Geometric morphometrics of vole (*Microtus californicus*) dentition as a new paleoclimate proxy: Shape change along geographic and climatic clines

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A B S T R A C T

Quaternary small-mammal fossils are widespread and have served as valuable paleoclimatic proxies through niche-modeling or otherwise relating presence-absence data to climatic data. This paper examines the potential for a new, more resolved type of paleoclimate proxy in the form of *Microtus* tooth shape, using an abundant California species, *Microtus californicus*. Geometric morphometrics and partial least squares analyses are used to find geographic signals in the shape of the first lower molar (m1) of this species. *M. californicus* m1s are relatively straight in the northwest, cooler, moister portion of California and more curved in the southeast, hotter, drier portion of the state. These tooth shape changes may be a result of selection related to different vegetation ultimately controlled by climate, and therefore diet, within the species' range. The pattern in m1 shape persists when phylogeographic hypotheses are taken into account, indicating that the climate signal is significant independent of intraspecific groupings. This method adds an important new tool to the suite of proxies available to reconstruct past climates at fine spatiotemporal scales and reveals a geographic/climatic signal that correlates with morphological variation across the range of *M. californicus*.

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1. Introduction

At a time when the world's climate is changing at the most rapid rates in millennia (Barnosky et al., 2003; Jansen et al., 2007), it is important to explore how impending climate change will affect communities and species. This paper approaches the issue by analyzing phenotype in relation to climatic gradients. Previous studies of the effects of climate change on biota have primarily examined changes in species ranges and community composition through past environmental disruptions (e.g. Graham et al., 1996; Hadly, 1996, 1999; Montuire, 1999; Lyons, 2003, 2005; Rodríguez, 2004; Hernández Fernández et al., 2007; Palombo, 2007; MacDonald et al., 2008) or by recent observational data that indicate how species have changed their ranges, abundance, or phenology over the past several decades (e.g. Visser and Both, 2005; Parmesan, 2006; Davis et al., 2008; Moritz et al., 2008). In some cases genetic response to climate change has been evaluated (Hadly et al., 2004).

Phenotypic responses to climate have been documented in some cases, including studies on the effects of past climate changes on body size (e.g. Dayan et al., 1991; Smith, 1995; Smith and Betancourt, 1998, 2003, 2006; Millien-Parra and Loreau, 2000; Millien, 2004;

Millien and Damuth, 2004; Blois et al., 2008; MacDonald et al., 2008; Blois and Hadly, 2009) and attempts to correlate specific morphological features to climate variables (e.g. Damuth et al., 2002; Fortelius et al., 2002; Legendre et al., 2005; Fortelius et al., 2006; Montuire et al., 2006; Krmpotic et al., 2009). Such demonstrations that morphology can correlate with climate are important because phenotype is the level on which selection acts. A decrease in phenotypic variability subsequently can reduce the ability of the species to adapt to new situations, and as such phenotypic variation metrics can offer a way to determine the ability of populations and species to survive change. Additionally, changes in the distribution of phenotypes within populations can be an indicator that genetic and distributional changes are underway.

This paper examines whether climate change selects for or against specific morphological features, and assesses the suitability for using morphological features of indicator mammals as climate proxies where other proxies may be unavailable (e.g., paleosols, pollen assemblages, plant macrofossils, tree rings, stromatolites, and stable isotopes). Methods for examining the relationship between morphology and climate are introduced. Finally, phylogeographic history is assessed as an alternative hypothesis for apparent correlation among morphology, geography, and climate.

Voles are integral in seed dissemination, soil mixture, decomposition, and as prey for terrestrial and avian predators (Huntly,

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1991; McLaren et al., 1998; Carey and Harrington, 2001). Past work on voles indicates that they have excellent potential as climate and ecosystem indicators. Hadly et al. (2004) suggested that even moderate shifts in climate can affect population sizes and genetic variation in some voles, and genetic and morphological variation in voles (*Microtus*) may be correlated with local climate factors (Davis, 1987; Conroy and Cook, 2000; Nappi et al., 2006). Piras et al. (2009) have shown that some components of fossil voles' (*Terricola savii*) first lower molar (m1) shape correlates with broad-scale, global average climate through time when compared to oxygen isotope data from Zachos et al. (2001).

Microtus californicus, the California vole, is a particularly suitable model for examining intraspecific climate change effects because it is abundant throughout California and common in the Quaternary fossil record. These voles occupy many climate regions throughout California today, resulting in a large range of conditions that could affect morphology. Within these climate regions, 17 subspecies have been recognized according to morphological characteristics including skull shape, body size, and pelage (Kellogg, 1918). However, no molecular work has yet verified the validity of these subspecific designations. Conroy and Neuwald (2008) and others (Gill, 1980, 1982; Modi, 1985) have found two phylogeographic groups within *M. californicus* that demonstrate disparate morphology, decreased fertility in crossed pairs, and genetic isolation (Fig. 1). These two groups appear to be divided along climatic clines within the state of California. These phylogeographic groups are used to test whether an intraspecific climate signal can be distinguished from a signal that is the result of population structure. Because there can be a correlation between tooth shape and climate in some voles (Piras et al., 2009), and because teeth are the most commonly preserved *M. californicus* fossil, the correlations between tooth morphology, geography, and climate in this important California species are explored.

Critical for understanding the correlation between dental morphology and climate are the rich variation and high complexity in the shape of vole teeth. Teeth are an animal's key interface with its food, so teeth both reflect and inform us about ecological and evolutionary change (Cuvier, 1825; Davis, 1987; Evans et al., 2007; Nappi et al., 2006; Osborn, 1907; Owen, 1840–1845). Because they develop early in ontogeny, their shapes differ for one of three reasons: ontogenetic toothwear, early changes in development, or genetic changes. Polly (2003) has shown that teeth have a complex, quickly-evolving form that varies significantly among populations and has a high additive genetic component. Due to their hardness, teeth are commonly preserved as fossils and provide a rich source of paleoenvironmental information in Quaternary fossil localities that lack other paleoclimate proxies. Indeed, teeth are by far the most abundant diagnostic element in the mammalian fossil record.

Previously, researchers have examined tooth shape using traditional morphometrics, distances between landmarks or ratios of those distances (Nappi et al., 2006). However, these methods require a priori assumptions about important landmarks, lack statistical power and often make it difficult to account for size effects (Zelditch et al., 2004). To overcome these issues, geometric morphometrics, a statistically powerful analytical method for determining changes in shape across objects independent of the effects of size and without a priori assumptions about which features are significant (Zelditch et al., 2004) are employed. The focus is on the m1 of *M. californicus*, the most diagnostic tooth for vole species (Wallace, 2006), and because of its relatively large size, one of the most common teeth preserved as fossils.

This paper tests whether the m1s of *M. californicus* can serve as a paleoclimate indicator. First, a possible correlation between tooth shape and geography that parallels climatic gradients in California is assessed. The intraspecific pattern of shape distribution are

examined to determine if the observed relationships are merely the remnant of a previously documented phylogeographic pattern from the two distinct *M. californicus* groups where geographic separation and drift have occurred, or whether climate patterns potentially drive the pattern within and between these two groups.
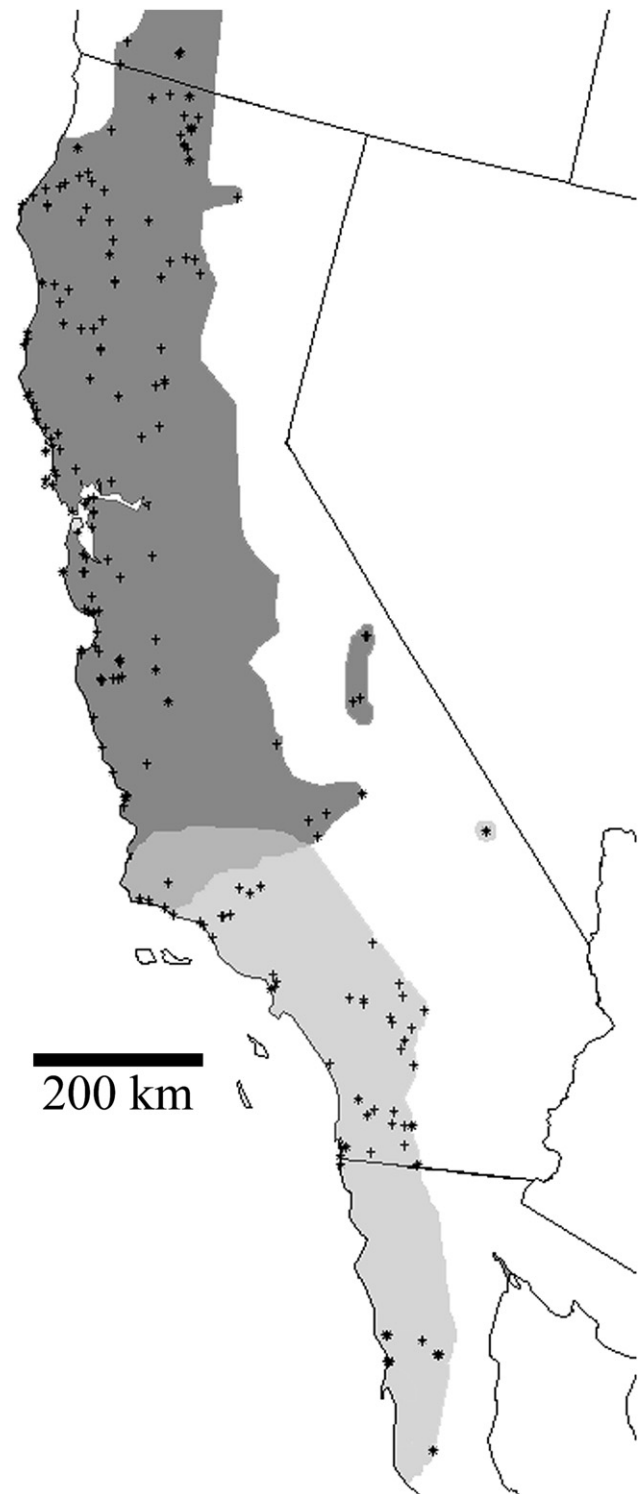

Fig. 1. Map of California and surroundings overlaid with the range of *M. californicus* phylogeographic groups (North = dark gray; South = light gray; Hybrid = medium gray; based on Conroy and Neuwald, 2008) and specimens used (black stars).

2. Materials and methods

2.1. Data collection

The m1s of 307 *M. californicus* specimens (146 female and 161 male) were obtained throughout the range of the species (Fig. 1) and include all 17 subspecies (Hall, 1981). Initially, right and left m1s were treated separately and were found to differ slightly but significantly in shape. To increase the signal-to-noise ratio, and so that the method can later be easily transferred to fossil *M. californicus* specimens available primarily as isolated teeth, only left m1s were used. All specimens are from the Museum of Vertebrate Zoology (MVZ) (Berkeley, USA) collections; only jaws with fully erupted m1s were used. Lower toothrows were digitally photographed using a Nikon D70s and AF Micro-NIKKOR 60 mm f/2.8D lens. Two-dimensional coordinates were digitized on Tiff images using tpsDig 2.10 (Rohlf, 2006a), including 21 landmarks after Wallace (2006) (Fig. 2).

Latitude and longitude coordinates for each specimen were taken from the MVZ database (http://arctos.database.museum). Climate variables included average daily maximum temperature and total precipitation per year, both averaged over the years 1971–2000 and at 1 sq km resolution (PRISM Group, 2008). These data were downloaded and imported into ARCMaps 9.2 (ESRI, Redlands, CA). Hawth's intersect points tool (Beyer, 2004) was used to determine the temperature and precipitation for each *M. californicus* specimen. It was not possible to pair climate data with collection dates for the individual specimens. Additionally, climate data were not available for specimens from Baja California, Mexico, which likely represents the hottest, driest portion of the species range.

2.2. Geometric morphometric analyses

Kendall (1977) defines shape as "all the variation that remains in the configurations of landmarks after removing differences in location, size and orientation." Geometric morphometrics is a method that directly compares shape differences among specimens. Generalized Procrustes analysis (GPA) was performed to superimpose landmark configurations and correct for non-shape variation (Rohlf and Slice, 1990). In a GPA, specimens are superimposed by translating the centroid of each specimen (x_L, y_L) to that of a mean specimen (x_c, y_c). Next, centroid size (CS), defined as:

$$CS = \sqrt{\sum \left[(X_L - X_C)^2 + (Y_L - Y_C)^2 \right]} \tag{1}$$

is normalized across specimens. Finally, specimens are rotated to minimize the overall summed squared distances between landmarks. The resulting size – and orientation – corrected landmark coordinates can then be used to compare shape differences between specimens.

The processes of translation, resizing and rotation each constrain the data, removing degrees of freedom and creating
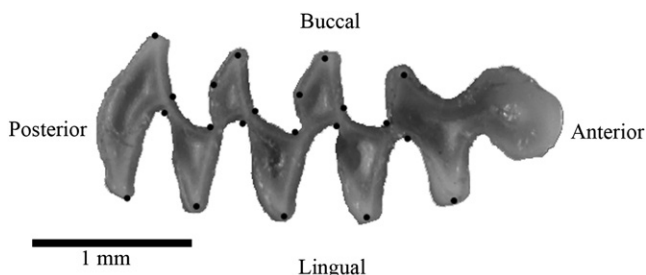

Fig. 2. Lower left first molar of *M. californicus* with landmarks.

a mismatch between the number of variables and the degrees of freedom (Rohlf and Corti, 2000; Zelditch et al., 2004). Therefore, several of the statistical analyses use partial warp and uniform component scores, which contain equal degrees of freedom and variables, rather than Cartesian coordinates. This gives the same results as if the Cartesian coordinates were used and the degrees of freedom were corrected manually (Rohlf and Corti, 2000; Zelditch et al., 2004). Partial warps and uniform components were calculated using standard geometric morphometric procedures (Bookstein, 1989, 1991; Zelditch et al., 2004).

Geometric morphometric and statistical analyses were performed using the following programs: tpsDig 2.10 (Rohlf, 2006a), Coordgen 6h (Sheets, 2000), DisparityBox 6i (Sheets, 2006a), manovaboard 6.4 (Sheets, 2006b), Standard6a (Sheets, 2001), tpsPLS 1.18 (Rohlf, 2006b), PLSMaker (Sheets, 2002), and JMP 7.0 (SAS Institute, Cary, NC).

To detect the amount of photographic error present in the analysis (Rohlf, 2003), a single specimen was photographed 20 times. The sum of squared errors (SSE) among these photographs was then compared to the SSE of the overall specimen pool. Variation due to photographic error represented 2.1 percent of total variation in tooth shape. Sexual dimorphism was tested using a 1-way multiple analysis of variance on shape variables (MANOVA), which revealed no significant difference in m1 shape between sexes ($P > 0.05$). Sexes were pooled for all other analyses.

2.3. PLS analyses

In order to determine the relationship between morphology, represented as 42 shape coordinates and centroid size, and geographic position, represented by latitude and longitude, it is necessary to reduce the dimensionality of the data. A two-block partial least squares (PLS) analysis, reduces dimensionality by creating new linear combinations of the independent and dependent variables, called singular axes. The axes are chosen to maximize the covariation between morphology and geography (Bookstein et al., 2003; Rohlf and Corti, 2000). First singular axes are similar to principal components, in that they do not actually alter the data; they simply represent a large number of variables with a much smaller set. Unlike principal components, in which axes are chosen to maximize the amount of variation described in a set of observations, PLS axes maximize covariation between two sets of variables. Thus, PLS isolates the components of the variation that are most relevant to the relationship of the two multivariate sets (i.e. morphology and geography). Although the variation represented by each of these axes may be small compared to the overall variation in the entire data set, it is important to consider the strength of the correlation between the data along the singular axes and whether that relationship is stronger than would be expected by chance. In this case, the overall amount of shape variation correlated to geography could be low, but the amount of variation along a particular axis of shape could be highly correlated with geography. Correlation coefficients (r) represent the strength of the association between morphology and the geographic variables along their singular axes. A null distribution for establishing the significance of the correlation coefficients was determined by performing 10 000 bootstrapping replicates, or random permutations, of the morphologic and geographic variables (Rohlf, 2006b). Comparison of the actual morphology–geography correlation to this null distribution provides a *P* value indicting whether the covariation between two singular axes is greater than expected by chance. As with principal components, the resulting significant singular axes are a reduced-dimension representation of morphology and geography. Each singular axis can then be independently used in regressions against new variables of interest not incorporated in the original model. In practice, this

allows extraction of a single axis of morphological change that is most significantly correlated with changes in geography.

2.3.1. Morphology and geography

A PLS was first performed with latitude and longitude as the independent variables, and shape and centroid size as the dependent morphological variables. For this analysis, geographic variables were considered a proxy for the climate variables, because climate stations are not densely distributed through all of the geographic space and climate data have not been paired with specimen collection dates. The validity of the correlation of geographic and climate variables is tested using methods described in 2.4. Following the PLS, a multiple linear regression was performed to determine the extent to which the two singular geographic axes could describe the variation within the singular shape axes with which they maximally covaried.

2.3.2. The effects of size versus shape

If an allometric relationship is present in *M. californicus* m1s, size may independently correlate with climate even though it has been removed from the shape analysis during the GPA. To control for the effect of size, shape variables were regressed onto centroid size. The residuals of this analysis were then added to the predicted specimen (taken from the regression model) at the mean centroid size of 3.38 mm (Sheets, 2001). A second PLS regression was performed of the size-normalized morphology data onto geography. A significant correlation coefficient in this analysis would reveal that shape alone is significantly correlated to geography.

To test if size plays a significant role independent of shape, the first size-normalized morphologic singular axis was extracted and a multiple regression of this axis and centroid size onto the first geographic singular axis was performed. If size significantly contributes to this regression, then it indicates that size provides information about geography independent of shape change.

2.4. Climate correlation

Once the PLS analysis determined the geographic axis that maximally covaries with the morphologic singular axis, the effectiveness of broad climate variables to predict this linear combination of geography variables (latitude and longitude) could be examined. The first geographic singular axis was extracted and a multiple regression of the precipitation and temperature variables onto this axis tested the climate-geography relationship.

2.5. Phylogeographic group effects

The two phylogeographic groups (Conroy and Neuwald, 2008) are distributed along a similar axis as the first geographic singular axis. Thus, it is important to determine whether there are differences along the morphological shape axis among phylogeographic groups, and whether any differences are the result of a factor other than geographic or climatic clines. Such a factor might involve geographic separation and subsequent drift within each group or another variable. An initial examination of the PLS graph (Fig. 3) reveals no distinct morphological breaks between the phylogeographic groups.

To analyze this question more rigorously, an analysis of variance (ANOVA) was performed on the first morphologic singular axis to examine whether m1 morphology differs between phylogeographic groups. Next, a linear regression was performed between morphology and climate within each of the two subspecies. These regressions analyze the first geographic singular axis and the first morphologic singular axis of the northern and southern phylogeographic groups separately. Finally, a multiple regression of the first morphologic singular axis, a binary variable encoding

phylogeographic group membership, and first geographic singular axis (Fig. 1) determines whether there is still a geographic signal across phylogeographic groups when accounting for any differences between groups.

3. Results

3.1. PLS analyses

3.1.1. Morphology and geography

The first morphologic singular axis and the first geographic singular axis are significantly correlated (PLS regression, r = 0.56;
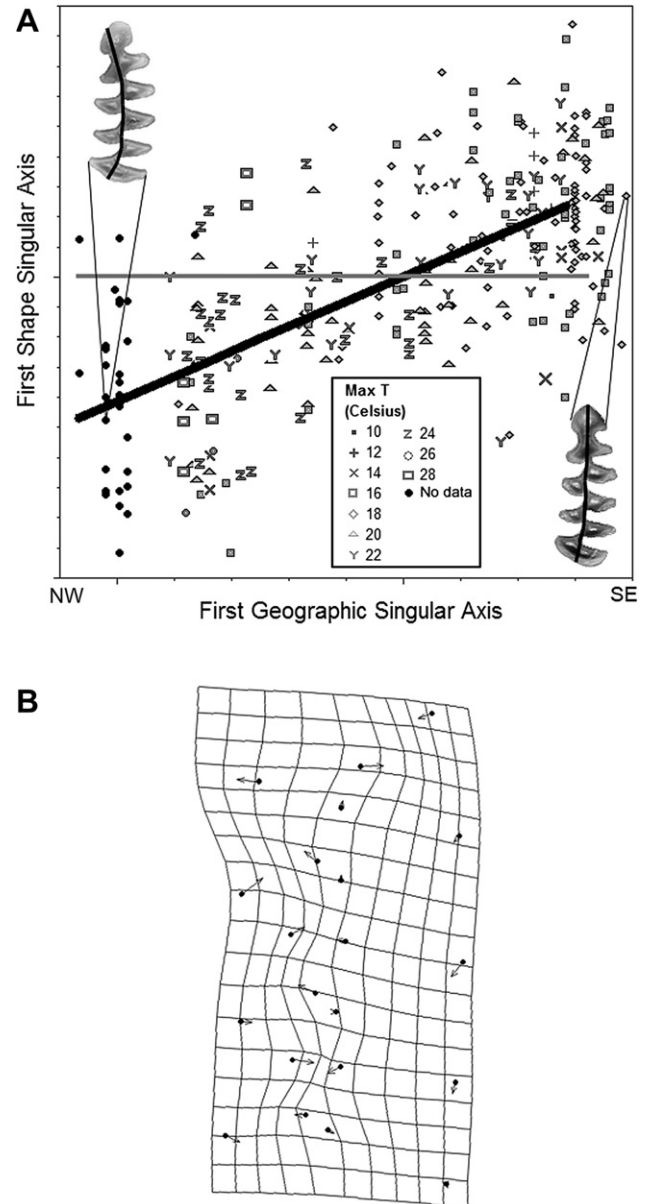

Fig. 3. A. A PLS Regression with a gray line representing the mean tooth shape. Specimens above the line are more curved than average, and specimens below the line are straighter than average. Specimens are designated by their maximum daily temperature values (in degrees Celsius). Individual teeth are expanded to highlight shape at each geographic extreme. A line traces the axis of the teeth from each geographic extreme. B. The warp diagram shows tooth shape change from northwest to southeast California. Points represent landmarks of teeth in the northwest extreme, and arrows represent the vector change to tooth shapes in the southeast extreme. Note the shortening of buccal triangles and overall increased curvature of tooth shape.

$P = 0.01$) (Fig. 3a), as are the second two singular axes (PLS regression, r = 0.35; $P = 0.04$). Combining both pairs of axes reveals the percentage of shape change described by the complete set of geographic variables. While geographic position describes only 4.3 percent of the variation in shape and centroid size, it describes 46.7 percent of shape change along the two morphologic singular axes. This predictive power is significantly higher than expected by chance (Permutation test, $P < 0.01$).

A warp diagram with vector projection (Fig. 3b) provides a visual demonstration of how shape changes along the axis of maximum covariation (Sheets, 2002). Along the first singular axis of shape change, the anterior portion of the tooth is curving inward (lingually). To adjust for this curvature, the triangles on the labial side of the tooth undergo a relative narrowing and lengthening. At the opposite geographic extreme, labial triangles are shorter and wider, and the overall tooth shape is straighter (Fig. 3).

3.1.2. The effects of size versus shape

An allometric relationship was detected in *M. californicus* m1s. When size-normalized morphology is used in the PLS, the correlation of the first and second two geographic singular axes is slightly lower (r = 0.50 and r = 0.32 respectively) than when non-normalized morphology is used. However, the PLS analysis is still significant ($P < 0.01$), indicating that shape covaries with geographic variables independent of size along similar morphological axes.

Also, size has a significant contribution independent of shape as determined by a multiple regression of size and the first shape singular axis back onto the first geographic singular axis (partial *t*–test of size on first geographic singular axis, $P < 0.01$). This indicates that in the original analysis both size and shape trend significantly across the first geographic axis.

3.2. Climate correlation

The two initial climate variables correlated significantly with the geographic axes along which dental morphology varied in the first analyses (multiple regression, $r^2 = 0.43$; $P < 0.01$). Both variables had significant independent effects across geography, as did their interaction term (partial *t*–test of precipitation on first geographic axis $P < 0.01$, partial *t*–test of temperature on first geographic axis $P = 0.03$, partial *t*–test of the cross product of precipitation and temperature on the first geographic axis $P < 0.01$). Temperature generally increased from northwest to southeast California, while precipitation generally decreased along the same geographic axis.

3.3. Phylogeographic group effects

An ANOVA revealed a significant difference in the first singular axis of m1 shape between northern and southern phylogeographic groups ($P < 0.01$). However, linear regression of the morphological axis along the first geographic singular axis was still significant within each of the two phylogeographic groups (north group: $P < 0.01$; $r^2 = 0.1$; south group: $P < 0.05$; $r^2 = 0.1$). When phylogeographic group membership is coded as a binary variable and combined with the continuous geographic variable in a multiple regression on shape, the north-south grouping does not provide significant correlation with morphology independent of the geography variable ($P = 0.057$). Hence geography is still a significant predictor of shape regardless of phylogeographic grouping. While a shape change can be detected between phylogeographic groups, this change can be entirely accounted for by the continuous geographic variable.

4. Discussion

4.1. PLS analyses

The combination of geometric morphometric and PLS analyses is generally used in one of two ways: to determine the extent to which morphological structures covary (e.g. Mitteroecker and Bookstein, 2007) or to determine the relative loadings of variables on singular axes (e.g. Rychlik et al., 2006). In these cases, an overall high percent of variation explained in the dependent and independent variables by the PLS regression are necessary for determining these relationships. However, the present study is focused on determining whether there is a singular axis of shape change that varies significantly with geographic, and eventually climate variables. Given the large number of parameters that could affect variation in dental morphology, the fact that only a small percentage of overall morphological change is accounted for by the two geographic variables is neither surprising nor unexpected. What is important is that there exists an axis of morphological change that is significantly correlated with geography, that shape change along this axis both is significant, independent of size, and is systematic (i.e. easily describable in the warp diagrams; Fig. 3b), and that climate parallels the geographic axis along which this shape change is most prominent.

4.1.1. Morphology and geography

Although geography describes only a relatively small, (although significant) 4.3 percent of overall shape change, it describes a relatively large percent, 46.7 percent, of shape change along those particular morphological axes. Because this morphologic singular axis is extracted as a linear combination of many component variables using a PLS analysis, the resulting correlation between morphology and geography must be tested to determine if is significantly greater than would be expected by chance fitting of a singular axis to "random," i.e. non-geographically correlated, variation in morphology. The P values for the correlation coefficients are the result of bootstrapping, which tests for this contingency. These tests are significant, revealing that the amounts of covariation between the first and second pairs of singular axes are greater than a random signal would be expected to produce.

The results of the PLS analysis indicate that both shape and size significantly covary with geography (Fig. 3a). Importantly, this shape change along the first geographic singular axis leads to a meaningful hypothesis of how shape covaries with climate. As we can see in Fig. 3b and from the description in 3.1.1, the shape change along the first singular axis indicates that tooth curvature gradually increases from northwest to southeast. Much of the variation in tooth shape along the first geographic singular axis occurs in the anterior portion of the tooth. This is consistent with a study by Guthrie (1965), which found that the anterior loop of *Microtus paroperarlus* and *Microtus pennsylvanicus* m1s are the most variable region of the toothrow. Unfortunately, because the anterior loop represents a curve, rather than discrete, homologous features, it requires the placement of semilandmarks (Bookstein, 1997) rather than landmarks, adding many more steps to the analysis. In the future, semilandmarks placed on the anterior loop may yield shape information that will be important for additional correlations with climate variables. However, preliminary results indicate that the placement of these semilandmarks will not change the overall conclusions of this analysis.

4.1.2. The effects of size versus shape

Independent PLS analyses and multiple regressions indicate that both size and shape independently covary with geographic variables. One interesting result of these analyses is that size increases

to the southeast portion of *M. californicus'* distribution. This trend is in opposition to Bergmann's rule, which states that within species of mammals individuals tend to be larger in cooler environments (Rensch, 1938). The observed trend is different from some other rodents, including *Spermophilus* and *Neotoma*, which adhere to Bergmann's rule (Smith and Betancourt, 2003; Blois et al., 2008). However in *Spermophilus*, large body size appears to be more highly correlated with increased precipitation rather than temperature since the last glacial maximum (Blois et al., 2008). In this and several other cases it has been proposed that body size may trend with precipitation because wetter places are more productive (Rosenzweig, 1968; James, 1970; Yom-Tov and Yom-Tov, 2005; Yom-Tov and Geffen, 2006; Medina et al., 2007; Blois et al., 2008). In contrast to all of these situations, *M. californicus* individuals with larger body size (as inferred from tooth size) are found in warmer, more arid environments that have lower overall primary productivity. According to a drought tolerance study performed by Church (1966), *M. californicus* has poor water conservation. Larger bodies would reduce the surface area to volume ratio of the vole, which is important for water conservation and possibly contributes to this reversal to the expectations of Bergmann's rule.

4.1.3. Climate correlation

Climate variables strongly correlate with the geographic axis that maximally covaries with tooth shape, even though topography was not taken into account. *M. californicus* teeth are relatively straight in the northwest, cooler, moister portion of California and relatively more curved in the southeast, hotter, drier portion of the state with intermediate forms in the central regions. The basis for this difference in curvature is unknown, but it may reflect different characteristics in dietary plants at the two climate extremes. The grasslands in northwest California are generally more mesic and fertile than those in the southwest (Schiffman, 2007). Across this gradient and within the range of *M. californicus*, the two major divisions of grasslands, north coastal grasslands and valley and south grasslands, encompass many smaller communities (Keeler-Wolf et al., 2007). Differences in grass type may reflect differential biomechanical pressures and therefore differential selection in dentition. These ideas remain to be tested.

More detailed microhabitat data including assessment of annual maximum and minimum temperatures, length of dry season, and vegetation types specific to each specimen's capture time would be desirable to further understand the underlying mechanisms of the climate-shape correlation. It may even be possible to examine the stable isotope composition of the actual teeth being analyzed (following Navarro et al., 2004) to corroborate the specific climate when and where the specimen was collected. Nevertheless, these results corroborate previous studies that have indicated that tooth morphology approximately parallels geography and climate gradients (Davis, 1987; Nappi et al., 2006) and indicate that tooth shape can be a reliable proxy to characterize the climate and potentially the microhabitat in which the specimen is located.

Microhabitat information beyond vertebrate community composition is often difficult to infer at terrestrial vertebrate fossil localities. Isotopic analyses can often yield important information about regional climate; however, this information can be easily lost through diagenetic or taphonomic processes (Chillon et al., 1994). Similarly, paleosols or floral assemblages can provide habitat information but are rarely preserved in conjunction with vertebrate fossils. This lack of climate reconstructions for most vertebrate localities limits our ability to interpret community responses to climate factors. The morphological differences reported here indicate that vole m1s can provide an important new climate proxy for terrestrial vertebrate localities. For *M. californicus*, it appears that larger, more curved teeth may reflect generally drier, warmer

climates found in the southeast portion of their range, whereas smaller, straighter teeth may be indicative of cooler, moister climates found in the northwest part of their range.

4.2. Phylogeographic group effects

Phylogeographic group effects had the potential to mimic or obscure climate signals. Along the first morphologic singular axis, the m1 is distinguishable between the northern and southern phylogeographic groups. However, tooth shape along this axis correlates with geographic variables beyond the phylogeographic grouping. Even when group effect is accounted for, a morphological cline is still detectable within both populations, indicating that location along the geographic axis, and potentially climate, is the dominant signal. Thus, it is unlikely that population isolation and subsequent drift are the only factors affecting m1 shape in this population. This is confirmed by a multiple regression, which indicates that only geography is significant when both phylogeographic group and geography are included in the analysis. Because group membership does not significantly affect the model, group effect should not weaken any resultant climate model.

Conroy and Neuwald (2008) showed that the division between northern and southern clades of *M. californicus* is convergent with the location of the Transverse Ranges, although the barrier appears incomplete because some hybridization occurs. Further, they showed that the climates experienced by each of these groups are different, because the northern clade experiences considerably more climatic variability than the southern one. The findings are consistent with this perspective, and suggest that a climate signal underlies the secondary clade divergence. A more detailed phylogeographic history would greatly improve this analysis, but that is beyond the scope of this paper.

4.3. Geometric morphometrics

Previous attempts to examine the correlation among climate, geography and *Microtus* m1s (Davis, 1987; Nappi et al., 2006) used traditional morphometric techniques, with linear measurements used to characterize the tooth's shape. Both papers (Davis, 1987; Nappi et al., 2006) concluded that populations in different climate regions can be distinguished based on relative length of the molar. Nappi et al. (2006) pointed out that degree of closure of the anterior loop is important, whereas Davis (1987) emphasized the percent of the population with additional triangles. However, neither analysis was able to attain a high-resolution model of how tooth shape changes with geographic and climatic variables.

In contrast, geometric morphometrics illustrates that for *M. californicus*, teeth vary across a geographic gradient correlated with climate such that they are relatively straight with narrow, long labial triangles in the northwest portion of California where maximum temperatures average 10° to 16 °C with greater than 10 cm of rainfall per year resulting in more northern coastal grass varieties (Keeler-Wolf et al., 2007). The teeth become increasingly curved with wider, shorter labial triangles in the southeast portion of California, reaching maximum curvatures where maximum temperatures average greater than 28 °C with less than 3 cm of rainfall per year resulting in warmer desert grass varieties (Keeler-Wolf et al., 2007). Thus, given a tooth, it becomes possible to use this model to place the tooth along the climate gradient, giving an approximation of the climate that existed when the animal was alive. In this way the method potentially provides a well-resolved paleoclimate proxy, rather than simply a broad classification method.

5. Conclusions

M. californicus teeth significantly covary with geographic variables along axes that reflect broad climate variables. The hypothesis that the observed pattern of morphological change represents a phylogeographic pattern unrelated to climate is thus rejected. Instead, there is a significant climate signal in the tooth morphology of *M. californicus*.

An important implication of this finding is that m1s of *M. californicus*, and potentially other *Microtus* species, as quantified using geometric morphometrics, provide a new paleoclimate proxy that can be used throughout Quaternary vertebrate sites wherever *Microtus* teeth are abundant. In general, *M. californicus* m1s are relatively straight in the northwest, cooler, moister environments and relatively more curved in the southeast, hotter, drier regions with intermediate forms in the central portion of the state. Phylogeographic biases can be accounted for statistically and do not affect the overall patterns once they are compared across all populations. In principle these same techniques could be applied to other vole taxa, or any common fossils, to obtain a paleoclimate signal where other proxies are unavailable. This method adds an important new tool to the suite of proxies available to reconstruct climate. Tracking the changes in morphology through future censuses may indicate where ongoing effects of global warming are becoming pronounced. The development of more detailed morpho-climate modeling may enable us to predict and, using historical data, to test the impacts climate change can have on selection and variation in key ecological indicator species.

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