Macro- and Microgeographic Variation in Metabolism and Hormone Correlates in Big Brown Bats (*Eptesicus fuscus*)

Christopher S. Richardson1,*
Tim Heeren2
Eric P. Widmaier1
Thomas H. Kunz1
1Department of Biology, Boston University, Boston, Massachusetts 02215; 2School of Public Health, Boston University, Boston, Massachusetts 02118

Accepted 5/19/2009; Electronically Published 9/16/2009

**ABSTRACT**

To better understand intraspecific variation in basal metabolic rate (BMR), we examined environmental, physiological, and/or cellular bases for residual variation in BMR in big brown bats, *Eptesicus fuscus*. We measured BMR and plasma levels of thyroid hormone (T3) and leptin in bats captured in maternity colonies in eastern Massachusetts (MA; northern population) and in Alabama and Georgia (ALGA; southern population) to assess macrogeographic (between- or among-population) and microgeographic (within-population) variation in those traits. After accounting for effects of body mass, stage of pregnancy, and within-population variation, bats from the northern population did not differ significantly in BMR, T3, or leptin values from those in the southern population. However, after accounting for the effects of body mass and stage of pregnancy, a test for differences in all traits among colonies from both populations was significant. For BMR, bats differed significantly among the northern colonies. Moreover, after removing the effects of body mass and stage of pregnancy, bats from the AL colony had significantly higher BMR than did bats from all other colonies except one in MA, and they had significantly higher T3 levels but lower leptin levels than did bats from two other colonies. The presence of among-colony and within-population variation for these traits suggests that proximate (nonevolutionary) factors (e.g., microhabitat differences such as roost type) play an important role in shaping intraspecific variation in BMR and its hormone correlates.

**Introduction**

A primary focus of ecological and evolutionary physiology has been the study of metabolism and thermoregulation, and small mammals have been common subjects (Jansky 1973; Grodzinski and Wunder 1975; Lynch 1986, 1994; Richardson et al. 1994). Allocation of energy to maintenance metabolism, growth and reproduction, and thermoregulation has been of particular interest (Thompson 1992; Wunder 1992). Rates of energy expenditure in organisms are believed to be critical in the evolution of other physiological and behavioral traits (Tomasi and Horton 1992; Lovegrove 2000; Speakman 2000; McNab 2002; Speakman and Thomas 2003). Basal metabolic rate (BMR), the rate of metabolism in resting, postabsorptive individuals at thermoneutrality, has been one of the most commonly measured energetic variables for comparative studies. This trait provides a standardized estimate of energy expenditure (Bartholomew 1982; McNab 1992, 2002).

Life-history traits, which include the size and number of offspring produced and how often an animal reproduces, determine how energy is expended during reproduction (Thompson 1992; Barclay and Herder 2003; Kunz and Orrell 2004). Reproduction entails a number of energetically costly activities such as courtship, mating, securing nest sites, and nourishing developing embryos (Bronson 1989; Thompson 1992). In addition to the direct energetic costs of reproduction, females may require an increased BMR (and body temperature) to facilitate optimal development of offspring or to increase the rate of milk production (Thompson 1992; Kunz and Hood 2000; Kunz and Orrell 2004). The greatest energy costs associated with reproduction in mammals occur during late pregnancy and lactation (Thompson 1992). During pregnancy, metabolic rate increases as embryonic mass increases in eutherian mammals, with the greatest energy cost being the maintenance of a gravid uterus (Kunz and Orrell 2004).

The most important factors influencing interspecific variation in mammalian BMR are not clear (McNab 1992; Lovegrove 2000; Speakman 2000; Speakman and Thomas 2003; Speakman et al. 2004; Cruz-Neto and Jones 2006). While most of the variation in BMR is associated with body mass, other factors also appear to be important (McNab 1992; Lovegrove 2000; Speakman et al. 2004; Johnstone et al. 2005). After removing the effect of body mass on BMR in mammals, in general, and in bats, in particular, considerable unexplained residual variation in BMR remains (McNab 1992; Speakman and Thomas 2003; Speakman et al. 2004; Johnstone et al. 2005). Phylogeny, diet, climate, organ morphology, hormones, life history, reproductive condition, and latitude are all known to affect BMR (McNab 1986, 1992; Speakman 2000; Speakman and Thomas 2003; Speakman et al. 2004; Cruz-Neto and Jones 2006).
In particular, we examined intraspecific variation in BMR and its hormone correlates in free-ranging big brown bats (Eptesicus fuscus). Our study was designed to assess what degree macrogeographic (between- or among-population) and microgeographic (within-population) variation contribute to intraspecific variation. This is important for understanding the extent to which proximate (non-evolutionary) factors (e.g., microhabitat differences such as roost type) shape intraspecific variation in BMR and its hormone correlates.

A complex array of subcellular, cellular, and hormonal factors affects metabolic rate (Denckla and Marcum 1973; Hulbert 1978, 1987, 2000; Barthalmolomew 1982; Hulbert and Else 2000). In particular, the thyroid hormones, triiodothyronine (T3) and thyroxine (T4), are important, as they can elevate metabolic rate within 12 h (Hulbert 1978; Silva 1995). The primary hormone secreted from the thyroid gland is T4, although it must be deiodinated to T3 to become metabolically functional (Gross et al. 1992).

Leptin is also associated with reproduction, with levels of plasma leptin increasing during pregnancy in all mammals, including bats, that have been studied to date (Henson and Castracane 2000; Kronfeld-Schor et al. 2001). Leptin resistance during pregnancy allows levels of maternal serum leptin to rise (Henson and Castracane 2006) while maintaining energy consumption to match increasing maternal metabolic demands (Kunz and Orrell 2004). Like thyroid hormones, leptin also affects energy expenditure and storage. However, the interactions (or lack thereof) between leptin and thyroid hormones and between leptin and BMR are not well understood (Johnstone et al. 2005; Considine 2006). Laboratory-bred, hypothyroid rats (Rattus norvegicus) provided with an energy-rich diet exhibited no increase in serum leptin levels (Curtio et al. 1999), whereas euthyroid rats provided with a similar energy-rich diet but given T4 injections experienced a 50% decrease in plasma leptin levels (Masaki et al. 1997).

FMW and the relationships between levels of plasma thyroid hormones and stages of pregnancy are not well understood. Previous studies on bats have not found a consistent association between plasma thyroid hormone levels and stage of pregnancy (Crichton and Krutzsch 2000).

BMR is variable among mammals (especially bats; McNab 1983; Speakman 2000; Speakman and Thomas 2003; Cruz-Neto and Jones 2006), which suggests that phylogeny is important. However, BMR also is highly variable within species (Speakman et al. 2004). Many studies have reported population (or macrogeographic) differences, including genetically based differences in metabolic traits (see Garland and Adolph 1991). Macrogeographic variation in BMR has been reported in mole rats (Haim et al. 1984). MacMillen and Garland (1989) found significant among-population variation in BMR in Peromyscus after analyzing a multispecies sample of 31 populations (based on literature values). Scheck (1982) reported genetically based population differences for BMR in the cotton rat (Sigmodon hispidus). Speakman (2000) and Speakman and Thomas (2003) reported positive correlations between BMR and latitude in small mammals. McNab and Bonaccorso (2001) found population differences in two small (<35 g) species of Pteropus, but these differences were due to altitudinal and not latitudinal differences. Milam-Dunbar (2009) found latitudinal population differences for torpid metabolic rate in migratory and nonmigratory bat species. In addition to population differences for metabolic traits, Hulbert et al. (1985) reported population differences for plasma thyroxine levels for several species of California rodents. Population differences within species have been reported for leptin as well, but only in humans (although not consistently) and domestic animals (Berg et al. 2003; Garte 2003). How often population differences in metabolism and hormone correlates are due to evolutionary adaptation to different environments is not clear (Garland and Adolph 1991).

Although macrogeographic differences have been reported for metabolism, T3, and leptin, few studies have examined intraspecific variation at the microgeographic scale (e.g., differences among subpopulation groups such as colonies). Interindividual variation or differences among individuals within a
population have been reported for BMR and leptin in humans (*Homo sapiens*) and for resting metabolic rate in laboratory mice (*Mus musculus*; Speakman et al. 2004; Johnstone et al. 2005). Interindividual variation can be used to determine the mechanisms underlying differences in performance or physiology (Bennett 1987). Phenotypic correlations between two traits (e.g., BMR and $T_3$) suggest that they may be functionally linked. While interindividual variation within a population exists for physiological traits (Bennett 1987), the prevalence of subpopulation group differences is not clear.

In general, bats are ideal models for investigating factors related to variation in BMR (Speakman and Thomas 2003; Cruz-Neto and Jones 2006). Bats are among the most diverse groups of mammals. Many species experience a broad range of climatic conditions and have evolved a variety of physiological and morphological traits (Cruz-Neto and Jones 2006). In particular, temperate-zone microchiropterans such as *E. fuscius* are excellent model species for investigating BMR and its hormonal correlates because they are heterothermic. Bats are facultative endotherms that are capable of physiological temperature regulation, but they do not consistently maintain an elevated body temperature (Bartholomew 1982; Willis et al. 2005). Heterothermic bats experience highly variable patterns of energy expenditure depending on whether torpor occurs in different reproductive stages and environmental conditions (Thompson 1992; Crichton and Krutzsch 2000; Willis et al. 2005). Additionally, during reproduction, when energy demand increases, temperate-zone bats are particularly sensitive to factors that influence metabolic rate, such as food availability and ambient temperature. When considering the effect of flight on metabolic rate, particularly in individuals that are pregnant, the range of physiological and metabolic changes a temperate-zone bat experiences is extraordinary among mammals (Lyman 1970; Thompson 1992). This makes temperate-zone microchiropterans ideal subjects for studying BMR and related physiological variables (Thompson 1992; Crichton and Krutzsch 2000; Willis et al. 2005). The big brown bat is an especially valuable species for studying the effects of microhabitat differences on metabolic rate and hormone correlates because they inhabit a wide range of roost types, including barns, trees, and rock crevices, and they exist in a variety of ecosystems throughout North America (Barbour and Davis 1969).

In Massachusetts (MA), individual *E. fuscius* arouse from hibernation in April and enter hibernation in October (Kurta and Baker 1990; T. H. Kunz, personal observation), whereas in Alabama (AL) and Georgia (GA), individuals arouse from hibernation in February and enter hibernation in late November (Mendonça et al. 1996; M. T. Mendonça, personal communication). In eastern North America, *E. fuscius* individuals give birth to two offspring following a 2-mo gestation period (Kurta and Baker 1990). Onset of parturition in southern populations occurs from mid- to late May, and bats in the most northern population start giving birth from mid- to late June. Females are easier to capture during warm months because they typically form maternity colonies (Kunz and Reynolds 2003), whereas males tend to be solitary and are difficult to find.

BMR increases following cold exposure (Feist and Rosenmann 1976; Hayes 1989; Wunder 1992; Speakman et al. 2004). In cold climates and after cold exposure, small mammals increase BMR, in part to maintain the increased mass of organs (e.g., heart, intestines, liver) required to sustain the increased energy demands that are due to cold stress (Wunder 1992; Speakman 2000; Kröl et al. 2003). In addition, because the growing season gets shorter and colder at higher latitudes, offspring in northern parts of their ranges have less time to grow and, thus, mothers need to invest more energy (i.e., they need a higher maternal resting metabolic rate) earlier in fetal development (Fujita 1986; Bronson 1989; Thompson 1992).

We tested the hypothesis that, in *E. fuscius*, macrogeographic variation in BMR would be greater than microgeographic variation. Because northern populations of big brown bats in North America experience colder temperatures than do southern populations, and because BMR increases in response to cold exposure, we predicted that bats from a northern population would have greater BMRs than would bats from a southern population. While the relationship between BMR, $T_3$, and leptin is complex, the general paradigm is that metabolism is positively related to $T_3$ and leptin activity. Thus, we predicted that plasma $T_3$ levels and plasma leptin levels should be higher in bats from a northern population than in bats from a southern population. Additionally, we tested the hypothesis that intraspecific variation in BMR would be correlated with intraspecific variation in plasma $T_3$ and plasma leptin levels.

**Material and Methods**

**Experimental Design and Sampling Procedures**

We captured female bats from each of seven maternity colonies in eastern MA from April 30 to June 10, 1997, and from April 29 to June 5, 1998, and we captured bats from a colony in AL and a colony in GA from March 25 to April 22, 1998 (Table 1). The AL and GA colonies are considered to be part of one population (ALGA), because they come from a similar geographic area and are located only 295 km apart. Both are located >1,770 km from the eastern MA colonies, which are all located within 80 km of one another.

We were limited to studying only two colonies for the southern population because of difficulty in finding accessible, large colonies for repeated sampling. We designed our study to ensure that we sampled approximately the same number of bats from each colony and at each stage of pregnancy. However, in practice, variability among colonies in capture success affected how many bats from each stage could actually be measured. Thus, we experienced some imbalance in each stage of pregnancy across all colonies. In particular, we were unable to capture any stage-2 pregnant bats from the AL colony or stage-1 pregnant bats from the Wilberham (MA) colony. Otherwise, the three stages of pregnancy were sampled for each colony. We assessed, by palpation, the stage of pregnancy (using size of fetus as a relative measure): none (fetus not detectable; 0),
Eptesicus fuscus hours (on the day of capture). Metabolic rate was measured for 5–6 h beginning at 1200–1300

Measurements of BMR (3–4 per group) in a simulated natural roost until metabolic

imize stress during transport, bats were housed in small groups

inside the can. Control runs, performed periodically, demon-

strated that the wood never changed the oxygen concentration of the chamber air. Perforated (to ensure adequate mixing of air inside the chamber) inlet and outlet tubes were inserted into the lid and sealed with silicone sealant. The tubes entered the top of the cavity and extended its full length to ensure adequate air flow within the wooden chamber. Bats were placed into the wooden chamber, and a piece of 6-mm hardware cloth screen mesh was placed over the opening of the chamber to contain bats during measurements. The lid was sealed airtight with silicone lubricant.

The lower critical temperature of big brown bats from eastern MA is 30°C (Stack 1985), but our preliminary analysis found that 30°C was within the thermoneutral zone for individuals from ALGA (i.e., no difference existed in resting metabolic rate between 30°C and 32°C). Six bats, each placed in a separate chamber within a different can, plus an empty control (baseline) airline, were sequentially sampled for oxygen concentration for 5 min every 35 min. Oxygen concentration was measured at 30°C in a positive-pressure, open-circuit respirometry system using calibrated upstream flowmeters (Brooks Sho-Rate; flow rate at 225–260 mL min

Metabolic rate was measured for 5–6 h beginning at 1200–1300 hours (on the day of capture). Eptesicus fuscus is generally post-absorptive by 1200 hours, and activity levels are minimal between 1300 and 1700 hours (Stack 1985). The chambers for measuring metabolic rates were designed to simulate natural roost conditions (Kunz and Kurta 1988). Each chamber was carved from a wooden beam and mounted on the inside of the lid of a 4-L paint can (with the interior of the can painted flat black). The wood cavity or simulated roost faced downward inside the can. Control runs, performed periodically, demon-

Hormone Analysis

We did not collect blood at the time of capture in order to minimize stress before BMR measurements. After measuring BMR, bats were immediately removed from simulated roosts

Table 1: Study sites in eastern Massachusetts (MA), Alabama (AL), and Georgia (GA)

<table>
<thead>
<tr>
<th>Colony</th>
<th>Location (Latitude, Longitude)</th>
<th>Year</th>
<th>Temperature (°F)*</th>
<th>Size b</th>
</tr>
</thead>
<tbody>
<tr>
<td>Byrd</td>
<td>Needham, MA (42.27°N, 71.27°W)</td>
<td>1997–1998</td>
<td>53, −3, 25</td>
<td>35</td>
</tr>
<tr>
<td>Sherborn Town Hall</td>
<td>Sherborn, MA (42.24°N, 71.38°W)</td>
<td>1997–1998</td>
<td>53, −3, 25</td>
<td>49</td>
</tr>
<tr>
<td>Coblyn</td>
<td>Sherborn, MA (42.22°N, 71.39°W)</td>
<td>1998</td>
<td>53, −3, 25</td>
<td>15–20</td>
</tr>
<tr>
<td>Fruitlands</td>
<td>Harvard, MA (42.50°N, 71.61°W)</td>
<td>1997</td>
<td>49, −3, 23</td>
<td>69</td>
</tr>
<tr>
<td>Wilson</td>
<td>Harvard, MA (42.50°N, 71.59°W)</td>
<td>1998</td>
<td>49, −3, 23</td>
<td>40–60</td>
</tr>
<tr>
<td>Graves</td>
<td>Wilberham, MA (42.15°N, 72.40°W)</td>
<td>1997</td>
<td>52, −5, 24</td>
<td>26</td>
</tr>
<tr>
<td>Talbot Courthouse</td>
<td>Talbotton, GA (32.67°N, 84.54°W)</td>
<td>1998</td>
<td>72, 16, 44</td>
<td>80–100</td>
</tr>
<tr>
<td>Bridge on Highway 106</td>
<td>Georgiana, AL (31.64°N, 86.74°W)</td>
<td>1998</td>
<td>73, 15, 44</td>
<td>50–70</td>
</tr>
</tbody>
</table>

* Maximum, minimum, and average temperatures in January 1997. Closest weather station is within 32 km of each colony.

b Colony size was estimated by flight count as bats first departed from roosts to forage at dusk or by visual census of roosting bats.

early (fetus diameter is ∼8 mm; 1), or middle (fetus diameter is ∼15 mm; 2; Racey 1988).

Females from the AL colony roosted in an expansion joint of a concrete bridge, whereas bats in all of the other colonies roosted in the attics of houses or in barns. To assess the thermal conditions of the bridge roost relative to those of the more typical building roosts, we measured roost temperature using Hobo temperature data loggers (Onset Computers) placed in the rafters of the attic of the GA colony and inside the expansion joint of the bridge of the AL colony. Temperature was recorded every 8 min for 45 d in March and April (during gestation). The temperature for each day is reported as the mean of the 8-min temperature values recorded each day. The variability for each day is represented by the standard error of all 8-min values recorded each day.

We measured BMR and assessed plasma T, and plasma leptin levels on a total of 167 adult female big brown bats at Boston University (Boston, MA) and Auburn University (Auburn, AL; Institutional Animal Care and Use Committee protocol 05-012). Up to six bats per day were captured using a mist net, a hoop net, or a gloved hand during their second foraging bout or shortly after they returned (typically, between 0000 and 0200 hours). Our sampling protocol was designed to minimize the probability that bats would abandon the site. Bats were transported to the laboratory (∼30–90 min after capture). To minimize stress during transport, bats were housed in small groups (3–4 per group) in a simulated natural roost until metabolic measurements were taken (Kunz and Kurta 1988).

Measurements of BMR

We did not collect blood at the time of capture in order to minimize stress before BMR measurements. After measuring BMR, bats were immediately removed from simulated roosts.
and 20–30 μL of blood was drawn from the caudal vein (in the interfemoral membrane or uropatagium). Blood was collected without anesthesia in heparinized microhematocrit capillary tubes that were sealed with citoose (see Kunz and Nagy 1988). Blood was then centrifuged for 5 min to separate plasma from formed elements. Plasma was immediately frozen at −20°C to prevent degradation of the hormone. We measured leptin concentrations in the plasma. Body mass was recorded immediately after blood was collected, and then all bats were given water ad lib and returned to the holding device. The following day, 75–100 μL of blood was collected, as previously described, to measure plasma T₃ levels (up to 150 μL of blood can be taken safely from each adult E. fuscus individual). After the second blood sample was collected, all bats were returned to the holding device and then released at the site of capture between 1900 and 2000 hours.

Leptin concentrations in plasma were quantified using a commercial radioimmunoassay kit (Linco, HL-81K) for human leptin. Heparin does not affect plasma leptin concentrations and does not interfere with leptin assays (Ma et al. 1996). In our experience, chiropteran peptide hormones, including leptin, exhibit the highest cross-reactivity with antibodies generated against homologous human peptides relative to peptides of other species (Kronfeld-Schor et al. 2000). Authentic human leptin and leptinlike immunoreactivity in bat plasma were diluted in parallel in the human leptin radioimmunoassay, and recovery of exogenous human leptin in bat serum was ~100% (Kronfeld-Schor et al. 2000). T₃ concentrations in plasma were quantified using a commercial radioimmunoassay kit (MP Biomedical, 06855158-R14). In preliminary tests, samples from bats were tested for parallelism with the T₃ standard curve, and we found that 30–40 μL of plasma was sufficient to detect plasma T₃ levels. The plasma concentrations of leptin and thyroid hormone reflect the balance between synthesis/secretion and metabolic clearance at any given time. Point measurements of plasma hormone levels by radioimmunoassay do not distinguish between these factors, and thus, changes in plasma concentration of hormone may reflect changes in secretion or metabolic clearance or both.

Statistical Analyses

Differences for each trait were examined between the northern (eastern MA) and southern (ALGA) populations (see Table 1). For each trait, we used a nested-design model to test for differences between populations. The primary grouping factor was population, a fixed effect. Colony was a random effect nested within population. If the population term was not significant, and if colony nested within population was significant, then we tested for differences among all colonies but without the population term in the model.

Before performing the nested-design model to test for population differences, we assessed each trait to determine the colony structure within each population. First, we tested for differences between years for two MA colonies. In general, reasons for differences between years are similar to reasons for differences between sites (e.g., difference in ambient temperature). For the sake of simplicity of comparisons and interpretation, and because we have not conducted a full experiment to separate year from location effects, if years differed within a colony then we treated each year as a different colony (otherwise, we pooled data for both years, treating the combined sample as one colony).

Next, we tested for differences between colonies from two MA towns. Where no significant differences between colonies within the same town were found, we pooled data to create one overall colony for the town for all subsequent analyses. To avoid confounding effects, data from the Cronin colony were pooled with those from the Coblyn colony (see Table 1) because (1) the two sites were <1.6 km apart (and thus they experienced similar local ambient environmental conditions), (2) the barns were structurally similar, and (3) only a few bats were captured in the Coblyn barn. Finally, we tested for differences among all colonies within each population.

We used random-effect, multiple-regression models to test for population differences, which accounted for colony differences within populations (i.e., nested-design model). Multiple-regression models with population and colony coded with one or more 0–1 dummy variables and appropriate covariates were used to compare populations, colonies, and years for all traits; this analysis is similar to ANCOVA, but it accounts for colony as a random effect.

For all regression models, the main covariates were body mass and stage of pregnancy (0, 1, or 2). Because body mass was positively associated with stage of pregnancy (R² = 0.45, P < 0.001, $R^2 = 0.40$), both covariates were included in the models. For all outcome variables or traits, model results with body mass and stage of pregnancy were similar to the results achieved using only body mass or stage of pregnancy, except for in two comparisons (also, results from the models with either BMR or log-transformed BMR on log-transformed body mass did not qualitatively differ from non-log-transformed models). For the two comparisons (BMR among colonies within the northern population and leptin between colonies within the southern population), significant interaction exists between colony and pregnancy stage and between colony and body mass. Multiple interactions involving the same variable can lead to problems of collinearity and misleading results (Kleinbaum et al. 1988). Thus, for those two comparisons, we report results for the model with body mass only and the model with stage of pregnancy only. Unless stated otherwise, for all other comparisons, we report only the results from models with both covariates (stage of pregnancy and body mass) that have significant $F$ scores and $P$ values for year and within-population colony terms. $F$ scores and $P$ values, regardless of significance, are reported for population terms and colony-nested-within-population terms. Additionally, we tested for interaction between year, colony, or population terms and covariates.

On the basis of our analyses (see "Results"), the AL colony appeared to be different from all other colonies for all traits. We performed exploratory post hoc, pairwise comparisons with Bonferroni adjustment between the AL colony and each MA
colony to assess the nature of the differences. For purposes of comparing the AL colony with the other colonies, adjusted means controlling for both body mass and stage of pregnancy were computed using an ANCOVA model without the population term. Because only bats in pregnancy stages 0 and 1 were captured at the AL colony, we did not include stage 2 in the post hoc comparisons.

For BMR, we judged three data points to be outliers because they were higher than the rest for their stage of pregnancy (42.4 mL O₂ h⁻¹ [Byrd Colony, Needham, MA; 1997; stage 2 of pregnancy; 20.10 g]; 46.3 mL O₂ h⁻¹ [Talbottown Colony, GA; stage 1 of pregnancy; 13.8 g]; 36.7 mL O₂ h⁻¹ [Wilson Colony, Harvard, MA; stage 2 of pregnancy; 14.7 g]; Table 2; see Table 1 for colony location). The outliers were not minimal values because the three bats never settled down in the metabolic chambers. For all comparisons, removing the outliers generally did not change the results (we only report results from models without the outliers).

Plasma T₃ levels were significantly related to the assay batch. We ran models adjusting for differences between T₃, assay batches. However, for all comparisons, our results using models with T₃, assay batch as a covariate were qualitatively similar to models without T₃, assay batch (we only report models without T₃, assay batch).

We performed a partial correlation to test the linear association between BMR and T₃, or leptin and between T₃ and leptin that controlled for the effect of colony differences. However, this does not treat colony as a random effect but as a fixed effect. We also accounted for the effects of body mass and stage of pregnancy when performing the partial correlation.

All statistical analyses were performed using SAS, version 6.12 (SAS Institute, Cary, NC). Independent variables (i.e., group dummy variables and covariates) were judged to be significant at P < 0.05. All post hoc, pairwise comparisons were judged to be significant at P < 0.002 for BMR, P < 0.003 for T₃, and P < 0.005 for leptin (for a single comparison, the Bonferroni-adjusted P values are 0.002 [= 0.05/28] for 28 comparisons, 0.003 [= 0.05/15] for 15 comparisons, and 0.005 [= 0.05/10] for 10 comparisons, respectively).

## Results

### Roost Temperature

The daily temperature inside the AL roost ranged from 12.86°C to 31.36°C, with an average of 23.3°C. The daily variability in temperature inside the AL roost ranged from 0.08°C to 0.82°C, with an average of 0.53°C. The daily temperature inside the GA roost ranged from 12.02°C to 27.42°C, with an average of 22.3°C. The daily variability in temperature inside the GA roost ranged from 0.06°C to 0.61°C, with an average of 0.29°C.

### BMR

For the Byrd colony (Needham, MA), BMR was significantly greater in 1997 than it was in 1998 (F₁,₅₀ = 9.85, P = 0.006; Table 2). For Sherborn, MA, BMR in the Cronin/Coblyn colony was significantly greater than it was in the Sherborn Town Hall colony (F₁,₃₀ = 5.05, P = 0.032; Table 2). In comparisons among all northern colonies, each year of the Byrd Colony and both Sherborn colonies (Cronin/Coblyn and Sherborn Town Hall) were treated separately, but the Harvard colonies (Fruitlands and Wilson) were pooled.

Within the northern population, in the comparison of all MA colonies, the colony and interaction (colony × covariate) terms were significant (stage of pregnancy–only model: colony: F₁,₄₂ = 2.40, P = 0.044; interaction: F₁,₄₂ = 2.51, P = 0.017; body mass–only model: colony: F₁,₄₂ = 3.35, P = 0.008; interaction: F₁,₄₂ = 3.36, P = 0.008). Significant interactions indicate that colonies within MA may have differed significantly, but the differences among colonies changed depending on the covariate value(s) (Tables 2, 3). For the southern population, the AL colony had significantly greater BMR values than did the GA colony (F₁,₄₃ = 14.36, P < 0.001; Table 2; Fig. 1).

We found no significant population differences in BMR.

<table>
<thead>
<tr>
<th>Colony</th>
<th>Stage of Pregnancy</th>
<th>N</th>
<th>BMR</th>
<th>N</th>
<th>BMR</th>
<th>N</th>
<th>BMR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Byrd 1997</td>
<td>0</td>
<td>6</td>
<td>10.75 ± .72</td>
<td>4</td>
<td>20.48 ± 4.60</td>
<td>4</td>
<td>31.18 ± 2.67</td>
</tr>
<tr>
<td>Byrd 1998</td>
<td>1</td>
<td>...</td>
<td>...</td>
<td>4</td>
<td>12.46 ± .89</td>
<td>3</td>
<td>23.27 ± 3.52</td>
</tr>
<tr>
<td>Sherborn Town Hall</td>
<td>2</td>
<td>14</td>
<td>14.93 ± 1.61</td>
<td>8</td>
<td>15.28 ± 1.94</td>
<td>3</td>
<td>24.48 ± 1.76</td>
</tr>
<tr>
<td>Cronin/Coblyn</td>
<td>3</td>
<td>3</td>
<td>20.98 ± 2.49</td>
<td>4</td>
<td>19.37 ± 3.00</td>
<td>3</td>
<td>27.38 ± 1.15</td>
</tr>
<tr>
<td>Fruitlands/Wilson</td>
<td>4</td>
<td>8</td>
<td>15.59 ± 2.53</td>
<td>18</td>
<td>20.96 ± .88</td>
<td>4</td>
<td>21.94 ± 1.73</td>
</tr>
<tr>
<td>Graves</td>
<td>5</td>
<td>6</td>
<td>12.61 ± 1.40</td>
<td>...</td>
<td>...</td>
<td>6</td>
<td>23.93 ± 1.00</td>
</tr>
<tr>
<td>Talbot Courthouse</td>
<td>6</td>
<td>9</td>
<td>15.90 ± 1.24</td>
<td>6</td>
<td>18.13 ± 1.08</td>
<td>5</td>
<td>22.19 ± .70</td>
</tr>
<tr>
<td>Bridge on Highway</td>
<td>7</td>
<td>17</td>
<td>24.13 ± 1.72</td>
<td>16</td>
<td>26.33 ± .65</td>
<td>...</td>
<td>...</td>
</tr>
</tbody>
</table>

* Three outliers were excluded (see text).

* Talbot Courthouse is in Georgia; Bridge on Highway 106 is in Alabama.
Intraspecific Variation in Metabolism in Big Brown Bats

Table 3: Mean ± SE body mass (g)* of female *Eptesicus fuscus* from colonies in Massachusetts, Georgia, and Alabama

<table>
<thead>
<tr>
<th>Colony</th>
<th>Stage of Pregnancy</th>
<th>0</th>
<th>Body Mass (g)</th>
<th>1</th>
<th>Body Mass (g)</th>
<th>2</th>
<th>Body Mass (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Byrd 1997</td>
<td></td>
<td>6</td>
<td>13.21 ± .51</td>
<td>4</td>
<td>16.12 ± .43</td>
<td>4</td>
<td>18.84 ± .46</td>
</tr>
<tr>
<td>Byrd 1998</td>
<td></td>
<td>...</td>
<td>...</td>
<td>4</td>
<td>14.97 ± .49</td>
<td>3</td>
<td>19.55 ± .87</td>
</tr>
<tr>
<td>Sherborn Town Hall</td>
<td></td>
<td>14</td>
<td>14.37 ± .27</td>
<td>8</td>
<td>15.65 ± .57</td>
<td>3</td>
<td>17.58 ± .53</td>
</tr>
<tr>
<td>Cronin/Coblyn</td>
<td></td>
<td>3</td>
<td>14.87 ± .84</td>
<td>4</td>
<td>14.95 ± .67</td>
<td>3</td>
<td>20.25 ± .67</td>
</tr>
<tr>
<td>Fruitlands/Wilson</td>
<td></td>
<td>8</td>
<td>14.71 ± .61</td>
<td>18</td>
<td>16.43 ± .29</td>
<td>4</td>
<td>19.29 ± .37</td>
</tr>
<tr>
<td>Graves</td>
<td></td>
<td>6</td>
<td>15.16 ± .57</td>
<td>...</td>
<td>...</td>
<td>6</td>
<td>17.21 ± .51</td>
</tr>
<tr>
<td>Talbot Courthouseb</td>
<td></td>
<td>9</td>
<td>14.84 ± .39</td>
<td>6</td>
<td>14.87 ± .32</td>
<td>5</td>
<td>16.92 ± .26</td>
</tr>
<tr>
<td>Bridge on Highway 106b</td>
<td></td>
<td>17</td>
<td>15.91 ± .41</td>
<td>16</td>
<td>17.44 ± .38</td>
<td>...</td>
<td>...</td>
</tr>
</tbody>
</table>

* Body mass was recorded after basal metabolic rate (BMR). Three BMR outliers were excluded.

b Talbot Courthouse is in Georgia; Bridge on Highway 106 is in Alabama.

Figure 1. Basal metabolic rate (ml O₂ h⁻¹) versus (A) body mass and (B) stage of pregnancy (values are means ± SE) for female *Eptesicus fuscus* from colonies in Alabama/Georgia (ALGA) and Massachusetts (MA). Open circles = bridge on Highway 106, Georgiana, AL (solid line); filled circles = Talbotton, GA (dashed line); filled triangles = MA (dotted line).

However, there were significant colony differences nested within populations (F₁,160 = 4.48, P < 0.001). Additionally, our test for differences among colonies (without population term) was significant (F₁,160 = 6.79, P < 0.001; Table 2). Thus, most of the variation in BMR among colonies is not explained by differences between populations (Fig. 1).

Post hoc, pairwise comparisons of the AL colony with each MA colony showed that, after controlling for body mass and stage of pregnancy, bats from AL had significantly higher BMR than did bats from each MA colony except one (Table 4; Fig. 1). Additionally, post hoc, pairwise comparisons of GA with each MA colony showed that GA bats did not differ significantly from MA ones.

**Hormones: T₃ and Leptin**

No significant differences were found in T₃ and leptin levels between years within a colony or between colonies within a town (we pooled data from the two Sherborn colonies and the two Harvard colonies to create one colony for each town). Within MA, we found no significant differences among colonies for T₃ or leptin.

In the southern population, bats from AL had significantly higher plasma T₃ levels than did those from GA (F₁,40 = 3.94, P = 0.054; Table 5; Fig. 2; also, P values from each model with one covariate were <0.02). In the southern population, in the comparison for plasma leptin levels between AL and GA, the colony and interaction (colony × covariate) terms were significant (stage of pregnancy–only model: colony; F₁,41 = 6.33, P = 0.016; interaction: F₁,41 = 6.63, P = 0.014; body mass–only model: colony: F₁,42 = 3.88, P = 0.056; interaction: F₁,42 = 5.75, P = 0.021). Colonies in AL and GA initially had similar leptin levels as functions of body mass and pregnancy stage, but, as body mass and stage of pregnancy increased, leptin levels diverged between the two colonies, increasing in GA while changing minimally in AL (Table 5; Fig. 3). Thus, over the range of interest for each covariate (i.e., stage of pregnancy = 0–2, and body mass = 10–20 g), leptin levels in bats from the AL and GA colonies diverged significantly.

There were no significant population differences for plasma T₃ or plasma leptin levels (T₃: F₁,4 = 4.02, P = 0.115; leptin: F₁,4 = 0.59, P = 0.485). However, we found significant colony
Among colonies (\textit{, } ). Post hoc, pairwise accounting for body mass, no significant differences were found and 1 of pregnancy. For bats at stage 0 of pregnancy, after we performed separate pairwise analyses for bats at stages 0 and 1, we found no population differences for BMR, \( T_3 \), or leptin. For BMR and \( T_3 \), results are for bats of pregnancy stages 0 and 1, while for leptin, results are for bats of pregnancy stage 1 only. \( \text{NA} \) = not available.

4). Finally, post hoc, pairwise comparisons of the GA colony versus the MA colonies found no significant differences. Differences between AL and other colonies cannot be explained by differences between populations. Thus, most of the variation among colonies cannot be explained by differences between populations.

### Correlations between BMR, \( T_3 \), and Leptin

After controlling for colony differences and accounting for body mass and stage of pregnancy, BMR was not significantly correlated with \( T_3 \) or leptin among individual bats. Also, \( T_3 \) was not significantly correlated with leptin after controlling for the same covariates.

### Discussion

After accounting for effects of body mass and stage of pregnancy, we found no population differences for BMR, \( T_3 \), or leptin, even though we found considerable intercolony variability within populations. Thus, most of the variation among colonies cannot be explained by population differences.

Macrogeographic or population differences for metabolic traits have been reported for several species of small mammals (see “Introduction”). However, the lack of macrogeographic differences in BMR that we observed is consistent with the results of Speakman and Thomas (2003). They concluded that BMR was not correlated with latitude among bat species and suggested that, while ambient temperature clearly varies with latitude, roost temperature does not. Typical temperate-zone bat species roosts, such as caves or barns, buffer local climatic conditions. Thus, roosts tend to moderate latitudinal differences in ambient temperature, minimizing a bat’s cold exposure.

---

**Table 4: Least square–adjusted colony means ± SE corresponding to ANCOVA models comparing all colonies of female \textit{Eptesicus fuscus}**

<table>
<thead>
<tr>
<th>Colony</th>
<th>BMR</th>
<th>( T_3 )</th>
<th>Leptin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Byrd 1997</td>
<td>16.59 ± 1.51*</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Byrd 1998</td>
<td>12.98 ± 2.41*</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Sherborn Town Hall</td>
<td>16.29 ± 1.02*</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Cronin/Coblyn</td>
<td>21.00 ± 1.78</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Sherborn\textsuperscript{b}</td>
<td>NA</td>
<td>46.96 ± 3.42**</td>
<td>64.40 ± 15.74**</td>
</tr>
<tr>
<td>Fruitlands/Wilson</td>
<td>18.58 ± 0.93*</td>
<td>36.74 ± 3.76*</td>
<td>84.09 ± 11.26*</td>
</tr>
<tr>
<td>Graves</td>
<td>13.61 ± 1.96*</td>
<td>43.59 ± 7.53</td>
<td>...</td>
</tr>
<tr>
<td>Talbot Courthouse</td>
<td>17.96 ± 1.22*</td>
<td>48.34 ± 4.09\textsuperscript{c}</td>
<td>80.48 ± 18.17*</td>
</tr>
<tr>
<td>Bridge on Highway 106</td>
<td>23.42 ± .89</td>
<td>57.41 ± 3.56</td>
<td>7.58 ± 11.84</td>
</tr>
</tbody>
</table>

Note. The adjusted colony means ± SE are from ANCOVAs (without population term in the model), with adjustments made to the grand mean values of body mass and stage of pregnancy for basal metabolic rate (BMR) and thyroid hormone \( T_3 \), and of body mass only for leptin. For BMR and \( T_3 \), results are for bats of pregnancy stages 0 and 1, while for leptin, results are for bats of pregnancy stage 1 only. \( \text{NA} \) = not available.

\textsuperscript{a} Three outliers were excluded (see text).
\textsuperscript{b} Sherborn is composed of pooled data from the Cronin/Coblyn colony and the Sherborn Town Hall colony.
\textsuperscript{c} Suggestive difference from Alabama colony bats (\( P < 0.05 \)) for model with stage of pregnancy only for bats of pregnancy stages 0 and 1 (see “Results” for comparison of the Alabama colony with the Georgia colony for all bats).

\textsuperscript{d} Statistically significant difference from Bridge on Highway 106 (Alabama) colony bats for respective trait (after Bonferroni adjustment: \( P < 0.002 \) for BMR, \( P < 0.003 \) for \( T_3 \), and \( P < 0.005 \) for leptin).

\textsuperscript{e} Suggestive difference from Alabama colony bats for respective trait (\( P < 0.05 \)).

---
(although bats do experience short-term cold exposure when foraging).

One colony was notable for being different from all the others. After removing the effects of body mass and stage of pregnancy, the AL colony had generally higher BMR, higher plasma $T_3$, and lower plasma leptin levels than did the other colonies (Table 3). Additionally, for reasons unknown, AL bats tended to be heavier than bats from other sites (Table 3). The relationship between BMR, leptin, and thyroid hormone is usually positive. Increasing adiposity normally leads to increased levels of circulating leptin, which signals (via the brain) to increase thyroid hormone and energy expenditure, thereby decreasing adiposity and body mass (Maffei et al. 1995; Considine et al. 1996; Johnstone et al. 2005). This, in turn, has the effect of reducing adiposity, which then results in a decrease in circulating levels of leptin. Thus, a higher BMR would be predicted to be associated with higher leptin and thyroid hormone levels, and leaner animals would be expected to have lower leptin levels. Our results suggest that, at least in AL big brown bats, these relationships do not hold in all circumstances. However, because we do not have lean-mass measurements for these bats, it is unknown whether the greater mass of the AL bats reflects increased adiposity or a greater skeletal muscle mass. This is important, because the relationship between adiposity, circulating leptin and thyroid hormones, and BMR is further influenced by changes in target-cell sensitivity to leptin that are known to occur in mammals as they increase or decrease body fat mass. AL bats may have hypothalamus neuronal cells that have higher levels of leptin receptors than do bats from the other colonies (Enriori et al. 2006). These hypothalamus cells may be more sensitive to circulating leptin in AL bats than those in the bats from other colonies. Thus, AL bats may need lower plasma leptin levels to maintain higher levels of thyroid hormone and greater metabolic rates than do bats from other colonies.

All colonies (except AL) roosted in enclosed wooden structures (e.g., in attics of houses or in barns), a roost type that is commonly used by temperate-zone bats (Kunz and Reynolds 2003). The AL colony was unique because its roost was an expansion joint in a precast concrete bridge. During March and April, while the average ambient temperatures were similar for the AL and GA colonies, the daily variation in roost temperature was different. The average daily temperature and average daily variability in temperature (see “Material and Methods”) were 23.3°C and 0.53°C, respectively, for the AL roost, and 22.3°C and 0.29°C, respectively, for the GA roost. Bridges do not appear to moderate temperatures like enclosed wooden structures do. Differences in thermal characteristics between the attic roost and the concrete bridge roost suggest that macrogeographic or even microhabitat differences are a more important influence on variability in BMR than are macrogeographic differences in ambient temperatures (i.e., it is generally colder in the north than the south).

In addition to the variability observed between the AL colony and all other colonies, BMR varied between years for one colony and among MA colonies, but not as markedly. However, $T_3$

---

Table 5: Plasma thyroid hormone $T_3$ (ng dL$^{-1}$) and plasma leptin (ng mL$^{-1}$) levels of female *Eptesicus fuscus* from colonies in Massachusetts, Georgia, and Alabama

<table>
<thead>
<tr>
<th>Colony, Parameter</th>
<th>Stage of Pregnancy</th>
<th>0</th>
<th>Mean ± SE</th>
<th>1</th>
<th>Mean ± SE</th>
<th>2</th>
<th>Mean ± SE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>N</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Byrd:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plasma $T_3$</td>
<td></td>
<td>5</td>
<td>32.8 ± 6.3</td>
<td>7</td>
<td>38.7 ± 7.1</td>
<td>6</td>
<td>36.1 ± 3.9</td>
</tr>
<tr>
<td>Plasma leptin</td>
<td></td>
<td>2</td>
<td>8.0 ± .7</td>
<td>4</td>
<td>65.9 ± 18.8</td>
<td>7</td>
<td>140.9 ± 15.7</td>
</tr>
<tr>
<td>Sherborn:*</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plasma $T_3$</td>
<td></td>
<td>12</td>
<td>38.2 ± 4.4</td>
<td>11</td>
<td>54.6 ± 7.0</td>
<td>5</td>
<td>43.0 ± 10.8</td>
</tr>
<tr>
<td>Plasma leptin</td>
<td></td>
<td>9</td>
<td>15.0 ± 3.9</td>
<td>7</td>
<td>57.0 ± 10.4</td>
<td>5</td>
<td>134.3 ± 27.9</td>
</tr>
<tr>
<td>Fruitlands/Wilson:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plasma $T_3$</td>
<td></td>
<td>7</td>
<td>35.7 ± 5.3</td>
<td>12</td>
<td>39.0 ± 5.2</td>
<td>3</td>
<td>25.9 ± 9.1</td>
</tr>
<tr>
<td>Plasma leptin</td>
<td></td>
<td>5</td>
<td>10.4 ± 1.9</td>
<td>13</td>
<td>83.0 ± 15.0</td>
<td>3</td>
<td>158.6 ± 37.7</td>
</tr>
<tr>
<td>Graves:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plasma $T_3$</td>
<td></td>
<td>5</td>
<td>40.8 ± 7.1</td>
<td>...</td>
<td>...</td>
<td>4</td>
<td>40.8 ± 9.9</td>
</tr>
<tr>
<td>Plasma leptin</td>
<td></td>
<td>1</td>
<td>24.0</td>
<td>...</td>
<td>...</td>
<td>3</td>
<td>76.2 ± 19.8</td>
</tr>
<tr>
<td>Talbot Courthouse:*b</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plasma $T_3$</td>
<td></td>
<td>9</td>
<td>48.5 ± 4.5</td>
<td>7</td>
<td>45.9 ± 6.4</td>
<td>5</td>
<td>38.3 ± 3.9</td>
</tr>
<tr>
<td>Plasma leptin</td>
<td></td>
<td>7</td>
<td>9.7 ± 1.4</td>
<td>6</td>
<td>65.2 ± 23.7</td>
<td>5</td>
<td>125.4 ± 11.5</td>
</tr>
<tr>
<td>Bridge on Highway 106:*b</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plasma $T_3$</td>
<td></td>
<td>16</td>
<td>53.4 ± 3.5</td>
<td>8</td>
<td>67.0 ± 4.2</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>Plasma leptin</td>
<td></td>
<td>13</td>
<td>10.3 ± .6</td>
<td>15</td>
<td>18.9 ± 7.0</td>
<td>...</td>
<td>...</td>
</tr>
</tbody>
</table>

*a* Sherborn is the pooled data from the Cronin/Coblyn colony and the Sherborn Town Hall colony.

*b* Talbot Courthouse is in Georgia; Bridge on Highway 106 is in Alabama.
and leptin only varied between the AL colony and all other colonies. The difference between BMR and the two hormones in the nature of among-colony variability suggests that (1) different proximate mechanisms may be operating on how T₃ and/or leptin affect BMR, (2) T₃ and leptin may be affected by different environmental factors than is BMR, and/or (3) BMR is different from T₃ and leptin in how it responds over time to environmental factors. At least proximately, T₃ and leptin appear to be linked with BMR at the microgeographic level. These are the first data for microgeographic or within-population variation for T₃ and leptin in bats.

After adjusting for colony differences and removing the effects of body mass and stage of pregnancy in big brown bats, the lack of any association in BMR, T₃, or leptin among individual bats was consistent with the findings of a study of humans (*Homo sapiens*). Johnstone et al. (2005) found that, after removing the effects of fat mass and fat-free (lean) mass, BMR was not associated with plasma leptin or plasma T₃ levels among individual humans within a single population. The difficulty in determining clear relationships among BMR, leptin, and T₃ is due to the confounding effects of body mass and fat mass on BMR and hormone levels. The positive association disappears when the shared variation in metabolism and hormones (due to body mass) is removed.

As expected, BMR increased with stage of pregnancy in big brown bats from all colonies and from both populations. Individuals of species with higher BMRs tend to have higher maternal metabolic rates and higher rates of biosynthesis during reproduction (Thompson 1992). At higher latitudes, during the spring and summer, periods of low temperatures and/or low food availability force pregnant bats to enter torpor, which delays embryonic development and extends gestation (Crichton and Krutzsch 2000). The lack of population differences in BMR in our study suggests that big brown bats from higher latitudes do not always require greater metabolic rates during pregnancy to compensate for the torpor-induced delay in embryonic development in colder climates. However, the differences in BMR between years (Table 2) suggest that environmental factors such as temperature may influence metabolic rate during reproduction. Levels of plasma T₃ in bats from the MA and GA colonies decreased with stage of pregnancy, which is typical of hibernating bat species (Crichton and Krutzsch 2000). However, T₃ levels increased in AL bats during pregnancy, which is typical of nonhibernating bat species (Crichton and Krutzsch 2000).
Our results suggest that the role of $T_3$ in reproduction may vary within bat species as well as among bat species. Levels of plasma leptin in bats from all colonies except the one in AL increased with stage of pregnancy. We found that bats from AL had an unexpectedly slow increase in leptin levels during pregnancy (Fig. 3), even as metabolic rate increased. This slow increase suggests that variation in placental leptin production does exist within a single species. AL bats may not experience hyperleptinemia with the associated leptin resistance during pregnancy (Enriori et al. 2006; Henson and Castracane 2006). It is also possible that this result reflects in part the constraints of using a qualitative assessment of pregnancy stage in this study (although our assessment method was consistently used on all bats from all colonies). Future research should examine the extent that intraspecific variation in reproduction, such as pregnancy, is linked to microgeographic variation in thyroid hormone and leptin function.

Our ability to generalize to other southern colonies is limited somewhat by the number of colonies sampled in the southern population. If we had included additional colonies from the southern population, we may have observed lesser or greater variability among colonies than is represented in our data. Future research should focus on sampling more southern colonies from enclosed wooden structures. This may help determine whether the AL bridge colony or the GA enclosed wooden structure colony is representative of colonies in this region.

The lack of macrogeographic differences in BMR and hormone correlates that we observed raises the question of how important macrogeographic variation is to intraspecific variation in small mammals, particularly bats. Macrogeographic variation in metabolic and thermoregulatory traits may reflect evolutionary adaptations to different thermal environments (Garland and Adolph 1991; Garland and Carter 1994). However, macrogeographic variation can also be the result of environmental effects, not just genetic differences (Garland and Adolph 1991). Because differences in selective pressures (e.g., differences in thermal environments) are not as great at the microgeographic level as compared with the macrogeographic level (see Table 1), microgeographic variation will tend to reflect variability in immediate environmental conditions (i.e., proximate factors). Additionally, within MA, differences in BMR among colonies changed as pregnancy developed (see findings on the interaction between colony and stage of pregnancy in “Results”), indicating that immediate environmental variation at the microgeographic level is an important influence. The high plasticity of metabolic traits in response to seasonal change (Wunder 1992; Speakman 2000) and the fact that physiological traits generally respond to a variety of environmental conditions (e.g., acclimation and acclimatization) suggest that much of the intraspecific variation (whether macro- or microgeographic variation) in metabolic traits is not genetically based (Garland and Carter 1994). Strong prior selection for metabolic traits may have occurred in small mammals, possibly including bats, which means that little additive genetic variance is left (i.e., little response to current selection pressures; Lynch 1994).

As a part of understanding the influences on intraspecific variation in metabolic traits, the question of resolution is important. Future studies on intraspecific variation in metabolic traits in mammals, including hormone activity, should assess to what degree macrogeographic and microgeographic variation each contribute to intraspecific variation. Our study was limited in that only two populations were examined and covered just one portion of the range of *Eptesicus fuscus*. For example, future work could compare populations of *E. fuscus* in the northwestern United States (e.g., Oregon) with populations of *E. fuscus* in the southwestern United States (e.g., southern California or Arizona). One environmental difference between these regions is that the northwest tends to be much wetter and has more moderate temperatures than the southwest, which tends to be drier and much hotter than anywhere else in the continental United States. Roosts (particularly enclosed wooden structures) in the southwest may not moderate the effects of high temperatures and low precipitation levels as well. The combination of higher temperatures and much drier conditions in the southwest compared with the northwest could be significant enough to require that bats and other small mammals in the southwest lower their metabolic rates to conserve water loss and minimize heat production during the hottest and driest months of the year (Schmidt-Nielsen 1997). Thus, differences in roost type or microhabitat differences in that case may matter less. Nonetheless, if microgeographic variation in metabolic traits is determined to be fairly extensive, then a major source of intraspecific variation would be due to environmental factors acting immediately. Future research should also examine the effect of roost type on variation in metabolism and hormone correlates in bats.

In conclusion, much of the among-colony, within-population variation in BMR, $T_3$, and leptin may be explained by environmental factors (acting recently and/or immediately). Clearly, a major source of intraspecific variation occurs at the microhabitat or colony level. In particular, microhabitat differences (e.g., roost type or diet quality) may be an important proximate (nonevolutionary) variable explaining why variation exists in physiological traits such as BMR, $T_3$, and leptin. Moreover, variability in $T_3$ and leptin may only affect variation in BMR when variability for BMR within populations (among colonies) is greatest (i.e., a coarse-grain effect).

**Acknowledgments**

We wish to thank R. Marsh and L. Kaufman for their comments and suggestions on early drafts of this manuscript. We wish to thank M. Mendonça for providing laboratory space for our measurements recorded at Auburn University. We also wish to thank the various assistants who helped in the laboratory and the field. Finally, we wish to thank all the landowners who allowed us to study their bat colonies. Funding for this study was provided in part from grants from Sigma Xi and the American Society of Mammalogists (to C.S.R.).
Literature Cited


Kronfeld-Schor N., C. Richardson, B.A. Silvia, T.H. Kunz., and E.P. Widmaier. 2000. Dissociation of leptin secretion and