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The effects of Quaternary environmental changes on Microtus distribution and morphology

Author
McGuire, Jennifer Lynn

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The effects of Quaternary environmental changes on *Microtus* distribution and morphology

By

Jennifer Lynn McGuire

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Committee in charge:

Professor Anthony D. Barnosky, Chair
Professor Craig Moritz
Professor Kevin Padian
Professor Steven R. Beissinger

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Abstract

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Dramatic climate changes are already occurring, yet their ecological impacts remain difficult to predict as we lack both a detailed understanding of how key indicator species will react as well as large-scale models that are ground-truthed using paleontological data. In my dissertation, I focused on these challenges. I examine how climate change not only changes species' size and range, but can elicit evolutionary responses in phenotype and alter population variation. Detailed reconstructions of species' paleontological ranges test predictions made by species distribution models (SDMs) as well as their underlying assumptions. Population-level analyses of shifts in morphological variation through time allow the prediction of future evolutionary responses and the assessment of susceptibility to environment stress due to reduced natural variation. I focus on the how climate changes might affect California's ecosystems, developing quantitative methods to look at how morphological shape changes across space and through time in ecologically important small mammals, *Microtus* (voles). Looking back at their response to paleoecological transitions, I make predictions about how they might react in the future.

In Chapter 1, I use geometric morphometrics of vole dentition to establish a potential new paleoclimate proxy. First, I test if *Microtus* morphology correlates with paleoclimate signals, using an abundant California species, *Microtus californicus*. Geometric morphometrics (quantitative shape analysis) and partial least squares (PLS) analyses reveal 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 reveals a geographic/climatic signal linked with morphological variation across the range of *M. californicus* and adds an important proxy to reconstruct past climates at fine spatiotemporal scales.

Another challenge addressed in my dissertation is that of species-level identification of fossils, which is necessary to maximally interpret the biostratigraphy, evolution, and paleoecology of Quaternary vertebrate localities. *Microtus* fossils typically

are preserved only as isolated teeth, making identification difficult. In Chapter 2, I distinguish between the five species of *Microtus* living in California today (*M. californicus, M. longicaudus, M. montanus, M. oregoni* and *M. townsendii*) using geometric morphometrics on only their m1. Discriminant analysis on the resulting projected shapes correctly classifies extant specimens of known species 95 percent of the time, enabling vole identification from several important Quaternary fossil localities. In addition, I demonstrate the importance of using jackknife metrics for evaluating discriminant analyses and make some initial identifications that recognize the first extralimital fossil *Microtus* specimens in California.

Using the identification methods established in Chapter 2, I then trace range shifts in Pacific-coast *Microtus* species throughout the Quaternary. I reinterpret fossil localities that contain *Microtus* specimens and compare Quaternary range change to patterns of species' range shifts over the last 100 years. I find range contractions in all five species examined, but individual congeners react differently and to different climate variables. Using these detailed range reconstructions based on newly identified fossils, I examine whether SDMs projected into the past can predict that past habitats would have been suitable for the fossils found therein. I find that nearly half of the extralimital fossil specimens, those fossils found outside the modern range of the species, are living in habitats in the past that were predicted by the SDMs. The species that are not predicted by the SDMs all have niche reconstructions that are strongly influenced by precipitation, leading to the hypothesis that paleoclimate reconstructions may be incorrectly modeling precipitation variables. In addition, I find that two species of *Microtus* reacted similarly to climate change earlier in the Quaternary as they have over the last 100 years. Although one species, *M. longicaudus*, has experienced much more rapid range contraction 100 years than previously in the Quaternary.

In my final chapter, I trace morphological variation in *M. californicus* m1s through time and across California over the last 45 ky. I compare the effects of climate change on this species at the population and species levels. I find that m1 shape is significantly correlated with mean annual precipitation in this species. As California has become increasingly arid, *M. californicus* appears to have lost some of the m1 variation that is correlated with high-precipitation climate regimes. Although, at the species level I have yet to see any evidence that climate change has resulted in a change in Quaternary range limits. However, future climate change is likely to stress the species to the extent that populations in increasingly wet or dry habitats may be extirpated.

Throughout my dissertation, I demonstrate the utility of using detailed paleontological data in examining the past and future effects of climate change. In being able to identify fossil specimens to the species level, we can begin to directly compare modern and paleontological ecology studies. Using those identifications, we can create accurate paleorange reconstructions that can be used to test predictive distribution models and explore the underlying factors maintaining range limits. Once a species is identified, high-resolution quantification of morphological features, such as teeth, using geometric morphometrics can allow us to trace shifts in variation through time. This allows us to see the history of a species variation so that we can determine whether it retains enough variation to react to future stress.

To Simey, my greatest critic and strongest supporter.

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CHAPTER 1

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

ABSTRACT

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. Here I examine 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*. I use geometric morphometrics and partial least squares analyses to find geographic signals in the shape of the first lower molar (m1) of this species. I found that *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*.

Keywords *Microtus californicus*, climate proxy, geometric morphometrics, partial least squares (PLS)

INTRODUCTION

At a time when the world's climate is changing at the most rapid rates in millennia (Barnosky *et al.*, 2003; Jansen, 2007), it is important to explore how impending climate change will affect communities and species. Here I approach this 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; Hernández Fernández *et al.*, 2007; Lyons, 2003, 2005; MacDonald *et al.*, 2008; Montuire, 1999; Palombo, 2007; Rodríguez, 2004) or by recent observational data that indicate how species have changed their ranges, abundance, or phenology over the past several decades (e.g., Davis *et al.*, 2008; Moritz *et al.*, 2008; Parmesan, 2006; Visser & Both, 2005). 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., Blois *et al.*, 2008; Blois & Hadly, 2009; Dayan *et al.*, 1991; MacDonald *et al.*, 2008; Millien, 2004; Millien & Damuth, 2004; Millien-Parra & Loreau, 2000; Smith & Betancourt, 1998, 2003, 2006; Smith *et al.*, 1995) and attempts to correlate specific morphological features to climate variables (e.g., Damuth *et al.*, 2002; Fortelius *et al.*, 2002; Fortelius *et al.*, 2006;

Krmpotic *et al.*, 2009; Legendre *et al.*, 2005; Montuire *et al.*, 2006). 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.

Here, I examine whether climate change selects for or against specific morphological features. I assess 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). I introduce methods for examining the relationship between morphology and climate. Finally, I assess phylogeographic history 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 (Carey & Harrington, 2001; Huntly, 1991; McLaren *et al.*, 1998). 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 (Conroy & Cook, 2000; Davis, 1987; 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; Gill & Bolles, 1982; Modi, 1985) have found two phylogeographic groups within *M. californicus* that demonstrate disparate morphology, decreased fertility in crossed pairs, and genetic isolation (Figure 1-1). These two groups appear to be divided along climatic clines within the state of California. I have used these phylogeographic groups 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, I explore the correlation between tooth morphology, geography, and climate in this important California species.

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, here I employ 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). I focus 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.

In this paper, I test whether the m1s of *M. californicus* can serve as a paleoclimate indicator. To do this, I first assess whether there is a correlation between tooth shape and geography that parallels climatic gradients in California. I then examine the intraspecific pattern of shape distribution 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.

MATERIALS AND METHODS

Data collection

The m1s of 307 *M. californicus* specimens (146 female and 161 male) were obtained throughout the range of the species (Figure 1-1) 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 60mm f/2.8D lens. Two-dimensional coordinates were digitized on Tiff images using tpsDig 2.10 (Rohlf, 2006a), including 21 landmarks after Wallace (2006) (Figure 1-2). 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.

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 & 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:

$$CS = \sqrt{\Sigma [(x_L-x_c)^2+(y_L-y_c)^2]} \qquad \text{(Eq. 1.1)}$$

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 a mismatch between the number of variables and the degrees of freedom (Rohlf & 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 & Corti, 2000; Zelditch *et al.*, 2004). Partial warps and uniform components were calculated using standard geometric morphometric procedures (Bookstein, 1989, 1991; Rohlf & Corti, 2000; 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, 2001b), 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.

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 analysis (PLS), 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 & 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.

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 above. 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.

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, 2001b). 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.

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.

Phylogeographic group effects

The two phylogeographic groups (Conroy & 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 (Figure 1-3) 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 (Figure 1-1) determines whether there is still a geographic signal across phylogeographic groups when accounting for any differences between groups.

RESULTS

PLS analyses

Morphology and geography

The first morphologic singular axis and the first geographic singular axis are significantly correlated (PLS regression, r=0.56; P=0.01) (Figure 1-3a), 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 (Figure 1-3b) 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 (Figure 1-3).

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.

Climate correlation

My two initial climate variables correlated significantly with the geographic axes along which dental morphology varied in my 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.

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.

DISCUSSION

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 & 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, in the present study, I am most interested in 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; Figure 1-3b), and that climate parallels the geographic axis along which this shape change is most prominent.

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, I must test if the resulting correlation between morphology and geography 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 (Figure 1-3a). 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 Figure 1-3b and from the description in the results, 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 *M. paroperarlus and M. 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.

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 (Blois *et al.*, 2008; Smith & Betancourt, 2003). 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 (Blois *et al.*, 2008; James, 1970; Medina *et al.*, 2007; Rosenzweig, 1968; Yom-Tov & Geffen, 2006; Yom-Tov & Yom-Tov, 2005). 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.

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.

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 detectible 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. My 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.

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 10º to 16º 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 º 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.

CONCLUSIONS

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

An important implication of this finding is that m1s of *Microtus 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.

FIGURES AND TABLES

FIGURE 1-1 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).

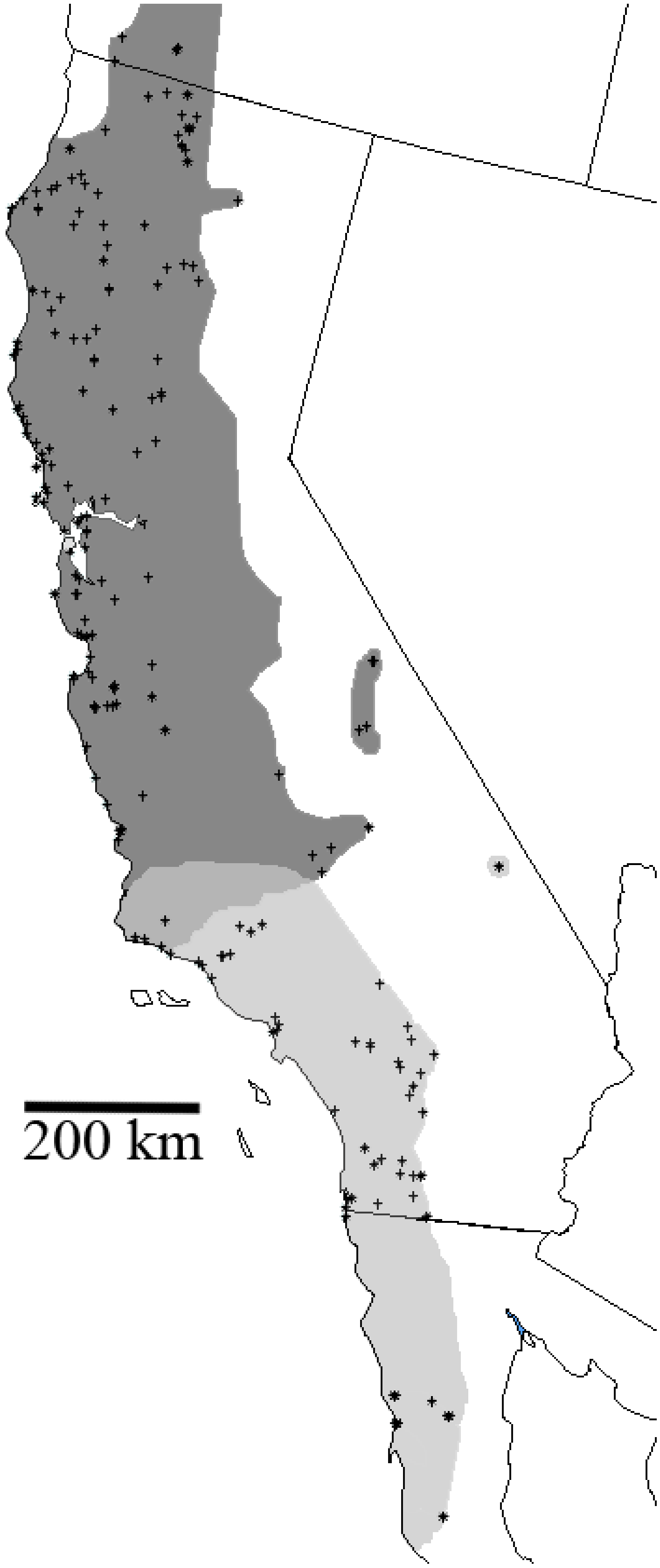

200 km

FIGURE 1-2 Lower left first molar of *M. californicus* with landmarks.

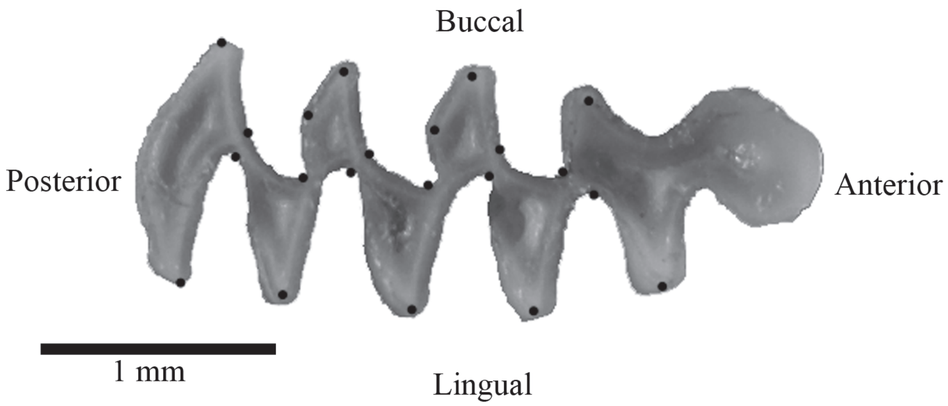
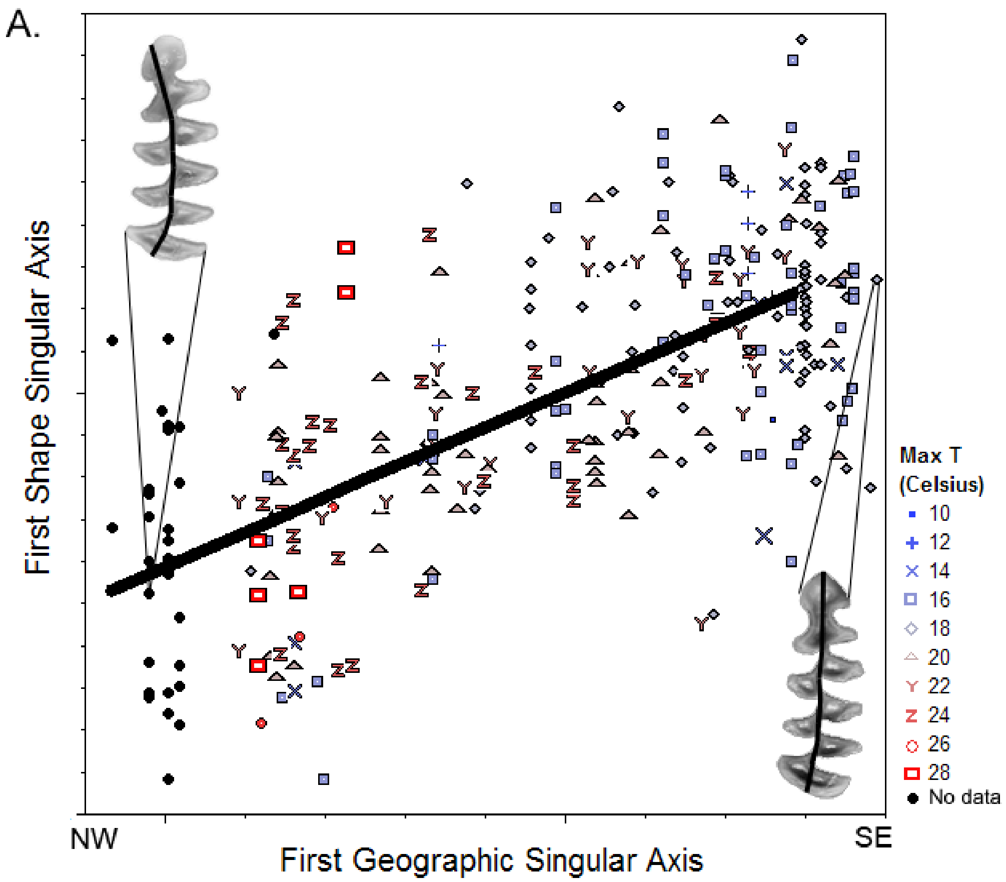
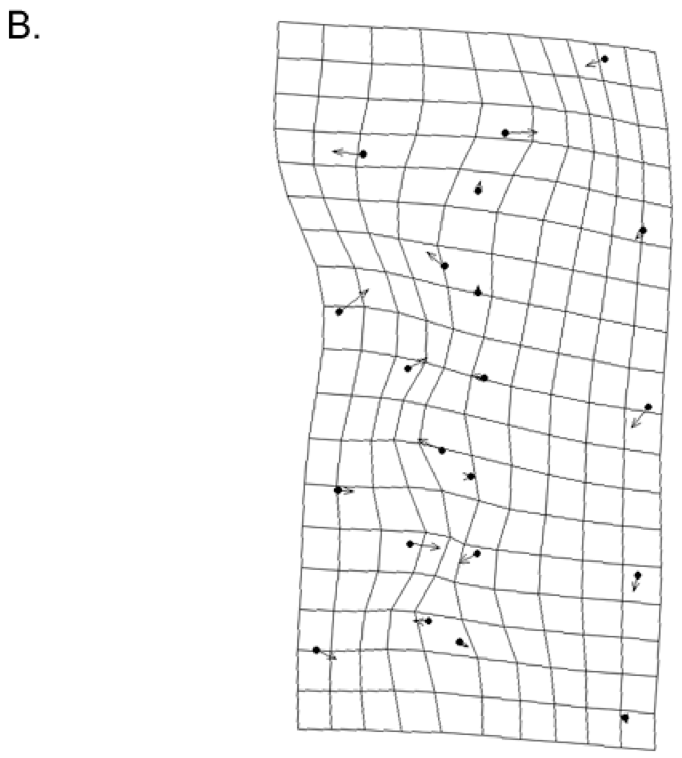

Buccal
Posterior
Anterior
1 mm
Lingual

FIGURE 1-3 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.


A.
First Shape Singular Axis
First Geographic Singular Axis
NW
SE
Max T (Celsius)
▪ 10
+ 12
× 14
□ 16
◇ 18
△ 20
Y 22
Z 24
○ 26
□ 28
● No data


B.

SUPPLEMENTARY TABLES AND FIGURES

TABLE 1-S1 Museum of Vertebrate Zoology (University of California, Berkeley) modern *M. californicus* specimens used in analyses.

Specimen Number	Subspecies
1766	sanctidiegi
2007	sanctidiegi
2199	sanctidiegi
2200	sanctidiegi
2201	sanctidiegi
2240	sanctidiegi
2439	sanctidiegi
2657	sanctidiegi
2789	sanctidiegi
2947	sanctidiegi
3076	sanctidiegi
3077	sanctidiegi
3078	sanctidiegi
3081	sanctidiegi
3082	sanctidiegi
3083	sanctidiegi
3404	sanctidiegi
3520	californicus
3645	californicus
3646	californicus
3664	aestuarinus
5198	sanctidiegi
6286	sanctidiegi
6287	sanctidiegi
6329	sanctidiegi
6330	kernensis
6332	kernensis
7002	kernensis
7003	californicus
7057	sanctidiegi
7381	sanctidiegi
7383	sanctidiegi
7384	sanctidiegi
7534	sanctidiegi
7536	sanctidiegi
7539	sanctidiegi
7670	sanctidiegi
7673	sanctidiegi
7674	sanctidiegi
8991	sanctidiegi
11546	constrictus
11586	constrictus
11627	constrictus
12671	sanctidiegi
12717	californicus
12727	aestuarinus
12804	eximius
12807	eximius
13393	eximius
13394	eximius
13395	eximius
13396	eximius
13397	eximius
13399	eximius
13400	eximius
13402	eximius
13403	eximius
13405	eximius
14099	californicus
15760	kernensis
15776	kernensis
16380	sanctidiegi
17385	vallicola
18159	aestuarinus
18160	aestuarinus
18232	aestuarinus
19002	sanctidiegi
20034	eximius
20058	eximius
20064	eximius
20065	eximius
20092	eximius
20101	eximius
20102	eximius
20846	eximius
24497	sanctidiegi
25551	californicus
25794	eximius
26374	vallicola
26383	vallicola
26385	vallicola
28268	vallicola
28269	vallicola
28270	mohavensis
28920	californicus
28922	californicus
28923	californicus
28925	californicus
28926	californicus
28928	californicus
28929	californicus
28932	californicus
28933	californicus
28934	californicus
29336	californicus
30508	eximius
34626	eximius
34631	eximius
35863	aequivocatus
35864	aequivocatus
35865	aequivocatus
35866	aequivocatus
35867	aequivocatus
35868	aequivocatus
35869	aequivocatus
35870	aequivocatus
35871	aequivocatus
35872	aequivocatus
35874	aequivocatus
35875	aequivocatus
35876	aequivocatus
35877	aequivocatus
35880	aequivocatus
35881	aequivocatus
35883	huperuthrus
35884	huperuthrus
35886	huperuthrus
35887	huperuthrus
35888	huperuthrus
35934	aequivocatus
36132	huperuthrus
36135	huperuthrus
36136	huperuthrus
36137	aequivocatus
36318	aequivocatus
36320	aequivocatus
36837	eximius
36862	eximius
36865	eximius
39639	sanctidiegi
39747	eximius
44509	sanctidiegi
44510	sanctidiegi
47196	californicus
47197	californicus
50236	aequivocatus
50237	aequivocatus
51600	eximius

51999	eximius
54417	sanctidiegi
54927	eximius
54928	eximius
57049	eximius
59095	eximius
59105	eximius
59106	eximius
59107	eximius
59108	eximius
59889	aestuarinus
60289	kernensis
65428	eximius
65430	eximius
65501	eximius
66806	stephensi
66807	stephensi
66808	stephensi
69507	eximius
69511	eximius
69512	eximius
69513	eximius
69514	eximius
69515	eximius
69516	eximius
69517	eximius
69519	eximius
69520	eximius
69522	eximius
69687	eximius
70129	scirpensis
70130	scirpensis
70160	californicus
73072	californicus
73073	californicus
73074	californicus
73076	californicus
73077	californicus
73078	californicus
73079	californicus
73080	californicus
74535	sanctidiegi
74697	stephensi
81363	sanctidiegi
83499	eximius
83519	sanpabloensis
84924	sanctidiegi
85151	sanctidiegi
86793	eximius
86794	eximius
86936	californicus
88214	eximius
88680	eximius
89453	eximius
89903	sanctidiegi
89904	sanctidiegi
90271	sanctidiegi
93549	eximius
93551	eximius
93552	eximius
93554	eximius
93561	eximius
93579	eximius
93895	aestuarinus
95743	eximius
95751	eximius
96275	eximius
96736	eximius
97529	californicus
98016	californicus
98017	californicus
98019	californicus
98020	californicus
98021	californicus
98022	californicus
99629	constrictus
99652	constrictus
100973	californicus
101086	eximius
101362	eximius
102651	aestuarinus
102696	eximius
102820	eximius
103901	californicus
103902	californicus
103903	californicus
103904	californicus
105890	eximius
105894	eximius
105898	eximius
105900	eximius
108773	californicus
108777	californicus
108778	californicus
108780	californicus
108781	californicus
108784	halophilus
108816	halophilus
112068	californicus
113230	eximius
114364	sanctidiegi
116243	Eximius
123548	sanctidiegi
125073	eximius
125904	sanctidiegi
126953	californicus
128796	californicus
128797	californicus
129075	californicus
132723	eximius
132724	eximius
132725	eximius
132726	eximius
132727	eximius
132728	eximius
136209	californicus
136673	eximius
148454	eximius
148455	eximius
148460	eximius
148461	eximius
148489	eximius
148490	eximius
148494	eximius
148503	eximius
149766	californicus
154399	eximius
154408	eximius
154443	eximius
158042	eximius
177622	californicus
177623	californicus
179051	eximius
179072	eximius
179073	eximius
183834	californicus
192086	eximius
198772	sanctidiegi
198787	californicus
200049	sanctidiegi
200053	sanctidiegi
200871	sanctidiegi
200873	sanctidiegi
200874	sanctidiegi
200876	sanctidiegi
206895	aestuarinus
206897	aestuarinus
206898	aestuarinus
208675	sanctidiegi
216053	sanctidiegi
216061	sanctidiegi
216077	sanctidiegi

CHAPTER 2

Identifying California *Microtus* species using geometric morphometrics documents Quaternary geographic range contractions

ABSTRACT

Paleontology can provide a deep-time dimension to observations about recent reactions of small mammals to climate change. Obtaining this perspective for voles (*Microtus*), a common and important constituent of North American mammal communities, has been difficult because species identification based on their dental remains is problematic. Here I demonstrate that geometric morphometrics and discriminant analyses can use commonly-fossilized dental features to reliably identify the five species of *Microtus* living in California today: *Microtus californicus* (California vole), *Microtus longicaudus* (long-tailed vole), *Microtus montanus* (montane vole), *Microtus oregoni* (Oregon vole), and *Microtus townsendii* (Townsend's vole). Analyses of landmarks on the lower first molar provide more accurate identification than those of the third upper molar, and it is important to use jackknife misidentification metrics to assess the precision of discriminant analyses. Addition of semilandmark curves on the first lower molar does not improve accuracy. The utility of these techniques is demonstrated by identifying *Microtus* specimens from two California fossil localities, Pacheco 2 and Prune Avenue, which provides the first evidence for extralimital presence of *M. longicaudus* at both localities. The presence of *M. longicaudus* at these low-elevation sites indicates that pronounced geographic range changes in this species that has been observed in California over the last hundred years also occurred during previous climate changes. Eventually it may be possible to ascertain whether current range changes are exceeding those that typified response to past climate changes.

Keywords *Microtus*, discriminant analysis, geometric morphometrics, jackknife, Quaternary, California, *Microtus longicaudus*

INTRODUCTION

California's small mammal species have shifted their ranges over the last hundred years as a result of climate change (Moritz *et al.*, 2008), but the significance of these shifts relative to a background rate of range changes is not well known. Such knowledge can only be gained by setting recent changes in a longer temporal context using the fossil record (Hadly & Barnosky, 2009). While we know that in general small mammals shifted their ranges in response to climatic changes throughout the Quaternary (Graham *et al.*, 1996; Guralnick, 2007; Lyons, 2003), and almost certainly earlier, few studies have documented range shifts in California (Blois & Hadly, 2009; Blois *et al.*, 2010), and none have documented shifts in one of the most abundant taxa, voles of the genus *Microtus.*

Rodents of the genus *Microtus* can provide essential information about how small mammals reacted to historic environmental changes, because they are abundant now, are well represented in the Quaternary fossil record, and are known to have experienced range changes in response to past and recent environmental changes. Some species remained in place during past environmental changes as dramatic as glacial-interglacial transitions at the Pleistocene-Holocene boundary that marked the end of the Last Glacial Maximum (LGM; 21 kya) (Blois *et al.*, 2010) others changed their ranges dramatically when faced with such major ecosystem shifts (Bell, 2004; Graham *et al.*, 1996; Guilday, 1962; Repenning, 1987; Wood & Barnosky, 1994). California *Microtus* species faced additional stress when they experienced the near-complete replacement of their main food supply, native grasses and fronds, when European invasives swept through the Pacific Coast starting just 200-300 years ago (Dallman, 1998).

Although California's fossil record contains abundant *Microtus* fossil specimens, they are primarily preserved as isolated teeth that have frustrated attempts to assign them to species, which is critical to understanding their detailed responses to environmental changes. Here, I describe techniques developed to identify, with only a single molar, five species of the genus *Microtus* that are common in the Pacific Coast region of the United States: *Microtus californicus* (California vole), *Microtus longicaudus* (long-tailed vole), *Microtus montanus* (montane vole), *Microtus oregoni* (Oregon vole), and *Microtus townsendii* (Townsend's vole). I then show that these techniques permit the discovery of previously undetectable geographic range extensions that place these species' reactions to current environmental changes in broader context.

Bell and Bever (2006) report that California's *Microtus* fossil specimens have historically been assigned to species based on current geographic affinity (Miller, 1971; Savage, 1951) when the specimens could only be confidently assigned to the genus level based upon morphological characters. Geography-based species identifications are clearly problematic if we want to understand species-level range changes. In fact, the California archeological locality Nightfire Island, on the northeastern border, contains the only example of a fossil *Microtus* species other than *M. californicus* in all of California (Zakrzewski, 1985, NEOMAP 2010, Paleobiology Database 2010). Nightfire Island specimens are identified as a *M. cf. montanus* (Sampson, 1985), and the locality is within the modern range of this species. The reason that it is difficult to distinguish between these five vole species using isolated teeth is that *Microtus* teeth contain so much intraspecific variation in dental characters (Barnosky, 1990; Bell & Repenning, 1999; Graham & Semken, 1987). In fact, some previously recognized characters commonly used for specific identification in *Microtus* have since been found unreliable as diagnostic features because they sometimes occur in other species (e.g., the posterolingual dentine field on the second upper molar, Bell & Repenning, 1999). Past work has demonstrated, however, that it is possible to distinguish between vole species if one considers tooth shape as a whole rather than individual characters (Smartt, 1977; Wallace, 2006), especially using discriminant analyses. For example, Smartt (1977) identified fossil *Microtus* species in New Mexico by quantifying tooth length, tooth width, and reentrant angle depths for each molar (see Figure 2-1 for molar nomenclature). He included *Microtus pennsylvanicus* (meadow vole), *Microtus mexicanus* (Mexican vole), *M. montanus* and *Microtus ochrogaster* (prairie vole) in his analysis; the first lower molar (m1) most often correctly identified to species (Smartt 1977).

In 2006, Steven Wallace demonstrated that geometric morphometrics could be used in conjunction with discriminant analyses to distinguish between two other species of *Microtus*, *M. pennsylvanicus* and *Microtus xanthognathus* (taiga vole), using the m1.

In the present study, I use geometric morphometrics and discriminant analyses to distinguish between the five species of the genus *Microtus* living in California today by examining a single molar. I report the results from discriminant analyses using the landmarks established by Wallace (2006) on the m1, landmarks and semilandmark curves on the m1, and landmarks on the third upper molar (M3). Further, I discuss the importance of using jackknife results to determine whether the sample size of the training set is large enough to build a precise discriminant analysis. The methods described herein allow accurate identification of many more fragmentary specimens than were previously recognizable to species.

Although all five species considered in this study are currently present in California, *M. californicus'* range covers the majority of the state, whereas *M. montanus* and *M. longicaudus* are restricted to the higher elevations of the Sierra Nevada and Cascade Mountains (*M. montanus* is also in the Klamath and Northern Coastal Ranges and *M. longicaudus* in the Transverse Ranges) and *M. oregoni* and *M. townsendii* are only present in the very northern portion of the state (Figure 2-2). *M. montanus* and *M. longicaudus* have been reported to have Pleistocene ranges that included lower elevation regions east of their modern range extending into the Great Plains (Hoffman & Jones, 1970; Stewart, 1978, 1987; Turner, 1974; Wallace, 2001), however these specimen identifications have not all been validated. Additionally, Moritz et al. (2008) report that *M. longicaudus* has experienced an approximately 600 meter upward movement of its lower range boundary in Yosemite National Park over the last hundred years, and *M. californicus* experienced an approximately 500 meter expansion of its upper elevational range into the same mountains. No recent range change is recorded for *M. montanus* in Yosemite National Park.

Once I establish discriminant methods for identifying *Microtus* species, I then apply the discriminant analysis to specimens from two fossil localities in Northern California, Pacheco 2 and Prune Avenue (Figure 2-2), to assess whether Californian *Microtus* species ranges were as mobile as the purported movement of the species east of the Rocky Mountains (Hoffman & Jones, 1970; Stewart, 1978, 1987; Turner, 1974; Wallace, 2001). Pacheco 2 is presumed to be Pleistocene in age, and Prune Avenue is from the Holocene (836-4,283 cal. yr BP). Both California localities are within grassland habitats of the Central Valley.

MATERIALS AND METHODS

Specimens used

Discriminant functions were built using training sets of 271 to 241 modern specimens of known identity from the Museum of Vertebrate Zoology (MVZ) (University of California, Berkeley, USA) collections, depending upon the analysis (see Table 2-1 & Table 2-S1). The total number of specimens examined varied between the three analyses because an increasing number of specimens had to be discarded due to breakage as the number of variables (landmarks) and surface area of the tooth perimeter examined increased. Specimens were selected evenly from throughout the geographic range of each species and included an approximately even number of males and females from each species. Only jaws with fully erupted molars were used, though specimens with juvenile skull sutures were included. All specimens used were curated as skin and skull preparations, and all field identifications were reconfirmed using the key in Figure 2-3 with ambiguous specimens excluded. Toothrows were digitally photographed using a Nikon D70s and AF Micro-NIKKOR 60 mm f/2.8D lens.

Given that any single fossil locality has a limited number of specimens with differing preservation quality, methods for examining both the m1 and M3 were initially explored, because these *Microtus* molars are both highly variable and have excellent potential for identification purposes (Barnosky, 1990; Bell, 2004; Bell & Bever, 2006; Guilday, 1982; Semken & Wallace, 2002; Smartt, 1977; Zakrzewski, 1985). Only left molars were used, though no significant differences were found between left and right molars, and right fossils could likely be reasonably identified judging from the discriminant analyses (Wallace, 2006). Once it was determined that the m1 was the more accurate molar for identification, and in view of the fact that it is the most common *Microtus* molar found in fossil collections, further analyses using semilandmark curves were focused on that tooth in an analysis hereafter referred to as m1-SL (Table 2-1).

Landmark selection

Two-dimensional landmark coordinates were digitized on Tiff images using tpsDig 2.10 (Rohlf, 2006a)(Table 2-1). Landmarks are points that can be described anatomically and are homologous between specimens (Bookstein *et al.*, 1985). Landmarks for m1 and m1-SL were based upon those selected by Wallace (2006) with the exception that the 4 landmarks that anchor semilandmark curves were moved to the outside of the enamel band (Table 2-1). Landmarks on the M3 are similar to those on the m1 with the exception of the posterior-most landmark, which is a type II landmark that represents the apex of the curve on the posterior loop (Bookstein, 1991). Semilandmarks in the m1-SL analysis were placed on the tooth's posterior loop and anterior cap (Table 1). Three semilandmarks and two anchor landmarks were placed on the posterior loop to delimit the tooth and measure the relative width of the posterior loop. Twelve semilandmarks and 2 anchor landmarks were placed along the anterior cap (Figure 2-1 & Table 2-1), an important feature that represents the highest amount of variation in this tooth (Bell & Bever, 2006). Semilandmark curves were drawn and initially subsampled in tpsDig 2.10 (Rohlf, 2006a), slid using bending energy in tps Relative Warps 1.45 (Rohlf, 2007), and subsequently subsampled in tps Utility 1.40 to the semilandmarks reported herein (Rohlf, 2008).

Geometric morphometric and discriminant 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 & 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 $CS = \surd\ \Sigma\ [(x_L-x_c)^2+(y_L-$

$y_c)^2$], was normalized across specimens. Finally, specimens were rotated to minimize the overall summed squared distances between landmarks. The resulting size- and orientation- corrected landmark coordinates could 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 a mismatch between the number of variables and the degrees of freedom (Rohlf & Corti, 2000; Zelditch *et al.*, 2004). Therefore, the discriminant analysis used partial warp and uniform component scores calculated using PCAGen 6p (Sheets, 2001a), rather than Cartesian coordinates, because they contain equal degrees of freedom and variables. This gives the same results as if the Cartesian coordinates were used and the degrees of freedom were corrected manually (Rohlf & Corti, 2000; Zelditch *et al.*, 2004).

One of the strengths of geometric morphometrics is that it is able to consider overall shape independent of size. Size-independence can be very important for certain types of analyses, for example when looking at functional traits or ontogenetic series. However, in the case of species identification, size is not a potentially confounding factor. Thus if it significantly contributes to identification, it can only be a helpful variable to include in the discriminant analysis. In this study, discriminant analyses were performed on the partial warp scores, uniform components and/or centroid sizes from the training set using JMP 7.0 (SAS Institute, Cary, NC), and the precision of each discriminant analysis was described with and without the inclusion of centroid size (Table 2-1). Centroid sizes were produced by Coordgen 6h (Sheets, 2000) and, in the case of the m1-SL, were calculated prior to semilandmark sliding and subsampling.

A discriminant analysis determines the canonical axes that maximally separate designated training groups. The standard misidentification rate tells the percent of specimens in the training group that are misidentified when these canonical axes are applied. This is the standard metric reported for many discriminant analyses (e.g., Smartt, 1977; Wallace, 2006). Here, jackknife misidentification rates are also reported. The specimens were jackknifed by removing each specimen, coding it as an unknown, and then determining whether it was correctly identified by the discriminant analysis before replacing it and repeating for all specimens. This metric can indicate how well the analysis correctly identifies unknown specimens compared to another discriminant analysis. However, jackknife misidentification rates do not necessarily indicate that unknowns will be misidentified that percent of the time. Rather they indicate the percent of specimens that may not be confidently identifiable. Additionally, training sets were subsampled to 20 specimens per species to determine the effects of sample size on standard misidentification rates and jackknife misidentification rates. Jackknife misclassification rates were calculated using CVAGen 6n (Sheets, 2005) and the R package MASS (R Development Core Team 2009, Venables & Ripley, 2002).

Identification of fossil specimens

Both Pacheco 2 and Prune Avenue are fossil localities from the East Bay region of Northern California that were salvaged by members of the University of California Museum of Paleontology (UCMP) during construction activities as houses were being built. Pacheco 2 is associated with another locality, Pacheco 1, which contains extinct megafauna, lending stronger evidence that it is Pleistocene in age (Tomiya *et al.*, in preparation). Prune Avenue has been radiocarbon dated to 836-4,283 cal. yr BP (Holocene) at the Lawrence Livermore National Laboratory CAMS facility using preparation procedures described by Brown *et al.*, 1988 and Bronk Ramsay *et al.,* 2004. Once the discriminant analysis was established, 26 fossil *Microtus* specimens from Pacheco 2 and 19 specimens from Prune Avenue were included as unknowns in the m1-SL discriminant analysis (see Table 2-S2).

Identification confidence indicates which of the five species a specimen is most closely associated with. If a specimen were to fall midway between two species, for example *M. californicus* and *M. longicaudus*, it will have 50% identification confidence for each species; however if a specimen falls the same distance away from *M. californicus* but not in the direction of another species, it will still have high confidence for *M. californicus* but might also have a large Mahalanobis distance (squared distance to the centroid of the *M. californicus* group). Therefore, if a specimen is a representative of a species not included in the training set, it would not necessarily be detected by identification confidence, but would have a high Mahalanobis distance and potentially have other purported unidentifiable specimens clustered around it in discriminant shape space. Only specimens with ≥95 percent confidence in identification were considered, and the Mahalanobis distance of the specimen must have fallen within two standard deviations of the species mean shape.

RESULTS

Discriminant analyses

In all cases except the subsampled analyses, the inclusion of centroid size improved the discriminant analyses. Therefore, all results refer to those analyses that include centroid size. Geometric morphometrics and discriminant analyses were better able to distinguish between the training set m1 specimens than the M3 specimens both according to the standard misidentification rates (m1= 5.2; M3= 15.1) and the jackknife misidentification rates (m1= 12.7; M3= 24). The first four canonical axes provide nearly complete separation of the training groups (first 3 most significant canonical axes shown in Figure 2-4). Shape separation of m1s appears to be based upon different relative widths of lingual and labial triangles, reentrant angle size, and relative curvature of the major axis of the tooth (Figure 2-4).

M3 specimens appeared to exhibit higher rates of misidentification when fewer specimens were included in the analysis according to the standard misidentification metric ($M3_{100}$=23.6; $M3_{271}$=15.1). However, the jackknife metric suggests that sample size does not strongly affect the misidentification rates ($M3_{100}$=22; $M3_{271}$=24). The m1 demonstrated a quite different effect. According to the standard misidentification metric, m1 specimens appeared to have higher misidentification when more specimens were included in the training set than when they were not ($m1_{100}$= 1; $m1_{251}$= 5.2). However, the jackknife misidentification rates illustrate that the m1 analysis with fewer specimens actually performed much more poorly than the one that included more specimens in the training set ($m1_{100}$= 40; $m1_{251}$= 12.7) (Table 2-1).

The m1-SL analysis did not show an improvement over the m1 analysis (m1= 12.7; m1-SL= 12.9) (Table 2-1). The m1-SL analysis showed the same pattern as the m1 analysis in that the standard misclassification metric indicated that the analysis with fewer specimens in the training set was more accurate than the one with more specimens ($m1\text{-}SL_{100}$= 0; $m1\text{-}SL_{251}$= 1.2), whereas the jackknife misclassification rates told the opposite story ($m1\text{-}SL_{100}$= 42; $m1_{251}$= 12.9) (Table 2-1).

Fossil identification

Pacheco 2 specimens were identified as 15 (58.5%) *M. californicus* specimens, 3 (11.5%) *M. longicaudus* specimens, and 8 (30%) unknown (Table 2-S2). Prune Avenue specimens were identified as 7 (37%) *M. californicus* specimens, 3 (16%) *M. longicaudus* specimens, and 9 (47%) unknown (Table 2-S2). No specimens with extreme Mahalanobis distances were present at either fossil locality. The total percent of unidentifiable specimens among both localities was 38%.

DISCUSSION

Separation of the modern training groups is strong under the m1-SL analysis, as can be seen in Figure 2-4. The jackknife results of the discriminant analysis indicate that the m1 analyses that include centroid size are superior at distinguishing between the 5 modern California *Microtus* species and identifying unknowns. However, there is no reason to increase the number of variables used to discriminate specimens by including semilandmark curves on the m1, because this does not improve the analysis. The M3 analysis has lower reliability, possibly because there are fewer homologous structures, and therefore landmarks, on this tooth. However it is also possible that there is little selection, or else selection for very similar function, upon this tooth within each species. The superiority of the m1 to the M3 for distinguishing between *Microtus* species parallels the results seen in Smartt (1977), indicating that whatever the reason for this pattern, it may be consistent across North American *Microtus* species.

Subsampling the training sets has demonstrated the importance of using jackknife metrics to compare the relative strengths of discriminant analyses. When few specimens are included in each group of the training set, the overall variation within each group may not be fully sampled. If this is the case, it will be easier to find canonical axes that will separate the groups well than when enough specimens are included to approach the full level of variation in that group. The standard misclassification metric, which describes how many specimens are misclassified when the canonical axes of maximum group separation are applied, can therefore make an analysis with very few specimens superficially appear to discriminate more accurately than one in which more specimens are included. However, when these specimens are jackknifed, identifications of those ‘unknowns’ are less accurate than the standard misclassification rate reported, and consistently favor the analysis that includes more specimens (Table 2-1). As such, simply reporting how many specimens of known identity are misclassified by the discriminant analysis is not sufficient to characterize the precision or repeatability of the analysis. To create a precise discriminant analysis that will assign identifications robustly to unknown specimens, enough specimens must be included in each group of the training set that the percent misclassified under jackknifing approaches a stable number as more specimens are included. This problem is exacerbated as more variables are included in the discriminant analysis, as can be seen in the case of the M3 analysis, which has very few variables and less difference between the accuracy of the subset and full set analyses.

The percent of unidentifiable fossil specimens from Pacheco 2 and Prune Avenue (38%) exceeds that predicted by the jackknife misidentification rate of the m1-SL training set (12.9%). However, as Wallace (2006) pointed out, one must be careful about the assumptions implicit in these analyses. The training set consists of *Microtus* specimens collected over the last hundred years; whereas the fossil specimens represent a time-averaged sample of unknown duration. Additionally, it is difficult to know whether morphological variation was the same in past populations as in modern species. As mentioned in the introduction, California *Microtus* species have weathered a large shift in grassland plant species in the Holocene (Dallman, 1998), and this change in food type could have affected the amount of dental variation present in the species. Although the number of identifiable specimens is lower than expected, the specimens still strongly cluster with modern species (Figure 2-4), and do not include any specimens with large Mahalanobis distances or clustering that would suggest the presence of species not included in the discriminant analysis.

The fossil *Microtus* identifications represent the first extralimital *Microtus* fossils in California, demonstrating that *M. longicaudus* occurred at lower elevation and approximately 160 km further west than the species occurs at a similar latitude today. The fossil localities are approximately 600 meters lower than *M. longicaudus'* lower elevational limit in Yosemite 100 years ago, and about 1200 meters lower than it is presently known in California. This corresponds with the observed range contractions of this species east of the Rocky Mountains over the same time period (Wallace, 2001) and gives a deeper time extension to the modern range shifts (Moritz *et al.*, 2008) of the species in California. While the exact age of the fossil specimens is unknown, palynological data from nearby Mono Lake (Figure 2-2) indicate that this region was approximately 2°C cooler and got approximately 200 mm more precipitation 2000 year ago as compared to today, and was cooler and moister yet during the LGM of the late Pleistocene (Davis, 1999). An upward, eastward range contraction of *M. longicaudus* is consistent with warming and drying since the LGM, and possibly also to anthropogenic landscape modification at the two fossil localities examined. Because we can not be sure whether range shifts was constant or fluctuated through time, an exact rate of range retraction for prehistoric *M. longicaudus* in California is not possible to determine. However, assuming the minimum age of the confidently dated extralimital *M. longicaudus* fossils, 836 cal. yrs BP, then the rate of range contraction from that time until 100 years ago was 0.82 m/yr. In comparison, the rate of elevational range contraction in Yosemite of 6 m/yr is considerably more rapid. Such observations highlight the need for additional identifications of fossil specimens to more accurately assess whether current range shifts are indeed faster than past ones.

Although *M. montanus* is also purported to have undergone a similar range contraction to *M. longicaudus* from east of the Rocky Mountains (Hoffman & Jones, 1970; Stewart, 1978, 1987; Turner, 1974; Wallace, 2001), there is no evidence of *M. montanus* at low-elevation Central Valley sites in California. This parallels the pattern of recent range contractions reported for Yosemite, where *M. montanus* has not changed in its elevational distribution in the same time that *M. longicaudus* moved upslope. Identification of additional specimens from more sites is needed to see if this apparent pattern is meaningful, and if so, what it implies about the nature of climate changes and species response in California compared to the eastern side of the Rocky Mountains.

CONCLUSIONS

The methods developed in this paper provide more accurate identifications than have hitherto been possible for fossil *Microtus* species of California. The same species occur in much of the western United States, so the method should be broadly applicable. Importantly, the method provides a way to for trace the response of *Microtus* species—abundant members western American mammal communities—to past environmental changes, which in turn is essential to interpreting the significance of their observed range shifts over the past century.

The geometric morphometric and discriminant analyses described herein indicate that the m1 is the best tooth to use for discrimination among *Microtus* species. Subsampling and jackknifing of the discriminant analyses show that it is imperative to report jackknife results when examining the precision of an analysis for identifying unknowns and for comparing discriminant analyses.

By identifying the species of *Microtus* from only two fossil localities, it becomes apparent that significant geographic range changes occurred since the Pleistocene in California. This has not been documented before, but this pattern is not unexpected given the marked range changes for voles suspected elsewhere. *M. longicaudus* occurred prehistorically in the Central Valley of California, 160 km away from its historic range (100 years ago) and 600 meters lower. There is as yet no evidence that *M. montanus* exhibited dramatic range reductions when *M. longicaudus* did, a pattern that is evident in how these two species have reacted through the last century of climate change in Yosemite. However, much more fossil data is needed to verify whether this pattern widely holds in the Sierras and further west, and to determine how observed rates of range retraction of *M. longicaudus* over the past century compare with earlier range shifts that accompanied climate change.

FIGURES AND TABLES

FIGURE 2-1 First lower molar of *Microtus* showing the morphological terminology used.

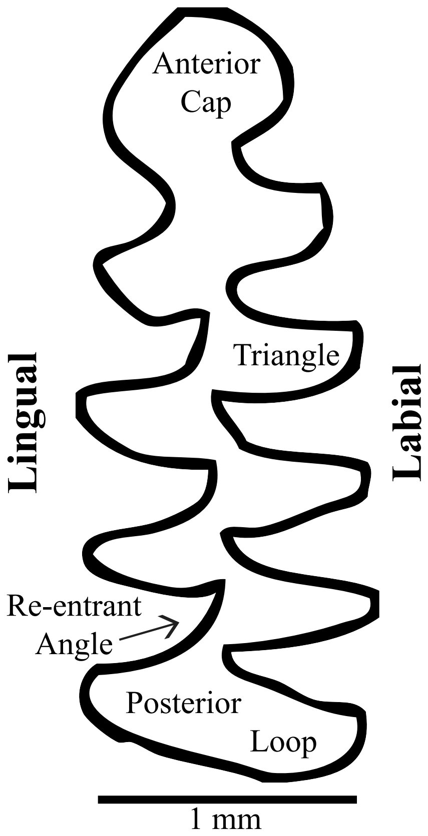

Anterior Cap
Triangle
Lingual
Labial
Re-entrant Angle
Posterior Loop
1 mm

FIGURE 2-2 Map of California showing the modern distribution of *M. californicus, M. longicaudus, M. montanus, M. oregoni,* and *M. townsendii*. The location of Pacheco 2 and Prune Avenue are also shown.

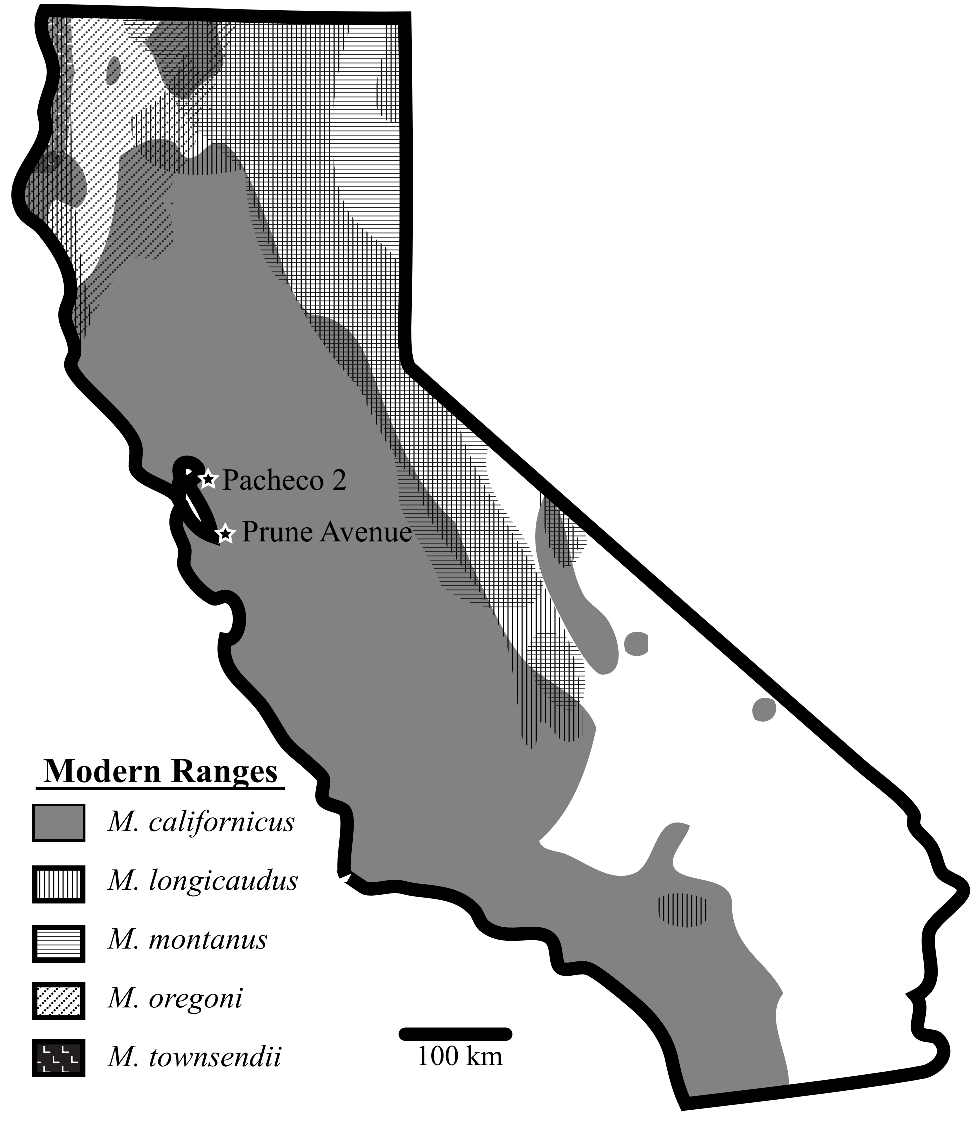

Pacheco 2
Prune Avenue
Modern Ranges
M. californicus
M. longicaudus
M. montanus
M. oregoni
M. townsendii
100 km

FIGURE 2-3 Key used to identify modern *Microtus* museum specimens from California. a. Figure demonstrating the appearance of incisive foramina that do and do not narrow posteriorly. b. Figure demonstrating a view from directly above the rostrum of the skull where incisors are and are not visible. (Sources consulted: Hall, 1981; Ingles, 1965; s, 2004; Maser & Storm, 1970; Verts & Carraway, 1998)

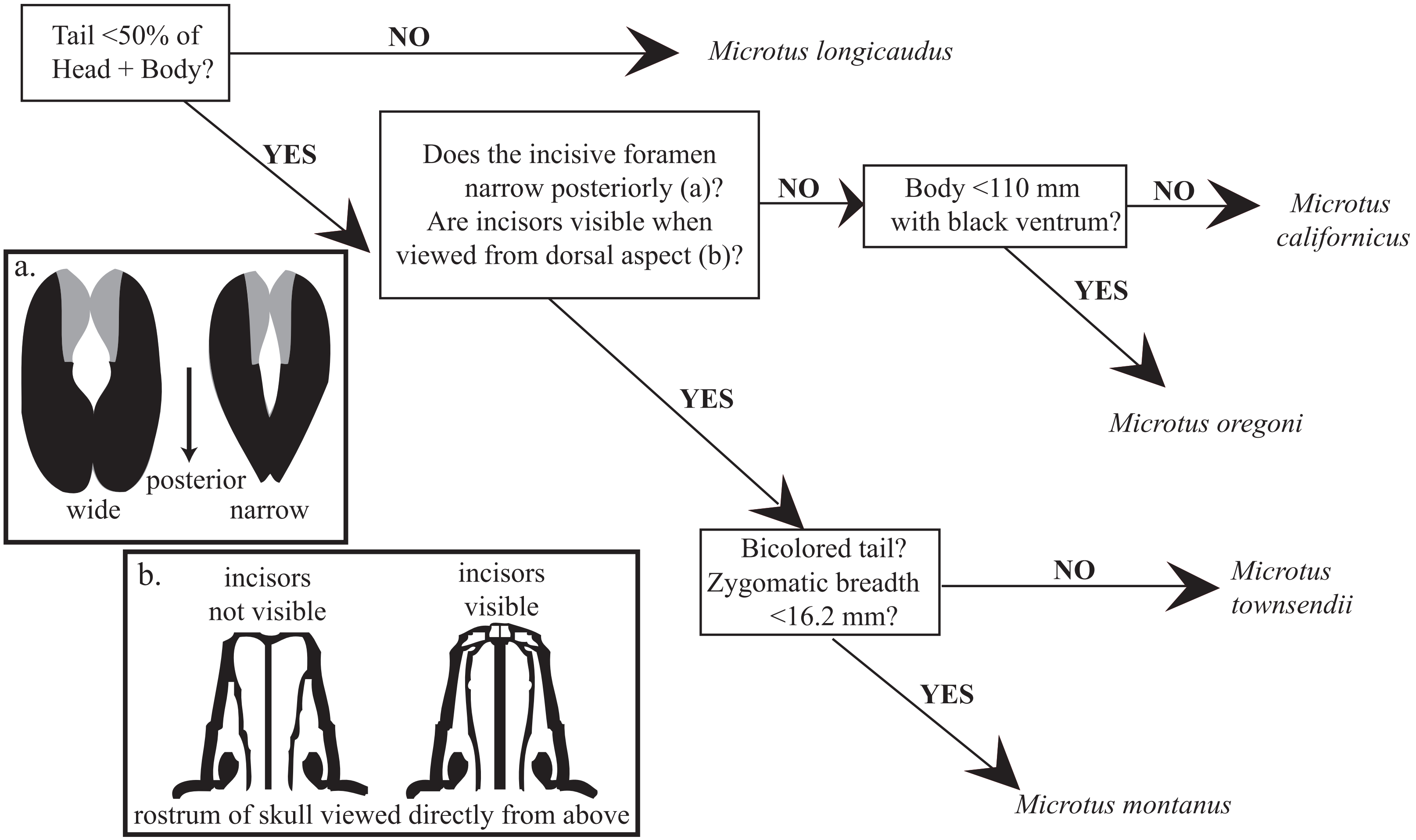

Tail <50% of Head + Body?
NO
Microtus longicaudus
YES
Does the incisive foramen narrow posteriorly (a)? Are incisors visible when viewed from dorsal aspect (b)?
NO
Body <110 mm with black ventrum?
NO
Microtus californicus
YES
Microtus oregoni
YES
Bicolored tail? Zygomatic breadth <16.2 mm?
NO
Microtus townsendii
YES
Microtus montanus
a.
posterior
wide
narrow
b.
incisors not visible
incisors visible
rostrum of skull viewed directly from above

FIGURE 2-4 Plot of discriminant analysis of m1-SL showing the first three canonical axes of training group separation. Fossil specimens are also plotted as points. Spheres represent the 95% confidence interval for the group mean. Example teeth for each species indicate the shape changes along the canonical axes.

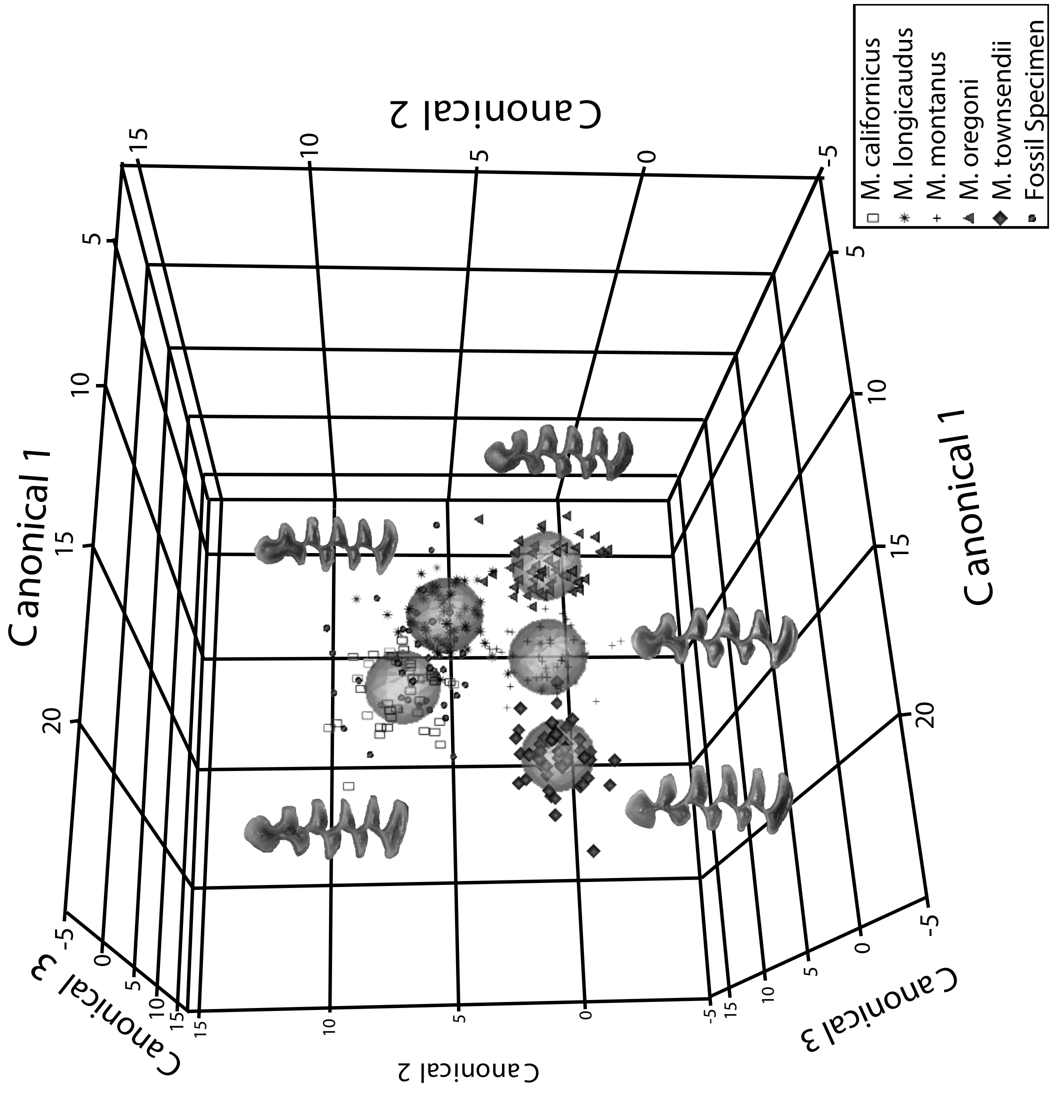
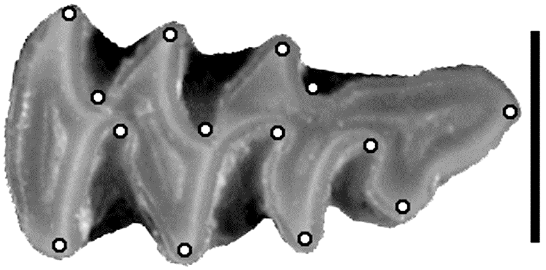
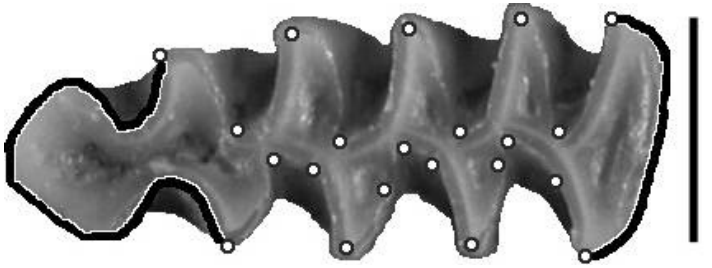

Canonical 1
Canonical 1
Canonical 2
Canonical 2
Canonical 3
Canonical 3
5
10
15
20
5
10
15
20
15
10
5
0
-5
-5
0
5
10
15
-5
0
5
10
15
15
10
5
0
-5
□ M. californicus
* M. longicaudus
+ M. montanus
▲ M. oregoni
◆ M. townsendii
● Fossil Specimen

Table 2-1 The results of the three discriminant analyses, M3, m1, and m1-SL, are compared and landmark and semilandmark placement are depicted. Landmarks are black-and-white points on the molars, and semilandmark curves are depicted as black lines along the anterior cap and posterior loop. Scale bars represent 1 mm. “*n*” is the number of *c, l, m, o*, and *t* representing *M. californicus, M. longicaudus, M. montanus, M. oregoni,* and *M. townsendii,* respectively.

Anterior Posterior	Total specimens examined	Centroid size included?	Percent mis-classification	Jackknife percent mis-classification
M3 with landmarks 	100: $n_{c,l,m,o,t}$=20	No	19	32
		Yes	23.6	22
	271: n_c= 46 n_l= 48 n_m= 49 n_o= 49 n_t= 49	No	18.8	29.5
		Yes	15.1	24
m1 with landmarks 	100: $n_{c,l,m,o,t}$=20	No	1	39
		Yes	1	40
	251: n_c= 46 n_l= 48 n_m= 49 n_o= 49 n_t= 49	No	9.1	20.7
		Yes	5.2	12.7
m1 with semilandmarks 	100: $n_{c,l,m,o,t}$=20	No	0	42
		Yes	0	42
	241: n_c= 46 n_l= 48 n_m= 49 n_o= 49 n_t= 49	No	3.7	19.1
		Yes	1.2	12.9

SUPPLEMENTARY TABLES AND FIGURES

TABLE 2-S1 Museum of Vertebrate Zoology specimens used to create discriminant analyses. "X" indicates in which analysis each specimen was included.

Specimen number	Species	m1	m1-SL	M3
13469	*M. longicaudus*	X	X	X
21099	*M. longicaudus*	X	X	X
21190	*M. longicaudus*	X		X
22388	*M. longicaudus*	X	X	X
23173	*M. longicaudus*	X	X	X
25014	*M. longicaudus*	X	X	X
25593	*M. longicaudus*	X	X	X
26409	*M. longicaudus*	X	X	X
26422	*M. longicaudus*	X	X	X
30816	*M. longicaudus*	X	X	X
30907	*M. longicaudus*	X	X	X
35063	*M. longicaudus*	X	X	X
35214	*M. longicaudus*			X
35224	*M. longicaudus*	X	X	X
37199	*M. longicaudus*			X
45858	*M. longicaudus*			X
46339	*M. longicaudus*	X	X	X
50909	*M. longicaudus*	X	X	X
51713	*M. longicaudus*	X	X	X
54419	*M. longicaudus*	X	X	X
54423	*M. longicaudus*	X	X	X
54823	*M. longicaudus*			X
54924	*M. longicaudus*	X	X	X
61270	*M. longicaudus*	X	X	X
64499	*M. longicaudus*	X	X	X
65221	*M. longicaudus*	X	X	X
65540	*M. longicaudus*	X	X	X
67060	*M. longicaudus*			X
69538	*M. longicaudus*	X	X	X
69558	*M. longicaudus*	X	X	X
71127	*M. longicaudus*	X	X	X
72400	*M. longicaudus*	X	X	X
74260	*M. longicaudus*	X	X	X
77535	*M. longicaudus*	X	X	X
79507	*M. longicaudus*	X	X	X
83922	*M. longicaudus*	X	X	X
86576	*M. longicaudus*	X	X	X
88687	*M. longicaudus*	X	X	X
96035	*M. longicaudus*	X	X	X
96036	*M. longicaudus*	X	X	X
96983	*M. longicaudus*	X	X	X
99283	*M. longicaudus*	X	X	X
103440	*M. longicaudus*	X	X	X
105523	*M. longicaudus*	X		X
109060	*M. longicaudus*	X	X	X
109327	*M. longicaudus*	X	X	X
118673	*M. longicaudus*	X	X	X
119354	*M. longicaudus*	X	X	X
122003	*M. longicaudus*	X	X	X
122005	*M. longicaudus*			X

126147	*M. longicaudus*	X	X	X
136903	*M. longicaudus*	X	X	X
148474	*M. longicaudus*			X
190188	*M. longicaudus*	X	X	X
196704	*M. longicaudus*	X	X	X
198766	*M. longicaudus*			X
202858	*M. longicaudus*	X	X	X
206525	*M. longicaudus*	X	X	X
15570	*M. montanus*	X	X	X
15633	*M. montanus*			X
24002	*M. montanus*	X	X	X
33722	*M. montanus*	X	X	X
36824	*M. montanus*	X	X	X
39748	*M. montanus*	X	X	X
40829	*M. montanus*	X	X	X
42048	*M. montanus*	X	X	X
45404	*M. montanus*	X	X	X
45416	*M. montanus*	X	X	X
45816	*M. montanus*	X	X	X
46590	*M. montanus*	X	X	X
50902	*M. montanus*	X	X	X
54789	*M. montanus*	X		X
59615	*M. montanus*	X	X	X
61280	*M. montanus*	X	X	X
64478	*M. montanus*	X	X	X
64711	*M. montanus*	X	X	X
64716	*M. montanus*	X	X	X
64922	*M. montanus*	X	X	
67671	*M. montanus*	X	X	X
68521	*M. montanus*	X	X	X
68750	*M. montanus*	X	X	X
68981	*M. montanus*	X	X	X
71110	*M. montanus*	X	X	X
72358	*M. montanus*	X	X	X
77464	*M. montanus*	X	X	X
77738	*M. montanus*	X	X	X
78051	*M. montanus*	X	X	X
78053	*M. montanus*	X	X	X
79421	*M. montanus*			X
79450	*M. montanus*			X
81522	*M. montanus*	X	X	X
83900	*M. montanus*	X	X	X
87790	*M. montanus*			X
88120	*M. montanus*	X	X	X
89964	*M. montanus*	X	X	X
93558	*M. montanus*	X	X	X
96372	*M. montanus*	X	X	X
96726	*M. montanus*	X	X	X
96735	*M. montanus*	X	X	X
112287	*M. montanus*	X	X	X
116451	*M. montanus*	X	X	X
116608	*M. montanus*	X	X	X
120809	*M. montanus*	X	X	X
122072	*M. montanus*	X	X	X

125219	*M. montanus*	X	X	X
126151	*M. montanus*			X
132861	*M. montanus*	X	X	X
134880	*M. montanus*	X	X	X
138668	*M. montanus*			X
147617	*M. montanus*	X	X	X
154039	*M. montanus*			X
154040	*M. montanus*	X	X	X
183846	*M. montanus*	X	X	X
206537	*M. montanus*	X	X	
206940	*M. montanus*	X	X	X
12348	*M. townsendii*	X	X	X
12428	*M. townsendii*	X	X	X
12435	*M. townsendii*	X	X	X
31069	*M. townsendii*	X	X	X
46453	*M. townsendii*	X	X	X
54432	*M. townsendii*	X	X	X
54433	*M. townsendii*	X	X	X
54434	*M. townsendii*	X	X	X
54435	*M. townsendii*	X	X	X
54436	*M. townsendii*	X	X	X
63495	*M. townsendii*	X	X	X
68823	*M. townsendii*	X		X
68824	*M. townsendii*	X	X	X
68825	*M. townsendii*	X	X	X
70435	*M. townsendii*	X	X	X
70436	*M. townsendii*	X	X	X
70437	*M. townsendii*	X	X	
70445	*M. townsendii*	X	X	X
83429	*M. townsendii*	X	X	X
83430	*M. townsendii*	X	X	X
83431	*M. townsendii*	X	X	X
88894	*M. townsendii*	X	X	X
94503	*M. townsendii*	X	X	X
94509	*M. townsendii*	X	X	X
94510	*M. townsendii*	X	X	X
94511	*M. townsendii*	X	X	X
94516	*M. townsendii*	X	X	X
94517	*M. townsendii*			X
94518	*M. townsendii*	X	X	X
94532	*M. townsendii*	X	X	X
94534	*M. townsendii*	X	X	X
94538	*M. townsendii*	X	X	X
94540	*M. townsendii*	X		X
94541	*M. townsendii*	X	X	X
94542	*M. townsendii*	X	X	X
94543	*M. townsendii*	X	X	X
96040	*M. townsendii*	X	X	X
96041	*M. townsendii*	X	X	X
96042	*M. townsendii*	X	X	X
96045	*M. townsendii*	X	X	X
96046	*M. townsendii*	X	X	X
101609	*M. townsendii*	X	X	X
134933	*M. townsendii*	X	X	X

134935	*M. townsendii*			X
134942	*M. townsendii*	X	X	X
134952	*M. townsendii*	X	X	X
183858	*M. townsendii*	X	X	X
183859	*M. townsendii*	X	X	X
190214	*M. townsendii*	X	X	X
216762	*M. townsendii*	X	X	X
216763	*M. townsendii*	X	X	X
216764	*M. townsendii*	X	X	X
216766	*M. townsendii*	X	X	X
20105	*M. oregoni*	X	X	X
20127	*M. oregoni*	X	X	X
20133	*M. oregoni*	X	X	X
20135	*M. oregoni*	X	X	X
20148	*M. oregoni*	X	X	X
44275	*M. oregoni*	X	X	X
46450	*M. oregoni*	X	X	X
46452	*M. oregoni*	X	X	X
54439	*M. oregoni*	X	X	X
54440	*M. oregoni*			X
54442	*M. oregoni*	X	X	X
57038	*M. oregoni*	X	X	X
60372	*M. oregoni*	X	X	X
87782	*M. oregoni*	X	X	X
87783	*M. oregoni*	X	X	X
87788	*M. oregoni*	X	X	X
88900	*M. oregoni*			X
88901	*M. oregoni*	X	X	X
94423	*M. oregoni*	X	X	X
94425	*M. oregoni*	X	X	X
94445	*M. oregoni*	X	X	X
94447	*M. oregoni*	X	X	X
94450	*M. oregoni*	X	X	X
94451	*M. oregoni*	X	X	X
94455	*M. oregoni*	X	X	X
94494	*M. oregoni*	X	X	X
94495	*M. oregoni*			X
94496	*M. oregoni*	X	X	X
96020	*M. oregoni*	X	X	X
96021	*M. oregoni*			X
96022	*M. oregoni*	X	X	X
96026	*M. oregoni*			X
96027	*M. oregoni*	X	X	
96028	*M. oregoni*	X	X	X
97516	*M. oregoni*	X	X	X
97518	*M. oregoni*	X	X	X
101862	*M. oregoni*	X		X
113249	*M. oregoni*	X	X	X
113251	*M. oregoni*	X	X	X
120608	*M. oregoni*	X	X	X
120609	*M. oregoni*	X	X	X
120610	*M. oregoni*	X	X	X
120612	*M. oregoni*	X	X	X
134901	*M. oregoni*	X	X	X

134902	*M. oregoni*	X	X	X
134905	*M. oregoni*	X	X	X
134906	*M. oregoni*	X	X	X
134910	*M. oregoni*	X	X	X
134915	*M. oregoni*	X	X	X
134918	*M. oregoni*	X	X	X
179087	*M. oregoni*	X	X	X
179088	*M. oregoni*	X	X	X
190202	*M. oregoni*	X	X	X
190203	*M. oregoni*	X	X	X
216761	*M. oregoni*	X	X	X
2790	*M. californicus*			X
2947	*M. californicus*	X	X	X
8991	*M. californicus*	X	X	
11586	*M. californicus*	X	X	X
12807	*M. californicus*			X
15760	*M. californicus*			X
15775	*M. californicus*	X	X	
15776	*M. californicus*			X
17385	*M. californicus*			X
18671	*M. californicus*	X	X	
23121	*M. californicus*			X
25001	*M. californicus*			X
26374	*M. californicus*			X
26383	*M. californicus*	X	X	
26385	*M. californicus*			X
28269	*M. californicus*	X	X	
28270	*M. californicus*	X	X	X
28922	*M. californicus*	X	X	
28923	*M.*	X	X	
28933	*M. californicus*	X	X	X
29336	*M. californicus*	X	X	X
35863	*M. californicus*	X	X	X
35884	*M. californicus*	X	X	
35887	*M. californicus*			X
36132	*M. californicus*			X
36136	*M. californicus*			X
36137	*M. californicus*	X	X	
36320	*M. californicus*	X	X	X
36865	*M. californicus*			X
44510	*M. californicus*	X	X	X
54927	*M. californicus*			X
59095	*M. californicus*	X	X	X
60289	*M. californicus*			X
65428	*M. californicus*	X	X	X
66806	*M. californicus*	X	X	X
69510	*M. californicus*	X	X	X
69522	*M. californicus*	X		X
70129	*M. californicus*	X		X
70130	*M. californicus*	X	X	X
73074	*M. californicus*	X	X	X
74697	*M. californicus*	X	X	X
83519	*M. californicus*	X	X	

84924	*M. californicus*	X	X	X
89453	*M. californicus*	X	X	
89904	*M. californicus*	X	X	
90271	*M. californicus*	X	X	
93895	*M. californicus*			X
96275	*M. californicus*	X	X	X
99652	*M. californicus*	X	X	X
101859	*M. californicus*	X		X
102696	*M. californicus*	X	X	X
103904	*M. californicus*	X	X	X
108768	*M. californicus*			X
108784	*M. californicus*	X	X	X
108816	*M. californicus*	X	X	X
113456	*M. californicus*			X
121605	*M. californicus*	X		
123685	*M. californicus*			X
129075	*M. californicus*	X	X	
132724	*M. californicus*	X	X	X
132727	*M. californicus*	X	X	X
148489	*M. californicus*	X	X	X
149766	*M. californicus*	X	X	X
154399	*M. californicus*	X	X	X
182146	*M. californicus*	X	X	
200047	*M. californicus*	X	X	
200053	*M. californicus*	X	X	X
200876	*M. californicus*	X	X	X
206897	*M. californicus*	X	X	
206898	*M. californicus*			X

TABLE 2-S2 Fossil specimens from the University of California Museum of Paleontology and their identifications based upon m1 tooth shape using geometric morphometrics and discriminant analyses. "X" indicates specimens with confident identifications based on prediction probability and Mahalanobis distances.

Specimen #	Locality	Predicted species	Prediction probability	Confident
v_190249	Pacheco 2	*M. longicaudus*	0.81	
v_190246	Pacheco 2	*M. californicus*	1.00	X
v_190244	Pacheco 2	*M. californicus*	0.99	X
v_190255	Pacheco 2	*M. longicaudus*	0.86	
v_190243	Pacheco 2	*M. californicus*	1.00	X
v_190242	Pacheco 2	*M. californicus*	1.00	X
v_190240	Pacheco 2	*M. californicus*	0.99	X
v_190251	Pacheco 2	*M. californicus*	0.99	X
v_190248	Pacheco 2	*M. longicaudus*	0.94	
v_190247	Pacheco 2	*M. californicus*	0.54	
v_190237	Pacheco 2	*M. californicus*	1.00	X
v_190236	Pacheco 2	*M. californicus*	1.00	X
v_190249	Pacheco 2	*M. californicus*	0.93	
v_190239	Pacheco 2	*M. californicus*	1.00	X
v_190463	Pacheco 2	*M. californicus*	0.85	
v_190465	Pacheco 2	*M. californicus*	1.00	X
v_190184	Pacheco 2	*M. californicus*	1.00	X
v_190199	Pacheco 2	*M. californicus*	1.00	X
v_190205	Pacheco 2	*M. californicus*	0.63	
v_190207	Pacheco 2	*M. californicus*	0.99	X
v_190208	Pacheco 2	*M. townsendii*	0.94	
v_190209	Pacheco 2	*M. longicaudus*	0.99	X
v_190211	Pacheco 2	*M. californicus*	1.00	X
v_190241	Pacheco 2	*M. longicaudus*	1.00	X
v_190245	Pacheco 2	*M. longicaudus*	1.00	X
v_190460	Pacheco 2	*M. californicus*	0.99	X
v_197576	Prune Avenue	*M. californicus*	1.00	X
v_197577	Prune Avenue	*M. californicus*	1.00	X
v_197578	Prune Avenue	*M. longicaudus*	0.97	X
v_197579	Prune Avenue	*M. californicus*	0.83	
v_197580	Prune Avenue	*M. californicus*	0.97	X
v_197581	Prune Avenue	*M. californicus*	0.88	
v_197582	Prune Avenue	*M. californicus*	1.00	X
v_197583	Prune Avenue	*M. longicaudus*	0.53	
v_197584	Prune Avenue	*M. longicaudus*	0.99	X
v_197585	Prune Avenue	*M. californicus*	1.00	X
v_197586	Prune Avenue	*M. longicaudus*	1.00	X
v_197587	Prune Avenue	*M. californicus*	1.00	X
v_197589	Prune Avenue	*M. longicaudus*	0.86	
v_197590	Prune Avenue	*M. californicus*	1.00	X
v_197591	Prune Avenue	*M. longicaudus*	0.74	
v_197592	Prune Avenue	*M. californicus*	0.86	
v_197593	Prune Avenue	*M. longicaudus*	0.97	X
v_197594	Prune Avenue	*M. californicus*	0.84	
v_197595	Prune Avenue	*M. californicus*	0.79	

CHAPTER 3

Using the paleontological record of *Microtus* to test species distribution models and reveal responses to climate change

ABSTRACT

Aim This study reconstructs the Quaternary distributions of five *Microtus* species (Rodentia: Arvicolinae) from the Pacific Coast of the United States using newly identified fossil specimens. I use these reconstructions to explore three hypotheses: (1) congeners react differently to climate change in a way that maintains genus-level range stability; (2) species distribution models (SDM) projected into the past are concordant with the empirical data on *Microtus* species distributions; and (3) recent patterns and rates of range change over the last 100 yrs of intense climate change are similar to those experienced by the same species since the Last Glacial Maximum (~21kya; LGM).

Location Specimens from 11 fossil localities in California, Oregon and Nevada are identified and evaluated.

Methods Geometric morphometrics and discriminant analyses are used to identify fossil *Microtus* specimens. SDMs for the five *Microtus* species are generated using 19 bioclimate variables and a maximum entropy modelling approach.

Results I confidently identify 144 *Microtus* fossils, including the first fossil *Microtus oregoni* and *Microtus townsendii.* Fossil range reconstructions indicate range contractions since the LGM in all five species, but the species react incongruously, maintaining a more or less stable distribution for the genus. Half of extralimital fossil specimens (i.e., those fossils found outside the present-day range of the species) are predicted by the SDMs. In Northern California, *Microtus montanus* and *Microtus longicaudus* show no range change and upward range contraction, respectively, throughout the Quaternary. The same reactions to climate as are seen in these two species over the last 100 years, although the rate of range contraction has greatly increased in recent time in *M. longicaudus*.

Main conclusions Pacific Coast *Microtus* species shifted their ranges since the LGM in response to different climate variables. In at least two of these species, *M. montanus* and *M. longicaudus*, recent climate responses (over the last 100 yrs) are concordant with those of warming at the LGM, but rates of elevational ascension increased in *M. longicaudus* over the last 100 years. Inconsistencies between LGM SDMs and Quaternary fossil ranges indicate potential problems with LGM precipitation reconstructions. In addition, recent range shifts in *Microtus californicus* do not appear to be the result of climate change, indicating that biotic or other abiotic variables not considered in the SDMs may be affecting present-day and past reconstructions. Overall, the study highlights the need for more, detailed, species-level palaeodistributions to put recent observations in a broader temporal context and examine the effectiveness of SDMs and LGM climate reconstructions.

Keywords *Microtus*, California, species distribution model, ecological niche, range, paleontology, Quaternary

INTRODUCTION

Monitoring efforts and predictive modelling provide us with data about how species are currently reacting to climate change and how we expect them to react in the future (e.g., Moritz *et al.*, 2008; Parmesan, 2006; Peterson *et al.*, 2002). Fundamentally important for both conservation and for understanding the interplay of climate change with species' ecology and evolution is to supplement these data with paleontological data (Hadly & Barnosky, 2009). This allows us to put short-term observations into a deeper-time perspective and to test the effectiveness of predictive models for individual species. For example, as temperatures have risen in recent times, species distributions have been shifting poleward and upward in elevation (Moritz *et al.*, 2008; Parmesan, 2006). Several Quaternary studies (2.58 mya-present) indicate that this may be a long-term trend, likely since the Last Glacial Maximum (LGM; *ca.* 21ka) (Graham *et al.*, 1996; Grayson, 2005; Guralnick, 2007). However, at the same time, a historical east-west precipitation gradient may be complicating this interpretation of North American range changes (Graham *et al.*, 1996; Lyons, 2003). These findings highlight the necessity of using detailed, species-level paleontological data and increasingly sophisticated methods to understand climatic impacts.

Unfortunately, we still face challenges in incorporating paleontological data into climate change research. Species-level fossil identifications are often difficult because fossil specimens are often fragmentary and not diagnostic to species. As a result, many paleoecological studies are performed at the genus level (e.g., Blois *et al.*, 2010; Hadly *et al.*, 2009), whereas most present-day monitoring and predictive studies are performed at the species level (e.g., Moritz *et al.*, 2008; Parmesan, 2006). A paleontological study linking these two levels of research by McGill *et al.* (2005) demonstrates community inertia at the genus level, and simultaneous extensive shifts in the species community through time, especially among congeners. The finding of stasis at the genus level and movement at the species level suggests that congeners may respond differently to a similar shift in climate so as to maintain similar genus-level distributions. Hadly *et al.* (2009) further augment these findings by demonstrating that genus-level ranges remain relatively constant through time, suggesting that this is the taxonomic level at which niche conservatism may occur. They go on to emphasize that congeneric competition and differential reactions to environmental changes may be the mechanisms necessary to maintain this genus range stability (Hadly *et al.*, 2009). *Microtus* was one of the genera found to be the most stable through time (Hadly *et al.*, 2009). Here I test the hypothesis that differential responses to climate change leads to genus-level range stability in a group of five *Microtus* (Rodentia: Arvicolinae) species from the West Coast of the United States: *Microtus californicus* (Peale, 1848)*, Microtus longicaudus* (Merriam, 1888), *Microtus montanus* (Peale, 1848), *Microtus oregoni* (Bachman, 1839)*,* and *Microtus townsendii* (Bachman, 1839).

To examine this hypothesis, we need to improve our ability to identify Quaternary fossil specimens to the species level with confidence. In previous publications (Chapter

2), I have taken a first step in this process by developing a method for identifying *Microtus*, a previously problematic group, in the fossil record. Here I present a detailed reconstruction of the geographic distributions of the five *Microtus* species living in California today at 11 Quaternary fossil localities. Next I construct species distribution models (SDMs), also known as species envelope models or environmental or ecological niche models (ENMs), for the LGM. I use modern times and the LGM as two time points that represent endpoints of the Quaternary climate spectrum. The LGM represents a relatively cool, moist period and the present-day represents a relatively hot, dry period in California (Davis, 1999). The fossil specimens and localities are all from time periods with climates that are intermediate between these two climates. Because SDMs use climate to reconstruct species distributions, the actual ranges of the species at intermediate climatic periods should therefore be intermediate between the predictions that SDMs would reconstruct for these two climatic extremes. Once I have constructed LGM SDMs, I then examine how the empirically derived fossil distributions compare to the distributions predicted by the SDMs.

Several instances of discordance have been reported in comparing empirically-documented fossil distributions with SDMs, indicating that SDMs do not always effectively predict prehistoric distributions of species (Davis *et al.*, in preparation; Guralnick & Pearman, 2010). Several possible explanations have been proposed for this discrepancy, including inaccurate LGM climate models and the evolution of individual species (Davis *et al.*, in preparation; Guralnick & Pearman, 2010; Jackson & Overpeck, 2000). Another likely explanation is that present-day realized niches of certain species are considerably smaller than their fundamental niches (Soberón, 2007), so reconstructions of species distributions will be inaccurate when projected into the past, when non-climatic factors might have changed the realized portion of the fundamental niche (Guisan & Thuiller, 2005). These results suggest the need to collect detailed species-level reconstructions of paleodistributions so that we can begin to test the predictive power of SDMs, tease apart the causes of any discrepancies, and determine which species have paleodistributions that are accurately predicted by SDMs.

Western *Microtus* species hold considerable potential for placing recent distributional shifts into a broader temporal context and for exploring the effectiveness of SDMs. Individuals of this genus are abundant in both present-day and Quaternary ecosystems and are well represented in the fossil record. As their abundance indicates, they play an important role in many ecosystems, influencing the structure of plant communities (Borchert & Jain, 1978) and providing sustenance for many small- to medium-sized predators (Huntly, 1991). In addition, three species of *Microtus* were recently surveyed in the Yosemite National Park region to determine how their distributions shifted over the last 100 years (Moritz *et al.*, 2008). Thus, we have the necessary data to compare rates and directions of distributional shifts through time as well as the paleontological data to create detailed fossil distributions for comparison with past SDMs.

According to the Yosemite study, two of the three *Microtus* species examined, *M. californicus* and *M. longicaudus,* have shifted their distributions over the last hundred years, while the other species, *M. montanus,* has maintained a stable distribution in the same region (Moritz *et al.*, 2008). If these changes are the direct result of climate change, this would indicate that recent changes in climate have been favorable to (range expansion), unfavorable to *M. longicaudus* (range retraction), and neutral for *M. montanus.* Reasonable alternative hypotheses are that climate change was detrimental to *M. longicaudus,* reducing its range, and this led to a competitive release that allowed *M. californicus* to move into previous *M. longicaudus* territory, or *vice versa* that *M. californicus* was favorably affected by the change in climate and outcompeted *M. longicaudus*. Whereas these different scenarios lead to similar present-day range changes, they arise from fundamentally different causes. Distinguishing between the causes driving distribution shifts in these species is critical both for understanding their ecology and predicting how they will further shift as climate changes accelerate.

By generating species-level paleontological range reconstructions for species of *Microtus* that have geographic ranges that are sympatric in some places and allopatric in others, we can begin to understand the history behind their present-day ranges and their reactions to climate. In this paper, I first apply geometric morphometrics and discriminant analyses to identify *Microtus* specimens from 11 western North American fossil localities to test whether individual congeners respond similarly or differently to past changes in climate in a way that maintains overall genus-level range. I construct LGM SDMs for each *Microtus* species, and I test if the modelled distributions predicted the presence of fossil populations. At the same time, I can compare which climate variables drive the paleontological range projections for each species and whether the determinant variables are consistent across species. Finally, I compare the directions and rates of *Microtus* distributional changes since the LGM to the present-day rates of distributional changes in Yosemite, directly testing the significance of these present-day changes in the context of the species' histories.

MATERIALS AND METHODS

Specimen sampling

Fossil specimen identification

Methods have been previously described by which geometric morphometrics and present-day *Microtus* specimens from throughout the range of each of the five species of interest were used to train a discriminant analysis for identifying fossil *Microtus* specimens (Chapter 2). Here, this discriminant analysis was applied to *Microtus* first lower molars (m1s) of fossil specimens from 11 fossil localities from California, Oregon, and Nevada (see Table 3-1 and Figure 3-1f).

Two potential problems could occur from identifying fossil specimens using a discriminant analysis built with present-day specimens. First, if the tooth shape of a species has changed through time, previously looking more similar to another species in the analysis, the shape of that specimen would appear intermediate between two species. In this case, the reported confidence for identification of that specimen represents the probability that the specimen belonged to each of the two potential species based on the training set, for example, 0.5 if it were exactly halfway between. For a specimen to be considered confidently identified, only specimens with ≥ 0.95 identification confidence were used. Second, if a specimen does not belong to any of the species included in the particular species), but its shape may fall far outside the range of shapes displayed by that species. The second criterion necessary for a specimen to be considered confidently identified is that the Mahalanobis distance of the specimen must fall within two standard deviations of the species' mean shape.

Fossil localities

Fossil localities examined are distributed throughout California, southern Oregon, and east-central Nevada. Their locations are shown in Figure 3-1f and ages and LGM climate information are described in Table 3-1.

To obtain dates for some localities, specimens were radiocarbon dated, and for others, dates were based on biostratigraphy. Fossil bone specimens from Prune Avenue and Samwell Cave were all dated at the Lawrence Livermore National Laboratory CAMS facility. The AMS radiocarbon preparation procedures for these specimens followed Brown *et al.*, 1988 and Bronk Ramsay *et al.,* 2004. Samples were decalcified with 0.25N HCl to obtain collagen, which was then gelatinized at 58 uC for 16 hours. The remaining solids were filtered out of the gelatin solution. The solution was then ultrafiltered to remove the 30-kDa fraction, which was freeze-dried and graphitized for AMS analysis. Dates for the Woodburn locality are radiocarbon dates taken from the literature (Campbell & Stenger, 2002). Dates for all other localities are biostratigraphic and were obtained from the databases at their respective museum holdings (see Table 3-1) or from NEOMAP (http://www.ucmp.berkeley.edu/neomap/, July 2010), a database of Quaternary fossil mammals compiled from the literature. All radiocarbon dates were calibrated with OxCal version 4.1.56 (Bronk Ramsay *et al.*, 2009), using the IntCal09 calibration curve (Reimer *et al.*, 2009).

Present-day specimens

Occurrence data are from the Arctos database (http://arctos.database.museum, July 2010), which includes natural history records from the University of Alaska Museum of the North, the Museum of Southwestern Biology, and the Museum of Vertebrate Zoology at the University of California, Berkeley. Duplicate specimens of a species from a locality were removed. Presence-only data were used, because absence data were not readily available for the species of interest.

Climate data

All climate layers were downloaded from the WorldClim dataset (Hijmans *et al.*, 2005, http://www.worldclim.org/, July 2010).

SDM climate data

Nineteen current (1950-2000) and LGM bioclimatic variables at a 2.5 arc-minute spatial resolution were used in SDM construction (Table 3-2). WorldClim bioclimatic variables are derived from 30-arc-second resolution monthly temperature and precipitation values and are selected to represent biologically meaningful variables including climatic annual trends, seasonality, and extremes (Hijmans *et al.*, 2005). LGM climate data available through WorldClim are calibrated and statistically downscaled from the PMIP2 LGM dataset (Braconnot *et al.*, 2007). These LGM data are derived from two general circulation models, the Community Climate System Model (CCSM) (Collins *et al.*, 2006) and the Model for Interdisciplinary Research on Climate (MIROC, version 3.2) (Hasumi & Emori, 2004).

Fossil locality climate data

Fossil localities were mapped using ArcGIS 9.3 (ESRI, Redlands, CA, USA). Current (1950-2000) mean annual temperature and mean annual precipitation layers at a 30 arc-second spatial resolution and LGM climate layers at a 2.5 arc-minute spatial resolution were extracted for each fossil locality. LGM data used here were derived from the CCSM (Collins *et al.*, 2006).

Holocene climate data

Holocene climate data were compiled from palynological, *Neotoma* midden, paleolimnology, soil core, and Holocene climate models in the literature (Boxt *et al.*, 1999; Diffenbaugh & Ashfaq, 2007; Kirby *et al.*, 2007; Mock & Brunelle-Daines, 1999; Thompson *et al.*, 1997).

Model reconstructions of LGM ranges

SDMs were built for the present-day data and then projected back in time to the Last Glacial Maximum (LGM), approximately 21 kya. Although few of the fossil localities are as old as the LGM, this time period represents one extreme in late Quaternary climate (cool and moist) with the present-day representing the opposite extreme (hot and dry). Since SDMs use these climate variables to reconstruct species ranges, modern and LGM predictions should bracket two extremes in species distributions. Therefore, the actual ranges of each species at a given time should be intermediate between the LGM and present-day predicted distributions, making these reconstructions relatively conservative.

Maxent version 3.3.2 (Phillips & Dudík, 2008) was used to construct SDMs of present-day and LGM species distributions using presence-only occurrence data. Although several programs are available to produce niche models, Maxent has been demonstrated to be among the most accurate of the programs that use presence-only (rather than presence-absence) data (Elith *et al.*, 2006; Phillips, 2008; Phillips *et al.*, 2004). However no studies have directly compared transference accuracy among presence-only programs (Pearson , 2007; Phillips, 2008). In order to create the models, the program contrasts presence records with pseudo-absence data resampled from the background study area. In the most recent versions of Maxent, the authors have programmed the default parameters to maximize results under a wide variety of circumstances (Phillips & Dudík, 2008). The default parameters were used with the exception that 25% of localities were reserved for model testing. Maxent provides a probability surface with grids on a log scale, ranging in value from essentially 0 to 100. This surface indicates the relative similarity of an area to the present-day realized niche of the species. Clamping was performed to determine whether any of the modelled regions were outside the values of the bioclimatic variables encountered during training (Hijmans & Graham, 2006). Present-day climate niches were then projected onto a set of LGM layers derived from the CCSM and the MIROC (described above) to create two LGM distribution hypotheses for each species. In addition, for each species, an analysis of variable contributions was performed to give a heuristic estimate of the relative contribution of each environmental variable to the present-day SDM (Phillips & Dudík, 2008). This analysis is used here to characterize the types of variables that are most important in determining the niche of each species. The relative importance of each variable cannot, however, be directly inferred from these results because the predictor variables are correlated.

Comparing fossil ranges and LGM modelled ranges

Fossil localities were plotted on the both the CCSM and the MIROC LGM paleogeographic distribution models. Extralimital fossil specimens, those fossils found outside the present-day range of the species, were then compared to both LGM models to determine whether the models predicted the suitability of that habitat for that species during the LGM. A 10% probability threshold was used, which predicts a larger distribution, and is therefore more conservative in this case than the often-used lowest presence threshold for all species examined (Pearson *et al.*, 2007). MIROC models generally predicted larger ranges for species during the LGM, and therefore more often predicted extralimital fossil ranges. MIROC models are shown in Figure 3-1f-j.

Comparing Quaternary and recent range shifts

Three *Microtus* species were included in the Yosemite survey: *M. californicus, M. longicaudus,* and *M. montanus* (Moritz *et al.*, 2008). Changes along the western edges of the Quaternary distributions of *M. montanus* and *M. longicaudus* and along the eastern edge of *M. californicus* were compared with elevational changes observed in the Yosemite resurvey project. Because *M. longicaudus* demonstrated a substantial elevational range contraction during both the Quaternary and over the last 100 years, rates of elevational range contractions were calculated for this species.

RESULTS

Microtus identification, fossil geographic range and comparison with SDMs

Of the 220 *Microtus* fossils, 144 specimens were confidently identified, 68 were rejected due to low identification confidence, six were rejected due to large Mahalanobis distances, and 2 were rejected for both reasons. Specimen identifications are found in Table 3-S1. The present-day range of the genus in Oregon, California, and Nevada covers nearly all portions of the states except the Mojave and Sonoran Deserts. The overall geographic range of Late Pleistocene to Holocene *Microtus* in these states, according to the fossil specimens, has not shifted through that time. However the individual species have shifted their ranges considerably. SDMs predict and fossil specimens demonstrate that past geographic ranges were larger than present-day ranges. Clamping was minimal with the exception of a small region of northwestern California for *M. longicaudus*, which does not affect the interpretations of predictions regarding its presence at fossil localities.

Microtus californicus

M. californicus was identified in nine of the eleven localities (Figure 3-1f). The only extralimital fossil specimen is from Woodburn in northern Oregon. The fossils from the Woodburn locality are approximately 11.5 ka. This locality is not predicted to have been suitable for *M. californicus* during the LGM and since that time has undergone approximately 2.4°C warming and a 94 mm increase in precipitation (Table 3-1) and since the early Holocene has experienced an increase in precipitation (Boxt *et al.*, 1999; Diffenbaugh & Ashfaq, 2007; Kirby *et al.*, 2007; Mock & Brunelle-Daines, 1999; Thompson *et al.*, 1997). In general, precipitation values had the most influence in ecological niche reconstruction (Table 3-2). Overall fossil *M. californicus* specimens exhibit range contraction at the northern edge of its range since 11.5 kya, and SDMs predict that habitat suitability has contracted throughout the range of the species, especially in the southeast portion of its range, since the LGM.

Microtus longicaudus

M. longicaudus was identified in eight of the eleven localities (Figure 3-1g). Specimens at four California localities were extralimital relative to the current distribution: Montezuma, Pacheco 2, Prune Avenue, and Palos Verdes. Montezuma, Pacheco 2, and Prune Avenue are in the San Francisco Bay area (SF Bay). Prune Avenue is dated to 836-4,283 cal. yr BP. Montezuma and Pacheco 2 are both from the late Pleistocene (~11-45 kya). Palos Verdes is from the Los Angeles region and is known only to be Rancholabrean in age (~11-300 kya) according to the Los Angeles County Museum (LACM) collection information (Table 3-1). All four extralimital fossil localities are projected to have been suitable for *M. longicaudus* during the LGM. Since that time, the SF Bay localities have undergone on average 2.9°C warming and a 196 mm decrease in precipitation, while Palos Verdes has undergone a 3.8°C warming and a 350 mm decrease in precipitation (Table 3-1). Prune Avenue, the only extralimital fossil locality of Holocene age, was accumulated during a relatively cold time compared to the rest of the Holocene (Kennett *et al.*, 2007). Temperature variables had the strongest effect on ecological niche reconstruction (Table 3-2). Overall, *M. longicaudus* fossil specimens demonstrate a late Holocene range contraction at the western edge of its range throughout California, and SDMs predict that habitats in the southwest and northern portion of its range become less suitable since the LGM.

Microtus montanus

M. montanus was identified in nine of the eleven localities (Figure 3-1c). The only extralimital fossil specimens are found in Woodburn in northern Oregon (11.5 kya).

However, when I examined present-day specimen data, I observed that Woodburn lies within a sparsely surveyed region between two areas that do contain *M. montanus* (Figure 3-S1). Additionally, the present-day SDM for *M. montanus* predicts that Woodburn, Oregon represents marginally suitable habitat for this species. Thus, it is uncertain whether *M. montanus* currently occupies this locality or not. Nevertheless, the Woodburn locality is projected to have been suitable habitat for this species during the LGM. Since the LGM (~21 kyr BP), Woodburn has undergone approximately 2.4°C warming and a 94 mm increase in precipitation (Table 3-1). Temperature values had the strongest effect on ecological niche reconstruction (Table 3-2). Overall, the fossil range of *M. montanus* exhibits either a slight eastward contraction or potentially no change at all along its western margin, and SDMs predict only slight habitat loss in the southwestern portion of the species range.

Microtus oregoni

The first known fossil specimens of *M. oregoni* were identified at two of the eleven localities (Figure 3-1d, i). The only extralimital fossil specimen is found in Paisley Caves in south-central Oregon and is dated to 12,581 ± 430 cal. yr BP (pers. comm., Dennis L. Jenkins). Paisley Cave is not projected to have been suitable habitat for *M. oregoni* during the LGM. Since that time, the locality has undergone approximately 5.9°C warming and an 11 mm decrease in precipitation (Table 3-1). In the late Pleistocene (10.9-13 kya), this region was warmer than the LGM but cooler and wetter than present day (Briles *et al.*, 2005). In general, precipitation of the coldest quarter has the strongest effect in ecological niche reconstruction (Table 3-2). Overall, fossil *M. oregoni* exhibits a westward range contraction, and SDMs predict that habitats in the south and cenral portions of California become less suitable and habitats in the northern Washington and southern Canada become more suitable for this species since the LGM.

Microtus townsendii

The first known fossil specimens of *M. townsendii* were identified at three of the eleven localities (Figure 3-1e, j). Extralimital fossil specimens are from Paisley Caves in south-central Oregon and Samwell Cave in northern California. The *M. townsendii* specimen from Paisley Cave is dated at approximately 9,000 cal. yr BP, and the specimen from Samwell Cave is dated at 0-1,505 cal. yr BP. Samwell Cave was projected to have been marginally suitable habitat for this species during the LGM, but Paisley Cave was not. Since the LGM, Samwell Cave has undergone approximately 3.7°C warming and a 336 mm decrease in precipitation and since approximately 9 kya is estimated to have become warmer and wetter or had no change in precipitation (Thompson *et al.*, 1997; Boxt *et al.*, 1999; Mock & Brunelle- 007; Kennett *et al.*, 2007; Kirby *et al.*, 2007). Since the LGM, Paisley Cave has undergone approximately 5.9°C warming and an 11 mm decrease in precipitation (Table 3-1), and approximately 9 kya it was relatively warm and dry (Briles *et al.*, 2005; Diffenbaugh & Ashfaq, 2007; Kennett *et al.*, 2007; Mock & Brunelle-Daines, 1999; Thompson *et al.*, 1997). In general, precipitation of the coldest quarter has the strongest effect in ecological niche reconstruction (Table 3-2). Overall, *M. oregoni* fossils exhibit a northwest range contraction from warmer or dryer to cooler or moister regions, and SDMs predict that habitats in the south and central portion of California have become less suitable for the species.

Comparisons with recent Yosemite range change

Microtus longicaudus

In the Yosemite resurvey project, *M. longicaudus* contracted the western edge of its range 614 m upward in elevation into the Sierra Nevada Mountains (Moritz *et al.*, 2008). If range contraction had occurred along the western edge of its range throughout the Quaternary, I would expect to see fossil specimens west of and lower than its current range, which fits with observations in this study. I do find *M. longicaudus* in 4 fossil localities west of its current range in California and approximately 600 m lower in elevation than the range limit 100 years ago. The range of *M. longicaudus* has contracted by a rate of 6.14 m/yr over the last 100 years. If we use the minimum age of the confidently dated extralimital *M. longicaudus* fossils, 836 cal. yrs BP, then the rate of range contraction from that time until 100 years ago was 0.82 m/yr. However, if the specimen is older, this rate may be slower, and if the species shifted back and forth over that time, the rate may be faster.

Microtus montanus

In the Yosemite resurvey project, *M. montanus* demonstrated no range change in the Yosemite region (Moritz *et al.*, 2008). If there were no range change during the Quaternary, I would expect *M. montanus* to be found in the same localities in the past and present. This is the case for *M. montanus* along the western edge of its range with the possible exception of a potential eastward contraction in northern Oregon.

Microtus californicus

In the Yosemite resurvey project, *M. californicus* expanded its range 505 m upward in elevation into the Sierra Nevada Mountains (Moritz *et al.*, 2008). If range expansion had occurred along the western edge of the range of *M. californicus* throughout the Quaternary, I would expect to see localities where *M. californicus* is currently present but that it did not inhabit in the past. Fossil sampling in the western range of this species is sparse and dating is insufficient to either support or reject the continuation of this trend throughout the Quaternary.

DISCUSSION

I find that throughout the Quaternary, all five of the *Microtus* species examined exhibited geographic range contractions, with the possible exception of *M. montanus* (Figure 3-1). However the individual species move in different directions, which resulted in an overall stable genus distribution. When I compare the location of these specimens to LGM

SDMs, even under the assumption of maximum cooling using LGM models, the SDMs do not predict the presence of some of the extralimital fossil specimens that we see—notably *M. californicus* (Figure 3-1f), *M. oregoni* (Figure 3-1i), and *M. townsendii* (Figure 3-1j). Interestingly, these three species are also the three species whose niche reconstructions are most strongly influenced by precipitation variables. One explanation for these findings is that LGM climate models may be inaccurate in their precipitation predictions. Another possibility is that these particular species may be vulnerable to biotic influences, and therefore have a smaller realized niche than fundamental niche in present-day times; thus, using climate values alone would not accurately project their distributions into the past (or the future). This latter possibility is supported by the present-day reactions to climate that we see in Yosemite populations of *M. californicus* and *M. longicaudus*. Because the niche models of these two species are influenced by different climatic variables, it is quite possible that the increase in minimum temperatures in the region (Moritz *et al.*, 2008) drove *M. longicaudus* into higher elevations, allowing *M. californicus* to fill in the vole habitats that were left unoccupied as *M. longicaudus* moved upslope. This indicates that competition may be an important limiting factor in the realized distributional space of *M. californicus*, although this hypothesis needs to be tested with further evidence. When I compare present-day and past reactions of the three *Microtus* species examined in the Yosemite study, I find that *M. longicaudus* and *M. montanus* seem to demonstrate the same pattern of movement in the recent past and in the Quaternary. Comparing the rates of elevational range ascension in *M. longicaudus*, I find that rates have increased more than sevenfold over the last hundred years.

Fossil identifications and distributions

The fossil identifications presented herein (Table 3-S1) contribute the first known specimens of *M. oregoni* and *M. townsendii* in addition to providing some of the first confident identifications of fragmentary specimens of the other three species. Although some tentative extralimital fossil specimens have previously been reported for *M. longicaudus* and *M. montanus* east of the Rocky Mountains (Hoffman & Jones, 1970; Stewart, 1978, 1987; Turner, 1974; Wallace, 2001), these are the first extralimital fossil specimens reported for these species from the western portion of their distributions (Figure 3-1g,h). For *M. californicus*, *M. oregoni,* and *M. townsendii* these are the first extralimital fossil specimens reported (Figure 3-1f,i,j). The discovery of these extralimital fossil specimens provides an excellent opportunity to examine how congeners react to similar climate pressures, to place recent observations of these species' reactions to climate change in a broader temporal context,
SDMs, gaining insight into the underlying factors driving species movements and the causes for underpredicted distributions from the SDMs.

Congener reactions to climate change

Since the LGM, I see range shifts in all of the species examined with the possible exception of *M. montanus.* The individual species, however, shift in different directions: *M. oregoni* (Figure 3-1d&i) and *M. townsendii* (Figure 3-1e&j) shift westward, *M. longicaudus* (Figure 3-1b&g) and *M. montanus* (Figure 3-1c&h) shift eastward (or possibly not at all in the case of *M. montanus*), and *M. californicus* (Figure 3-1a&f) shifts southward. In addition, every fossil locality is stable in *Microtus* occupancy but not in particular species occupancy. These findings support the previous findings of McGill *et al.* (2005) that indicate genus-level stability at individual localities while species actively move as climates change and the predictions of Hadly *et al.* (2009) that differential reactions to climate change may maintain genus-level range stability. The fact that I find the distribution of the genus to remain essentially static implies either that (1) species climate niches abut and as one species contracts due climate change the other expands, (2) that congeneric competition is limiting some species ranges (as suggested by Hadly *et al*, 2009), or (3) some combination of the two. Once I pair this species-level fossil analysis with LGM SDMs, we may gain more insight into the central cause.

Fossil ranges and LGM modelled ranges

Of the nine fossil occurrences from outside the present-day range of the species, SDMs were able to predict that the habitats for five of the fossil localities were suitable for that species during the LGM. Although the sample sizes for testing the SDMs are small, the models are clearly not adequately predicting ranges for the other four species. To determine the potential distribution of the species in the past, Maxent takes the realized niche of the species, determines the environmental parameters of that space, and then projects them onto the LGM climate layers (Phillips *et al.*, 2006). If the current realized niche space is much smaller than the fundamental niche space for a species, and therefore non-environmental factors play an important role in determining the species realized distribution, then any climate-projected species distributions are likely to be smaller than their actual ranges. The same issue may occur if the LGM climate data contains systematic inaccuracies, if the species has evolved through time (i.e., its fundamental niche has changed), or if the species is incompletely sampled (Davis *et al.*, in preparation; Guralnick & Pearman, 2010; Phillips, 2008).

If we assume niche constancy through time and that sampling and climate data are sound, the accuracy of SDMs in predicting past geographic ranges can give us an idea of the relative importance of environmental variables versus biotic variables (i.e., those limiting the realized niche) in driving a species' reaction to climate change. If a projected potential distribution does not match the species actual realized distribution in the past, then we can infer that factors other than the incorporated environmental variables are affecting that species' present-day realized distribution. Thus, present-day species ranges that underpredict LGM distributions may be more strongly affected by competition, human impacts, predation, disease, and/or unincorporated environmental factors (e.g., soil type or fire) than those whose distributions are well-modelled. If this is the case, it would indicate that incorporating biological interactions into species distribution models for these species may be necessary to accurately capture their future distributional shifts in response to climate change (Guisan & Thuiller, 2005).

The three species whose extralimital paleodistributions were not predicted by the SDMs all have niches reconstructions that are most strongly affected by precipitation variables. This suggests that the LGM climate layers may be inaccurate in their precipitation data. Inconsistencies in LGM climate models have been previously suggested (Davis *et al.*, in preparation; Jackson & Overpeck, 2000), and Pausata *et al.*

(2009) demonstrated that seasonal and spatial variability in atmospheric pressure can lead to the misinterpretation of proxy signals in the development of past climate models. To examine whether this is causing inconsistencies in SDMs, many more species and sites need to be modelled in a similar manner to determine whether those species with inconsistent fossil distributions and LGM SDMs have niche reconstructions that are driven by precipitation variables. Given the current data and sample sizes, no solid conclusion can be drawn as to the cause of these inconsistencies. However integration of these data with recent distributional shifts may shed some light on the factors at work in some of the species.

Quaternary and recent range shifts

Paleodistributions reconstructed from the fossil record support the hypothesis that the species of *Microtus* surveyed in the Yosemite project (Moritz *et al.*, 2008) exhibited similar response to warming over the last 100 years as they did to the warming that characterized the LGM-Holocene transition. *M. montanus* has maintained a relatively stable distribution throughout the Quaternary as well as over the last 100 years. *M. longicaudus* contracted its range upward 600 m in elevation from at least 836-100 years ago, and then again over the last 100 years. Thus it has experienced a rapid increase in the rate of ascension into the Sierra Nevada Mountains that parallels the increase in temperature changes seen over the last 100 years.

According to Moritz *et al.* (2008), the Yosemite region has undergone 3.9°C increase in January minimum temperatures and a 5.3°C increase in July. Given that the niche reconstruction of *M. longicaudus* is primarily dependent upon temperature variables (Table 3-2) and 836 to 4,283 ya is projected to have been colder than present-day temperatures (Kennett *et al.*, 2007), this ascension is to be expected. However, the subsequent expansion of the range of *M. californicus* into the region previously occupied by *M. longicaudus* is not necessarily expected (see Table 3-2). Millar *et al.* (2004) report "multidecadal variability in… precipitation" in the same region over the last 100 yrs, showing an overall increase in precipitation from 1951 to 2000 as compared to 1900 to 1950. However, *M. californicus* generally inhabits regions of lower precipitation than *M. longicaudus* (Table 3-S2). Thus it seems likely that the expansion of *M. californicus* into a region previously, but no longer, occupied by is not necessarily due to changes in climate. Several other possibilities exist as to the potential cause of this expansion. As Hadly *et al.* (2009) predicted for genera with stable ranges, it is possible that competitive release occurred when *M. longicaudus* contracted its range, allowing *M. californicus* to move into the region, thus maintaining stability in the genus range. However, Spaeth (2009) has shown that three *Microtus* species, including *M. longicaudus* and *M. montanus*, actually demonstrate convergent morphology in regions of sympatry, hypothesizing that they actually subdivide the habitat to avoid competition. Thus the hypothesis of congener competitive release still remains to be tested further as other possibilities may exist to explain this pattern. The Yosemite region was specifically selected to minimize anthropogenic effects (Moritz *et al.*, 2008), and *M. californicus* and *M. longicaudus* have similar predators so these are likely not the causes of the observed pattern. However, fire succession, disease, or even random fluctuations in range over a short timescale are all possibilities.

Neither the recent upward expansion of the range of *M. californicus* nor its southward range contraction during the Holocene seems to be related to climate factors considered in the SDM. Therefore the underprediction of its realized distribution during the LGM may have to do not with climate, but with a shift in overall ecological structure since that time. Nonetheless, it suggests the importance of incorporating the presence of potential competitors into SDMs (Guisan & Thuiller, 2005) and demonstrates that SDMs may not be accurate for predicting this species' future distributions. Additionally, the combination of correctly predicted LGM distributions for and the observed acceleration in range contraction both strongly suggest that climate, and temperature variables in particular, have strongly influence this species' distribution. Although this species has been able to contract its range to keep up with temperature changes thus far, it is not clear whether it will be able to do so if temperatures continue to increase at increasing rates. A resurvey of the full range of the species would shed light on the extent of the effect that has already been wrought, and further monitoring is suggested to determine whether the species will be able to handle future accelerations in climate change. Finally, the results herein highlight the utility of incorporating paleontological data with ecological observations and SDMs. Without a third timepoint, it would have been difficult to interpret the relevance of the rate of range contraction that we are seeing in *M. longicaudus*. Further, the integration of the fossil record with SDMs can be important for determining whether species' future distributions can be confidently modelled and for recognizing the errors present in the climate models underlying the creation of SDMs.

CONCLUSIONS

In general, this study highlights the utility of integrating species-level paleontological analyses with recent ecological monitoring and SDMs to determine the mechanisms underlying reactions to climate change and refining the accuracy of SDMs for modelling species' reactions to climate change. I find that Pacific Coast *Microtus* species have maintained the same genus distribution in the region since the LGM by shifting their ranges in response to different climate variables. As a result, since the LGM, *M. californicus* contracted its range southward, *M. longicaudus* contracted its range eastward and upward in elevation, *M. montanus* likely maintained a stable range in the western portion of its distribution, and *M. oregoni* and *M. townsendii* contracted their ranges westward. When compared to LGM SDMs, the presence of many extralimital fossil specimens were not predicted. These analyses of LGM SDMs point to potential issues with precipitation variables in LGM climate models. Again, similar comparisons across many more species could give a more definitive indication as to whether this really is the reason that SDMs are not always accurate for LGM distributions. However, it should be noted that biotic and anthropogenic effects on present-day realized niches should also always be considered as potential reasons for inaccurate SDMs. The reactions to recent climate change seen in *M. longicaudus* and *M. montanus* over the last 100 years are actually continuations, and acceleration in *M. longicaudus*, of their reactions to warming since the LGM. Because *M. longicaudus* appears to be sensitive to temperature changes, continued monitoring and broader surveying of the overall species distribution are recommended to determine its ability to react to future, accelerated warming. These findings emphasize that the addition of deeper time points to a study can only enhance our understanding of species reactions climate change by putting it in a broader context and further exploring the underlying causes of its reactions. Similar studies performed across a broad spectrum of species could explain how organisms respond to climate change at different taxonomic levels, and reveal if we should consider shifting conservation emphases to include the maintenance of genera (Hadly & Barnosky, 2009; Hadly *et al.*, 2009).

FIGURES AND TABLES

FIGURE 3-1 Species distribution models for the present (a-e) and the Last Glacial Maximum based on MIROC climate layers (f-j). Red indicates a probability threshold of over 25%. Light gray represents a probability threshold of 10-25%. White points (a-e) represent present-day specimens used for model construction. Solid black stars (f j) indicate localities where fossil specimens of the species are found, white stars (f-j) indicate localities where fossil specimens of the species are not found, and the gray star (g) indicates that 1 of the 2 localities in that region contain fossil specimens of that species. Numbers (f) correspond with locality names and data listed in Table 3-1.

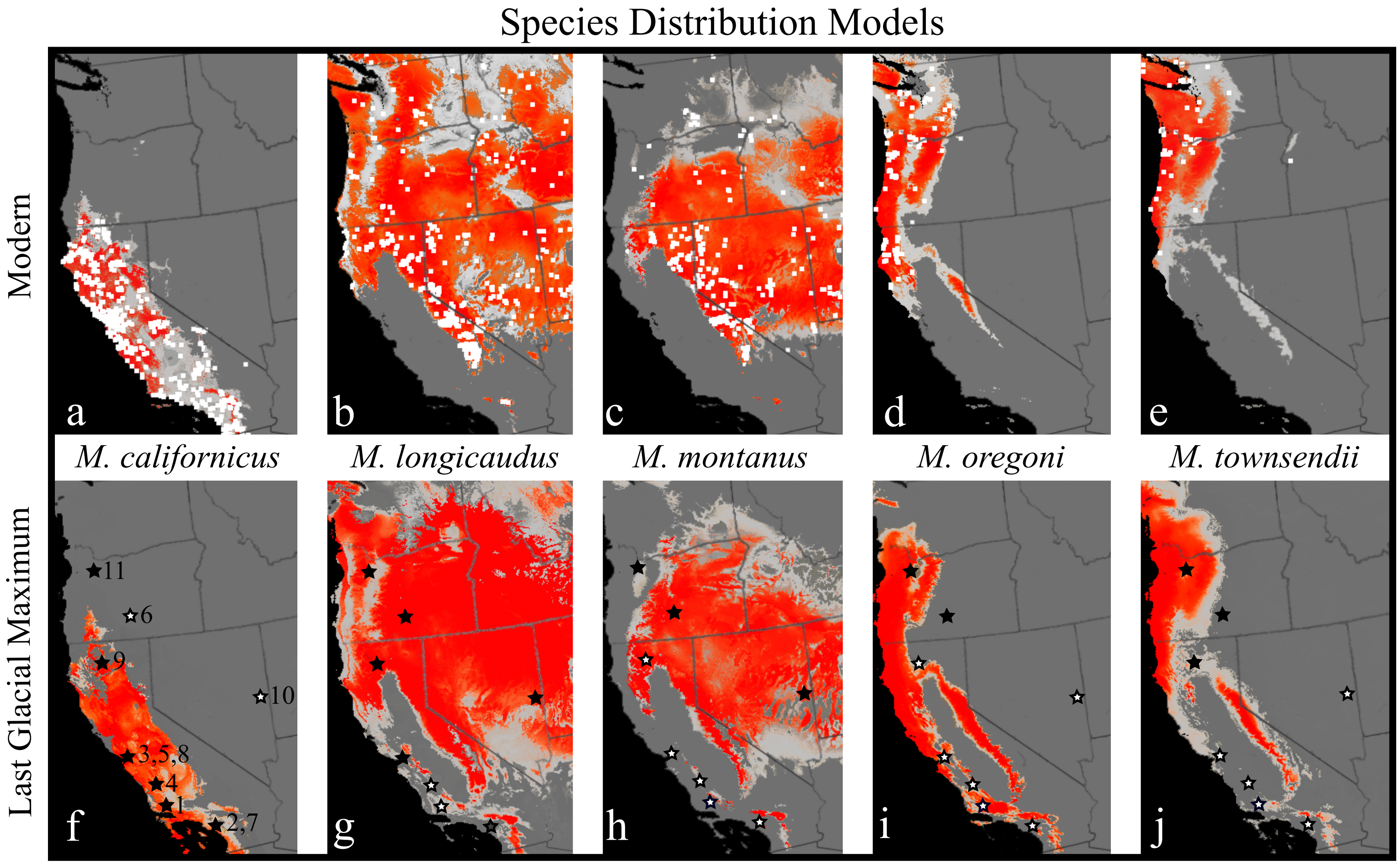

Species Distribution Models
Modern
Last Glacial Maximum
a
b
c
d
e
M. californicus
M. longicaudus
M. montanus
M. oregoni
M. townsendii
f
g
h
i
j
11
6
9
10
3,5,8
4
1
2,7

TABLE 3-1 Fossil localities, the species of *Microtus* identified at each, the ages of each, and climate data for each. Asterisks (*) next to species indicate extralimital fossils at each locality. Sources for dates are as follows: [1]From FAUNMAP Database; [2]From UCMP Database; [3]From LACM Database; [4]Pers. comm. Dennis L. Jenkins, 2010; [5]Radiocarbon dating performed by author (see Materials & Methods); [6]Campbell & Stenger, 2002

Locality	**Species**	**Specimen ages**	**LGM Climate**	**Present-day Climate**
(1) Carpinteria CIT 139	*M. californicus*	*ca.* 10,000-40,000 yrs (biostratigraphic)[1]	12.7°C 759 mm	15.9°C 354 mm
(2) Emery Borrow LACM 7053 LACM 6689	*M. californicus*	*ca.* 11,000-300,000 yrs (biostratigraphic)[2]	14.0°C 645 mm	17.8°C 343 mm
(3) Montezuma V-71001 V-6312	*M. californicus*	*ca.* 11,000-45,000 yrs (biostratigraphic)[3]	11.8°C 825 mm	14.7°C 609 mm
	**M. longicaudus*			
(4) McKittrick Brea CIT 138	*M. californicus*	0-37,011 cal. yr BP[1]	12.8°C 526 mm	16.2°C 279 mm
(5) Pacheco 2 V-78027	*M. californicus*	*ca.* 11,000-30,000 yrs (biostratigraphic)[3]	12.0°C 665 mm	14.9°C 487 mm
	**M. longicaudus*			
	**M. townsendii*			
(6) Paisley Cave 35LK3400	*M. longicaudus*	*ca.* 1,300-11,000 yr BP[4]	2.5°C 299 mm	8.4°C 288 mm
	M. montanus	*ca.* 500-10,500 yr BP[4]		
	**M. oregoni*	12,151-13,011 cal. yr BP[4]		
	**M. townsendii*	*ca.* 9,400 yr BP[4]		
(7) Palos Verdes LACM 3877	*M. californicus*	*ca.* 11,000-300,000 yrs (biostratigraphic)[2]	14.2°C 710 mm	18.0°C 360 mm
	**M. longicaudus*			
(8) Prune Avenue V-5301	*M. californicus*	836-4,283 cal. yr BP[5]	12.2°C 554 mm	15.2°C 361 mm
	**M. longicaudus*			
(9) Samwell Cave V-65217	*M. californicus*	0-8,275 cal. yr BP[5]	11.4°C 1910 mm	15.1°C 1574 mm
	M. longicaudus	6,520-7,523 cal. yr BP[5]		
	**M. townsendii*	0-1,505 cal. yr BP[5]		
(10) Smith Creek LACM 251	*M. longicaudus*	0-29,959 cal. yr BP[1]	-1.1°C 352 mm	9.0°C 238 mm
	M. montanus			
(11) Woodburn UO-3859 UO-3038 UO-3867	**M. californicus*	11,278-11,842 cal. yr BP[6]	8.9°C 936 mm	11.3°C 1030 mm
	M. longicaudus			
	*?*M. montanus*			
	M. oregoni			
	M. townsendii			

TABLE 3-2 The top three climate variables that contribute to species niche reconstructions in SDMs and the percent model contribution are listed for each species. All climate variables used in SDM construction include the following: (1) Annual mean temperature, (2) Mean diurnal temperature range, (3) Isothermality, (4) Temperature seasonality, (5) Maximum temperature of the warmest month, (6) Minimum temperature of the warmest month, (7) Temperature annual range, (8) Mean temperature of the wettest quarter, (9) Mean temperature of the driest quarter, (10) Mean temperature of the warmest quarter, (11) Mean temperature of the coldest quarter, (12) Annual precipitation, (13) Precipitation of the wettest month, (14) Precipitation of the driest month, (15) Precipitation seasonality, (16) Precipitation of wettest quarter, (17) Precipitation of the driest quarter, (18) Precipitation of the warmest quarter, (19) Precipitation of the coolest quarter.

Species	Climate Variable	% Model Contribution
M. californicus	Precipitation of the Warmest Quarter	34.2
	Precipitation of the Coldest Quarter	24.1
	Isothermality	15.4
M. longicaudus	Mean Annual Temperature	32.2
	Isothermality	23.5
	Temperature Seasonality	12.4
M. montanus	Isothermality	34.8
	Minimum Temperature of the Coldest Month	17
	Mean Temperature of the Coldest Quarter	10.8
M. oregoni	Precipitation in the Coldest Quarter	64.3
	Mean Temperature in the Coldest Quarter	20.3
	Mean Temperature in the Warmest Quarter	10.8
M. townsendii	Precipitation in the Coldest Quarter	53.8
	Mean Temperature in the Coldest Quarter	16.9
	Mean Temperature in the Warmest Quarter	13.7

SUPPLEMENTARY TABLES AND FIGURES

FIGURE 3-S1 Numbered markers represent the number of *Microtus* at each locality. Red markers are locations that contain *M. montanus*. The circled region is the region of interest, Woodburn, OR. Due to insufficient sampling in this region, it is difficult to assess whether *M. montanus* is currently present at this locality. Data are from the Arctos database (http://arctos.database.museum, August 2010).

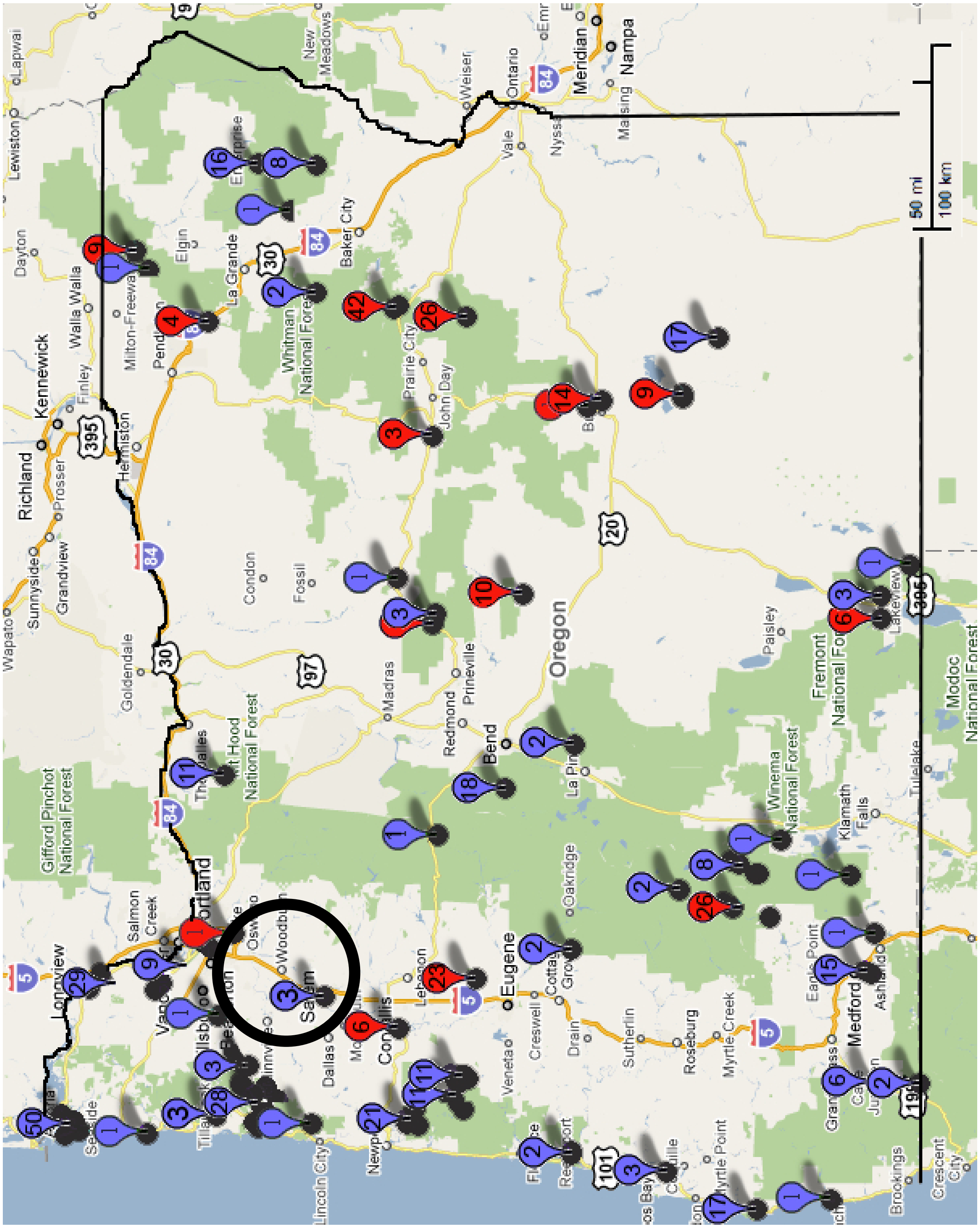

Lewiston
Lapwai
Dayton
Kennewick
Richland
Walla Walla
Milton-Freewater
Finley
Hermiston
Prosser
Grandview
Sunnyside
Wapato
Goldendale
Gifford Pinchot National Forest
Longview
Salmon Creek
Portland
Oswego
Woodburn
Salem
Hillsboro
Dallas
Corvallis
Lebanon
Eugene
Cottage Grove
Creswell
Veneta
Drain
Sutherlin
Roseburg
Myrtle Creek
Oakridge
Seaside
Tillamook
Lincoln City
Newport
Reedsport
Coos Bay
Myrtle Point
Brookings
Crescent City
Grants Pass
Medford
Ashland
Eagle Point
Klamath Falls
Tulelake
Winema National Forest
Fremont National Forest
Modoc National Forest
Lakeview
Paisley
La Pine
Bend
Redmond
Prineville
Madras
Mt Hood National Forest
The Dalles
Condon
Fossil
Pendleton
La Grande
Elgin
Enterprise
Baker City
Whitman National Forest
Prairie City
John Day
Burns
Vale
Nyssa
Ontario
Weiser
New Meadows
Meridian
Nampa
Oregon
5
84
30
97
20
395
101
199
50 mi
100 km
50
29
9
1
3
28
1
3
21
11
11
6
23
2
2
3
17
1
6
2
15
1
1
8
26
2
1
18
2
11
3
1
10
1
3
6
1
14
9
17
3
42
26
2
4
9
1
1
16
8

TABLE 3-S1 Fossil specimens identified by discriminant analyses and their associated localities. The repositories for the different localities are indicated by locality numbers: “CIT” and “LACM” = Los Angeles County Museum, “V-“ = University of California Museum of Paleontology, “UO” and Paisley Cave = Museum of Natural and Cultural History at the University of Oregon.

Species	Localities	Specimen Numbers
Microtus californicus	(1) Carpinteria CIT 139	CIT-139E, CIT-139F, CIT-139H, CIT-139I
	(2) Emery Borrow LACM 7053, LACM 6689	100095, 100096, 100124, 100126, 105631, 64664, 66541, 66544, 66547, 66585, 66587
	(3) Montezuma V-71001, V-6312	92279, 92282, 92283, 91240, 91248
	(4) McKittrick Brea CIT 138	CIT-138A, CIT-138C, CIT-138F, CIT-138G, CIT-138H, CIT-138I, CIT-138L, CIT-138M, CIT-138N, CIT-138Q, CIT-138R, CIT-138S, CIT-138T, CIT-138U, CIT-138V
	(5) Pacheco 2 V-78027	190246, 190244, 190243, 190242, 190240, 190251, 190237, 190236, 190239, 190465, 190184, 190199, 190207, 190211, 190460
	(7) Palos Verdes LACM 3877	610, 613, 631, 635, 636, 639, 643 20580A, 20580C, 20580F, 20580T, 20580AD, 20580AE, 20580AF, 20580AG, 20580AI, 20580AN, 20580AP, 20580AQ, 20580AR, 20580AS, 20580AV, 20580AW, 20580AX, 20580AY, 20580BB, 20580BE, 20580BG, 20580BN, 20580BO, 20580BU, 20580BV, 20580BY, 3877Surface1, 3877Surface2, 3877TD295A, 3877TD295B
	(8) Prune Avenue V-5301	197576, 197577, 197580, 197582, 197585, 197587, 197590
	(9) Samwell Cave V-65217	1423, 1443, 1471, 1526
	(11) Woodburn UO-3859, UO-3038, UO-3867	F-48009
Microtus longicaudus	(3) Montezuma V-6312	91245
	(5) Pacheco 2 V-78027	190209, 190241, 190245, 190248
	(6) Paisley Cave 35LK3400	5-12-QC-7-6b, 5-5-QC-29-1b, 5-5-QA-6-2b, 5-6-QB-10-7b
	(7) Palos Verdes LACM 3877	20580B, 20580V, 20580AC
	(8) Prune Avenue V-5301	197578, 197584, 197586, 197593
	(9) Samwell Cave V-65217	1495
	(10) Smith Creek LACM 251	14277L
	(11) Woodburn UO-3859, UO-3038, UO-3867	F-48001
Microtus montanus	(6) Paisley Cave 35LK3400	5-12-QA-16-2a, 5-6-QA-38-1b, 5-6-QA-38-1c, 5-12-QA-22-ASHa, 5-5-QC-7-2b, 5-5-QC-2-1b, 5-12-QC-8-5a, 5-12-QC-9-6c, 5-6-QA-11-2a, 5-6-QB-10-7a
	(10) Smith Creek LACM 251	14277I, 14277P
	(11) Woodburn UO-3859, UO-3038, UO-3867	F-48004, F-37350, F-37354a

Microtus oregoni	(6) Paisley Cave 35LK3400	5-5-QD-31-1b
	(11) Woodburn UO-3859, UO-3038, UO-3867	F-40616
Microtus townsendii	(5) Pacheco 2 V-78027	190208
	(6) Paisley Cave 35LK3400	5-6-QA-36-1a
	(9) Samwell Cave V-65217	1430
	(11) Woodburn UO-3859, UO-3038, UO-3867	F-48006, F-38566, F-40538

Table 3-S2 Modern climate range for each species. Data collected from WorldClim database (Hijmans *et al.*, 2005, http://www.worldclim.org/, July 2010) modern (1950-2000) mean annual precipitation and mean annual temperature values at 30-arcsecond resolution.

Species	**Variable**	**Mean**	**SD**	**Min.**	**Max**
Microtus californicus	Temperature (˚C)	9.6	8.3	-5.8	23.9
	Precipitation (mm)	1089	608	35	2240
Microtus longicaudus	Temperature (˚C)	-	12.7	-22.6	21.6
	Precipitation (mm)	1965	1097	77	4145
Microtus montanus	Temperature (˚C)	6.1	8.1	-10.1	20
	Precipitation (mm)	1517	825	90	2976
Microtus oregoni	Temperature (˚C)	4.4	6.9	-10.1	16.1
	Precipitation (mm)	1824	900	265	3385
Microtus townsendii	Temperature (˚C)	3.9	6.6	-10.1	14.9
	Precipitation (mm)	1843	889	304	3385

CHAPTER 4

Climate acts at multiple taxonomic scales to influence morphology, reduce phenotypic variation, and limit species ranges in the California vole (*Microtus californicus*).

ABSTRACT

Aim This study uses population-level paleontological and neontological data to examine how the morphological variation of *Microtus californicus* (Rodentia: Arvicolinae) has changed throughout the Quaternary. I test three hypotheses: (1) that *M. californicus* tooth shape is correlated with precipitation; (2) that the overall Quaternary warming and drying trends in California caused certain *M. californicus* morphotypes to shift northwest through time; (3) that Quaternary climate change led to an overall reduction in morphological variation. Finally, I discuss how shifts in the distribution of variation may affect range limits as a whole and the potential of *M. californicus* to react to future climate change.

Location California.

Methods Geometric morphometrics and partial least squares (PLS) analyses were used to test the hypothesis of a correlation between *M. californicus* tooth shape and mean annual precipitation and to determine the axis of tooth shape that correlates with latitude and longitude. These correlations were used to predict the relative locations of morphotypes in the Quaternary versus the present day. Disparity analyses and a MANOVA were performed to examine changes in variation and whether any shift in mean tooth shape occurred.

Results Currently *M. californicus* tooth shape is significantly correlated with mean annual precipitation along a roughly northwest-southeast gradient, and this pattern is also present in fossil specimens. However, distribution of morphotypes shifted as California became warmer and drier. Fossil morphotypes that were previously found throughout California are now isolated to northern California. When time averaging is taken into account, *M. californicus* demonstrates a 12% reduction in variation from fossil to present-day specimens.

Main conclusions *M. californicus* morphotype distribution is strongly affected by changes in precipitation. Since the late Pleistocene, as California has become drier and hotter, past high-precipitation morphotypes have become isolated to northern California. This resulted in the loss of some morphotypes associated with higher precipitation, decreasing morphological variation. Although climate has structured morphological variation at the intraspecific level, I find no evidence that Quaternary changes in precipitation have yet affected the overall range of the species. However, as climate continues to change, additional pressures may result in some range contraction in both northern and southern portions of the *M. californicus* range.

Keywords *Microtus californicus*, California, range limit, distribution, paleontology, Quaternary, morphological variation, geometric morphometrics, partial least squares (PLS)

INTRODUCTION

Understanding the factors that drive the distributions of organisms at different taxonomic levels is necessary for predicting how they will react to future climate change. Studies of geographic range limits performed at coarse taxonomic scales, for example, the family or the genus level, typically reflect mechanisms that manifest over long time scales and help identify the underlying causes of range stability or instability (e.g., Blois *et al.*, 2010; Hadly *et al.*, 2009; Roy *et al.*, 2009). Most range limit studies are focused at the species level in attempts to understand how range limits are maintained (e.g., Arif *et al.*, 2007; Case & Taper, 2000; Holt, 2009) or to predict past or future species ranges (e.g., Davis *et al.*, in preparation; Guralnick & Pearman, 2010; Hill *et al.*, 2002; Peterson *et al.*, 2002; Sykes *et al.*, 1996; Waltari *et al.*, 2007). The scale of these studies makes them especially informative for conservation where modelling of predicted effects and policy decisions focus on species distinctions. At the intraspecific level, researchers examine the distribution of variation within a species to gauge the potential for adaptation or range expansion (e.g., Eckert *et al.*, 2008; Garner *et al.*, 2004; Pujol & Pannell, 2008). The factors that limit distributions at these different scales typically include abiotic limitations, biotic interactions (competition, predation, or anthropogenic effects), inability to disperse, and restricted gene flow or adaptability. An outstanding question is how these factors interact across scales to shape how species react to past and future climate change. Yet combining high-resolution intraspecific measures of population variation across a deep temporal scale remains challenging.

One way that intraspecific variation has been analyzed is through phylogeographic reconstructions of population structure and variation (e.g., Conroy & Neuwald, 2008; Costa & Schlupp, 2010; Pujol & Pannell, 2008; Wroblewska, 2008). Such studies help infer which populations are most closely related and locate regions of high or low variation. A series of snapshots of past ranges and their internal variation can also trace how the populations moved across the landscape through time, and directly correlate these movements to historical changes. These snapshots can be obtained from the paleontological record, either through ancient DNA or by tracing specific phenotypes through time (e.g., Barnosky *et al.*, 2003; Ramakrishnan & Hadly, 2009). The benefit of using phenotypic data is that the phenotype is the level of variation on which selection acts, has an underlying genetic basis so reflects both genetic and ecophenotypic change, and is tractable to study in the many cases where ancient DNA is not preserved. Here I

whether climate change may elicit a biotic response other than obvious shifts in range limits.

Dentition provides excellent phenotypic traits for tracing population-level variation through time, because teeth have a high additive genetic component and complex, quickly evolving form that significantly varies among populations (Polly, 2003). In addition, because they develop early in ontogeny, the phenotypic plasticity of teeth is limited to predictable wear patterns (Viriot *et al.*, 1993). Because teeth are an organism's key interface with its energetic input, they strongly reflect the ecological and evolutionary context of the species (Davis, 1987). And perhaps most importantly, teeth are one of the main elements preserved in the fossil record, providing ample sample sizes for examining changing variation through time.

Here, I explore the factors that affect distributions at different taxonomic scales using a rich paleontological record to provide a historical context. I integrate information about interspecific range limits with intraspecific paleontological data to trace changes in dental variation in response to climate change since the late Pleistocene (~300 kya). To understand how historical factors have led to the distribution of phenotypes within a species, several questions must be explored (1) Does the modern pattern of variation correlate with environmental variables? This can be the first clue that a given environmental factor is driving changes in variation. (2) As a given environmental variable shifts in the past, does the pattern of variation shift? If so, the correlation between the environmental variable and the pattern of variation becomes stronger, and can suggest the nature of the relationship between the two variables. (3) Do environmental shifts result in an overall change in the amount of variation present? Understanding how an environmental variable changes the overall amount of variation provides a contextual understanding of the current state of the species and tells us how we would expect changes in that environmental variable to affect the species in the future. Answering these questions creates a picture of past and present intraspecific variation, which in turn can help identify the potential of populations in different regions of the species range to react to climate change.

I address these questions by examining the California vole, *Microtus californicus*, (Rodentia: Arvicolinae; Peale, 1848), an excellent study species in this regard because its teeth are very abundant in the Quaternary fossil record, and are identifiable to the species level using recently developed discriminant shape analyses (Chapters 2 & 3). *M. californicus* is very abundant in present-day ecosystems, where it influences the structure of plant communities (Borchert & Jain, 1978) and provides food for predators (Huntly, 1991). Critical for this study, previous research on *M. californicus* has demonstrated a correlation between present-day geographic clines and the shape of its first lower molar (m1) (McGuire, 2010). Because these geographic clines correlate with climate clines across California, m1 shape in this species may be driven by changes in climate (McGuire, 2010). The climate variable implicated as being most important in determining this species' range in several other studies is precipitation (Chapter 3; Church, 1966). Here, I test the hypothesis that m1 shape correlates with mean annual precipitation across the range of *M. californicus*. I use semilandmark geometric morphometric analyses in conjunction with partial least squares (PLS) analyses to determine whether one or more singular axes of shape variables correlates with precipitation.

Using geometric morphometrics and discriminant analyses, I have previously identified 101 *M. californicus* Quaternary fossil m1s from 7 California localities (Table 4-1 & Table 4-S2; Chapters 2 & 3). In concert with morphometric data from historic populations, these fossil specimens are used to test ideas about how precipitation influences morphology using the following logic. First I document a correlation between precipitation and morphology in present-day specimens. I then determine whether the same pattern prevails in the fossil specimens, by determining whether trends in fossil morphotypes agree with previously published paleoclimatic proxy data that infer past precipitation gradients. Since the Last Glacial Maximum (21kya; LGM), each of the included fossil localities has undergone warming and drying (Chapter 3). According to palynological data, paleolimnology, *Neotoma* midden evidence, and paleoclimate models, since 9 kya southern California has become drier, suggesting that morphotypes now found in wetter areas should have prevailed there in the past (Boxt *et al.*, 1999; Diffenbaugh & Ashfaq, 2007; Kirby *et al.*, 2007; Mock & Brunelle-Daines, 1999; Thompson *et al.*, 1997). Northern California and Oregon have had no change in precipitation or have become wetter over the same time period (Boxt *et al.*, 1999; Diffenbaugh & Ashfaq, 2007; Kirby *et al.*, 2007; Mock & Brunelle-Daines, 1999; Thompson *et al.*, 1997). These paleoclimate data suggest that present-day northern morphotypes should (1) exhibit morphotypes characteristic of the moister parts of the species range; (2) possibly not exhibit much change with respect to the past; and (3) should resemble those found further to the southeast from time intervals that were similarly moist.

The overall effect, if *M. californicus* m1 shape correlates with precipitation, should have been a general northwesterly shift of morphotypes through time as California became drier. A possible corollary of expecting morphotypes to have shifted to the northwest as aridity increased is that the entire species range shifted to the northwest. However, previous work suggests a southwest range contraction, rather than a northwest range expansion throughout the Quaternary (Chapter 3). Alternatively, high-precipitation morphotypes may have been lost in regions of increasing aridity, consistent with a southwest range retraction. Here, I will test whether Quaternary climate change led morphotypes to shift across the landscape, remain in place and lose variation, or not respond. To do this, I compare the relative positions of m1 morphotypes during the Quaternary and the present and examine the difference in overall shape variation in fossil versus present-day specimens.

MATERIALS AND METHODS

Morphological and climate data

Morphological data

The m1s of 297 modern and 101 fossil *M. californicus* were used. All modern specimens are from the Museum of Vertebrate Zoology (MVZ; Berkeley, USA), and were evenly sampled from throughout the range of the species (Figure 4-1 & Table 4-S1). Fossil specimens are from 7 different localities and are currently located at either the University of California Museum of Paleontology (UCMP; Berkeley, USA) or the Los Angeles County Museum of Natural History (LACM; Los Angeles, CA; Table 4-1; Figure 4-1; Chapter 3, Table 4-S2). Specimens used from two of the localities, Prune Avenue and Samwell Cave, are entirely Holocene in age (11kya-present). Specimens from four of the localities—Emery Borrow, Montezuma, Pacheco 2, and Palos Verdes—are not well constrained in age, but are from the Rancholabrean (~300-11kya). One of the localities, McKittrick Brea, likely contains specimens from approximately 37 kya to the present. Specimens from these localities were photographed using a Nikon D70s and AF Micro NIKKOR 60mm f/2.8D macro lens.

Geometric morphometrics

In order to trace fine-scale dental variation through time, it is necessary to use a high-resolution method for quantifying shape. Geometric morphometrics does just that, allowing quantification of shape by placing coordinates on homologous landmarks. The coordinates of each specimen can then be compared, accounting for the effects of size. For this analysis, left m1s were digitized on Tiff images using tpsDig 2.10 (Rohlf, 2006a), including 21 landmarks and 15 semilandmarks (after Chapter 2 semilandmark placement). Semilandmarks are a type of landmark that is defined relative to other features. They are generally used to define curved regions that do not contain strictly homologous structures (Zelditch *et al.*, 2004). Semilandmarks were included in this analysis to capture regions of high variation, especially the anterior portion of the *Microtus* m1s (Bell & Bever, 2006). Semilandmark curves were drawn and initially subsampled by length using tpsDig 2.10 (Rohlf, 2006a). Semilandmarks were slid using bending energy in tps Relative Warps 1.45 (Rohlf, 2007) and subsampled again in tps Utility 1.40 (Rohlf, 2008).

Generalized Procrustes analysis (GPA) was performed to superimpose landmark configurations and correct for non-shape variation. The GPA translates the centroid of each specimen to that of a mean specimen, normalizes the centroid across specimens, and rotates the specimens to minimize the overall summed squared distances between landmarks (Rohlf & Slice, 1990). The resulting size- and orientation-corrected landmark coordinates are then used to compare shape differences among specimens.

Climate data

To determine present-day precipitation values, each modern specimen and fossil locality was first mapped using ArcGIS 9.3 (ESRI, Redlands, CA, USA; Figure 4-1). A modern mean annual precipitation layer with 30 arc-second spatial resolution was downloaded from the WorldClim dataset (Hijmans *et al.*, 2005; http://www.worldclim.org). Present-day (1950-2000) climate data were then extracted for each modern *M. californicus* specimen and fossil locality.

Precipitation morphotypes

A two-block PLS analysis was used to reduce the dimensionality of the shape variables. This technique creates new linear combinations of the multidimensional variables, called singular axes, that maximize the covariation between dependent and independent variables (Bookstein *et al.* . In this case the variables used were m1 shape variables and mean annual precipitation. The program tpsPLS (Rohlf, 2006b) used 10,000 bootstrapping replicates of the m1 shape and precipitation variables to create a null distribution, which was then used to establish the significance of the correlation coefficients (r) for each singular axis (see McGuire, 2010 for further discussion).

Once the singular axes of modern m1 shapes were established, the PLS was used to estimate past precipitation values based on fossil m1 morphology. The ranges of morphologically estimated precipitation values for each fossil locality were then compared to the modern precipitation values at each locality in JMP 8.0 (SAS Institute, Cary, NC). The projected trends in morphologically derived paleoprecipitation estimates to present-day precipitation values were then compared to paleoclimate trends compiled from published data (Boxt *et al.*, 1999; Diffenbaugh & Ashfaq, 2007; Kirby *et al.*, 2007; Mock & Brunelle-Daines, 1999; Thompson *et al.*, 1997) to determine whether they recorded similar geographic and temporal climate trends.

Morphotype shifts through time

The first singular m1 shape axis from the precipitation PLS cannot be directly used in an analysis to reconstruct latitude and longitude, because error propagation across multiple prediction analyses results in too much noise to create accurate estimations. Therefore, in order to explore the relative distribution of morphotypes through time, another PLS was performed that included modern m1 shape variables as the dependent variable and latitude and longitude as the independent variables. The PLS was then used to determine the location of the present-day population that each fossil locality most resemble based on their m1 morphology. These PLS analyses were performed in JMP 8.0.

Structure of modern and Quaternary variation

The variances in overall m1 shape were compared between fossil and modern specimens. Due to sampling limitations, fossil localities did not fully sample Pleistocene variation, whereas modern sampling is performed throughout the range of the species. This effect would be expected to decrease variation in fossil samples with respect to modern ones. However, fossil localities also are time-averaged, which has the opposite effect on variation, theoretically averaging a wide range of morphologies from many different time slices at a single locality, falsely inflating palaentological variation. Hunt (2004) demonstrated that in small mammals inflation of variation due to time averaging is probably a more important bias—in that study time averaging over 100 ky demonstrated an approximate 3% decrease in variation from fossil to modern populations. If the Pleistocene specimens are completely sampled and represent the full ~300 ky timespan suggested (Table 4-1), then a 9% decrease in variation from fossil to modern populations (i.e., 3% per 100 ky) due to time averaging would be expected given no overall change in variation.

Two components of variation structure were considered: mean shifts and relative variation. To determine whether mean m1 shape shifted, Manovaboard 6.4 (Sheets, 2006b) was used to perform a 1-way MANOVA on shape variables as grouped by age (modern or fossil). DisparityBox 6i (Sheets, 2006a) was used to examine variation in overall m1 shape in fossil and modern specimens. DisparityBox uses disparity calculations established by Foote (1993), comparing the distance of each specimen to the centroid of that group of specimens (d): var.=$\sum (d_i^2)/(N-1)$. Two hundred bootstraps (resampling with replacement) were performed to determine the confidence intervals for each variation calculation.

RESULTS

Precipitation morphotypes

This PLS analysis demonstrates that the first singular m1 shape axis and mean annual precipitation are significantly correlated (PLS regression, r=0.55; P<0.01; Figure 4-2), as are the second two singular axes (PLS regression, r=0.23; P<0.01). The predictive power of the PLS is significantly higher than expected by chance (Permutation test, P<0.01). A deformation diagram indicates the relative shape change along the first singular m1 shape axis by indicating vectors of relative change at each landmark or semilandmark (Figure 4-2). Molars are relatively more straight and robust in drier climates and more curved and gracile in wetter climates.

While the PLS cannot confidently estimate the precipitation conditions experienced by a single fossil specimen, it can provide a reliable estimate when considered across local populations. At each fossil locality, it establishes a range of values that is representative of that population overall. Morphologically estimated precipitation values for fossil localities generally indicated more precipitation in the past than in the present (Figure 4-3). Past estimated precipitation values were significantly higher than modern precipitation values (t test, P<0.01) for Prune Avenue, Palos Verdes, Pacheco 2, McKittrick, and Emery Borrow; indistinguishable for Montezuma (t test, P=0.47); and significantly lower for Samwell Cave (t test, P<0.01).

The relative changes in precipitation estimated from precipitation-correlated morphotypes agree with those estimated by independent paleoclimate proxy studies. Chapter 3 indicates that each of the included fossil localities has become drier since the LGM. Of the sites that date back to the LGM—Emery Borrow, McKittrick, Montezuma, Pacheco 2, and Palos Verdes—all of the localities except for Montezuma are in agreement with this trend (Figure 4-3). Palynological, *Neotoma* midden, and paleolimnology data demonstrate that since 9 kya, southern California has become drier while northern California and Oregon have had no change in precipitation or become wetter over the same time period (Mock & Brunelle-Daines, 1999; Thompson *et al.*, 1997). These data are consistent with those of Kirby *et al*. (2007) and Boxt *et al*. (1999), who examined sediment cores from southern California, and with Diffenbaugh *et al.* (2007), who used nested climate models to demonstrate a decrease in precipitation in most of California with the exception of the northwest. The localities that are entirely Holocene in age—Samwell Cave and Prune Avenue—both agree with these estimations (Figure 4-3). Prune Avenue results indicate that the locality has become drier through the Quaternary while Samwell Cave, which is in northern California (Figure 4-1), indicates a trend towards increasing precipitation (Figure 4-3).

Morphotype shifts through time

This PLS analysis captures components of shape that show significant variation across the geographic range of *M. californicus*. The first singular m1 shape axis and the first singular latitude/longitude axis are significantly correlated (PLS regression, r=0.69; P<0.01), as are the second two singular axes (PLS regression, r=0.53; P<0.01), and the third two singular axes (PLS regression, $r=0.37$; $P<0.01$). The predictive power of the PLS is significantly higher than expected by chance (Permutation test, $P<0.01$).

Half of the localities contain fossil morphotypes that have shifted significantly through time (Figure 4-4). Emery Borrow, McKittrick, Palos Verdes, and Samwell Cave all show that fossil morphotypes are significantly more similar to present-day morphotypes in a different geographic region (t test, $P<0.01$); and Montezuma (t test, $P_{lat}=0.34$, $P_{long}=0.08$), Pacheco 2 (t test, $P_{lat}=0.76$, $P_{long}=0.04$), and Prune Avenue (t test, $P_{lat}=0.98$, $P_{long}=0.75$) do not show significant shifts in morphotypes through time.

Structure of modern and Quaternary variation

Fossil and modern *M. californicus* demonstrated a difference in overall mean m1 shape (MANOVA, $P<0.01$). Fossil specimens had 21% higher variance than modern specimens (Foote disparity, $P<0.01$), more than twice the amount that would be expected due to time averaging over 300 ky (9%). Given that the fossil specimens have considerably patchier geographic sampling than the modern specimens, this is likely an underestimation of the actual change in variation through time. The distribution of shape values along the first principal component demonstrates a shift in mean and difference in variance between the fossil (Figure 4-5a) and modern (Figure 4-5b) specimens. The deformation diagram indicates the relative shape change along the first principal component from fossil to modern specimens (Figure 4-5c). Fossil molars demonstrate relatively more complex, high-domed anterior caps than the modern specimens.

DISCUSSION

M. californicus m1 shape significantly covaries with modern mean annual precipitation, having relatively more gracile, curved morphotypes in wetter regions and more robust, straight morphotypes in drier regions (Figure 4-2). Paleoprecipitation values based on fossil morphologies largely agree with paleoclimate proxies from the literature (Boxt *et al.*, 1999; Diffenbaugh & Ashfaq, 2007; Kirby *et al.*, 2007; Mock & Brunelle-Daines, 1999; Thompson *et al.*, 1997). Most of the fossil localities had higher precipitation in the past than in the present, with the exception of Samwell Cave and Montezuma (Figure 4-3). However, this correlation between morphotype and precipitation does not indicate a uniform shift of all the morphotypes to the northwest as the state became warmer and drier. Instead, morphotypes that were once distributed throughout the range of the species are now isolated to the northern portion of the species range, near the San Francisco Bay area (SF Bay; Figure 4-4), leading to an overall reduction morphological variation of 21% from fossil to modern specimens and a corresponding shift in the overall mean shape from having more complex to simpler anterior caps (Figure 4-5).

Possible cause of correlation between precipitation and morphotypes

-precipitation morphotypes that are curved and gracile to low-precipitation morphotypes that are straight and robust (Figure 4-2) may well reflect the differences in food sources available in these different habitats. In grasslands, the predominant habitat of *M. californicus*, precipitation correlates with overall productivity and the relative abundance of certain grasses and forbs (Murphy, 1970; Pitt & Heady, 1978; Zavaleta *et al.*, 2003). Differences in the relative roughness or toughness of the grasses and forbs growing under different precipitation regimes could drive a change in overall tooth shape. Alternatively, tooth shape may be genetically linked to some other precipitation-dependent trait. It is unknown whether the link between precipitation and morphology relates to differences in annual precipitation or seasonal distribution of precipitation. Establishing this link is in theory tractable with more detailed analysis of the modern situation, but is the beyond the scope of the present study.

Regardless of the cause, the correlation between m1 shape and precipitation seems to have existed since the late Pleistocene, as evidenced by the similarities in trends recorded by paleoclimate proxies from the literature (Boxt *et al.*, 1999; Diffenbaugh & Ashfaq, 2007; Kirby *et al.*, 2007; Mock & Brunelle-Daines, 1999; Thompson *et al.*, 1997) and determined based on morphologically estimated paleoprecipitation values. The only one of the seven sites that did not agree with climate trends recorded in the literature was Montezuma (Figure 4-3). It is possible that the Montezuma fossil locality, which does not have well-constrained associated dates, includes more Holocene or modern specimens than has previously been realized, or these results may be due to the small sample size of fossils at the locality.

Morphotype shifts through time

Data presented here reject the hypothesis that morphotype has simply shifted northwest as climate has change in California. Rather, the data indicate that all fossil morphotypes are now only found in the present-day SF Bay region. It is possible that there is a northwest shift in the southern fossil morphotypes through time (Figure 4-4). However, the SF Bay fossil morphotypes show little to no movement through time, and the Samwell Cave fossil morphotypes actually shift from northeast to southwest (Figure 4-4). These findings could result from dealing with localities of different ages. Samwell Cave is Holocene in age, and the northwestern portion of California experienced no change in precipitation or an increase in precipitation over that time period. This fits with expectations that the fossil morphotypes should be those of drier (generally more southerly) climates. However, the patterns of SF Bay morphotypes fit less cleanly with the expectation of high-precipitation morphotypes shifting consistently to the northwest, unless all three localities—Montezuma, Pacheco2, and Prune Avenue—actually consist of primarily Holocene specimens. This possibility has already been discussed for Montezuma, and Prune Avenue is Holocene in age. This leaves the age of Pacheco 2 to be considered. The dating of Pacheco 2 as Pleistocene is the result of its close stratigraphic affiliation with Pacheco 1, which contains Pleistocene megafaunal remains (Tomiya *et al.*, in preparation). This Pleistocene age association is, therefore, also tenuous.

Structure of modern and Quaternary variation

The overall trend in how morphotypes shift (Figure 4-4) is consistent with the hypothesis that morphological variation was reduced *in situ* as climate changed, leading in a shift in the overall mean morphology. The statistical tests demonstrate the loss of variation through time, and the geographic pattern of remaining variation indicates that higher-precipitation morphotypes are limited to the northern portion of the range of *M. californicus* (Figure 4-4). Also, despite considerably less complete spatial sampling in the fossil record, and taking time averaging into account, I still found a 12% reduction in variation from fossil to modern specimens. This reduction in variation was also accompanied by a shift in mean overall shape that corresponds with the relative change in the shape of the anterior cap from a more complex to a simpler form (Figure 4-5). This axis of shape change (Figure 4-5c) was different from the singular m1 shape axis that correlates with precipitation (Figure 4-2). Nonetheless, the fossil-to-modern shape shift resulted in the loss of the relatively high-domed anterior cap that is associated with higher precipitation regimes (Figure 4-5c & Figure 4-2). This indicates the potential loss of high-precipitation morphotypes. It appears that as regions of aridity spread throughout California as the Quaternary proceeded, the morphotypes that are now associated with high-precipitation regions were lost in the more southerly portions of the species range.

Intra- and interspecific distribution

The establishment of a correlation between *M. californicus* m1 shape and mean annual precipitation, the agreement between m1 precipitation signals and paleoclimate proxy data from the literature, and morphotype shifts and loss of variation all demonstrate that precipitation is likely a driving factor in the overall intraspecific structure of variation. A similar pattern is reflected in a phylogeographic study of *M. californicus* by Conroy and Neuwald (2008). They describe two lineages of *M. californicus* based upon molecular phylogeographic markers that form a small hybrid zone just south of the McKittrick fossil site (Figure 4-1) and are divided by the Transverse Range. Conroy and Neuwald (2008) point out that the present-day northern lineage is in a region of higher climatic variability than the southern lineage, which was also supported by the clustering of majority of former morphotypes within the range of the northern lineage (Figure 4-4). The paleontological data presented herein suggests that the present-day northern lineage may consist of the remnants of the morphological variation that was once predominant in the majority of the past populations along the California coastline. The present-day morphological variation that remains in the southern lineage either originated in a region not sampled by the fossil data or was previously present at low concentrations within the fossil populations (Figure 4-4).

At the species level, climate seems to have had much less of an effect than at the intraspecific level. In the Yosemite region of its range, *M. californicus* has recently (over the last 100 years) expanded its range upward in elevation and eastward (Moritz *et al.,* 2008), but this expansion does not appear to be the result of regional precipitation changes over that time per (Chapter 3; Moritz *et al.*, 2008). In addition, from approximately 11 kya to the present, the northern range of *M. californicus* has contracted southward despite very little change in precipitation occurring in that portion of its range relative to many of the other fossil localities examined (Chapter 3). These shifts in range were likely the result of some other factor such as interspecific competition, fire succession, disease, or an unaccounted for abiotic factor.

Despite the lack of apparent affect of climate on Quaternary range limits, the internal climate dynamics of *M. californicus* have the potential to affect species-level range by differentially distributing phenotypic variance throughout the range of the species. Many authors have previously pointed out that the differential distribution of genetic variance within a species can restrict range limits by affecting the potential for gene flow dynamics (e.g., Gaston, 2009; Kirkpatrick & Barton, 1997). If the southern populations have actually lost the high-precipitation range of morphotypes, its ability to adapt to future climate change may be depleted. The northern lineage may still retain some the variation necessary to adapt to climate change, though not necessarily to the extent that it could during the Pleistocene, given that at least 12% of m1 variation has been lost when time averaging is taken into account.

Models project that California will become warmer over the next 100 yrs, and that precipitation will increase in the north and decrease in the south (Meehl *et al.*, 2007). Since the highest-precipitation morphotypes may have been lost, increases in precipitation could challenge some northern populations as precipitation regimes exceed the scope of the variability that remains in this species. Further, it is unclear whether the southern populations can endure additional reductions in precipitation, given their already-reduced morphological variability, which probably is underlain by reduced genetic variability. Although this species is currently abundant, continued monitoring is recommended, especially endangered populations such as *M. californicus scirpensis* (Neuwald, 2002), which lives in some of the most arid portions of the species' range.

Although Quaternary climate change does not appear to have caused a range reduction in *M. californicus*, it did lead to the reduction of overall variation within the species. These Quaternary changes set the stage as we now move into a period of even more rapid climate change. This overall reduction in variation has left the species in a more vulnerable position than it was in during the Pleistocene. The fact that *M. californicus*, a species that is incredibly abundant in modern and past California ecosystems, has experienced such a drastic reduction in morphological variation should increase our wariness about the possible status of many other species that are less abundant and potentially less resilient.

Botkin *et al.* (2007) refer to the discord between the predicted rise in endangered species resulting from present-day climate change and the relatively few species that became extinct during previous glacial-interglacial transitions as the "Quaternary conundrum." However, when we examine how the past has set the stage for the present and a future that includes unique climatic conditions, the conundrum disappears. As evidenced by *M. californicus*, it is likely that in many species a reduction in variation and population restructuring caused by earlier Quaternary climate change may limit their ability to adapt to the extent that they will not be able to weather future change.

CONCLUSIONS

Precipitation is a major driving factor in structuring *M. californicus* m1 variation. As California's precipitation has changed throughout the Quaternary, the geographic structure of m1 variation has shifted across the landscape. The majority of fossil variation now is found only in the present-day northern range of the species, while the decrease in precipitation in the southern portion of the state decreased variation and led to predominance of a dry-adapted southern lineage. These Quaternary decreases in precipitation, and the subsequent loss of morphotypes, have resulted in an overall reduction in the morphological variation in the m1 of *M. californicus*. Overall, these Quaternary changes have left the species more vulnerable to future environmental changes, and potentially clustered the majority of the species' m1 variation into the northern portion of its range. When examined with respect to the overall species range, the projected changes in precipitation may stress all regions of this species' range, as precipitation increases in the north in the face of lost high-precipitation morphotypes and continues to decrease in the south where populations may already be at their morphological limits.

FIGURES AND TABLES

Figure 4-1 *M. californicus*
the present-day range of *M. californicus* in light grey. Modern specimens used are indicated by small black asterisks. Fossil localities are indicated by large stars. Letters correspond with locality names and data listed in Table 4-1.

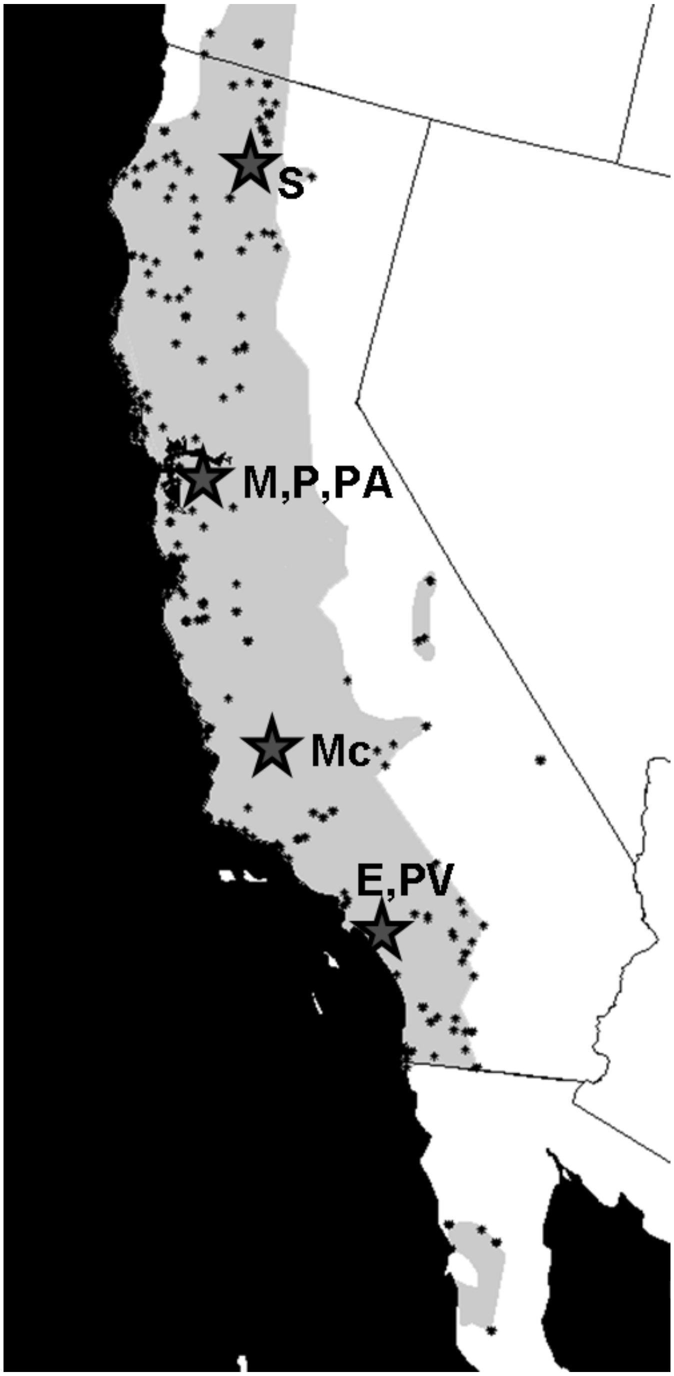

S
M,P,PA
Mc
E,PV

Figure 4-2 PLS plot of the first singular axis of m1 shape and precipitation (cm). An orthogonal line is shaded from black (dry) to grey (wet). These colors also correspond with the two end members of the warp diagram, which represents the relative shapes of teeth from each extreme of the graph.

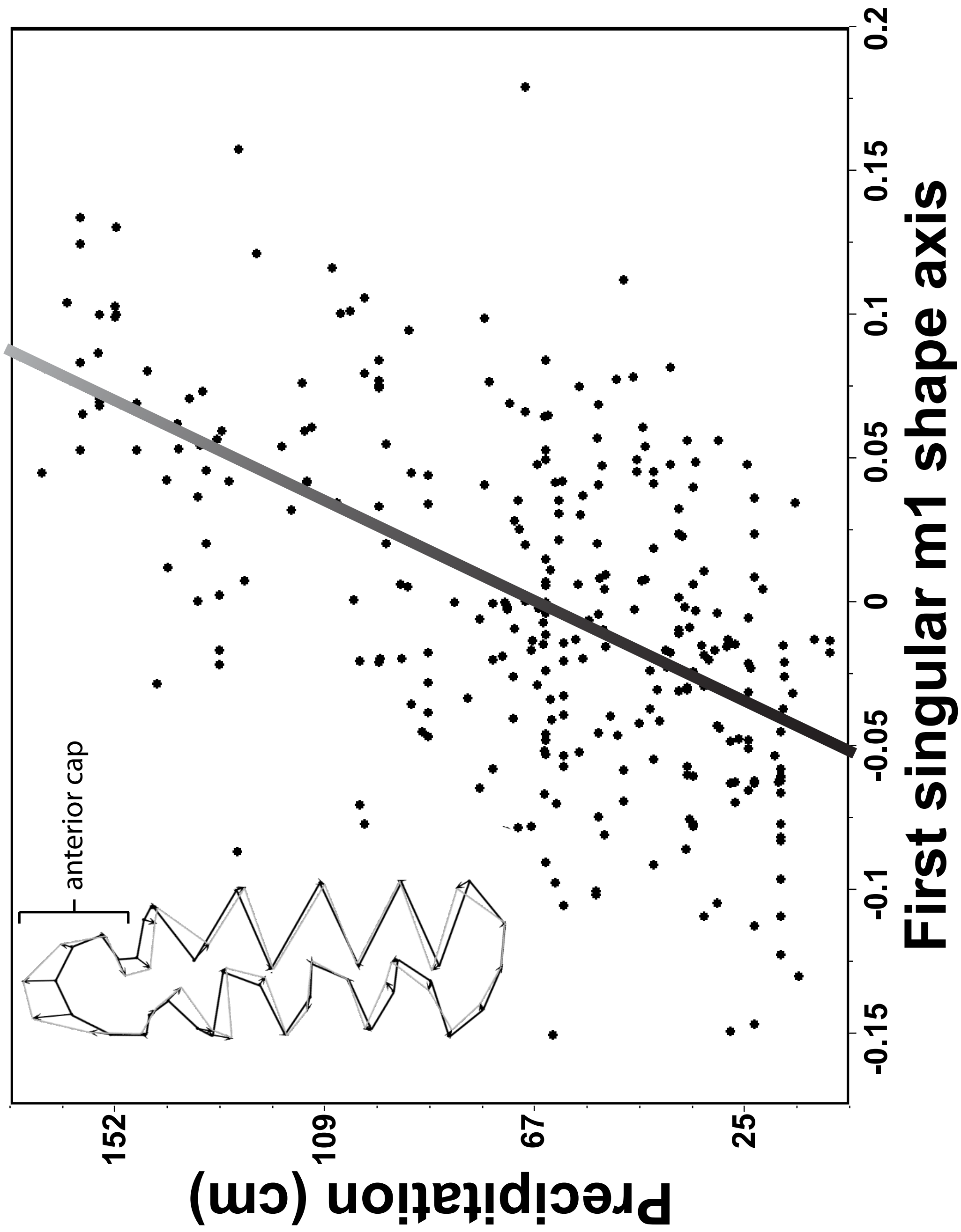
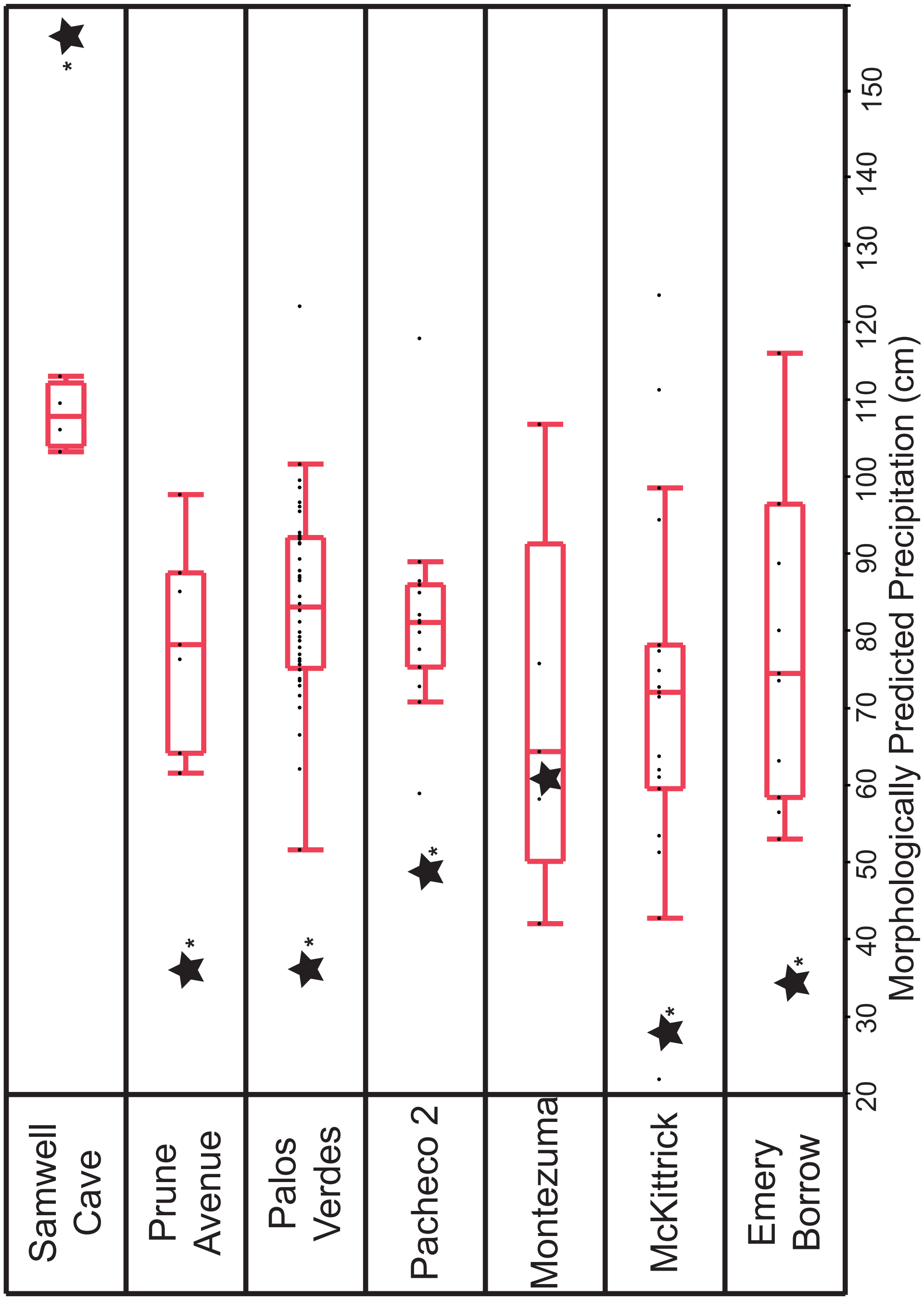

Precipitation (cm)
152
109
67
25
-0.15
-0.1
-0.05
0
0.05
0.1
0.15
0.2
First singular m1 shape axis
anterior cap

Figure 4-3 Box plots of morphologically estimated precipitation. The midline of the box represents the mean, the leading and end edges of the box are 25th and 75th quartiles, respectively, and the whiskers represent the 95 percent confidence interval of the mean. Black stars indicate present-day mean annual precipitation values at each fossil locality, and asterisks indicate which stars are significantly different than the morphologically estimated precipitation values at that locality.



Samwell Cave
Prune Avenue
Palos Verdes
Pacheco 2
Montezuma
McKittrick
Emery Borrow
20
30
40
50
60
70
80
90
100
110
120
130
140
150
Morphologically Predicted Precipitation (cm)
*

Figure 4-4 Loss of variation in southern populations through time. Three maps of California with arrows indicating the relative positions of precipitation morphotypes from the late Pleistocene to the present and from the Holocene to the present. Stars indicate fossil localities, asterisks (*) indicate significant values for localities that have lost fossil morphotypes, and letters correspond with locality names and data listed in Table 4-1. Grey shading in "Present" map indicates the modern range of *M. californicus*.

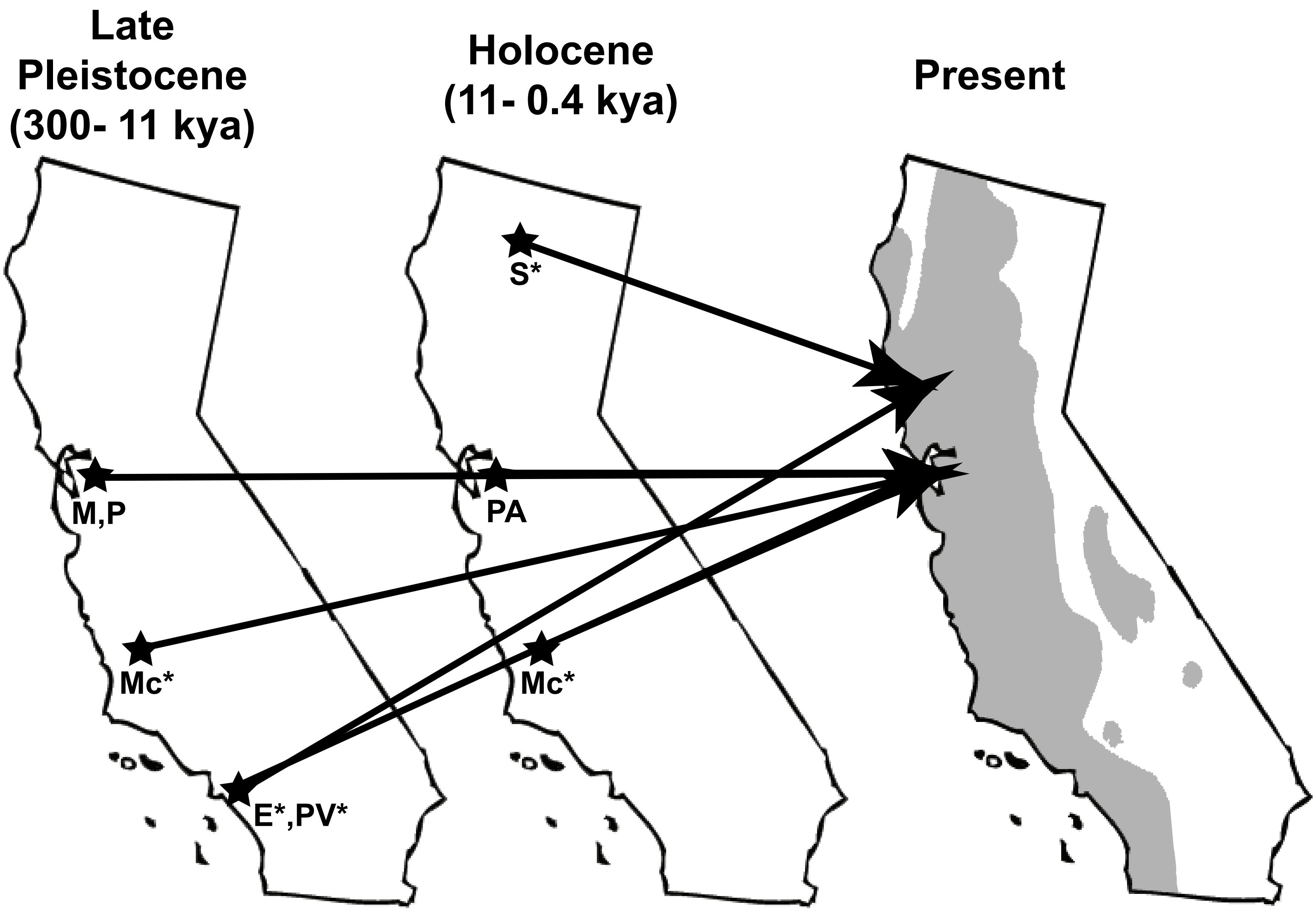

Late Pleistocene (300- 11 kya)
Holocene (11- 0.4 kya)
Present
S*
M,P
PA
Mc*
Mc*
E*,PV*

Figure 4-5 a. & b. Distributions of modern and fossil first principal components of tooth shape with standard error whiskers and quartile box plots above. Mean is shifted to the left ($p<0.01$) and variance is greater ($F<0.01$) in fossil specimens relative to modern specimens. c. Relative shape change between fossil and modern specimens along the first principal component.

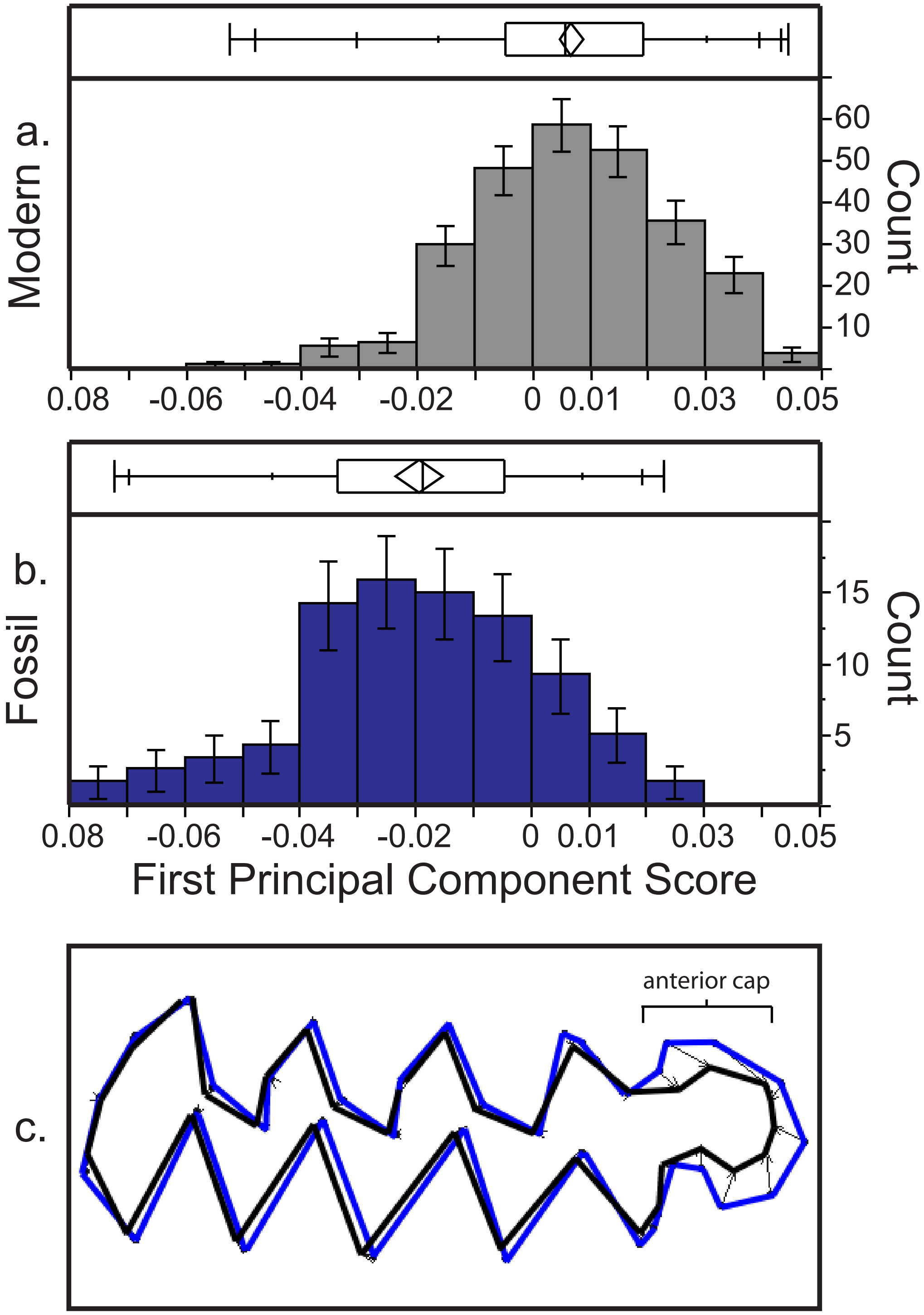

Modern a.
Count
60
50
40
30
20
10
0.08
-0.06
-0.04
-0.02
0
0.01
0.03
0.05
Fossil b.
Count
15
10
5
0.08
-0.06
-0.04
-0.02
0
0.01
0.03
0.05
First Principal Component Score
c.
anterior cap

TABLE 4-1 List of localities, ages of each and numbers of specimens from each. Ages were attained from the following sources: [1]From FAUNMAP Database; [2]From UCMP Database; [3]From LACM Database; [4]Chapter 3

Locality	**Locality Number**	**Specimen ages**	**Number of Specimens**
Emery Borrow (E)	LACM 7053 LACM 6689	*ca.* 11,000-300,000 yrs (biostratigraphic)[2]	11
Montezuma (M)	V-71001, V-6312 (UCMP)	*ca.* 11,000-45,000 yrs (biostratigraphic)[3]	5
McKittrick Brea (Mc)	CIT 138 (LACM)	0-37,011 cal. yr BP[1]	19
Pacheco 2 (P)	V-78027 (UCMP)	*ca.* 11,000-30,000 yrs (biostratigraphic)[3]	15
Palos Verdes (PV)	LACM 3877	*ca.* 11,000-300,000 yrs (biostratigraphic)[2]	40
Prune Avenue (PA)	V-5301 (UCMP)	836-4,283 cal. yr BP[4]	7
Samwell Cave (S)	V-65217 (UCMP)	0-8,275 cal. yr BP[4]	4

SUPPLEMENTARY TABLES AND FIGURES

TABLE 4-S1 Museum of Vertebrate Zoology (University of California, Berkeley) modern *M. californicus* specimens used in analyses.

Specimen Number	Subspecies
1766	sanctidiegi
2007	sanctidiegi
2199	sanctidiegi
2200	sanctidiegi
2201	sanctidiegi
2240	sanctidiegi
2439	sanctidiegi
2657	sanctidiegi
2789	sanctidiegi
2947	sanctidiegi
3076	sanctidiegi
3077	sanctidiegi
3078	sanctidiegi
3081	sanctidiegi
3082	sanctidiegi
3083	sanctidiegi
3404	sanctidiegi
3520	californicus
3645	californicus
3646	californicus
3664	aestuarinus
5198	sanctidiegi
6286	sanctidiegi
6287	sanctidiegi
6329	sanctidiegi
6330	kernensis
6332	kernensis
7002	kernensis
7003	californicus
7057	sanctidiegi
7381	sanctidiegi
7383	sanctidiegi
7384	sanctidiegi
7534	sanctidiegi
7536	sanctidiegi
7539	sanctidiegi
7670	sanctidiegi
7673	sanctidiegi
7674	sanctidiegi
8991	sanctidiegi
11546	constrictus
11586	constrictus
11627	constrictus
12671	sanctidiegi
12717	californicus
12727	aestuarinus
12804	eximius
12807	eximius
13393	eximius
13394	eximius
13395	eximius
13396	eximius
13397	eximius
13399	eximius
13400	eximius
13402	eximius
13403	eximius
13405	eximius
14099	californicus
15760	kernensis
15776	kernensis
16380	sanctidiegi
17385	vallicola
18159	aestuarinus
18160	aestuarinus
18232	aestuarinus
19002	sanctidiegi
20034	eximius
20058	eximius
20064	eximius
20065	eximius
20092	eximius
20101	eximius
20102	eximius
20846	eximius
24497	sanctidiegi
25551	californicus
25794	eximius
26374	vallicola
26383	vallicola
26385	vallicola
28268	vallicola
28269	vallicola
28270	mohavensis
28920	californicus
28922	californicus
28923	californicus
28925	californicus
28926	californicus
28928	californicus
28929	californicus
28932	californicus
28933	californicus
28934	californicus
29336	californicus
30508	eximius
34626	eximius
34631	eximius
35863	aequivocatus
35864	aequivocatus
35865	aequivocatus
35866	aequivocatus
35867	aequivocatus
35868	aequivocatus
35869	aequivocatus
35870	aequivocatus
35871	aequivocatus
35872	aequivocatus
35874	aequivocatus
35875	aequivocatus
35876	aequivocatus
35877	aequivocatus
35880	aequivocatus
35881	aequivocatus
35883	huperuthrus
35884	huperuthrus
35886	huperuthrus
35887	huperuthrus
35888	huperuthrus
35934	aequivocatus
36132	huperuthrus
36135	huperuthrus
36136	huperuthrus
36137	aequivocatus
36318	aequivocatus
36320	aequivocatus
36837	eximius
36862	eximius
36865	eximius
39639	sanctidiegi
39747	eximius
44509	sanctidiegi
44510	sanctidiegi
47196	californicus
47197	californicus
50236	aequivocatus
50237	aequivocatus
51600	eximius

51999	eximius
417	sanctidiegi
54927	eximius
54928	eximius
57049	eximius
59095	eximius
59105	eximius
59106	eximius
59107	eximius
59108	eximius
59889	aestuarinus
60289	kernensis
65428	eximius
65430	eximius
65501	eximius
66806	stephensi
66807	stephensi
66808	stephensi
69507	eximius
69511	eximius
69512	eximius
69513	eximius
69514	eximius
69515	eximius
69516	eximius
69517	eximius
69519	eximius
69520	eximius
69522	eximius
69687	eximius
70129	scirpensis
70130	scirpensis
70160	californicus
73072	californicus
73073	californicus
73074	californicus
73076	californicus
73077	californicus
73078	californicus
73079	californicus
73080	californicus
74535	sanctidiegi
74697	stephensi
81363	sanctidiegi
83499	eximius
83519	sanpabloensis
84924	sanctidiegi
85151	sanctidiegi
86793	eximius
86794	eximius
86936	californicus
88214	eximius
88680	eximius
89453	eximius
89903	sanctidiegi
89904	sanctidiegi
90271	sanctidiegi
93549	eximius
93551	eximius
93552	eximius
93554	eximius
93561	eximius
93579	eximius
93895	aestuarinus
95743	eximius
95751	eximius
96275	eximius
96736	eximius
97529	californicus
98016	californicus
98017	californicus
98019	californicus
98020	californicus
98021	californicus
98022	californicus
99629	constrictus
99652	constrictus
100973	californicus
101086	eximius
101362	eximius
102651	aestuarinus
102696	eximius
102820	eximius
103901	californicus
103902	californicus
103903	californicus
103904	californicus
105890	eximius
105894	eximius
105898	eximius
105900	eximius
108773	californicus
108777	californicus
108778	californicus
108780	californicus
108781	californicus
108784	halophilus
108816	halophilus
112068	californicus
113230	eximius
114364	sanctidiegi
116243	eximius
123548	sanctidiegi
125073	eximius
125904	sanctidiegi
126953	californicus
128796	californicus
128797	californicus
129075	californicus
132723	eximius
132724	eximius
132725	eximius
132726	eximius
132727	eximius
132728	eximius
136209	californicus
136673	eximius
148454	eximius
148455	eximius
148460	eximius
148461	eximius
148489	eximius
148490	eximius
148494	eximius
148503	eximius
149766	californicus
154399	eximius
154408	eximius
154443	eximius
158042	eximius
177622	californicus
177623	californicus
179051	eximius
179072	eximius
179073	eximius
183834	californicus
192086	eximius
198772	sanctidiegi
198787	californicus
200049	sanctidiegi
200053	sanctidiegi
200871	sanctidiegi
200873	sanctidiegi
200874	sanctidiegi
200876	sanctidiegi
206895	aestuarinus
206897	aestuarinus
206898	aestuarinus
208675	sanctidiegi
216053	sanctidiegi
216061	sanctidiegi
216077	sanctidiegi

TABLE 4-S2 Fossil specimens used in analyses and their associated localities. “CIT” and “LACM” localities are accessioned at the Los Angeles County Museum, “V-“ localities are accessioned at the University of California Museum of Paleontology. “UO” specimens are accessioned at the Museum of Natural and Cultural History at the University of Oregon.

Localities	Specimen Numbers
Carpinteria CIT 139	CIT-139E, CIT-139F, CIT-139H, CIT-139I
Emery Borrow LACM 7053, LACM 6689	100095, 100096, 100124, 100126, 105631, 64664, 66541, 66544, 66547, 66585, 66587
Montezuma V-71001, V-6312	92279, 92282, 92283, 91240, 91248
McKittrick Brea CIT 138	CIT-138A, CIT-138C, CIT-138F, CIT-138G, CIT-138H, CIT-138I, CIT-138L, CIT-138M, CIT-138N, CIT-138Q, CIT-138R, CIT-138S, CIT-138T, CIT-138U, CIT-138V
Pacheco 2 V-78027	190246, 190244, 190243, 190242, 190240, 190251, 190237, 190236, 190239, 190465, 190184, 190199, 190207, 190211, 190460
Palos Verdes LACM 3877	610, 613, 631, 635, 636, 639, 643, 657, 664, 667, 20580A, 20580C, 20580F, 20580T, 20580AD, 20580AE, 20580AF, 20580AG, 20580AI, 20580AN, 20580AP, 20580AQ, 20580AR, 20580AS, 20580AV, 20580AW, 20580AX, 20580AY, 20580BB, 20580BE, 20580BG, 20580BN, 20580BO, 20580BU, 20580BV, 20580BY, 3877Surface1, 3877Surface2, 3877TD295A, 3877TD295B
Prune Avenue V-5301	197576, 197577, 197580, 197582, 197585, 197587, 197590
Samwell Cave V-65217	1423, 1443, 1471, 1526
Woodburn UO-3859, UO-3038, UO-3867	F-48009

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