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Small-mammal isotope ecology tracks climate and vegetation gradients across western North America

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Stable carbon, nitrogen, hydrogen and oxygen isotopes have been used to infer aspects of species ecology and environment in both modern ecosystems and the fossil record. Compared to large mammals, stable isotopic studies of small-mammal ecology are limited; however, high species and ecological diversity within small mammals presents several advantages for quantifying resource use and organism–environment interactions using stable isotopes over various spatial and temporal scales. We analyzed the isotopic composition of hair from two heteromyid rodent species, *Dipodomys ordii* and *Perognathus parvus*, from localities across western North America in order to characterize dietary variation in relation to vegetation and climatic gradients. Significant correlations between the carbon isotopic composition ($\delta^{13}C$) of these species and several climatic variables imply that seasonal temperature and precipitation control the composition and distribution of dietary resources (grass seeds). Our results also suggest a moisture influence on the nitrogen isotopic composition ($\delta^{15}N$) of heteromyid diets. Population- and species-level variation in $\delta^{13}C$ and $\delta^{15}N$ values record fine-scale habitat heterogeneity and significant differences in resource use between species. Using classification and regression-tree techniques, we modeled the geographic variation in heteromyid $\delta^{13}C_{diet}$ values based on 10 climatic variables and generated an isotope landscape model ('isoscape'). The isoscape predictions for $\delta^{13}C_{diet}$ differ from expectations based on observed C_4 distributions and instead indicate that *D. ordii* and *P. parvus* record seasonally abundant grass resources, with additional model deviations potentially attributed to geographic variation in dietary selection. The oxygen and hydrogen isotopic composition of *D. ordii* is enriched relative to local meteoric water and suggests that individuals rely on highly evaporated water sources, such as seed moisture. Based on the climatic influences on vegetation and diet documented in this study, the isotopic composition of small mammals has high potential for recording ecological responses to environmental changes over short and long time scales.

Stable isotopes have become a significant tool for ecologists and paleoecologists alike for investigating relationships among resource use and availability and with environmental conditions across a variety of temporal and spatial scales (Koch 1998, Cerling et al. 2003). Stable isotopes preserved in fossil enamel and bone have provided the basis for dietary inferences of extinct species and documented major vegetation and climatic shifts from the local to the regional scale (Lee-Thorp and van der Merwe 1987, Bryant et al. 1996, Cerling et al. 1997). Applications to modern mammals include tracking of migration patterns and foraging ecology in the context of recent climate change and quantification of the isotopic niche in individual- to community-level comparisons (Koch 2007, Newsome et al. 2007). Small mammals, which are abundant and diverse in both modern and ancient ecosystems, have emerged as useful indicators of local environmental conditions with fine-scale variation in their stable isotopes recording aspects of habitat heterogeneity (Gehler et al. 2012, Hynek et al. 2012) and of niche partitioning over evolutionary timescales (Kimura et al. 2013). However, large gaps in our knowledge of isotope ecology remain across broad spatial scales, seasonal climates, and complex landscapes. Small mammals, studied along geographic and climatic gradients, offer promising insights about local and regional processes influencing mammalian isotopic composition and underlying variation in vegetation and climatic conditions.

Here, we investigate stable isotopes of carbon, nitrogen, oxygen, and hydrogen in hair of two modern heteromyid rodents, *Dipodomys ordii* and *Peroganthus parvus*, in order to document their dietary ecology and resource use in relation to vegetation and climatic gradients across 25 localities in western North America (Fig. 1). The geographic distributions of these two wide-ranging species encompass diverse

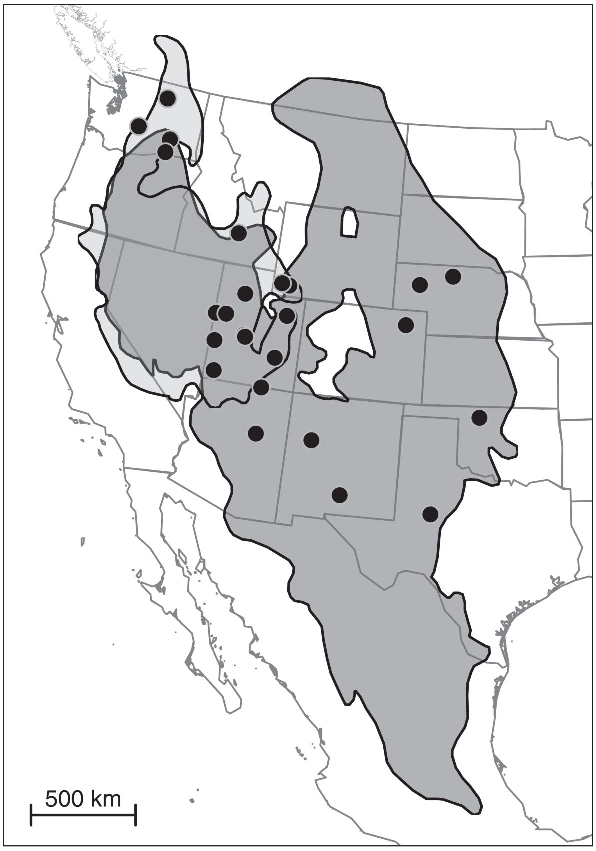

Figure 1. Geographic ranges of *Dipodomys ordii* and *Perognathus parvus* are shown in dark and light gray, respectively, with sample localities as black circles (n = 25).

environmental conditions and significant habitat heterogeneity, including Great Plains grasslands, the southwestern deserts, and the Great Basin. The rodent family Heteromyidae is a mammal radiation within western North America with high species and ecological diversity. The Heteromyidae have been studied as a model system to analyze ecological processes such as sympatric niche differentiation (Brown and Lieberman 1973) and the role of landscape history and biogeography in shaping regional diversity (Kelt 1999). *Dipodomys ordii* and *P. parvus* have small home ranges (less than 1 ha and 0.4 ha, respectively), forage on the ground, collect grass seeds in their cheek pouches for long-term storage in underground caches, and have highly efficient water-use strategies to conserve moisture in desert environments (MacMillen and Hinds 1983). The larger, bipedal individuals of *D. ordii* prefer less vegetated microhabitats and incur greater physiological costs due to water loss while foraging in the summer, whereas the smaller, quadrupedal individuals of *P. parvus* tend to forage beneath and around vegetation and can enter torpor during the cold season (Brown and Lieberman 1973, Price 1978). Differences in body size, locomotion, microhabitat selection and seasonal foraging ability provide a basis for differences in spatial and temporal resource use and microhabitat selection. Strong gradients in temperature, precipitation, and seasonal climate conditions across western North America provide the landscape context for evaluating the isotope ecology of these small mammals.

For herbivorous mammals, such as heteromyids, carbon isotope values, expressed as $\delta^{13}C$, of body tissues reflect the isotopic composition of vegetation ingested during formation of those tissues, thereby reflecting diet as well as the types of vegetation available for consumption (DeNiro and Epstein 1978, Hynek et al. 2012). Cool-season C_3 grasses and warm-season C_4 grasses fractionate carbon from the atmosphere through distinct photosynthetic pathways, resulting in average $\delta^{13}C$ values of ca –26.5‰ and –12.5‰, respectively, under pre-industrial atmospheric CO_2 conditions (Farquhar et al. 1989, Cerling et al. 1997). Through differences in photosynthetic pathway and plant physiology, climate acts as a primary control on the distribution and abundance of C_3 and C_4 grasses and on large-scale variation in $\delta^{13}C$ values of vegetation across the modern landscape, as documented by observations as well as statistical and mechanistic models (Teeri and Stowe 1976, Ehleringer et al. 1991, Tieszen et al. 1997).

While nitrogen isotopic composition often signifies animal trophic position, $\delta^{15}N$ values can also vary among individuals from the same primary-consumer level due to differences in local environment and isotopic composition of vegetation consumed (DeNiro and Epstein 1981, Ambrose 1991). Factors that influence the nitrogen isotopic composition of the local ecosystem (i.e. 10s of km^2 or less) include prior land use, species-level differences in plant $\delta^{15}N$, mycorrhizal associations, and climate (Evans 2001, Pardo and Nadelhoffer 2010). Although global and regional analyses document significant negative correlations between mean annual rainfall and plant $\delta^{15}N$, this relationship exhibits considerable variation around the general trend (Handley et al. 1999, Amundson et al. 2003) and is often masked by regional to local soil heterogeneity, processes of N-deposition and loss, and plant nutrient utilization (Prado and Nadelhoffer 2010).

Oxygen and hydrogen isotope ratios, expressed as $\delta^{18}O$ and δ^2H, record the isotopic composition of water sources consumed by animals, which indirectly reflect the isotopic composition of local meteoric water (Bryant et al. 1996). The isotopic composition of meteoric water is determined by latitude, elevation, temperature, and distance from coast, leading to predictable spatial patterns (Dansgaard 1964, Rozanski et al. 1993, Bowen 2010). The relationship between $\delta^{18}O$ and δ^2H in precipitation at the global scale is expressed by the global meteoric water line; the slope and intercept of this line at the local scale vary with changes in atmospheric humidity (Gat 1996, Kendall and Coplen 2001). Sources of oxygen and hydrogen that influence the isotopic composition in tissues of herbivorous mammals include drinking water and plant water, which are subject to fractionation processes such as evaporation in relation to aridity of the local environment, and atmospheric O_2 from respiration (Kohn 1996).

In this study, we compared geographic variation in heteromyid isotopic composition with climatic variables important for heteromyid ecology, grass productivity, and water fractionation processes over a broad spatial scale. Utilizing $\delta^{13}C$ and $\delta^{15}N$ records of dietary composition, we tested for significant differences in resource use between *D. ordii* and *P. parvus* in local ecosystems and over climate and vegetation gradients. We performed a novel application of classification and regression-tree (CART) methods to isotopic data to evaluate the relative importance of 10 climatic variables for predicting δ^{13}C values. We utilized the CART model to predict a carbon isoscape of heteromyid diet, which provided the basis for predicting C_3 and C_4 grass abundance across the landscape. In order to test the validity of rodent δ^{13}C composition as a vegetation proxy, we evaluated the isoscape model with our observed δ^{13}C values and proportions of C_4 grasses in local grass floras (Paruelo and Lauenroth 1996). Finally, we compared δ^{18}O and δ^{2}H of *D. ordii* with the isotopic composition of local meteoric water to infer water resource use. Our results enabled us to evaluate the potential for vegetation reconstruction, climate inference, and assessment of resource use based on rodent isotopic composition.

Material and methods

Collection and analyses

Hair samples were collected from 158 museum specimens of *Dipodomys ordii* (n = 89) and *Perognathus parvus* (n = 69) from the Univ. of Michigan Museum of Zoology and the Natural History Museum of Utah. Specimens were selected to represent a wide geographic distribution of localities (n = 25; Fig. 1) and a minimum of four adult individuals per species per locality (Supplementary material Appendix 1). Most individuals from each locality were collected within the same month and year, thereby representing a population sample of co-occurring individuals. Approximately 5 mg of hair per individual were collected and analyzed for the isotopic composition of the individual's diet since the last molt. Because the timing and frequency of molting varies geographically, and molting intervals for localities in this study are unknown, the isotopic composition of heteromyid hair samples may represent average seasonal to annual diet (Desha 1967, Speth 1969). Hair samples were rinsed with a 2:1 chloroform:methanol mixture to remove any surface contaminants and then homogenized, and samples for oxygen and hydrogen analysis were equilibrated with local atmospheric conditions prior to analysis. Samples were analyzed for carbon and nitrogen by an elemental analyzer, interfaced with a continuous-flow isotope ratio mass spectrometer. For oxygen and hydrogen analysis, samples were pyrolyzed with a temperature conversion/elemental analyzer and analyzed on a continuous-flow mass spectrometer. All analyses were performed at the Stable Isotope Ratio Facility for Environmental Research Laboratory at the Univ. of Utah. Isotope values are expressed as δ values, $\delta x = 1000\ (R_{sample} / R_{standard} - 1)$, where x is ^{13}C, ^{15}N, ^{2}H and ^{18}O, and R_{sample} and $R_{standard}$ are the molar ratios of the heavy to light isotopes (e.g. ^{13}C/^{12}C) for the sample and standard. δx is expressed in per mil (‰) relative to international standards: Vienna Pee Dee Belemnite for carbon, atmosphere for nitrogen, and Vienna Standard Mean Ocean Water for oxygen and hydrogen. Measurement standard deviations for δ^{13}C and δ^{15}N were ≤ 0.2‰ for standards and average errors were 0.1‰ for samples under replicate analyses. Analytical precision for δ^{2}H was ≤ 1.5‰ and for δ^{18}O was ≤ 0.2‰.

The carbon isotopic values of museum specimens collected from 1922 to 1968 were corrected for changes in the δ^{13}C value of atmospheric CO_2 based on the year of collection (Leuenberger 2007). For each locality, 10 climatic variables were extracted from the WorldClim dataset (Hijmans et al. 2005), using the geostatistical raster package (Hijmans 2015), in R ver. 3.1.2 (<www.r-project.org>). Temperature and precipitation data represent annual means as well as seasonal and extreme variations (e.g. mean annual precipitation and maximum temperature during the warmest month), composite variables (e.g. mean temperature during the wettest/driest quarter and precipitation during the warmest/coldest quarter), and mean diurnal range (calculated as the mean of monthly maximum minus minimum temperatures). The 10 climatic variables were selected because they exhibit strong spatial gradients across western North America (Supplementary material Appendix 2 Fig. A1), are biologically meaningful (e.g. physiological costs for heteromyids from seasonal extremes in temperature, strong effect of moisture on primary productivity and availability of seed resources), and overall, have low to moderate correlations. Precipitation variables were the most strongly correlated; however, because of significant variation in seasonal precipitation across the localities, it was important to include these variables.

Statistical analyses

We analyzed geographic variation in heteromyid isotopic composition in relation to latitude and longitude using Pearson's correlation tests. Utilizing the Student's t-test and the F-test, we assessed differences in mean and variance in the isotopic composition of *D. ordii* and *P. parvus* samples among and within localities where the two species co-occur. Because climate values across the heteromyid localities are not normally distributed, we used Spearman's rank correlation analysis to assess significant relationships between the 10 climatic variables and each isotope ratio. Using CART machine learning techniques, we additionally examined the relative importance of each climatic variable for heteromyid carbon isotopic composition by conducting conditional forest analysis of 1000 decision trees through the party package in R (Strobl et al. 2008). CART and random forest analyses are useful tools for non-parametric regression and prediction, and can test the relative importance of a large number of predictor variables while accounting for multivariate interactions (Breiman 2001, Strobl et al. 2009). Conditional forest analysis has the additional advantage of being able to cope with correlated predictor variables by calculating the conditional permutation importance of each variable, which takes into consideration importance contingent on other variables (Strobl et al. 2008). We produced a heteromyid-diet isoscape for western North America based on the variable importance outcome of the conditional forest analysis and bioclimatic layers using the raster package (Hijmans et al. 2005, Hijmans 2015). The isoscape was adjusted to reflect an isotopic enrichment of 3.2‰ from diet to hair (Sponheimer et al. 2003). However, a range of enrichment values has been documented for small mammals based on taxon, diet consumed, and other factors and the use of a different value would uniformly shift the predicted diet isoscape up or down accordingly. The isoscape was restricted to the combined geographic range of the two species studied. Model δ^{13}C predictions based on all individuals were then compared to the average isotopic composition of population samples at each locality to assess local fit. We then converted the modeled carbon isotopic values to relative abundances of C_4 grasses using a two-component mixing model, with pure C_3 and pure C_4 end-member values of –26.7‰ and –12.5‰, respectively (average pre-industrial grass $\delta^{13}C$ values; Cerling et al. 1997). Predicted C_4 grass abundances based on heteromyid diets were then compared with observed proportions of C_4 grasses within local grass floras (Supplementary material Appendix 2, Table A1), calculated from data compiled by Paruelo and Lauenroth (1996).

Finally, we evaluated the oxygen and hydrogen isotopic composition of *D. ordii* in comparison to the isotopic composition of local precipitation across a subset of localities spanning a steep gradient in isotopic composition of local water sources (Bowen 2010; localities = 8, individuals = 39). We tested for

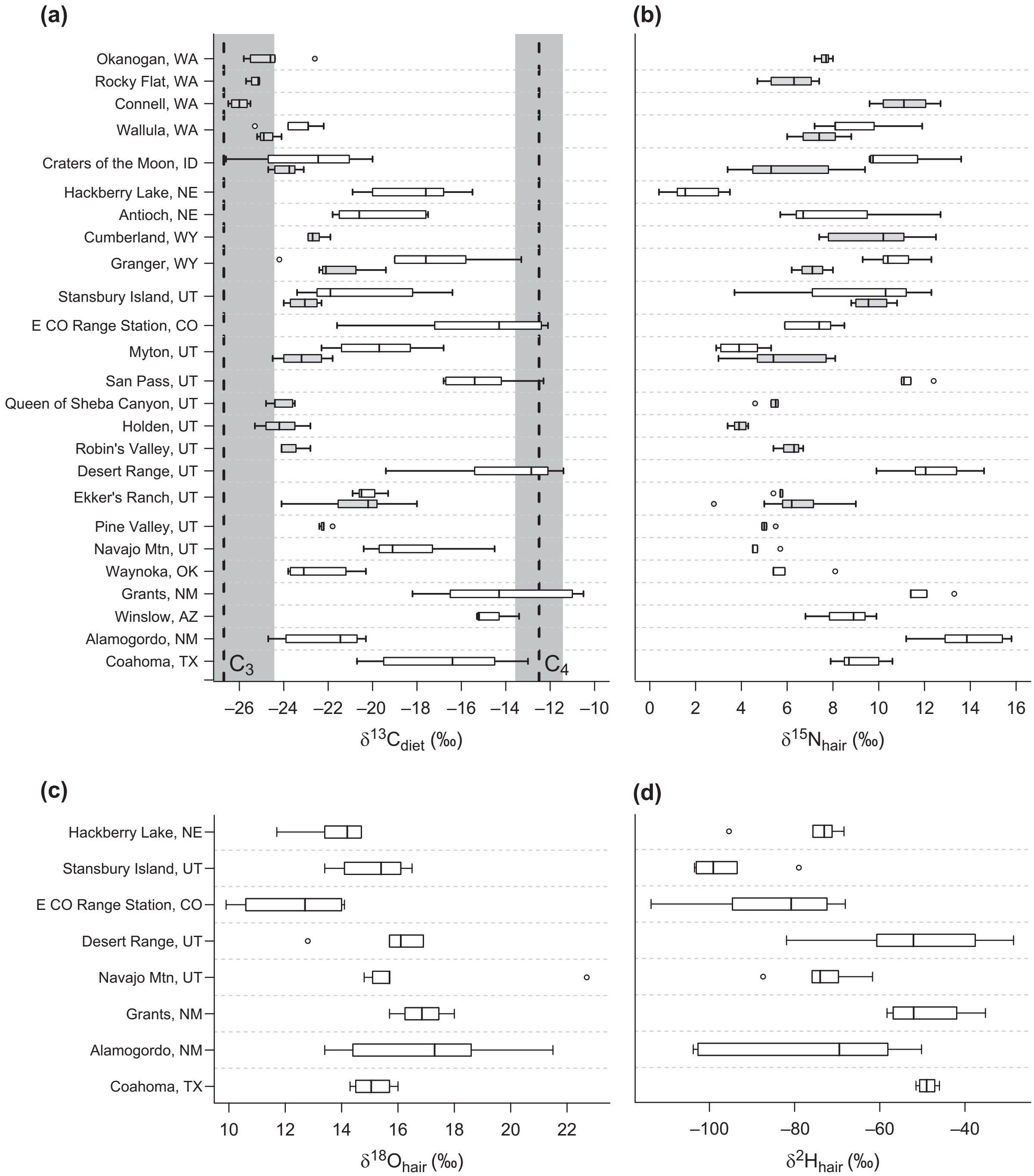

Figure 2. Isotopic composition of heteromyid samples. (a) Carbon isotopic composition of diet ($\varepsilon^*_{hair\text{-}diet}$ = 3.2 ‰ from Sponheimer et al. 2003) with average (vertical dashed lines) ± 1 standard deviation (gray bands) isotopic composition of C_3 and C_4 grasses (Cerling et al. 1997) and (b) nitrogen isotopic composition of hair, with *D. ordii* samples (n = 89) shown in white and *P. parvus* samples (n = 69) shown in light gray for 25 localities, listed from higher to lower latitude. (c) Oxygen and (d) hydrogen isotopic composition of *D. ordii* samples (n = 39) from eight localities, listed from higher to lower latitude.

significant correlation between the oxygen and hydrogen isotopic composition of *D. ordii* using a Pearson's correlation test, and regressed δ^2H_{hair} on $\delta^{18}O_{hair}$. We compared results from the regression analysis to global and local meteoric water lines from Coplen and Kendall (2000) in order to evaluate the isotopic offset between local water sources and *D. ordii* hair, as well as the overall similarity in the trends of these two datasets. We performed all analyses using the R software environment for statistical computing, ver. 3.1.2.

Data deposition

Data available from the Dryad Digital Repository: < http://dx.doi.org/10.5061/dryad.33b9s > (Smiley et al. 2015).

Results

Variation in $\delta^{13}C$ and $\delta^{15}N$ between species and in relation to climatic gradients

The carbon isotopic composition of the two wide-ranging heteromyid species, *Dipodomys ordii* and *Perognathus parvus*, varies from –23.4‰ to –7.3‰ across western North America. Assuming a carbon isotopic enrichment of 3.2‰ between diet and hair (Sponheimer et al. 2003), this range corresponds to individual diets composed of exclusively C_4 vegetation, diets of mixed vegetation, and diets of primarily C_3 vegetation (Fig. 2a). We found that $\delta^{13}C_{diet}$ is negatively correlated with latitude ($r = –0.45$, $p < 0.001$) and positively correlated with longitude ($r = 0.37$, $p < 0.001$), tracking expected geographic variation in C_3 and C_4 vegetation (Teeri and Stowe 1976, Paruelo and Lauenroth 1996) and implying a greater contribution of C_4 grasses to the heteromyid diets of more southern and eastern populations. There are significant differences in $\delta^{13}C_{diet}$ of *D. ordii* and *P. parvus* (across all localities: $p < 0.001$; within localities: $p = 0.014$) and in isotopic variance within population samples ($p < 0.001$). Across the sites, *D. ordii* individuals are enriched in ^{13}C relative to *P. parvus* (with one exception: Ekker's Ranch, UT), and *D. ordii* has greater variability within population samples than *P. parvus* (Fig. 2a).

We evaluated the relationship between $\delta^{13}C_{diet}$ and climatic variables for *D. ordii* and *P. parvus* individually as well as in combination, and found significant correlations between carbon isotopic composition and several climatic variables (Table 1). $\delta^{13}C_{diet}$ tends to be positively correlated with temperature variables and negatively correlated with precipitation variables across the sample sets. The highest correlation coefficients correspond to seasonal variables that represent interactions between temperature and precipitation, such as mean temperature during the wettest quarter (i.e. rainy season temperature) and precipitation during the coldest quarter. Seasonal variables have strong trends in relation to longitude (Supplementary material Appendix 2 Fig. A1) and contrast localities with warm rainy seasons in the east versus localities with cold rainy seasons in the west. These composite variables encompass both temperature (e.g. cross-over temperature, Ehleringer 1978) and precipitation (e.g. precipitation minimum, Still et al. 2003) thresholds that are important for C_4 grass dominance during the growing season.

The nitrogen isotopic composition of *D. ordii* and *P. parvus* varies by 15.4‰ across localities and up to 9‰ within population samples (Fig. 2b). Correlation coefficients for the relationship between $\delta^{15}N_{hair}$ and latitude and longitude are both non-significant. While differences between *D. ordii* and *P. parvus* are significant ($p < 0.001$), their $\delta^{15}N$ values overlap somewhat and samples of co-occurring species do not exhibit a consistent offset in isotopic composition. Correlations between climatic variables and $\delta^{15}N$ values are generally weaker than with $\delta^{13}C$ values (Table 1). All significant correlations between $\delta^{15}N$ and precipitation variables are negative, suggesting a moisture control on nitrogen isotopic composition of these rodents.

Isoscape and C_4 grass abundance based on heteromyid $\delta^{13}C$

Using CART conditional forest analysis, we assessed the relative importance of interacting climatic variables in predicting the isotopic composition of heteromyid diets across western North America (Supplementary material Appendix 2 Table A2). Our model explains 69.7% of the variance in the data, and has an average error of ± 2.23‰ on the validation (out-of-bag) dataset. Precipitation during the coldest quarter and mean temperature during the wettest quarter had the highest variable importance scores (> 1.5) in our

Table 1. Spearman's correlation coefficients (r) of isotopic variables with 10 climatic variables, T = temperature, P = precipitation. Bold values are significant at $p < 0.05$, *$p < 0.01$, **$p < 0.001$.

	$\delta^{13}C_{hair}$ r			$\delta^{15}N_{hair}$ r			δ^2H_{hair} r	$\delta^{18}O_{hair}$ r
Variable	*D. ordii* (n = 89)	*P. parvus* (n = 65)	Combined (n = 154)	*D. ordii* (n = 89)	*P. parvus* (n = 65)	Combined (n = 154)	*D. ordii* (n = 39)	*D. ordii* (n = 39)
Mean diurnal range	**0.40****	**0.25**	**0.31****	**0.41****	**−0.46****	0.12	**0.43***	0.20
T seasonality	−0.01	**0.44****	**0.21***	**−0.56****	0.10	**−0.30****	**−0.48***	**−0.45***
Maximum T, warmest month	−0.04	0.13	**0.33****	0.17	0.13	**0.25***	0.14	0.22
Minimum T, coldest month	−0.15	−0.24	−0.01	0.18	**0.26***	**0.25***	−0.18	**0.40**
Mean T, wettest quarter	0.13	**0.54****	**0.57****	−0.03	0.04	0.07	**0.50***	**0.37**
Mean T, driest quarter	**−0.28***	**−0.44****	**−0.37****	0.18	0.20	0.08	−0.17	**0.43***
Mean annual precipitation	**−0.26**	−0.18	**−0.19**	−0.17	**−0.34***	**−0.21***	−0.14	**−0.53****
P, driest quarter	−0.09	0.00	**−0.30****	−0.06	**−0.41****	**−0.28****	0.13	0.13
P, warmest quarter	−0.07	**0.25**	0.16	−0.19	**−0.31***	−0.12	−0.07	**−0.51***
P, coldest quarter	**−0.44****	**−0.37***	**−0.62****	0.06	**−0.27***	−0.14	−0.02	**0.37**

model. Although these two variables are significantly correlated, both were included in the analysis because winter precipitation contributes to long-term soil moisture, whereas temperature during the rainy season reflects growing season conditions. Mean diurnal range of temperature also had relatively high variable importance (1.09). Several other climatic variables had intermediate variable importance scores (> 0.39), while mean annual precipitation and warmest quarter precipitation had the lowest variable importance in our model (< 0.16).

Using the CART model, we generated a heteromyid-diet isoscape and compared the predicted values for $\delta^{13}C_{diet}$ against the sample average $\delta^{13}C_{diet}$ measured for each locality (Fig. 3a). For most localities, measured minus predicted $\delta^{13}C_{diet}$ values fell within analytical error of our model (Supplementary material Appendix 2 Table A3). Localities that exceed the error range ($\pm$ 2.23‰) tend to have large variance and are in areas of high topographic and climatic complexity. The CART model paired with an isotope mixing model predicts a high to low longitudinal gradient in C_4 grass abundance according to heteromyid diet from the Great Plains to the Pacific Northwest (Fig. 3b). This geographic trend compares well with measured percent C_4 grasses within the total grass flora calculated from Paruelo and Lauenroth (1996); however, model predictions of C_4 grasses based on heteromyid isotopic composition tend to predict greater C_4 abundance compared to measured values with two notable exceptions: Arizona and the eastern edge of *D. ordii*'s geographic range.

δ^2H and δ^{18}O in relation to climate and local water sources

The hydrogen and oxygen isotopic composition of *D. ordii* individuals ($n = 39$) from eight localities in the southwest (Fig. 2c–d) are negatively correlated with latitude (δ^2H: $r = -0.41$, $p < 0.01$; δ^{18}O: $r = -0.41$, $p < 0.01$) and longitude (δ^{18}O: $r = -0.36$, $p < 0.03$). δ^2H and δ^{18}O values are positively correlated with mean temperature during the wettest quarter (Table 1) and decrease from western to eastern, inland localities, matching expected climate influences and spatial patterns (Dansgaard 1964, Rozanski et al. 1993, Bowen 2010). However, due to the small number of localities and high within-sample variability (e.g. up to 50‰ for δ^2H), correlations between climatic variables and δ^2H and δ^{18}O of *D. ordii* are generally non-significant.

Dipodomys ordii δ^2H and δ^{18}O values were significantly correlated ($r = 0.56$, $p < 0.001$) and assessed by linear regression ($r^2 = 0.31$, $p < 0.001$) in order to compare with the global meteoric water line (GMWL) and local meteoric water lines (LMWL), which document the influence of evaporative processes in arid western states (Fig. 4). The low slope of the linear regression (4.71) suggests that *D. ordii* is utilizing water sources that have been further evaporated from local water sources, such as leaf-derived seed moisture. The high variance around this relationship and an average isotopic enrichment from meteoric water to heteromyid hair of ~25‰ for δ^{18}O and ~30‰ for δ^2H likely reflects the combined isotopic composition of ingested food, water, and

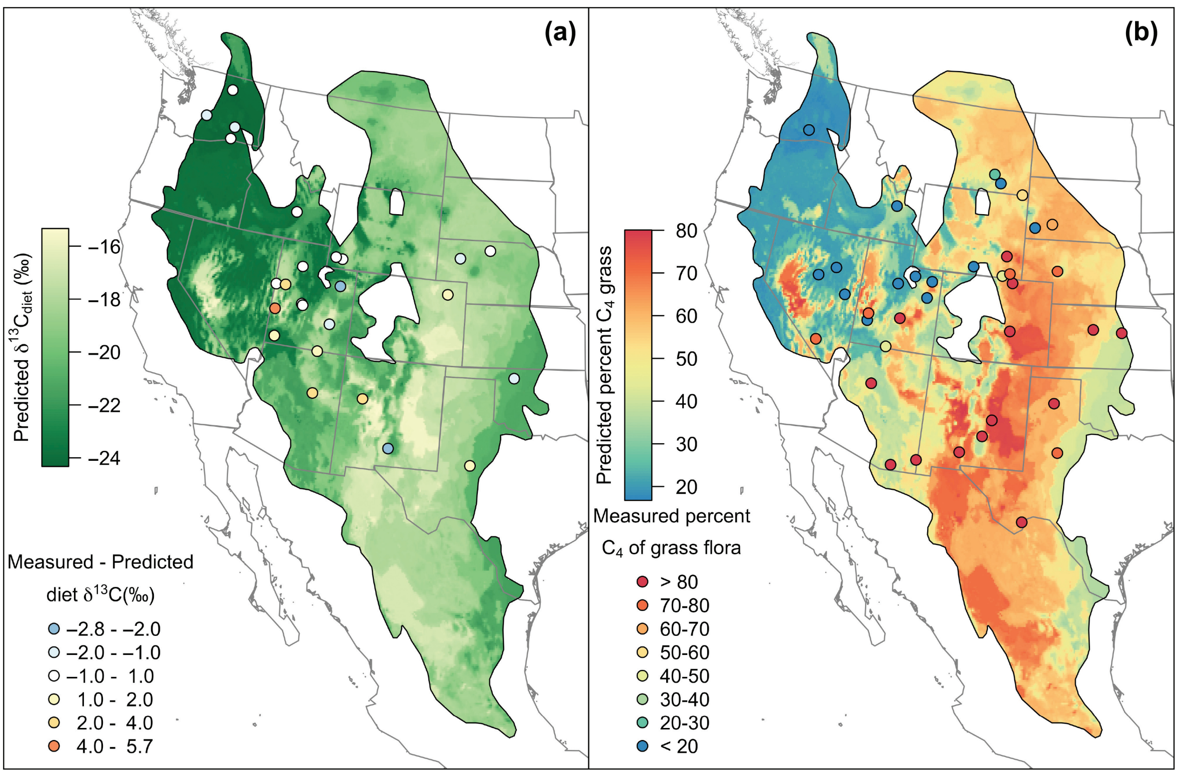

Figure 3. (a) Geographic variation in the carbon isotopic composition (isoscape) of *D. ordii* and *P. parvus* diets across their combined geographic range. $\delta^{13}C_{diet}$ values are predicted based on a conditional forest model, and the color of the filled circles represents the difference between measured and predicted $\delta^{13}C_{diet}$ values. (b) Percent C_4 grass abundance within the grass flora derived from the modeled isoscape, with the color of the filled circles representing the measured percent C_4 of the grass flora calculated from Paruelo and Lauenroth (1996).

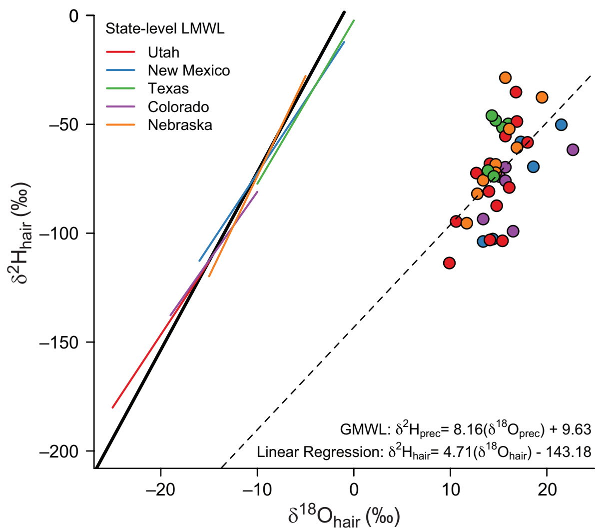

Figure 4. Oxygen and hydrogen isotopic composition of *D. ordii* specimens ($n = 39$) and corresponding global meteoric water line (GMWL: black solid line) and local meteoric water lines (LMWL) from Coplen and Kendall (2000). The relationship between δ^2H and $\delta^{18}O$ in *D. ordii* is expressed as a linear regression (black dashed line).

inspired air, and biological fractionation during metabolism and assimilation into tissue.

Discussion

This study spans a diversity of biomes, physiographic configurations, and environmental conditions, ranging from the topographically complex high-desert biome of the Basin and Range Province to the low-relief Great Plains grassland biome. Spatial variation in the isotopic composition of granivorous small mammals from study localities records differences in diet and water sources and reflects the associated variation in isotopic signatures of local vegetation and precipitation across the landscape. For *Dipodomys ordii* and *Perognathus parvus*, much of this variation is correlated with geographic and climatic gradients. The more enriched carbon isotopic composition of samples from southern and eastern localities indicates a greater contribution of C_4 grasses to the diet and therefore a greater presence of C_4 on the landscape in these regions. The $\delta^{13}C_{diet}$ values of several *D. ordii* individuals in southern localities overlap with the average $\delta^{13}C$ of C_4 grasses. In contrast, $\delta^{13}C_{diet}$ values for some *P. parvus* individuals in the northern most localities imply a pure C_3 diet. The carbon isotopic composition of most *D. ordii* and *P. parvus* individuals reflects a diet of mixed C_3 and C_4 grass seeds with variation in the relative contribution of these two dietary resources between species and across their geographic ranges.

A significant positive correlation between rainy season temperature and $\delta^{13}C_{diet}$ ($r = 0.57$, $p < 0.001$) implies that localities with a warm growing season show an increased contribution of C_4 grasses to heteromyid diets. A significant negative correlation between precipitation during the cold quarter and $\delta^{13}C_{diet}$ ($r = -0.62$, $p < 0.001$) implies greater C_3 grass within the diets of heteromyids from localities with higher winter precipitation and a spring growing season due to snow melt (e.g. eastern Washington). Compared to mean or extreme temperature and precipitation variables, the strong significant correlations and high relative importance of these composite climatic variables (Table 1) likely signify that heteromyid carbon isotopes track seasonal differences in the availability of C_3 and C_4 biomass for consumption and mirror the $\delta^{13}C$ value of more abundant growing-season resources rather than the mean annual value. Temporal separation of cool-season C_3 and warm-season C_4 grass production occurs across the temperate grasslands of North America, changing resource availability for these small herbivores seasonally (Collatz et al. 1998, Still et al. 2003). In addition to seed abundance, foraging behavior of heteromyids is also influenced by factors such as seed moisture content and air temperature (Brown and Lieberman 1973, Price 1978). Therefore, seeds collected and stored for year-round consumption may not represent the annual average proportion of C_3 or C_4 grasses.

The dietary signal from $\delta^{15}N$ varies by more than 15‰ across the landscape, and unlike $\delta^{13}C$, is not correlated with geographic gradients (Fig. 2b). Isotopic variation of up to 9‰ within single localities is better explained by local differences in vegetation composition and growth habits (including rooting depth, nutritional strategies, and fungal associations), moisture levels, and N-deposition than by differences in trophic position (Evans 2001, Pardo and Nadelhoffer 2010). Physiological mechanisms, such as catabolism, lactation, and increasing urea concentration as a water-conserving strategy, could additionally contribute to some of the variation among individuals from the same locality (Koch 2007). At the regional scale, we found significant negative correlations between heteromyid $\delta^{15}N$ values and both mean annual and seasonal precipitation variables (Table 1). These correlations follow global patterns attributed to greater N-losses (N lost through volatilization, denitrification, and leaching is typically depleted in ^{15}N) at drier sites, leading to ^{15}N-enrichment of residual soil and plant nitrogen pools (Handley et al. 1999, Amundson et al. 2003). However, there is considerable variability associated with this relationship in both global studies and within our data, suggesting that microhabitat conditions and local processes leading to spatial heterogeneity in soil and foliar $\delta^{15}N$ can overprint this pattern (Bowen 2010, Pardo and Nadelhoffer 2010).

While large-scale spatial patterns in $\delta^{13}C$ and $\delta^{15}N$ of *D. ordii* and *P. parvus* can be broadly attributed to environmental and climatic gradients, significant differences in isotopic composition between the species suggest that species diverge in their resource and microhabitat use. Across the localities sampled, *P. parvus* generally consumes a greater proportion of C_3 grasses than *D. ordii*, while differences in diet inferred from $\delta^{15}N$ composition could relate to microhabitat selection across an isotopically heterogeneous local landscape (Flaherty and Ben-David 2010). Signficant isotopic niche differentiation between co-occuring *D. ordii* and *P. parvus* conforms to documented resource and microhabitat partitioning among heteromyid species with differences in body size, locomator modes, and foraging behavior (Brown and Lieberman 1973, Price 1978). Within-species variance can additionally serve as a proxy for isotopic niche width

(Newsome et al. 2007), and significantly greater $\delta^{13}C$ variablity in *D. ordii* suggests that the larger kangaroo rat is a more generalist feeder, sampling both C_3 and C_4 grasses within their local ecosystems to a greater degree than in *P. parvus*.

Individuals from the same population with diets dominated by either C_3 or C_4 grasses also document the proximity of these two vegetation types within the local environment. High within-locality variation in isotopic composition implies that small mammals present several advantages over large mammals for recording local habitat heterogeneity. Whereas large mammals represent a temporally and spatially averaged isotopic signal from the landscape, small mammals with relatively small home ranges (e.g. 1 ha or less) can provide fine-scale estimates of the spatial and seasonal variation in vegetation (Hynek et al. 2012). Additionally, through consumption of small amounts of biomass and rapid rates of metabolism and tissue formation, there is little time for dietary mixing and a high probability of recording isotopic end-members within the rodent diet (Podlesak et al. 2008). Population-level analyses of rodent assemblages can be ideal for capturing spatial and temporal heterogeneity in resources, such as C_3 and C_4 grasses, for ecological and paleoecological studies. However, high within-locality variability in heteromyid $\delta^{13}C_{diet}$ and potential species-level dietary selection also suggests that it may be necessary to sample several individuals and species in order to accurately reconstruct the relative abundance of C_3 and C_4 grasses in an ecosystem.

The modeled $\delta^{13}C_{diet}$ isoscape shows strong geographic and topographic gradients, high isotopic heterogeneity in the intermontane west, and agrees, within error, with the average isotopic composition of samples from most localities (Fig. 3a). High within-site variability in carbon isotopic composition at Desert Range, UT ($\delta^{13}C_{diet}$ range = 8‰) could explain relatively poor model predictions at this site (residual = 5.7‰). The C_4 grass-abundance map generated from the heteromyid-diet isoscape mirrors trends in C_4 grass abundance documented by observations (Paruelo and Lauenroth 1996) and predicted by plant physiology (Ehleringer 1978). Across most of the geographic range of *D. ordii* and *P. parvus*, our model tends to predict slightly higher C_4 grass abundance than observed in the total grass flora. One plausible explanation for this pattern is based on our assigned isotopic end-member values; due to relatively low mean annual precipitation (less than 600 mm year^{-1}) across localities, an arid-C_3 end-member value may be more appropriate (Diefendorf et al. 2010), which would systematically lower C_4 estimates. Alternatively, the greater C_4 grass abundance predicted in our model could reflect higher seasonal abundance compared to mean annual abundance of C_4 grasses, suggesting the need for temporally dynamic models of resource isotopic composition. In two regions, Arizona and the eastern edge of *D. ordii*'s geographic distribution, our model predicts lower C_4 grass abundance than observed. The southwestern desert and Great Plains biomes tend to receive most of their rainfall in the summer, whereas the majority of localities used to generate this model receive higher winter rainfall. More complete geographic sampling and a greater diversity of heteromyid species should improve the model and more accurately characterize C_4 grass distributions across a variety of seasonal temperature and precipitation conditions.

While model construction and assumptions may explain the apparent departure of predicted $\delta^{13}C_{diet}$ and C_4 distribution from measured values within certain regions, differences in dietary preference among species and land-use history are also compatible with the observed patterns. In general, the isoscape model supports the importance of seasonally available grass resources to the heteromyid diet. However, in low-resource environments, such as the desert southwest, isotopic composition may additionally reflect variation in the degree of dietary selection across a species range. Results from this analysis demonstrate a potential strategy for distinguishing resource availability (influenced by climate and habitat characteristics) from resource use (influenced by ecological interactions and microhabitat selection). The long history of land-use, and in particular rangeland disturbance, across much of western North America is also relevant. As all sites are within or near publicly managed or protected lands (Bureau of Land Management and United States Forest Service), the indirect influence of grazing or direct influence of introduced grass species could potentially be a confounding factor at some localities and should be the subject of its own study in the future.

In order to characterize spatial variation in water resource use by heteromyids, we evaluated δ^2H and $\delta^{18}O$ for *D. ordii* only to control for any fractionation differences due to body size and physiology when ingested water and inspired air are assimilated into animal tissue (Fig. 4; Bryant and Froelich 1995, Kohn 1996). Warm and arid *D. ordii* localities tend to have higher rates of surface-water evaporation and plant transpiration, increasing the δ^2H and $\delta^{18}O$ values of water and plant matter that a small mammal may ingest (Helliker and Ehleringer 2002). LMWLs in arid ecosystems of New Mexico, Utah, Colorado and Texas document the effect of low relative humidity, with slopes ranging from 6.3 to 7.5 (Coplen and Kendall 2000). The low slope (4.71) of the linear regression line for δ^2H_{hair} and $\delta^{18}O_{hair}$ suggests that highly evaporated water sources, such as plant moisture stored in seeds, are utilized by *D. ordii* and recorded in their isotopic composition. From these results, in addition to significant positive correlations between both δ^2H and $\delta^{18}O$ in *D. ordii* and mean temperature during the rainy season, we infer that the hydrogen and oxygen isotopic composition of small mammals can track climatic gradients and water sources across the modern landscape. However, high variability in the isotopic composition of *D. ordii* and taxon-specific enrichment values limit their application to paleoenvironmental studies until more work is done to sample a greater variety of climatic conditions and improve our understanding of the unique physiology of these desert-adapted rodents (Podlesak et al. 2008, Gehler et al. 2012).

Conclusions

The carbon isotopic composition of two herbivorous rodents, *D. ordii* and *P. parvus*, is influenced by climate and vegetation gradients across western North America. Seasonal temperature and precipitation variables are the strongest correlates with $\delta^{13}C_{diet}$ in these species and can be used to predict variation in carbon isotopic composition and C_4

grass distributions according to heteromyid diet across the landscape. Significant negative correlations between $\delta^{15}N_{hair}$ and precipitation variables suggest a moisture control on the nitrogen isotopic composition of dietary resources and environment. The differences in $\delta^{13}C$ and $\delta^{15}N$ values between *D. ordii* and *P. parvus* imply differences in resource and possibly microhabitat use, with *D. ordii* consuming a higher proportion of C_4 grasses than co-occurring *P. parvus*. In general, the oxygen and hydrogen isotopic composition of *D. ordii* is positively correlated with temperature variables and negatively correlated with precipitation variables. In comparison to LMWLs, the slope of the relationship between $\delta^{18}O$ and $\delta^{2}H$ in *D. ordii* suggests these rodents are acquiring water from highly evaporated moisture sources, such as seeds.

This study assesses small-mammal isotopic composition over broad geographic and climatic gradients, and highlights the potential for small mammals, such as heteromyids, to record changes in vegetation and water sources across the landscape. The stable isotope ecology of small mammals can be used to assess dietary selection, local habitat heterogeneity, seasonal variation in resource availability, and environmental moisture conditions. Results from this study have several implications for studying the fossil record of small mammals, including quantifying differences in resource use among fossil species and fine-scale variation in vegetation that may not be captured by records from large mammals, preserved soil organic matter, and carbonates. However, due to high individual variation in isotopic composition, moderate sample sizes of small mammals ($n \geq 4$) are needed to accurately assess the average composition of dietary and water sources. Just as modern heteromyid rodents track spatial variation in resources and climate across the modern landscape, fossil heteromyids should also record changing resource use, vegetation composition, and climate through geologic time.

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Supplementary material (available online as Appendix oik-02722 at <www.oikosjournal.org/appendix/oik-02722>). Appendix 1–2.