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Evidence of cryptic individual specialization in an opportunistic insectivorous bat

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Habitat use and feeding behaviors of cryptic animals are often poorly understood. Analyses of stable isotope ratios in animal body tissues can help reveal an individual's location and resource use during tissue growth. We investigated variation in stable isotope ratios of 4 elements (H, C, N, and S) in the hair of a sedentary species of insectivorous bat (*Eptesicus fuscus*) inhabiting a chemically complex urban landscape. Our objective was to quantify population-level isotopic variation and test for evidence of resource specialization by individuals. Bats were sampled over 3 annual molt cycles at maternity roosts in buildings and variance components analysis was used to test whether intraindividual isotopic variation among molts differed from interindividual variation, after controlling for year and roost-group effects. Consistent with prior evidence that *E. fuscus* is opportunistic in its habitat use and foraging at the population level, we observed wide population-level variation for all isotopes. This variation likely reflects the chemical complexity of the urban landscape studied. However, isotopic variation among years within marked individuals was lower than variation among marked individuals within year for all isotopes, and carbon signatures indicated resource specialization by roost groups and individuals. This is the 1st study to examine variation in stable isotope ratios of individual wild bats over multiple years. Although our results suggest this population tends toward opportunistic habitat use or prey selection, or both, during molt periods, results also indicate that individuals and groups of bats composing the population might be habitat or dietary specialists—a novel finding for insectivorous bats.

Key words: carbon, Chiroptera, deuterium, diet, *Eptesicus fuscus*, habitat use, hydrogen, nitrogen, stable isotopes, sulfur

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Habitat use and feeding behaviors of cryptic animals are often poorly understood. This is particularly true for insectivorous bats, all of which are capable of moving at least several kilometers from their roosts during nocturnal foraging bouts. Because it is difficult to directly observe bats at night, most of what we know of their habitat use and feeding behaviors comes from indirect methods of observation. Such methods include acoustic monitoring of echolocation calls (Lacki et al. 2007; Parsons and Szewczak 2009), radiotracking (Amelon et al. 2009; Lacki et al. 2007), and visual or genetic analysis of feces or stomach contents (Clare et al. 2011; Whitaker et al. 2009). Major limitations of these techniques are that they either do not provide any information on behaviors of individuals (e.g., acoustic monitoring), or they only provide information on individuals over very short periods of time (Sullivan et al. 2006), such as hours in the case of fecal analysis or days (approximately 7–14 days) in the case of radiotracking. Furthermore, the latter methods for assessing individual behaviors are labor intensive and it is difficult to resample individuals, particularly if investigators are interested in characterizing variation in diet or habitat use over longer periods of time.

Most existing methods of studying habitat use and foraging by bats require generalizing data gathered over short periods from individuals to estimate population-level means and variances. Considering the potential for individual specialization, even in animal populations that show great variation in habitat use and feeding (Bolnick et al. 2002, 2003), it is

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important to move beyond simple summary statistics for populations and determine whether and how such variation might be biologically structured (i.e., nonrandom patterns of variance influenced by the biology of individual animals), such as if cryptic specialization by individuals occurs. Specialization by individuals may occur on the scale at which selection acts to influence evolutionary trajectories of a population, perhaps influencing fitness and population vital rates in variable environments (Bolnick et al. 2002, 2003). Although the ecological concepts of niche partitioning and competitive relationships structuring species assemblages have been previously explored in bats (e.g., Findley 1993), cryptic specialization in resource use has never been documented among individuals composing a population of insectivorous bats.

Stable isotope analysis offers an opportunity for studying the feeding behaviors of individual bats over longer periods of time than traditional methods allow (Martínez del Rio et al. 2009). The stable isotope composition of animal body tissues can help researchers infer resource assimilation during tissue growth, sometimes uncovering subtle ecological information such as predominant food types, habitats used, and individual-level foraging habits. Isotopic analysis has produced new information about habitat use and feeding ecology across a diverse group of mammals (see reviews by Crawford et al. [2008] and Kelly [2000]), including bats (e.g., Fleming et al. 1993; Siemers et al. 2011; Voigt and Kelm 2006; Voigt and Speakman 2007; York and Billings 2009). Stable isotopes offer a variety of biological markers with which to study the ways individual bats feed and move through landscapes.

We analyzed the stable isotope values in hair of big brown bats (*Eptesicus fuscus*) from 2 maternity roost groups in buildings within the city of Fort Collins, Colorado. With the exception of a predilection for beetles (e.g., Brigham 1990; Whitaker 1995), prior evidence indicates that *E. fuscus* is opportunistic in its habitat use and foraging at the population level (Duchamp et al. 2004; Kurta and Baker 1990; Sullivan et al. 2006). We predicted the spatially and isotopically heterogeneous urban landscape of Fort Collins would be a useful backdrop against which to study factors driving isotopic variation in *E. fuscus* and provide the ''isotopic resolution'' to detect fine-scale habitat use or prey selection by big brown bats living in the city. Bats were sampled over the course of 3 annual molt cycles, including a subset of marked individuals that were repeatedly sampled. Our objectives were to quantify variation in stable isotope values of δ^2H, $\delta^{13}C$, $\delta^{15}N$, and $\delta^{34}S$ at the population level, then to quantify intra- and interindividual variation among years to determine how individuals might contribute to population-level variation, and whether isotopes reveal evidence of cryptic individual specialization in habitat use or feeding behaviors, or both.

Materials and Methods

Study area.—We studied a population of bats inhabiting buildings in the city of Fort Collins, Larimer County, Colorado, during the summers of 2007 and 2008. Fort Collins encompasses approximately 140 km^2, has a human population of about 140,000, has an elevation of about 1,500 m, and is situated at the transition between the Great Plains and Rocky Mountains. The city and immediate surroundings comprise a rich mosaic of heterogeneous landscape features, which represent a chemically complex isotopic landscape, or isoscape (West et al. 2010). Prior research indicates that bats roosting in city buildings during summer are capable of potentially accessing much of the city and surrounding habitats each night while foraging (O'Shea et al. 2011). Potential contributing factors to isoscape complexity include approximately 2.4 km^2 of parks and 120 km^2 of natural areas dispersed among commercial and residential neighborhoods (City of Fort Collins, www.fcgov.com/visitor/fcfacts.php, accessed 1 November 2011); a long history of agriculture (McWilliams and McWilliams 1995) and active croplands scattered around the periphery of the city; importation of materials into the urbanizing landscape from around the globe; grasslands of the Great Plains to the east; a steep elevational gradient and coniferous forests at the western edge of the city; a river and creek that bisect the city and support riparian vegetation, a network of irrigation canals, natural and human-made ponds and wetlands, and a sewage-treatment facility; and a large reservoir (approximately 7.60 km^2) on the west margin of the city that stores water diverted from the western slope of the Rocky Mountains under the continental divide through the Colorado–Big Thompson Project. These features contribute to a wide range of spatial and temporal variation in landscape δ^2H (e.g., elevation and diverse seasonal water sources), $\delta^{13}C$ (e.g., C_3 [forbs] versus C_4 [grasses] plant communities and aquatic versus terrestrial plant communities), $\delta^{15}N$ (e.g., widespread and variable fertilizer use and sewage-treatment facility), and $\delta^{34}S$ (e.g., various geologies and aquatic habitats) across the city. Quantitative characterization of the isotopic composition of potential resources used by insectivorous bats in Fort Collins was beyond the scope of this project. Despite being impractical to characterize, such patchy isoscapes offer an opportunity to study potential specialization in prey and habitat use by nonmigratory insectivorous bats because their high mobility renders them capable of readily interacting with the full range of variation in the isoscape (O'Shea et al. 2011).

Study species.—The big brown bat is a small (11- to 23-g) insectivore that is not known to migrate long distances (>100 km) and hibernates during the winter (Kurta and Baker 1990; Neubaum et al. 2006). Females of this species have a propensity to form maternity colonies in anthropogenic structures and are one of the most common bats encountered in buildings across temperate zones of North America (Barbour and Davis 1969; Kurta and Baker 1990; Neubaum et al. 2007a). We worked with a well-studied population of big brown bats inhabiting buildings of Fort Collins. This population was the focus of previous, intensive ecological and demographic studies (e.g., see Ellison et al. 2007; George et al. 2011; Neubaum et al. 2006, 2007a, 2007b; O'Shea et al.

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2010, 2011). Colonies sampled were composed mostly of reproductive females that gather during spring and summer to communally birth and raise their young, although adult males were occasionally captured and sampled. Adult males are less common in the city during summer and tend to occur in greater proportions at higher elevations of the adjacent mountains (Neubaum et al. 2006; O'Shea et al. 2011).

Bat sampling.—More than 4,000 *E. fuscus* were individually marked by subdermal insertion of permanent passive integrated transponder tags (following Wimsatt et al. 2005) as part of earlier studies. We took advantage of the large number of bats still carrying passive integrated transponder tags and sampled hair of both marked and unmarked individuals from 2 discrete roosting groups at the north and south ends of the city. Previous monitoring of roost entrances for passive integrated transponder–tagged bats demonstrated high fidelity of females to these 2 groups, with no movement of individuals between groups (O'Shea et al. 2011; T. J. O'Shea, United States Geological Survey, pers. comm.). The northern roost group consisted of a colony that used a building and a park picnic pavilion situated approximately 400 m from each other, which were within 300–700 m of the Poudre River and approximately 7.5 km upstream from the sewage-treatment plant. In general, the northern roosting group was situated amidst what is essentially an urban forest of mature trees that is contiguous with the wetlands and riparian areas of the nearby Poudre River. The southern roost group used an old wooden house in an industrializing area of former agricultural use, and was situated more than 2 km from the Poudre River, approximately 4.5 km downstream from the sewage-treatment plant, and 9.25 km from the northern group. The landscape surrounding the southern roost was not forested like that of the northern roost, and bats would have had to travel farther than the northern group to access forests and riparian habitats.

Bats were captured in harp traps and mist nets as they exited their roosts during the evening (Kunz et al. 2009). Each individual was held in a separate clean cloth bag placed within a paper cup prior to processing and marked bats were identified by passing a handheld, passive integrated transponder–reading device (Power Tracker IV; Avid Inc., Norco, California) around each cup. We recorded sex, reproductive condition, and relative age (adult or young of year) using standard methods (Brunet-Rossinni and Wilkinson 2009; Racey 2009). Because bats were last tagged with passive integrated transponders in 2005, all marked individuals in this study were adults at the time of sampling. Small samples of hair (approximately 10 mg) were clipped with scissors from the midscapular region of the dorsal pelage, as close to the base of hair as possible without risk of cutting the skin. Hair samples were stored in clean 20-ml glass vials and all bats were released after sampling, typically within 1 h of capture. Like other species of temperate-zone insectivorous bats (Constantine 1957, 1958; Quay 1970), *E. fuscus* presumably completes a single molt each year during late summer (Phillips 1966). Based on observations of hair regrowth after sampling recaptured bats with passive integrated transponder tags, we estimated the molting period for reproductive females to occur during July with some individuals still molting into the 1st week of August (see ''Results''). We therefore defined a ''molt year'' as the period from the 2nd week of August to the following June. Although fieldwork was only conducted during the summers of 2007 and 2008, bats sampled in early summer of 2007 were presumably still in the prior year's pelage; thus, we were able to sample 3 annual molt cycles (2006–2008). Capture and sampling of bats followed guidelines of the American Society of Mammalogists (Sikes et al. 2011) and animal protocols were approved by the Institutional Animal Care and Use Committee of the United States Geological Survey Fort Collins Science Center *(Standard Operating Procedure 01-01 for the Capture, Handling, Marking, Tagging, Biopsy Sampling, and Collection of Bats).* Bats were captured under authority of a scientific collecting license issued by the Colorado Division of Wildlife (07TR738A3 and 08TR2010).

Sample preparation and isotopic analysis.—We analyzed stable hydrogen (δ^2H), carbon (δ^{13}C), nitrogen (δ^{15}N), and sulfur (δ^{34}S) isotope composition of hair for each individual sampled. Hair samples were cleaned using a 2:1 choloroform : methanol solution, air dried, and weighed into silver (δ^2H; approximately 0.5 mg) or tin (δ^{13}C, δ^{15}N, δ^{34}S; approximately 2.0 mg with 1.5 mg of V_2O_5 added to sulfur samples) capsules. Carbon, nitrogen, and sulfur isotope ratios were measured using an elemental analyzer (δ^{13}C and δ^{15}N—Carlo Erba NC 2500; CE Elantech Inc., Lakewood, New Jersey; and δ^{34}S—Costech ECS 4010; Costech Analytical Technologies Inc., Valencia, California) interfaced to an isotope ratio mass spectrometer operated in continuous-flow mode (δ^{13}C and δ^{15}N—Micromass Optima; Micromass United Kingdom Ltd., Manchester, United Kingdom; and δ^{34}S—Thermo-Finnigan Delta Plus XP; Thermo Scientific, Bremen, Germany; Fry et al. 1992; Giesemann et al. 1994). For δ^2H, samples were allowed to air equilibrate to ambient laboratory conditions for at least 2 weeks prior to analysis (Wassenaar and Hobson 2003). Following equilibration, samples were pyrolyzed at 1,425°C in a high-temperature elemental analyzer (Thermo-Finnigan TC/EA; Thermo Scientific, Bremen, Germany) interfaced to an isotope ratio mass spectrometer (Thermo-Finnigan Delta Plus XL; Thermo Scientific) operated in continuous-flow mode. Isotope ratios were reported in delta (δ) notation, expressed as parts per thousand (‰). Nonexchangeable δ^2H values were reported relative to Vienna Standard Mean Ocean Water (VSMOW) following normalization to calibrated keratin standards (Wassenaar and Hobson 2006); δ^{13}C and δ^{15}N were reported relative to Vienna Pee Dee Belemnite (VPDB) and air using primary isotopic standards (United States Geological Survey 40 and 41, δ^{13}C = −26.24‰ and 37.76‰, δ^{15}N = −4.52‰ and 47.57‰). δ^{34}S values are reported relative to Vienna Canyon Diablo Troilite (VCDT) following normalization with NBS127 and IAEA-SO6 (δ^{34}S = 21.1‰ and −34.05‰, respectively). Analytical error and sample precision were ±4‰ for δ^2H and ±0.2‰ for δ^{13}C, δ^{15}N, and δ^{34}S. To avoid potential systematic bias during analysis, sample order was randomized in all cases and quality control and assurance verified by repeated

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Table 1.—Mean (95% *CI*) values of stable isotope ratios of hydrogen (δ^2H), carbon (δ^{13}C), nitrogen (δ^{15}N), and sulfur (δ^{34}S) measured in the hair of big brown bats (*Eptesicus fuscus*) sampled from 2 maternity roost groups in buildings at Fort Collins, Colorado, by sex and age (male and female juveniles pooled). Bats were sampled over 3 annual molts and sample includes individuals captured multiple times.

Group	*n*	δ^2H	δ^{13}C	δ^{15}N	δ^{34}S
Adult females	189	−95 (−96 to −94)	−21.7 (−21.9 to −21.4)	11.6 (11.4 to 11.7)	−10.1 (−10.5 to −9.7)
Juveniles	65	−90 (−91 to −89)	−21.4 (−21.6 to −21.1)	12.2 (12.1 to 12.4)	−9.2 (−9.9 to −8.4)
Adult males	9	−79 (−85 to −73)	−20.0 (−20.9 to −20.4)	10.4 (9.8 to 11.0)	−3.6 (−4.7 to −2.5)

analyses of an in-house keratin standard, as well as primary standards analyzed as unknowns.

Data analysis.—To quantify the variance components of isotopic values, we fit a linear mixed model independently for each isotope with molt year and roost group as fixed effects, and individual bat as a random effect. We limited this analysis to marked bats that had been sampled over at least 2 molts and for which we had data on all 4 isotopes; all were adult females. We fit the mixed model using restricted maximum likelihood (REML) as implemented by the function lme in the nlme package (version 3.1-97) in R statistical software version 2.8.0 (R Development Core Team 2010). We report the proportion of variance attributed to each of the random effects (among and within individual bats) after fitting the mixed effects model using the function varcomp in the package ape (version 2.7-2). Code is available from the authors. Statistical significance was set at $P \leq 0.05$.

Results

Confirmation of molt and population-level isotopic variation.—We sampled big brown bats on a total of 27 nights from June through August of 2007 and 2008. Evidence of prior hair clipping was not apparent in most individually marked bats sampled before August each year and then subsequently recaptured ($n = 61$). Molt progressed quickly during July, but in 13 individuals we observed hair still growing into previously sampled areas during the 1st week of August, whereas 19 others sampled during the latter period had already molted. Of 172 captures of unmarked bats during August, only 11 (6%) showed evidence of prior sampling, all within the 1st week of the month and none thereafter. In contrast, during June and July, 24% of 242 unmarked bats captured at roosts showed signs of previous sampling. Individually marked bats sampled in summer and then captured again prior to August ($n = 34$) showed no evidence of hair regrowth, nor did bats sampled during mid- to late August and then recaptured in June and early July of the following year ($n = 6$). These observations support our assumptions that *E. fuscus* molts once annually, mostly in July, and that hair sampling does not seem to induce the regrowth of hair before the molt period. We analyzed a total of 263 hair samples for all 4 isotopes from both unmarked and marked bats; this sample included repeat captures of marked individuals and possible recaptures of unmarked bats. The range of isotope values (means, ranges) was broad across all bats analyzed, including δ^2H ($\bar{X} = -93‰$, range = −121‰ to −70‰), δ^{13}C ($\bar{X} = -21.6‰$, range −28.9‰ to −18.9‰), δ^{15}N ($\bar{X} = 11.7‰$, range 8.9‰ to 14.7‰), δ^{34}S ($\bar{X} = -9.7‰$, range −17.6‰ to −1.8‰). For all isotopes measured, 95% confidence intervals (95% *CI*s) of sample means for adult females and males did not overlap, and 95% *CI*s of δ^2H and δ^{15}N did not overlap between adult females and juveniles (Table 1). Because of these sex and age differences, sample-size issues, and known differences in the thermoregulatory strategies and ecologies of male and female temperate-zone bats during summer (Grinevitch et al. 1995; Weller et al. 2009), adult males and juveniles were excluded from further analysis. We chose to focus our analysis on reproductive females in order to limit the potential influence of individual physiological state on the isotopic composition of hair. Unlike male bats that generally tend to roost alone and use torpor more sporadically (reviewed by Weller et al. 2009), reproductive female *E. fuscus* in buildings of Fort Collins are exposed to the same general microclimates within roosts during the day, most adults reproduce, and births tend to be synchronous within the population (George et al. 2011; O'Shea et al. 2010, 2011). When all isotopic data from adult females ($n = 189$) were plotted against each other, certain outliers from the different roost groups trended into different areas of ''δ-space'' (Fig. 1).

Variance components analysis of recaptured bats.—A total of 49 marked adult females were recaptured and sampled over 2 ($n = 39$) or 3 ($n = 10$) molts. Fifteen of these 49 bats were associated with the southern roost group and the remaining 34 were associated with the northern roost group. Model residuals were normally distributed and homoscedastic, as assumed by the mixed linear model we fit to the data. After adjusting the population isotope means for the fixed effects of year and roost group, most of the remaining variance in all 4 isotopes was attributed to differences among individual bats. Variance among individuals was nearly twice as much for δ^2H than within individuals (Table 2). The mean values for δ^2H in the 2 roost groups were not different (Welch 2-sample *t*-test; $t_{89} = 0.775$, $P = 0.44$). Nearly 85% of the variation in δ^{13}C was accounted for by differences among individuals; this was almost 6 times as great as the amount of variation that could be attributed to differences within individuals. The mean value of δ^{13}C for the northern group was lower than that for the southern group ($t_{103} = -6.843$, $P < 0.0001$). Nearly three-fourths of the total variation in δ^{15}N could be described by differences among individuals, an amount that was about 3 times as large as that for the within-individual level. The mean of δ^{15}N for the northern group was lower than that for the southern group ($t_{41} = -3.368$, $P = 0.002$). δ^{34}S was similar to

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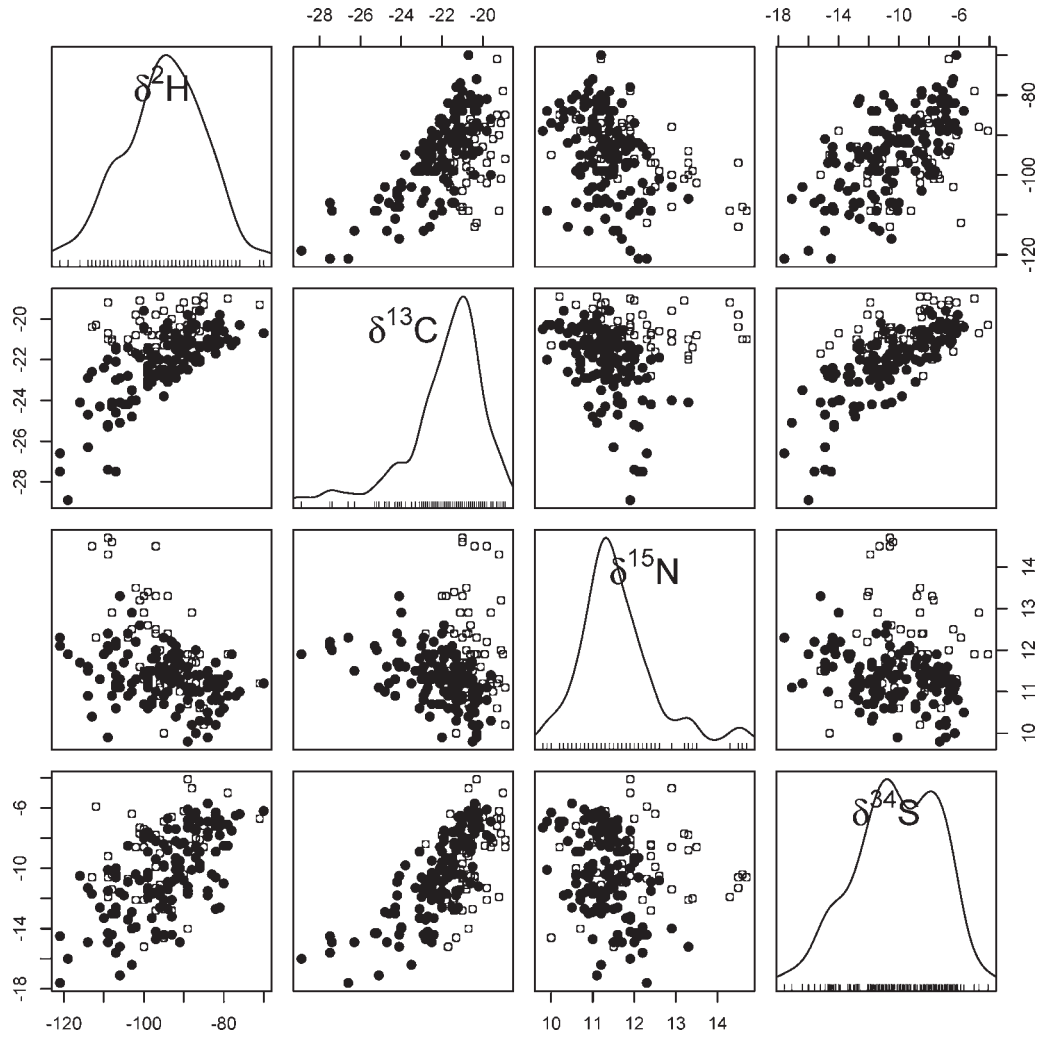


Fig. 1.—Stable isotope ratios of hydrogen (δ^2H), carbon (δ^{13}C), nitrogen (δ^{15}N), and sulfur (δ^{34}S) in hair of reproductive female big brown bats (*Eptesicus fuscus*) roosting in buildings of Fort Collins, Colorado, sampled over 3 molts (2006–2008; $n = 189$ samples). Bats were sampled from roosting groups on north (closed dots) and south (open circles) ends of the city. Density histograms for single isotopes appear on the diagonal and hash marks indicate distribution of data along a line scaled for each isotope. Off-diagonal panels show bivariate plots for each pair of isotopes and axis labels follow from the diagonal labels.

δ^2H; variance among individuals accounted for just over twice as much variance as did that within individual bats (Table 2). The mean δ^{34}S for the northern roost group was lower than that for the southern roost group ($t_{83} = -2.233$, $P = 0.03$).

Discussion

Consistent with the assumption that *E. fuscus* is opportunistic in habitat use and foraging (Brigham 1990; Duchamp et al. 2004; Kurta and Baker 1990; Sullivan et al. 2006), we observed wide population-level variation for all isotopes. We did not characterize bat physiology or the isotopic composition of the landscape and potential prey, all of which can influence isotopic variation in animal tissues (Bearhop et al. 2004; Flaherty and Ben-David 2010; Newsome et al. 2007). The wide variation observed across isotopes in hair could have been attributable to differential prey selection by individuals, spatial and temporal differences in the isotopic composition of insects fed upon or habitats used by bats, assimilation differences among individual bats, or some combination of those factors. The ranges of isotopic variation observed across the population during the molt period (July) were equivalent to ranges quantified in other systems as indicating tissue growth across several degrees of latitude (δ^2H [e.g., Britzke et al. 2009; Cryan et al. 2004]), assimilation of both C_3 and C_4 plant signatures (δ^{13}C [e.g., Sullivan et al. 2006]), as well as feeding at multiple tropic levels (δ^{15}N [e.g., Siemers et al. 2011; Voigt and Kelm 2006]) and among divergent geologies and distances from marine environments (δ^{34}S [Zazzo et al. 2011]). This potentially inflated variation likely reflects the isotopic complexity of the urbanized landscape studied, including unique geologic features and the mobility and opportunistic insectivorous feeding of *E. fuscus*.

Big brown bats are known to feed on a variety of insects in other regions—mostly coleopterans, hemipterans, and homopterans (Sullivan et al. 2006; Whitaker 1995). Fecal analysis of

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Table 2.—Results of variance components analysis for stable isotope ratios of hydrogen (δ^2H), carbon (δ^{13}C), nitrogen (δ^{15}N), and sulfur (δ^{34}S) in the hair of reproductive adult female big brown bats (*Eptesicus fuscus*; $n = 49$) sampled from roosts in Fort Collins, Colorado. A linear mixed model was fit using molt year and roost group as fixed effects, and individual bat as a random effect. We limited the variance analysis to marked adult females that had been sampled over at least 2 molts and for which we had data on all 4 isotopes ($n = 49$ individual bats; $n = 107$ individual hair samples). Cell values are the percentages of variance that can be attributed to differences among individual bats (the random effect of individual) and within individual (residual variance).

	δ^2H	δ^{13}C	δ^{15}N	δ^{34}S
Among individuals (%)	66	84	73	72
Within individual (%)	34	16	27	28

guano from *E. fuscus* sampled beneath roosts in Fort Collins over 3 summers (2002–2004) revealed a local diet of coleopterans, hymenopterans, hemipterans, dipterans, and lepidopterans during the molt period; considerable annual variation in overall diet composition was observed among years (E. Valdez, United States Geological Survey, pers. comm.). With so many potential prey items that are ephemeral in availability and occurring across what is likely an isotopically patchy environment, it would be extremely difficult to accurately measure prey isotope compositions and relate them in a meaningful way (e.g., mixing models) to isotopic values of bat tissues. Further, we lack an understanding of the isoscape inhabited by *E. fuscus* in Fort Collins. However, isotopic signatures among groups and individual bats within the population can be useful for identifying underlying broadscale patterns of spatial habitat use and feeding.

Differences in the isotopic composition of hair were apparent between adult male and female big brown bats. Isotopic differences between adult males and females are not surprising because temperate zone insectivorous bats often exhibit remarkable sex differences in use of habitats, roosts, and thermoregulatory strategies during the summer months (Weller et al. 2009). Sex differences in behaviors of *E. fuscus* are common during summer, with males more often roosting alone, occupying higher-elevation habitats in mountainous regions, and using daytime torpor more frequently than females (Hamilton and Barclay 1994; Neubaum et al. 2006). Capture sampling of bats throughout Fort Collins during summer revealed a predominance of females in the city (Neubaum et al. 2006; O'Shea et al. 2011). Our isotope results indicate that male *E. fuscus* captured in the city might consistently forage on different prey resources or in different areas than reproductive females. Alternatively, sex-specific energy allocation strategies (e.g., growing hair with stored versus recently acquired nutrients), potential sex differences in molt timing (Cryan et al. 2004; Quay 1970), or systematic biases in assimilation associated with frequent torpor use also could lead to the isotopic differences observed between sexes. Although sample size for males was low, their δ^2H values were more positive than those of reproductive females, contrary to the expected pattern if males sampled in Fort Collins had been regularly foraging at higher elevations of the adjacent mountains. However, small numbers of adult males regularly occur in maternity colonies in Fort Collins (O'Shea et al. 2011) and likely remain at low elevations during summer.

Much remains to be learned about the influence of torpor on isotopic discrimination in body tissues (Siemers et al. 2011). Several hypotheses have been proposed for explaining causes of sex differences in the summer distributions of bats, ranging from divergent needs of males and reproductive females to competitive exclusion of males from female areas (Weller et al. 2009). Our results suggest that stable isotope analysis might be a useful way of studying the processes that influence sex differences in seasonal ecologies of bats. For example, isotopes could be used to determine if adult males and reproductive females exploit the same food resources while co-occurring in certain habitats.

Volant juvenile bats showed wide isotopic variation and their δ^2H and δ^{15}N values tended to be higher than reproductive females. This suggests that, like adults, they presumably forage across a broad range of prey types or habitats, or both, and, as indicated by δ^{15}N values, may derive some of their nutrients for molting into winter pelage from their mothers, as is known to occur in other mammals (Dalerum et al. 2007). Because of the potential ontogenetic and sex differences in behavior and physiology that could influence isotope values of body tissues, we limited our analysis of intra- and interindividual isotopic variation to a demographic group in which behavioral, physiological, and environmental differences were likely minimized—reproductive females.

Several characteristics of reproductive female big brown bats make them good candidates for studying isotopic variation among and within individuals in a population. First, large numbers of individuals show high fidelity to the same daytime roosts, forage from the same starting points each night, and are thus equally likely to have access to the same habitats and food resources. Second, bats in maternity colonies generally experience the same microclimate conditions, which are typically warm and promote consistent euthermia (torpor less likely). Third, most adult females breed and the timing of pregnancy and birth in big brown bats in Fort Collins tends to be fairly synchronous (George et al. 2011; O'Shea et al. 2010, 2011). Prior sampling revealed median parturition dates varying from 10 to 16 June over a 4-year period, with nearly all births occurring within a 30-day period (T. J. O'Shea, pers. comm.), meaning that most females are likely in a similar energetic and physiological condition during the molt period. For these reasons, it is reasonable to infer that observed variance in isotopic signatures of individual reproductive females is less likely attributable to physiological differences than differences in habitat use or feeding. However, we do not have sufficient data on the timing of parturition in the individuals we sampled to assess whether there was a relationship between isotopic variance and birth timing. Future investigations into the relationship between birth

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timing and isotopic composition of molted hair could help us better understand the influence of individual physiology on isotope incorporation.

Considering only the individually marked reproductive females, variance components analysis provided evidence of biological structure in the population. For interpreting the results from the variance components analysis, we refer to any differences accounted for at the among-roost level as attributed to fine-scale spatial patterns, because the roost groups were spatially distinct at the local level, but not at the regional level. We refer to differences that are accounted for at the within-roost (among individual) level as fine-scale population patterns, because varying habits of individual bats that compose the population generate these differences. Finally, we define differences at the within-bat level as fine-scale spatial and population patterns because these differences are generated by consistent spatial and foraging habits of individual bats.

Isotopic variation within marked individuals was lower than variation among individuals for all isotopes, and $\delta^{13}C$ values were especially indicative of resource specialization by roost groups and individuals—a novel finding for this opportunistic species. Differences between roost groups were responsible for proportionally higher variance in $\delta^{13}C$ than were differences within bats, indicating that fine-scale spatial differences in the foraging habits of northern and southern roost groups might exist (e.g., predominately terrestrial versus aquatic feeders). Evidence for differences among roosts in other isotopes was weaker, although the larger sample that included unmarked bats indicated outliers from each roost group trended into different areas of δ-space (Fig. 1). Within each roost group, patterns of variance in $\delta^{13}C$ within individual bats indicate that they also may have used only a small portion of the available foraging habitat or food resource from year to year. Because year was fit as a fixed effect in our model, the variance components that we examined related to effects that were considered after adjusting for general year-to-year variation in mean isotope values. Therefore, individual dietary preference is a more likely explanation than specialized habitat use for the observed low variance of $\delta^{13}C$ within individuals. Relatively higher within-individual variance components for $\delta^{2}H$, $\delta^{15}N$, and $\delta^{34}S$ may imply that these elements are influenced more strongly by landscape level factors (variable water sources, fertilizer supplements, and insect life history) that can vary more systematically from year to year than does $\delta^{13}C$.

Wide isotopic variation in hair at the population level suggests that the population of reproductive female *E. fuscus* we studied tends toward opportunistic habitat use or prey selection, or both, during the molting period. However, examination of isotope data from recaptured bats indicated that individuals and groups of bats composing the population might be habitat or dietary specialists. Radiotracking studies of *E. fuscus* in central Indiana documented foraging in agricultural land, wooded areas, and urban zones, with little evidence of high fidelity to particular foraging sites (Duchamp et al. 2004). In Fort Collins, 16 reproductive female big brown bats tracked during the summer of 2004 tended to forage in natural areas along the creek and river, but showed little evidence of fidelity to particular foraging sites (O'Shea et al. 2011). However, the variance components analysis is interpreted from a relative perspective. Therefore, although radiotelemetry does not suggest fidelity to foraging sites, individual bats may nonetheless forage in a much narrower isotopic range (e.g., prefer specific feeding areas or types of prey) than is available to them across the larger landscape around the city of Fort Collins. Individuals exhibiting a wide range of specialization may compose the opportunistic population of big brown bats in Fort Collins. As discussed above, it is difficult to uncover subtle patterns of long-term habitat use and feeding behaviors of individual bats with traditional methods like radiotracking or fecal analysis, which are limited in temporal and spatial resolution. Although our stable isotope results only offer a glimpse into the potential and relative feeding and habitat-use dynamics of reproductive females during the molt period, the patterns we observed are intriguing and may shed light on how selection acts upon bat populations that appear to be very opportunistic in foraging and habitat use.

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