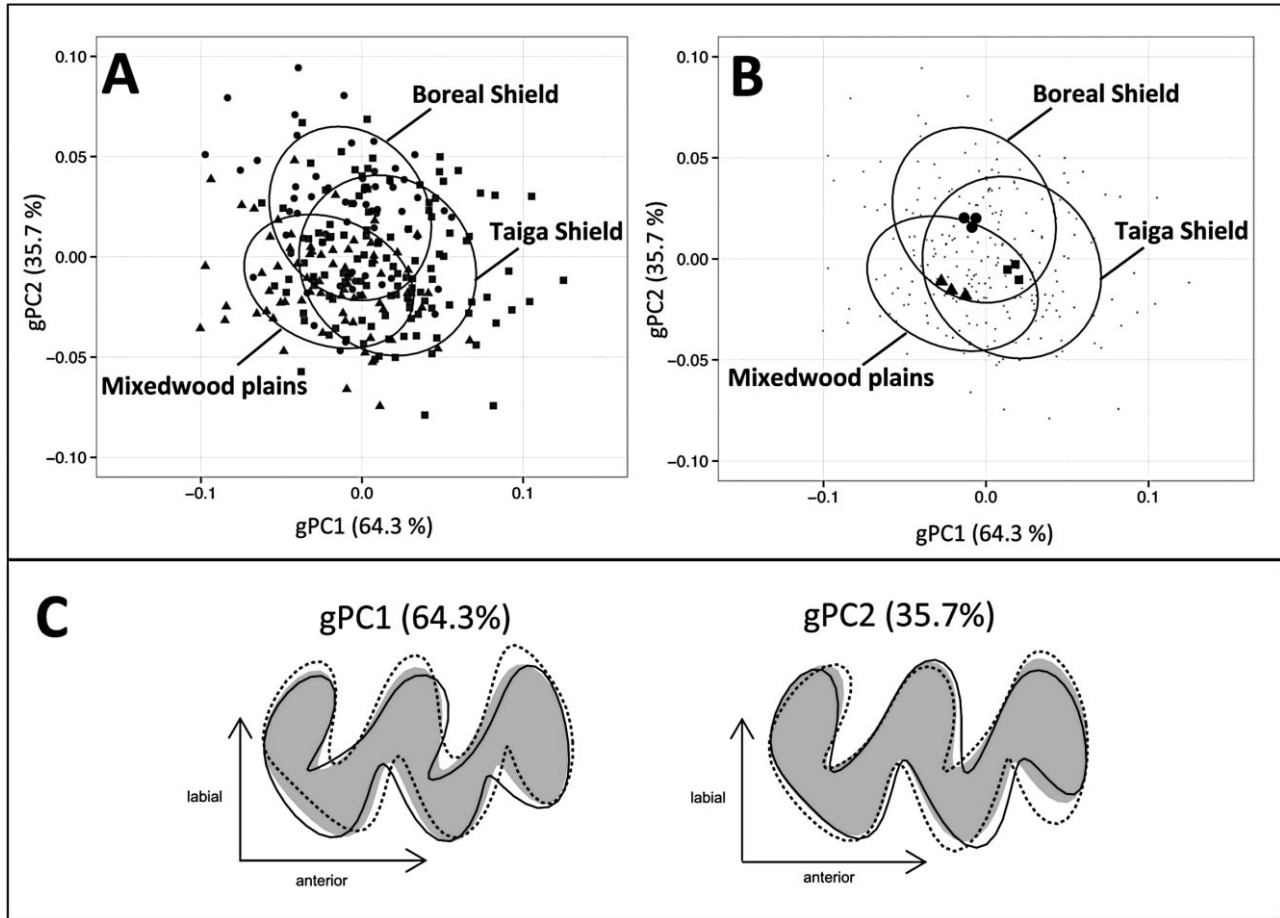


Figure 4. Variation in first upper molar shape in different ecozones represented by a between-group PCA; ellipses define the 95% confidence interval of groups; triangles: Mixedwood Plains, circles: Boreal Shield, squares: Taiga Shield. A, individual data points; B, mean by locality. C, tooth reconstruction along the first two axes of the between-group PCA; the grey shading on the tooth shape reconstructions represents the mean shape, while solid lines and dotted lines represent high score (+3 SD) and low score (−3 SD) shapes, respectively.

The first pair of PLS axes of the two-block PLS analysis accounted for 94.38% of the total covariation between skull shape and the environment ($P < 0.0001$). Scores of the two blocks were highly correlated ($r = 0.62$, $P < 0.0001$, Fig. 7A). Loadings of environmental variables on the environment axis indicated that minimum temperature of the coldest month, mean temperature of the warmest and coldest quarters, mean annual temperature, maximum temperature of the warmest month and net primary productivity contributed the most to the shape variation correlated with environment (Table 4). Both temperature and precipitation seasonality had a positive loading on the environment axis (Table 4). Higher scores on the first shape axis were thus associated with a lower temperature with increased seasonality, and less productive areas. The main environment-related shape change in skulls revealed by the two-block PLS was an increase in the proportion of the

temporal fossa, mainly due to a more rostral position of the anterior part of the zygomatic arch, as the environment becomes colder, less productive and more seasonal (Fig. 7B).

DISCUSSION

SIZE

We found morphological variation in the first upper molars of southern red-backed voles to be strongly affected by tooth wear. Previous studies have also identified a major effect of wear on tooth size and shape in the bank vole *Myodes glareolus* (Fominykh *et al.*, 2010; Guérécheau *et al.*, 2010; Ledevin *et al.*, 2010b). According to these authors, voles trapped in different seasons (e.g. spring and autumn) differ in the degree of wear due to population dynamics. Voles captured in the spring are typically old overwintered

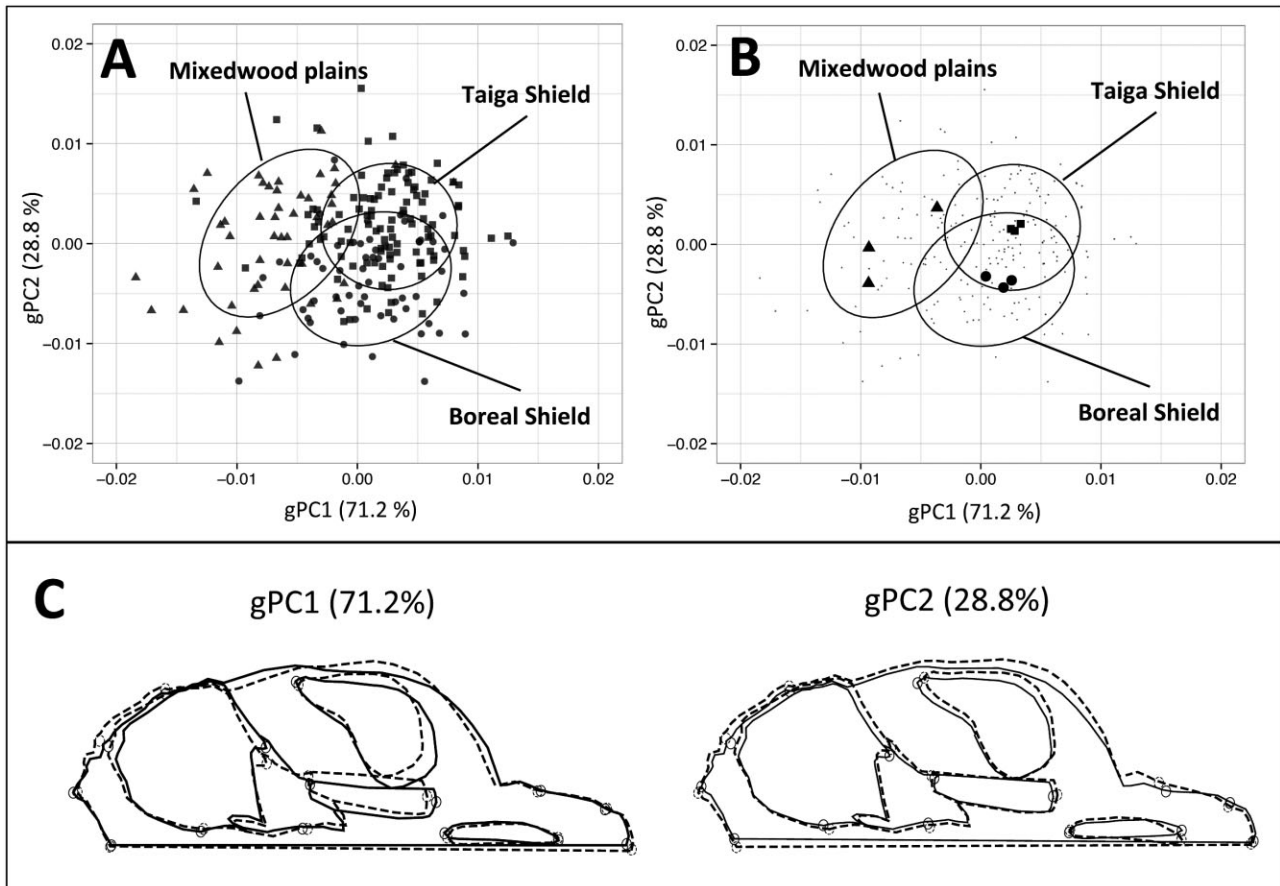


Figure 5. Variation in skull shape in different ecozones represented by a between-group PCA; ellipses define the 95% confidence interval of ecozone means; triangles: Mixedwood Plains, circles: Boreal Shield, squares: Taiga Shield. A, individual data points; B, mean by locality. C, skull reconstruction along the first two axes of the between-group PCA; the solid and dotted lines on the skull shape reconstructions represent high score (+3 SD) and low score (−3 SD) shapes, respectively.

individuals with much worn teeth, while in the summer the population is dominated by voles born within the year with less worn teeth. Our work thus confirms the importance of taking into account confounding factors for the study of tooth morphological variation in voles, and more generally in herbivorous mammals with ever-growing cheek teeth. In our study we would not have detected a larger size in teeth of voles from the Mixedwood Plains if wear had not been controlled for.

The size of the first upper molar was mostly affected by temperature during winter, as well as temperature and precipitation seasonality. Skull size was heavily influenced by the 11 environmental variables used in our analyses. We did not observe a Bergmannian pattern of size variation (Rensch, 1938) in our study area. We found instead a ‘u-shaped’ pattern, with voles from the Mixedwood Plains and the Taiga Shield being on average larger in the size of their skull than voles from the Boreal Shield. Voles from the Mixedwood

Plains also had larger teeth than voles from higher latitudes, thus opposing Bergmann’s rule.

Precipitation had more influence on skull size than temperature, indicating that thermoregulation was not the central mechanism driving skull size variation. However, the relationship between precipitation and skull size was opposite to what should be expected: skulls were larger in drier environments. Precipitation has commonly been associated with plant productivity, and therefore size in herbivorous mammals is expected to be positively correlated with precipitation (Yom-Tov & Geffen, 2006; Cardini *et al.*, 2007; Pergams & Lawler, 2009). The negative correlation between size and precipitation, together with the low explanatory power of net primary productivity, indicated that resource availability was not a major driver of size in voles in Québec. This is in disagreement with the resource rule proposed by McNab (2010), and is opposite to what has been observed in European shrews (Ochocinska & Taylor,

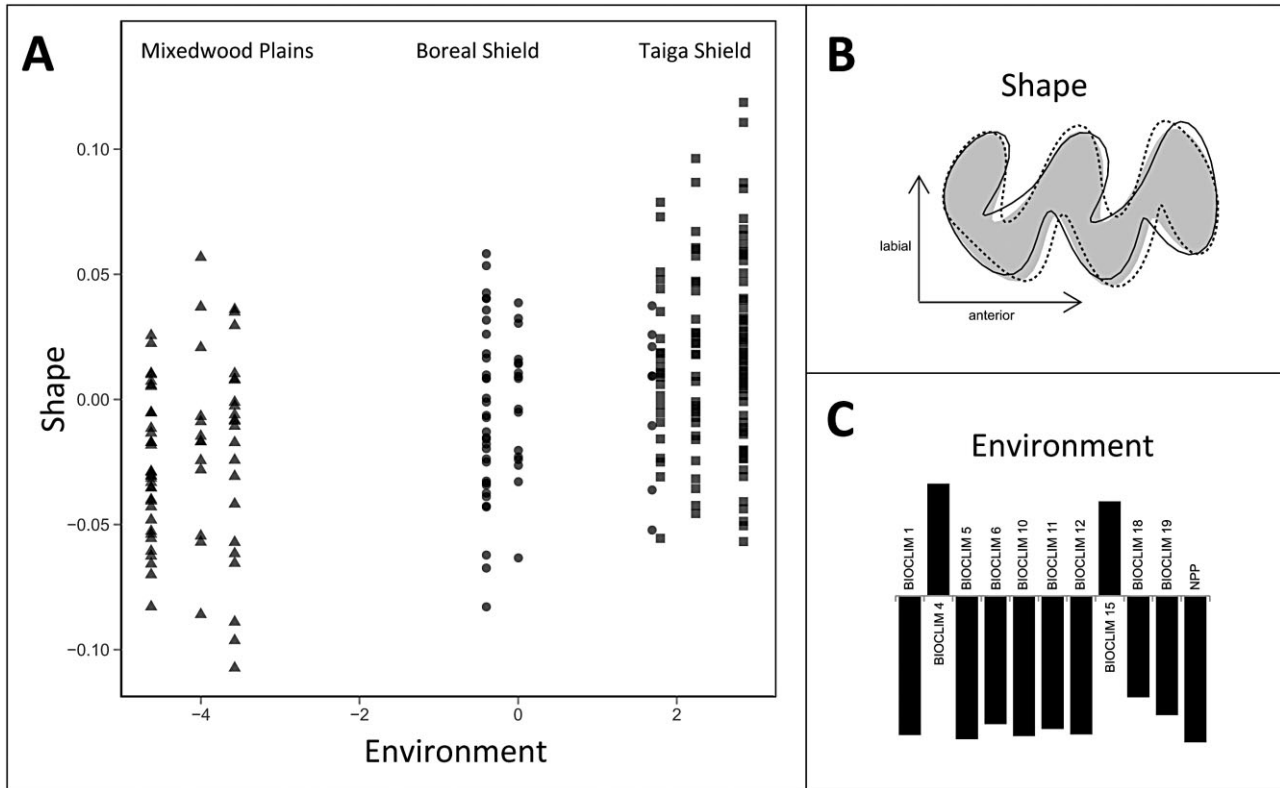


Figure 6. A, two-block partial least squares (PLS) analysis showing the first upper molar shape variation (y -axis) associated with climate variables and net primary productivity (referred to as the environment, x -axis). B, tooth shape reconstruction; the grey shading represents the mean shape, while solid lines and dotted lines represent high score (+3 SD) and low score (-3 SD) shapes, respectively. C, loadings of the environmental variables on the environment axis of the PLS; variable abbreviations as in Table 2.

Table 4. Contribution (%) of environmental variables to size variation in the first upper molar and the skull obtained through a hierarchical partition analysis; loadings and corresponding percentage contribution in parentheses of the environmental variables on the first environmental axis obtained from a two-block PLS analysis between shape and the environment

| Bioclimatic variable | % Contribution (size) | | Loadings on LV1 (shape) | |
|----------------------|-----------------------|-------|-------------------------|----------------|
| | M1 | Skull | M1 | Skull |
| BIOCLIM1 | 9.00 | 9.21 | -0.33 (10.73%) | -0.35 (12.46%) |
| BIOCLIM4 | 12.08 | 5.17 | 0.27 (7.00%) | 0.31 (9.48%) |
| BIOCLIM5 | 7.86 | 9.00 | -0.34 (11.38%) | -0.35 (12.35%) |
| BIOCLIM6 | 12.75 | 6.57 | -0.30 (9.13%) | -0.36 (11.29%) |
| BIOCLIM10 | 7.83 | 10.10 | -0.33 (10.91%) | -0.35 (12.46%) |
| BIOCLIM11 | 11.00 | 7.88 | -0.31 (9.81%) | -0.35 (11.97%) |
| BIOCLIM12 | 7.40 | 14.81 | -0.33 (10.62%) | -0.25 (6.07%) |
| BIOCLIM15 | 10.79 | 3.26 | 0.22 (4.96%) | 0.28 (7.66%) |
| BIOCLIM18 | 7.02 (n.s.) | 18.44 | -0.24 (5.70%) | -0.08 (0.64%) |
| BIOCLIM19 | 6.74 | 7.77 | -0.28 (7.88%) | -0.22 (4.89%) |
| Mean NPP | 7.50 (n.s.) | 7.77 | -0.34 (11.88%) | -0.33 (10.63%) |

The description of bioclimatic variables is given in Table 2; n.s.: $P > 0.05$.

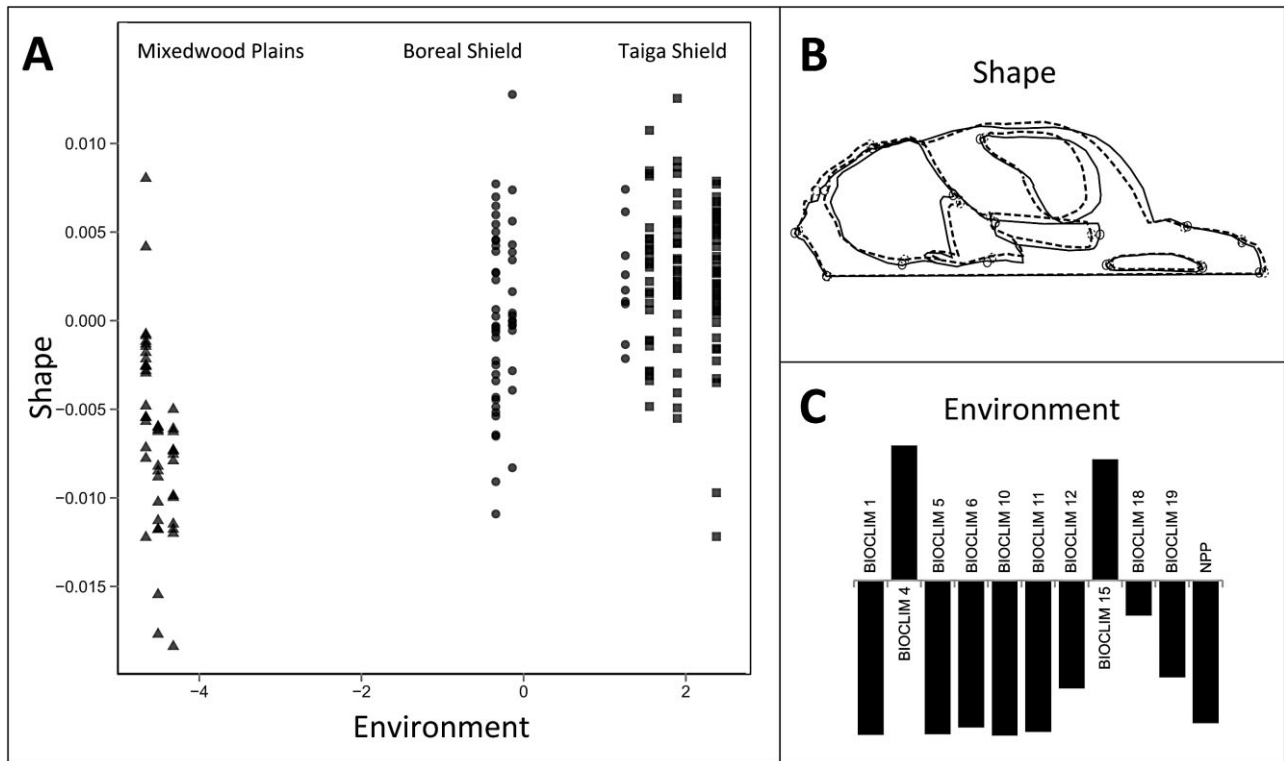


Figure 7. A, two-block partial least squares (PLS) analysis showing the skull shape variation (y-axis) associated with climate variables and net primary productivity (referred to as the environment, x-axis). B, skull reconstruction; the solid and dotted lines represent high score (+3 SD) and low score (-3 SD) shapes, respectively. C, loadings of the environmental variables on the environment axis of the PLS; variable abbreviations as in Table 2.

2003), masked shrews (Yom-Tov & Yom-Tov, 2005), desert rodents (Yom-Tov & Geffen, 2006) and vervet monkeys (Cardini *et al.*, 2007). Martínez & Di Cola (2011) observed that skull size of *Graomys* mice in Argentina was not correlated with precipitation or normalized difference vegetation index (NDVI; a proxy for primary productivity), but rather with the minimum temperature of the coldest month. Monteiro *et al.* (2003) did not detect a correlation between environmental factors and the skull size of punaré rats in Brazil, despite the wide range of variation in the primary productivity between their study sites. In summary, it seems that although resource availability is an important factor influencing size in mammals, it is not always the main factor behind intraspecific size variation.

McGuire (2010) also found a negative correlation between tooth size and precipitation in California voles (*Microtus californicus*), and suggested that this trend might be related to better water conservation in larger bodied voles. A larger body mass has also been suggested as an adaptation for water conservation in birds (James, 1970). Southern red-backed voles indeed have high water requirements (Getz, 1962,

1968; McManus, 1974). Therefore, it is possible that the inverse correlation between skull size and precipitation is partly due to a water conservation strategy in southern red-backed voles.

SHAPE

We found that voles from different ecozones had different tooth shapes, suggesting that differences in the local habitat and floral composition have an important effect on tooth shape. The diet of southern red-backed voles is composed of fungi, forbs, roots, bark, berries, ferns, mosses, grasses, lichens and invertebrates, the proportion of items ingested shifting in different environments according to their availability (Schloyer, 1977; Merritt & Merritt, 1978; Martell, 1981; Merritt, 1981; Norrie & Millar, 1990). The proportions of dietary items available are different in environments as distinct as the Mixedwood Plains, the Boreal Shield and the Taiga Shield, and therefore we expect the diet of an opportunistic generalist to differ substantially in these ecozones. Martell (1981) found that voles in neighbouring coniferous and mixed-wood forest stands in northern Ontario all

used lichens and fungi as their major food source, but voles from the mixed-wood stands ate primarily fungi, while those in the coniferous stands ate primarily lichens. Martell attributed this difference to the availability of these food items in the two forest types. Given that the primary function of teeth is to process food, it is likely that tooth shape varies as voles use food resources, either through local adaptation or phenotypic plasticity.

As the climate becomes colder and drier we found a trend of a greater rotation along the antero-posterior axis of the tooth (labial triangles leaning forward and lingual triangles leaning backward), and deeper re-entrant angles in the first upper molar. McGuire (2010) also found a climate-related trend on the curvature of first lower molars of California voles, with teeth being more curved along the antero-posterior axis of the tooth in cooler and more humid climates. Although the depicted shape variation patterns differ (as do the climate gradients), both this study and ours indicate the tendency of climate to influence the overall shape of teeth and not only the shape of specific tooth parts. Piras *et al.* (2010) also observed deeper re-entrant angles in the teeth of *Terricola savii* in colder and drier environments. The studies by McGuire (2010) and Piras *et al.* (2010) suggest that the climate is influencing tooth shape through its effect on vegetation and diet, a link that we also invoke here although this has not yet been tested directly.

Skull shape was significantly different between ecozones, and most of this difference was manifest on the zygomatic arch. The zygomatic arch is closely related to mastication muscles, as the area of origin of the masseter and defining the lateral limits of the temporal fossa where the temporalis is placed. The shape variation related to climate and productivity revealed in the PLS analysis was also mostly concentrated on the zygomatic arch. This suggests that differences in skull shape may be due to different feeding habits in different environments. As the climate becomes colder and drier it is likely that soft food items such as fungi or berries will be less available, and that more developed mastication muscles will be necessary to process tougher food. The compressive force needed to crush hard food is exerted by the temporalis muscle (Turnbull, 1970; Kesner, 1980; Satoh, 1997), and a broader zygomatic arch provides space for a more developed temporalis. At the same time, a broader zygomatic arch also provides more area for the origin of the masseter muscle, which is responsible for the propalinal jaw movement for grinding food items (Turnbull, 1970; Kesner, 1980; Satoh, 1997). Kiliaridis, Engstrom & Thilander (1985) demonstrated that rats fed soft and hard diets differed in skull shape due to bone remodelling, which suggests that the pattern we observed may be partly due to phenotypic

plasticity. On the other hand, the strong relationship between skull morphology and diet in rodents demonstrated by Samuels (2009) suggests that part of the skull shape variation we observed could be due to inherited adaptations to different feeding habits.

The grouping we observed could be interpreted in a few different ways. The separation between the southern populations and the other populations could reflect the phylogeography of the species. Most of the Québec region was covered by the Laurentide ice sheet until less than 10 kya (Carlson *et al.*, 2008). Voles colonized this area as the ice sheet retreated and suitable habitats became available, which could have happened differently from a simple south to north expansion. Based on the analysis of mitochondrial DNA markers, Runck & Cook (2005) hypothesized that three refugial vole populations colonized Canada in the post-glacial period. It is likely that members of the eastern clade identified by these authors colonized southern Québec and expanded their range northwards. This re-colonization process may have occurred via a series of successive founder events (e.g. Gracia *et al.*, 2013; Schulte *et al.*, 2013), a process that may also have played a role in other species occurring in Quebec (Ledevin & Millien, 2013). These successive founder events would have further structured populations spatially. In addition, around 9 kya, a land corridor running between the Great Lakes and the retreating Laurentide ice sheet was formed (Dyke & Prest, 1987; Carlson *et al.*, 2008). Moving through this corridor, voles from the central clade also had access to central Québec and could have colonized central and northern Québec as habitats became available. This hypothesis could be tested through molecular analysis – namely a phylogeographical study based on mitochondrial DNA, as done for example in Renvoisé *et al.* (2012) – but unfortunately at this time we do not have a molecular phylogeny of the populations studied available. Another possibility is that this grouping pattern is habitat driven, and both the separation of the southern populations from other populations and the grouping of the three northernmost populations would reflect morphological adaptations to the Mixedwood Plains, the Boreal Shield and the Taiga Shield, as discussed above.

CONCLUSIONS

The distinct ecological and environmental conditions present in the three ecozones studied are reflected in differing skull and tooth morphologies in red-backed voles. Temperature and food resource availability are not the major drivers of red-backed vole size in our study area, perhaps because water is a more important limiting resource than food for this species. Tooth wear and skull size are more important predictors

than environmental factors in tooth and skull shape variation, but wear-independent tooth shape and size-independent skull shape seem to be at least partly related to feeding habits in different ecozones.

ACKNOWLEDGEMENTS

All field sampling procedures were approved by the McGill Animal Care Committee (AUP 5420), the Québec government (SEG permits 2008051501400SF, 2009051501400SF, 2010051501100SF and 2011051501400SF), and the Department of Environment and Conservation of Newfoundland and Labrador (Scientific Research Permit IW2011-25). Funding was provided by Northern Scientific Training Program grants and a Quebec Centre for Biodiversity Science grant to R.B.S.-L., and a McGill University Start-up grant to V.M. R.B.S.-L. was funded by NSERC, FQRNT and McGill University fellowships. We are indebted to Dr S. Wang for NPP data, to the MRNF of Québec for some of the specimen collection, and to the Gault Nature Reserve crew for access to the reserve. Particular thanks go to A. Howell and J. Gaitan for help with fieldwork. We are grateful to the six anonymous reviewers whose suggestions helped improve the manuscript.

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APPENDIX 1

TOOTH WEAR CLASSIFICATION SCHEME

Tooth wear can greatly influence the shape of molars in arvicoline rodents (Guérécheau *et al.*, 2010; Ledevin *et al.*, 2010b). As a vole grows older and its teeth are worn down, the shape of the complex occlusal surface suffers important alterations (Abe, 1973). The effect of age and therefore wear on molar shape can be of the same magnitude as phylogeographical or population cycle factors (Fominykh *et al.*, 2010; Guérécheau *et al.*, 2010; Ledevin *et al.*, 2010b). To control for shape variation due to wear, we classified teeth into five different wear stages. The criteria used were the number of dentinal space isthmuses open, the continuity of the enamel crest on the edges of triangles, and the presence of dentin in the re-entrant angles between triangles. The reasoning for the use of these criteria is that as teeth are worn down the open isthmuses that connect dentin basins decrease in width and ultimately close (Abe, 1973), the enamel at the external edges of triangles is disproportionately worn out leading to a rupture of the enamel crest, and the height of the tooth decreases to a point where the occlusal surface reaches the base of the tooth characterized by the presence of dentin in the re-entrant angles. In this sense, if all isthmuses were open a score of 0 was attributed to the tooth; if two or three isthmuses were open the score was 1; if one or no isthmus was open the score was 2. If the enamel crest was ruptured or much thinned at the external vertices of triangles 1 additional point was scored. If at least three out of four re-entrances were filled with dentin 1 point was added. The result was a score from 0 (not worn) to 4 (very worn) for each tooth analysed (Fig. A1).

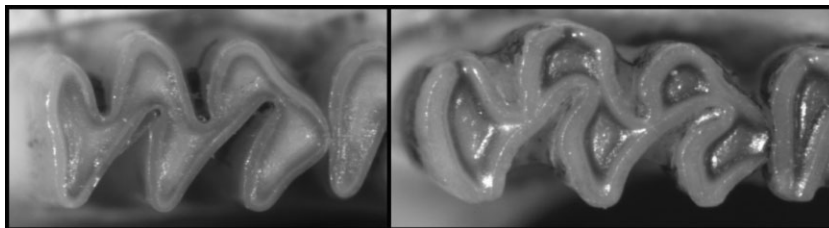


Figure A1. Example of the classification of the first upper molars according to wear stage. The specimen on the left has a score of 0 (very little or no wear) and the specimen on the right has a score of 4 (very worn).