

Hibernation in bats (Mammalia: Chiroptera) did not evolve through positive selection of leptin

Margaret E. Lazzeroni¹  | Frank T. Burbrink² | Nancy B. Simmons³

¹Richard Gilder Graduate School, American Museum of Natural History, New York, New York

²Division of Vertebrate Zoology, Department of Herpetology, American Museum of Natural History, New York, New York

³Division of Vertebrate Zoology, Department of Mammalogy, American Museum of Natural History, New York, New York

Correspondence

Margaret E. Lazzeroni, Richard Gilder Graduate School, American Museum of Natural History, New York, NY.
Email: mlazzeroni@amnh.org

Funding information

Earth Institute Travel Grant; Theodore Roosevelt Memorial Grant; Ecology Evolution and Environmental Biology Department's MA Student Travel Grant from Columbia University

Abstract

Temperature regulation is an indispensable physiological activity critical for animal survival. However, relatively little is known about the origin of thermoregulatory regimes in a phylogenetic context, or the genetic mechanisms driving the evolution of these regimes. Using bats as a study system, we examined the evolution of three thermoregulatory regimes (hibernation, daily heterothermy, and homeothermy) in relation to the evolution of leptin, a protein implicated in regulation of torpor bouts in mammals, including bats. A threshold model was used to test for a correlation between lineages with positively selected *lep*, the gene encoding leptin, and the thermoregulatory regimes of those lineages. Although evidence for episodic positive selection of *lep* was found, positive selection was not correlated with lineages of heterothermic bats, a finding that contradicts results from previous studies. Evidence from our ancestral state reconstructions suggests that the most recent common ancestor of bats used daily heterothermy and that the presence of hibernation is highly unlikely at this node. Hibernation likely evolved independently at least four times in bats—once in the common ancestor of Vespertilionidae and Molossidae, once in the clade containing Rhinolophidae and Rhinopomatidae, and again independently in the lineages leading to *Taphozous melanopogon* and *Mystacina tuberculata*. Our reconstructions revealed that thermoregulatory regimes never transitioned directly from hibernation to homeothermy, or the reverse, in the evolutionary history of bats. This, in addition to recent evidence that heterothermy is best described along a continuum, suggests that thermoregulatory regimes in mammals are best represented as an ordered continuous trait (homeothermy \leftarrow \rightarrow daily torpor \leftarrow \rightarrow hibernation) rather than as the three discrete regimes that evolve in an unordered fashion. These results have important implications for methodological approaches in future physiological and evolutionary research.

KEYWORDS

ancestral state reconstruction, bats, hibernation, homeothermy, leptin, thermoregulation, torpor

1 | INTRODUCTION

In mammals, the evolution of endothermy—the use of metabolically produced heat to maintain T_b (body temperature)—has enabled survival of many different taxa across a variety of ecological niches and ecosystems (Fristoe et al., 2015; Hillenius & Ruben, 2004; Scholander, Hock, Walters, & Johnson, 1950). Homeothermy is defined by maintenance of a relatively constant T_b over time (Ivanov, 2006), and endothermy and homeothermy are frequently linked in mammals. Endothermic heterothermy, characterized by a combination of metabolically produced heat and the use of torpor—a physiological state characterized by the reduction in metabolism, and subsequently T_b , below basal levels—has enabled the survival of mammalian taxa globally, especially in extremely cold climates, highly variable climates, or regions with periodic constraints on food and water availability (Geiser & Stawski, 2011; Geiser & Turbill, 2009; Tattersall et al., 2012). The duration of discrete torpor bouts is often used to define the two traditionally recognized forms of heterothermy in mammals: hibernation and daily heterothermy, also known as daily torpor (Geiser, 2004; Ruf & Geiser, 2015). Hibernators are capable of multiday torpor bouts and drastic T_b reduction, while daily heterotherms exclusively use shorter (<24 hr), shallower torpor bouts (Geiser, 2004; Ruf & Geiser, 2015). However, this binary categorization of heterothermy has been debated, with some authors arguing that hard boundaries between hibernation and daily torpor do not exist (Boyles et al., 2013; Canale, Levesque, & Lovegrove, 2012; Geiser & Ruf, 1995; Ruf & Geiser, 2015; van Breukelen & Martin, 2015). As new methods are developed, support for a continuum of heterothermic abilities has been growing but a consensus has not been reached (Boyles, Bennett, Mohammed, & Alagaili, 2017; Levesque, Nowack, & Stawski, 2016).

Although the common ancestor of mammals was traditionally presumed to be homeothermic (Crompton, Taylor, Jagger, & a, 1978), some recent authors have argued against this interpretation on physiological and/or behavioral grounds (Grigg, Beard, & Augee, 2004; Lovegrove, 2012). Grigg et al (2004) suggested that endothermic heterothermy may be ancestral to homeothermy in mammals because endothermic heterothermy would provide a reasonable intermediate along the transition from ectothermy to full endothermic homeothermy, a shift that would have required a many-fold increase in metabolism (Geiser & Stawski, 2011).

Transitions to new thermoregulatory regimes over evolutionary time require physiological changes. Leptin, a hormone encoded by the *lep* gene, influences thermoregulation and satiety by signaling to the brain the degree of adiposity available for energy intake (Dodd et al., 2014; Enriori, Sinnayah, & Simonds, 2011; Kaiyala, Ogimoto, & Nelson, 2015; Zhang et al., 2011). Due to the role it plays in regulating satiety and metabolic activity, leptin concentrations may also be impacted by diet (Clarke & Connor, 2014; Harvey et al., 2000; Weigle et al., 2005). Leptin function is correlated with post-hibernation weight gain in arctic ground squirrels (Boyer et al., 1997), pre-hibernation weight gain in male woodchucks (Concannon, Levac, & Rawson, 2001), and feeding activity of at least one homeothermic

bat (*Scotophilus heathii*; Srivastava & Krishna, 2008). Exon 3 of *lep* may be particularly important because it contains 29 amino acid variants with functional significance (Yuan et al., 2011). Some hibernating bats show structural alterations in exon 3 of *lep* that may cause leptin to become more physiologically active (He et al., 2010). Outside of the relationship leptin has with food intake and temperature regulation, leptin has a mechanistically independent function in initiating torpor bouts in some species (Stehling, Doring, & Ertl, 1996; Swoap, 2008). As part of a complex matrix of gene–gene interactions, sympathetic activation of white adipose tissue during fasting triggers a dramatic decrease in circulating leptin which is thought to initiate a torpor bout (Swoap, 2008). Entrance into torpor for Siberian hamsters is triggered by leptin (Freeman, Lewis, & Kauffman, 2004), and regulation of T_b is impacted by leptin in homeothermic rats (Stehling et al., 1996).

Bats (Chiroptera) represent a useful model for comparative evaluation of the evolutionary genomics of thermoregulatory regimes because they are a large monophyletic group with nearly 1,400 extant species, and show considerable variation in thermoregulatory regimes across the phylogeny including homeothermy, daily torpor, and hibernation (Lyman, 1970; Simmons, 2005; Stawski, Willis, & Geiser, 2014). Bats are also among the most diverse mammalian clades with respect to diet, with various lineages specialized as insectivores, carnivores, frugivores, nectarivores, omnivores, and even sanguivores (Fenton & Simmons., 2015; Hill & Smith., 1984).

Consistent with studies on other mammals, leptin in bats influences torpor abilities, feeding activity, and energy balance (Banerjee, Udin, & Krishna, 2011; He et al., 2010; Kronfeld-Schor, Richardson, & Silvia, 2000; Srivastava & Krishna, 2008; Yuan et al., 2011; Zhu et al., 2014). Ancestral state reconstruction based on the evolution of the *lep* gene (Yuan et al., 2011) and circumstantial evidence based on the abundance of tropical and subtropical heterothermic bats (Cory Toussaint, McKechnie, & Merwe, 2010; Geiser & Stawski, 2011; Liu & Karasov, 2011; Stawski, Turbill, & Geiser, 2009; Turbill, Law, & Geiser, 2003) have been used to suggest that the most recent common ancestor of bats was heterothermic. However, it is unclear if this ancestor used hibernation or daily torpor.

To better understand the unique evolutionary history of thermoregulation, we examined the relationship between thermoregulatory regime of extant taxa and selection on *lep* exon 3. Yuan et al. (2011) found that leptin has undergone positive selection in heterothermic bat lineages, and therefore associated it with the evolution of torpor. However, by using a *lep* exon 3 gene tree instead of a species tree, Yuan et al. (2011) effectively treated two nonindependent variables (branch length and *lep* selection) as independent. Coalescence theory suggests that the use of gene trees to calculate ω , a measure of positive selection, may bias results because gene trees do not necessarily mirror species trees, and may have alternative branching patterns and lengths compared to the species tree (Diekmann & Pereira-Leal, 2016). To reexamine the findings of Yuan et al. (2011), we hypothesized that *lep* exon 3 has undergone episodic positive selection in heterothermic lineages. Episodic positive selection is defined as positive selection which occurs in a subset of lineages. We

TABLE 1 Overview of the thermoregulatory regimes and diet of bat species included in this study

Taxa	TR	TR reference	Diet	Diet reference	Accession #	Loan Institution: ID
Bats						
<i>Anoura geoffroyi</i> ^a	Daily Torpor ^b	Audet and Thomas (1997), Avila-Flores and Medellin (2004), Stawski et al. (2014)	Insectivorous	Baker, Jones, and Carter (1976), Ortega and Alarcón-D (2008)	GU2308833	
<i>Carollia perspicillata</i>	Daily Torpor	Audet and Thomas (1997)				
<i>Dermanura gnoma</i> ^a	Daily Torpor ^b	Audet and Thomas (1997), Avila-Flores and Medellin (2004), Stawski et al. (2014)	Frugivorous	Solari et al. (2009), Rojas, Vale, Ferrero, and Navarro, (2011)	GU2308832	
<i>Erophylla bombifrons</i>	Daily Torpor	Rodriguez-Duran (1995)				
<i>Eumops perotis</i>	Daily Torpor	Leitner (1966)				
<i>Glossophaga soricina</i>	Daily Torpor	Rasweiler (1973)				
<i>Lasiurus seminolus</i>	Daily Torpor	Genoud (1993)				
<i>Macroglossus minimus</i> ^a	Daily Torpor	Bartels, Law, and Geiser (1998), Geiser (2006)	Nectarivorous	Nowak (1994), Hollar and Springer (1997)		AMCC: CEF801
<i>Megaloglossus woermanni</i>	Daily Torpor	Kulzer and Storf (1980)				
<i>Monophyllus redmani</i>	Daily Torpor	Rodriguez-Duran (1995)				
<i>Mops condylurus</i>	Daily Torpor	Maloney, Bronner, and Buffenstein (1999)				
<i>Myotis daubentonii</i>	Daily Torpor	Dietz and Kalko (2006)				
<i>Nycteris thebaica</i>	Daily Torpor	Cory Toussaint and McKechnie (2012)				
<i>Nyctimene albigenter</i>	Daily Torpor	Bartholomew, Dawson, and Lasiewski (1970)				
<i>Nyctimene robinsoni</i>	Daily Torpor	Geiser (2006)				
<i>Pteropteryx macrotis</i>	Daily Torpor	Genoud, Bonaccorso, and Anends (1990)				
<i>Pternotus davyi</i>	Daily Torpor	Czenze and Dunbar (2017)				
<i>Rhinolophus megaphyllus</i>	Daily Torpor	Young (2001)				
<i>Scotophilus dinganii</i>	Daily Torpor	Jacobs, Kelly, Mason, and Stoffberg (2007)				
<i>Sturnira lilium</i>	Daily Torpor	Audet and Thomas (1997)				
<i>Syconycteris australis</i> ^a	Daily Torpor	Geiser (2006)	Nectarivorous	Altringham (1996), Hollar and Springer (1997), Courts (1998)		Smithsonian: 585524
<i>Tadarida teniotis</i> ^a	Daily Torpor	Arlettaz et al. (2000)	Insectivorous	Freeman (1979), Rydell and Arlettaz (1994), Whitaker and Karataş (2009)	GU2308839	
<i>Taphozous australis</i>	Daily Torpor	Kulzer, Nelson, McKean, and Möhres (1970), Geiser (2006)				
<i>Barbastella barbastellus</i>	Hibernation	Pohl (1961), Russo et al. (2017)				

(Continues)

TABLE 1 (Continued)

Taxa	TR	TR reference	Diet	Diet reference	Accession #	Loan Institution: ID
<i>Chaerophon plicatus</i> ^a	Hibernation	Vivier and Van Der Merwe (2007), Yuan et al. (2011)	Insectivorous	Freeman (1979), Bohmann et al. (2011), Kusuminda and Yapa (2017)	GU230836	
<i>Chalinolobus gouldii</i>	Hibernation	Stawski and Currie (2016)				
<i>Eptesicus fuscus</i> ^b	Hibernation	Brack (2007)	Insectivorous	Agosta (2002)	NW_007370746	
<i>Hipposideros armiger</i> ^a	Hibernation	Liu and Karasov (2011)	Insectivorous	Whitaker and Karataş (2009), Weiterings, Wardenaar, Dunn, and Umponstira (2015)	NW_017731447	
<i>Lasionycteris noctivagans</i>	Hibernation	Izor (1979)				
<i>Lasiurus borealis</i>	Hibernation	Dunbar and Tomasi (2006)				
<i>Lasiurus cinereus</i>	Hibernation	Cryan (2003), Willis, Brigham, and Geiser (2006)				
<i>Miniopterus fuliginosus</i> ^a	Hibernation	Kimura and Uchida (1983), Yuan et al. (2011)	Insectivorous	Hu, Wei, Zhu, Wang, and Zhang (2011)	GU230844	
<i>Miniopterus natalensis</i> ^a	Hibernation	Van Der Merwe (1973)	Insectivorous	Naidoo, Mackey, and , Schoeman MC, (2011)	NW_015504548	
<i>Miniopterus schreibersii</i>	Hibernation	Kulzer et al. (1970), Geiser (2006)				
<i>Myotis adversus</i>	Hibernation	Kulzer et al. (1970)				
<i>Myotis brandtii</i> ^a	Hibernation	Villanueva-Cañas et al. (2014)	Insectivorous	Vaughan (1997), Whitaker and Karataş (2009)	NW_005360677	
<i>Myotis davidii</i> ^a	Hibernation	Villanueva-Cañas et al. (2014)	Insectivorous	Zhang et al. (2013)	NW_006296405	
<i>Myotis leibii</i>	Hibernation	Best and Jennings (1997)				
<i>Myotis lucifugus</i> ^a	Hibernation	Brack (2007)	Insectivorous	Belwood and Fenton (1976)	NW_005871121	
<i>Myotis myotis</i>	Hibernation	Pohl (1961), Harmata (1987), Koteja, Jurczyszyn, and Woloszyn (2001)				
<i>Myotis nattereri</i>	Hibernation	Hope and Jones (2012)				
<i>Myotis ricketti</i> ^a	Hibernation	Zhang et al. (2014)	Piscivorous	Ma et al. (2003)	GU230846	
<i>Myotis septentrionalis</i>	Hibernation	Brack (2007)				
<i>Myotis sodalis</i>	Hibernation	Brack (2007)				
<i>Myotis velifer</i>	Hibernation	Tinkle and Patterson (1965), Riedesel and Williams (1976)				

(Continues)

TABLE 1 (Continued)

Taxa	TR	TR reference	Diet	Diet reference	Accession #	Loan Institution: ID
<i>Myotis vivesi</i>	Hibernation	Salinas, Herrera, Flores-Martínez, and Johnston (2014)				
<i>Nyctalus noctula</i>	Hibernation	Ransome (1990), Arlettaz et al. (2000)				
<i>Mystacina tuberculata</i>	Hibernation	Czenze, Brigham, Hickey, and Parsons (2017a)				
<i>Nyctophilus geoffroyi</i>	Hibernation	Turbill et al. (2003)				
<i>Nyctophilus gouldi</i>	Hibernation	Turbill et al. (2003)				
<i>Pipistrellus pipistrellus</i>	Hibernation	Kayser (1964), Kulzer (1965)				
<i>Plecotus auritus</i>	Hibernation	Eisentraut (1956)				
<i>Rhinolophus ferrumequinum^a</i>	Hibernation	Park, Jones, and Ransome (1999); Chen, Yuan, and Sun (2008)	Insectivorous	Vaughan (1997), Whitaker and Karataş (2009)	GU230845	
<i>Rhinolophus hipposideros</i>	Hibernation	Harmata (1987)				
<i>Rhinopoma microphyllum^a</i>	Hibernation	Levin and Kronfeld-schor (2012), Stawski et al. (2014)	Insectivorous	Sharifi and Hemmati (2002)	GU230830	
<i>Scotophilus heathii^a</i>	Hibernation	Rashid, Irfan, Nadeem, and Shabbir (2016)	Insectivorous	Jacobs, Eick, Schoeman, and Matthee (2006)	GU230843	
<i>Tadarida aegyptiaca</i>	Hibernation	Geiser and Stawski (2011)				
<i>Tadarida brasiliensis</i>	Hibernation	Herreid (1963), Herreid and Schmidt-Nielsen (1966)				
<i>Taphozous melanopogon^a</i>	Hibernation	Kulzer (1965)	Insectivorous	Hu et al. (2011), Weterings et al. (2015)	GU230842	
<i>Carollia brevicauda^a</i>	Homeothermy	Avila-Flores and Medellín (2004)	Frugivorous	Fleming (1991)	GU230829	
<i>Cynopterus sphinx^a</i>	Homeothermy ^b	Banerjee, Meenakumari, and Krishna (2007), Stawski et al. (2014)	Frugivorous	Ruby, Nathan, Balasingh, and Kunz (2000)	GU230842	
<i>Dobsonia viridis^a</i>	Homeothermy ^b	Stawski et al. (2014)	Frugivorous	Bonaccorso, Winkelmann, Dumont, and Bat (2002)	GU230840	
<i>Eidolon helvum^a</i>	Homeothermy	Zaidan (1980)	Frugivorous	Richter and Cumming (2006)	GU230838	
<i>Eonycteris spelaea^a</i>	Homeothermy	Krutzsch (1979)	Nectarivorous	Fenton (1990), Bumrungsri et al. (2013)	GU230848	
<i>Macroderma gigas</i>	Homeothermy	Lyman (1970)				
<i>Mormoops blainvilli</i>	Homeothermy	Bonaccorso et al. (1992), Rodríguez-Duran (1995)				
<i>Nyctimene major^a</i>	Homeothermy	Bartholomew et al. (1970)	Frugivorous	Freeman (1995)		AMCC: PRS2767

(Continues)

TABLE 1 (Continued)

Taxa	TR	TR reference	Diet	Diet reference	Accession #	Loan Institution: ID
<i>Pteronotus parnellii</i> ^a	Homeothermy	Bonaccorso et al. (1992); Stawski et al. (2014)	Insectivorous	Fenton (1990), Brigham (1991), Rojas et al. (2011)	GU230831	
<i>Pteronotus personatus</i>	Homeothermy	Bonaccorso et al. (1992), Stawski et al. (2014)				
<i>Pteronotus quadridens</i>	Homeothermy	Rodriguez-Duran (1995)				
<i>Pteropus alecto</i> ^a	Homeothermy ^b	McNab and Bonaccorso (2001)	Herbivorous	Zhang et al. (2013)	NW_006434839	
<i>Pteropus giganteus</i> ^a	Homeothermy	Kulzer (1965)	Frugivorous	Nowak (1994), Vaughan (1997)	GU230837	
<i>Pteropus hypomelanus</i> ^a	Homeothermy	Ochoa-Acuña and Kunz (1999)	Frugivorous	Heard and Whittier (1997)		AMCC: PER 1
<i>Pteropus vampyrus</i> ^a	Homeothermy	McNab and Armstrong (2001)	Herbivorous	Stier and Mildenstein (2005)	NW_011888814	
<i>Rousettus aegyptiacus</i> ^a	Homeothermy	Noll (1979)	Frugivorous	Korine, Izhaki, and Arad (1999)	NW_015494499	
<i>Rousettus leschenaultii</i> ^a	Homeothermy ^b	Noll (1979), Stawski et al. (2014)	Frugivorous	Raghuram, Thangadurai, and Gopukumar (2009)	GU230847	
Outgroups						
<i>Capra hircus</i> ^a	Homeothermy	Kaciuba-Usciz, xliexo, Jessen, Feistkorn, and Brzezinska (1987)	Folivorous	Genin and Pijoan (1993)	AM114397	
<i>Galeopterus variegatus</i> ^a	Homeothermy		Herbivorous	Dzulhelmi and Abdullah (2009)	NW_007735418	
<i>Homo sapiens</i> ^a	Homeothermy	Mekjavic and Eiken (2006)	Omnivorous		D63519	

Notes. TR: thermoregulatory regime.

^aIncluded in the dataset for the tests of positive selection of *lep* exon 3. ^bSpecies-specific thermoregulatory regime is undocumented in the literature. The most likely thermoregulatory regime was inferred based on associated literature and family-level summaries of thermal physiology (Stawski et al., 2014).

tested this hypothesis in the context of a species tree for relevant bat taxa. We also reconstructed the ancestral thermoregulatory regimes of bats with revised species-level data compared to previous family-level analyses (Yuan et al., 2011). By evaluating the number of transitions between regimes across the phylogeny through stochastic character mapping, we also evaluated the validity of categorizing thermoregulatory regimes as three discrete character states. Finally, to address other known effects of leptin, we tested for correlation between lineages with positively selected *lep* exon 3 and the diets of those lineages.

2 | MATERIALS AND METHODS

2.1 | Taxon sampling

To test for positive selection of *lep* exon 3, 31 species of bats with known thermoregulatory regimes were sampled for the *lep* exon 3 gene, including representatives from nine families (Table 1). Thirteen of these species hibernate, five use daily torpor, and 13 are homeothermic (Table 1). *Lep* exon 3 sequences for 27 of these species were downloaded from GenBank, and the other four, *Pteropus hypomelanus*, *Nyctimene major*, *Macroglossus minimus*, and *Syconycteris australis*, were sequenced in the Sackler Institute for Comparative Genomics. *Lep* exon 3 sequences for three outgroups were also included, *Homo sapiens* (human), *Capra hircus* (goat), and *Galeopterus variegatus* (Sunda colugo; Table 1). Species-specific data on thermoregulatory regimes and dietary preferences were taken from the primary literature (Table 1, dietary preferences only listed for species in this dataset). The resulting dataset included 34 taxa, which we used for analyses of positive selection of *lep* exon 3 and models evaluating the relationship between positive selection of *lep* exon 3 and thermoregulatory strategies or diet.

A larger dataset including 76 bat taxa and three outgroups was used for the ancestral state reconstruction of thermoregulatory regimes. Here, we were not limited by the requirement of having leptin sequences, which allowed for greater coverage of the phylogeny. Species were selected for the ancestral state reconstruction based on availability of data describing their thermoregulatory regime and presence in the published phylogeny that was used in the analyses (i.e., Amador, Arévalo, & Almeida, 2016). Data on the thermoregulatory regime of each species were taken from the primary literature by searching databases (Google Scholar and Web of Science) between the dates of September 2015 and August 2018, using keywords "bats," "thermoregulation," "thermoregulatory regimes," "temperature regulation," "hibernation," "torpor," "daily torpor," "daily heterothermy," "heterothermy," "homeothermy," "metabolism," and "Chiroptera." These data are summarized in Table 1.

DNA was extracted and *lep* exon 3 was amplified and sequenced for four bat species for the tests for positive selection—*Pteronotus hypomelanus*, *Nyctimene major*, *Macroglossus minimus*, and *Syconycteris australis*. All laboratory work was conducted in the Sackler Institute for Comparative Genomics. DNA was extracted from either wing punches or tissues of museum specimens (Table 1) using the Qiagen

DNeasy Blood and Tissue Extraction Kit. In order to amplify *lep* exon 3 in these species, a primer pair (F1—AGAAGGGAGGGAGGACTCAAC, R1—GCTTCAGCACCCAGGGCTG) was developed on the flanking region of the consensus sequence from a multiple alignment of published *lep* sequences from *Rousettus leschenaultii*, *Pteronotus giganteus*, *Eonycteris spelaea*, *Eidolon helvum*, *Dobsonia viridis*, and *Cynopterus sphinx* which was made by eye in Geneious (Kearse et al., 2012).

The polymerase chain reaction (PCR) was carried out using illustra™ puReTaq Ready-To-Go PCR Beads Kit. Amplification was performed in a 25 μ l reaction volume. This consisted of 20.7 μ l nuclease-free water, 0.3 μ l bovine serum albumin, 1 μ l 10 \times solution of forward primer, 1 μ l 10 \times solution of reverse primer, 2 μ l template, and one bead containing recombinant puReTaq DNA polymerase. PCR conditions were as follows: an initial denaturation phase at 95°C for 5 min, 25 cycles with a denaturation phase at 95°C for 30 s, an annealing phase at 57°C for 30 s, and an extension phase at 72°C for 45 s. The wells were then stored in a refrigerator at 4°C. PCR products were purified with AgenCourt AMPure XP. PCR products were sequenced using Sanger sequencing (Smith & Hood, 1987) following protocol from the BigDye® Terminator v3.1 Cycle Sequencing Kit.

Sequences were assembled and edited within Geneious (Kearse et al., 2012). Ends of forward and reverse sequences were trimmed with an error set to 0.01. Trimmed regions were ignored and forward and reverse sequences were assembled using de novo assembly, as outlined by the Geneious manual (Biomatters, 2017). The forward and reverse sequences of *lep* for *Nyctimene major* did not assemble through de novo assembly. Therefore, these were mapped to the reference sequence from which the primers were built. Reads were then manually edited to maximize the coverage and identity between the forward and reverse sequences. *Lep* exon 3 was trimmed to the open reading frame (ORF), and stop codons were removed from the tail. ORFs from all species in the dataset were then aligned by eye in Geneious (Kearse et al., 2012).

2.2 | Branch-sites under positive selection

To identify lineages that have experienced episodic positive selection of *lep* exon 3, we ran a mixed-effects model of evolution (MEME) to detect a subset of branch-sites under episodic positive selection (Murrell et al., 2012). MEME uses a fixed-effect model to explain the distribution and variation of ω across sites, and a random-effects model to explain variation in the distribution of ω across branches (Kosakovsky Pond et al., 2011; Murrell et al., 2012; Nielsen & Yang, 1998). MEME was used here because other tests, which usually average ω over branches and sites, often miss positive selection when it occurs in a subset of branch-sites (Yang & Nielsen, 2002). When evaluating if a branch-site is under positive selection, MEME considers the specific model of molecular evolution, which here was the TRN93 model (Tamura & Nei, 1993), differences in codon frequencies, and ω .

The test for branch-sites under episodic positive selection, MEME, was performed within the DataMonkey web server (Delpont,

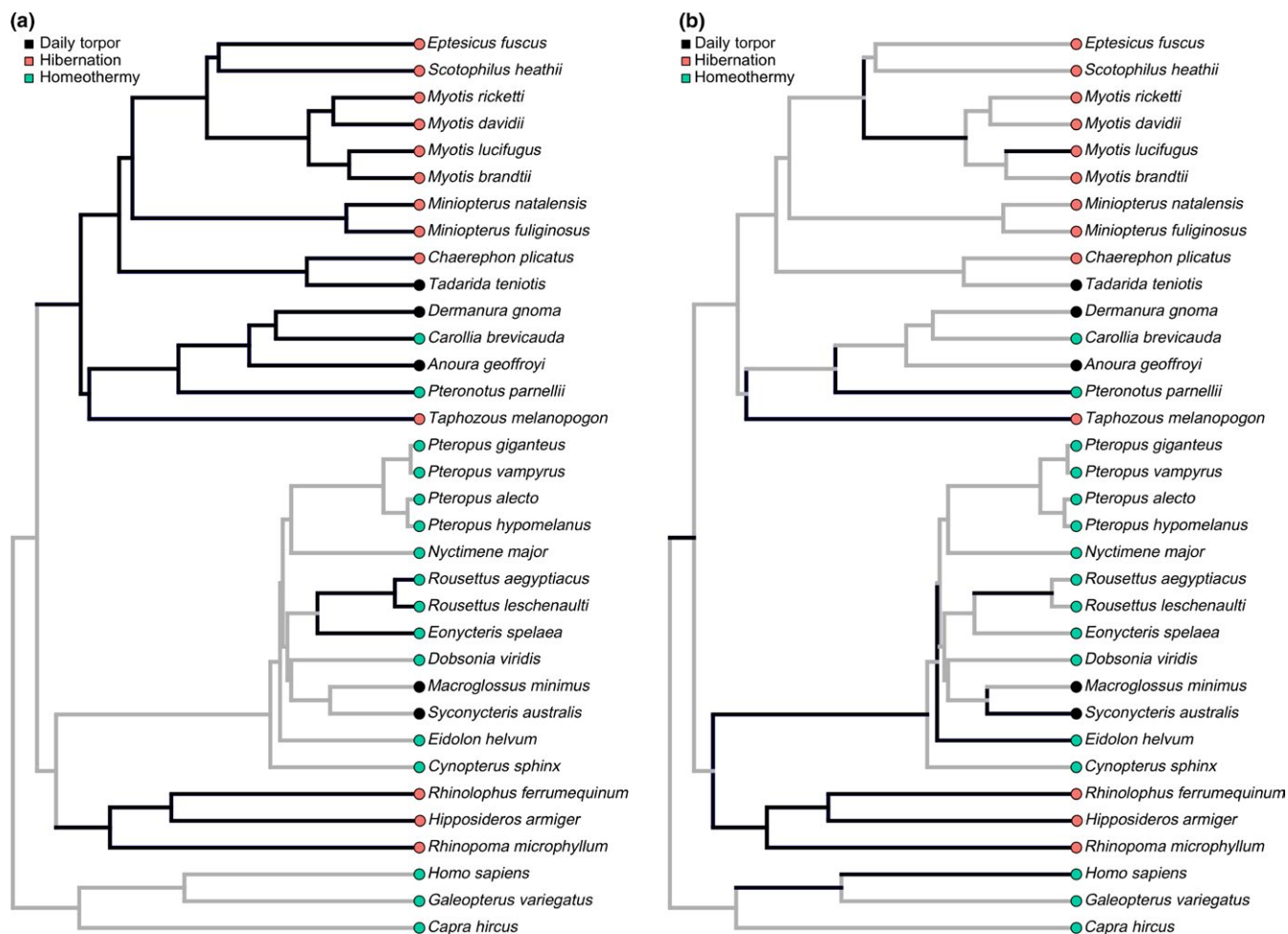


FIGURE 1 BUSTED hypotheses tests for positive selection of *lep* exon 3 across bats with different thermoregulatory regimes (black = daily torpor; pink = hibernation; green = homeothermy). Topology of tree generated from Amador et al. (2016). Bolded lineages represent the lineages tested for positive selection (foreground branches). Gray lineages represent the background. (a) Foreground branches correspond with those found to have evidence of positive selection by Yuan et al. (2011). (b) Foreground branches represent lineages detected by MEME to have evidence of positive selection

Poon, Frost, & Kosakovsky Pond, 2010) using the aligned *lep* exon 3 sequences, the automatic substitution model selection tool, and a user-specified tree from Amador et al. (2016). This 807 taxa phylogeny was calibrated using 44 key fossils, inferred using nine nuclear and mitochondrial genes, and shows support for the majority of currently recognized bat clades (Amador et al., 2016). The Amador et al. (2016) tree was chosen for this study because it represents the most genus- and species-level diversity, 90% and 64%, respectively, compared to other phylogenies (Amador et al., 2016). The tree was pruned using the *ape* package in R (Paradis, Claude, & Strimmer, 2004) to only include the 31 bats and three outgroup taxa in our dataset. The model of molecular evolution that best fit these data was determined to be the TRN93 model (Tamura & Nei, 1993) using the automatic function available in DataMonkey (Delpont et al., 2010).

The significance threshold was set to 0.1 and a log-ratio test (LRT) was performed, comparing the alternative model to the null model. The alternative model allows for positive selection in a subset

of branch-sites, while the null model does not allow for positive selection in a subset of branch-sites. For each branch, an empirical Bayes factor (EBF) for having $\omega > 1$ was calculated with an associated posterior probability.

Mixed-effects model of evolution only detects branch-sites under episodic positive selection, not lineages with gene-wide positive selection. Therefore, to confirm that *lep* exon 3 is indeed under positive selection in the branches detected by MEME, we tested for gene-wide episodic positive selection using BUSTED, a branch-site unrestricted statistical test for episodic positive selection (Murrell et al., 2015). BUSTED uses a LRT to detect evidence of episodic positive selection, when the rate of non-synonymous to synonymous substitutions at branch-sites is transiently greater in the foreground branches compared to background (Murrell et al., 2015). Foreground branches are the lineages hypothesized to be under positive selection, and the background branches are all other branches in the phylogeny. This model assumes that the gene evolves under the general time reversal model (Tavaré, 1986).

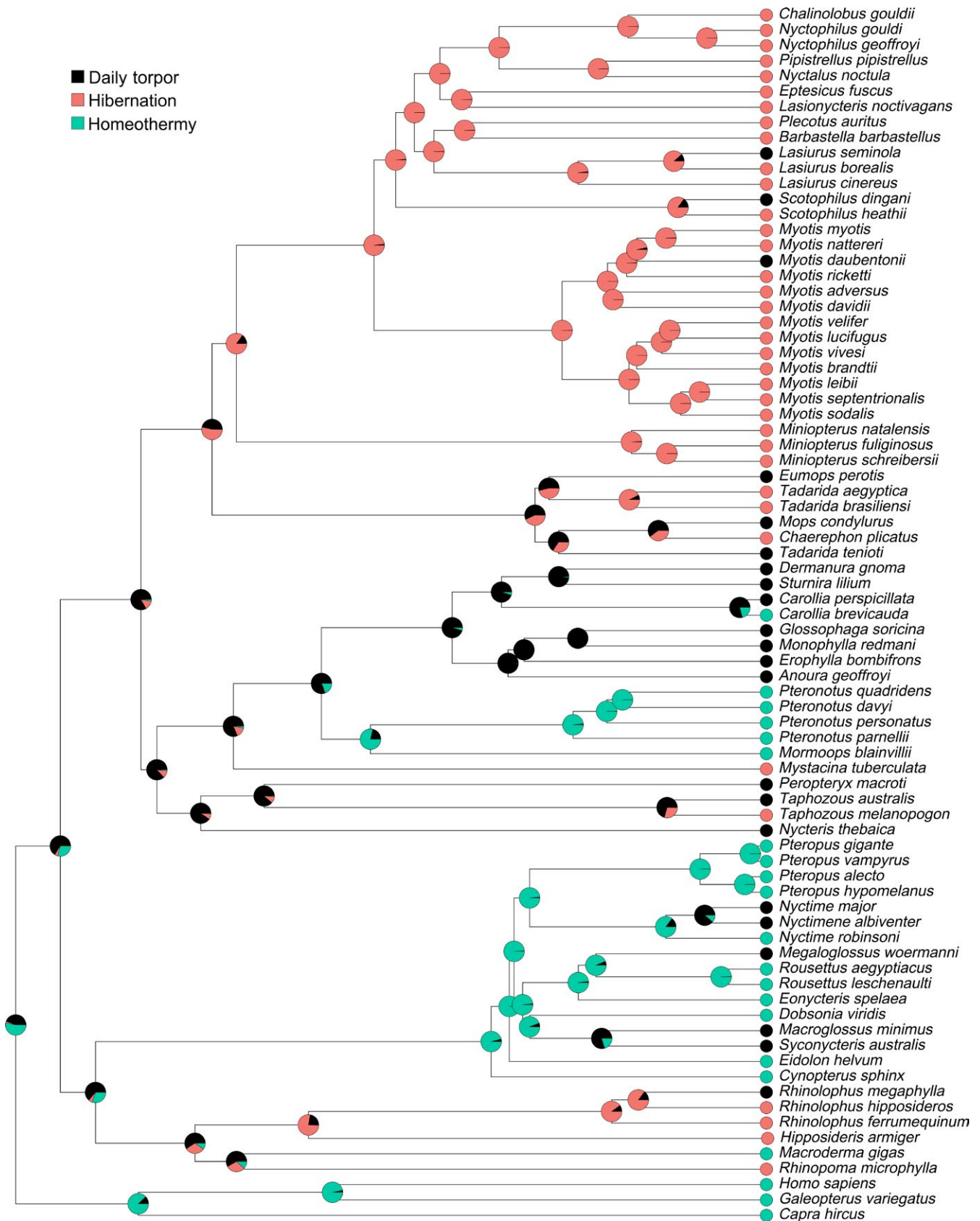


FIGURE 2 Ancestral state reconstruction under the Mkn model. Topology of tree generated from Amador et al. (2016). Pie charts represent the marginal likelihoods of each thermoregulatory regime at a given node

We also used BUSTED to test for episodic positive selection by binning branch-sites into three ω categories representative of either purifying, neutral, or positive selection. Purifying selection is defined by having a $\omega < 1$, indicating that there is strong selection to maintain the sequence over evolutionary time. Neutral selection is defined by having a ω approximately equal to 1, indicating that there is neither strong selection for the maintenance of a sequence over time nor selection for changes to that sequence. In the unconstrained model, both foreground and background branches can evolve under positive selection. In the null models, neither foreground nor background branches are allowed to evolve through positive selection. This analysis required, as input, the same alignment of *lep* exon 3 sequences used in the MEME analysis, and the pruned, user-specified phylogeny from Amador et al. (2016).

Two analyses to test for gene-wide positive selection were performed. In the first analysis, we tested for selection along branches where evidence for positive selection was previously detected (Figure 1a; Yuan et al., 2011). In the second analysis, we selected foreground branches based on lineages with evidence for positive selection from the MEME results (Figure 1b). Here, branches were considered foreground if they met the following criteria based on the MEME output: A branch had at least one codon site with an EBF > 3 for having $\omega > 1$, and a posterior probability > 0.25 for having $\omega > 1$. These criteria conform with the guidelines for interpreting EBF values from Kass and Raftery (1995). In addition to the LRT calculation, Akaike information criteria (AIC) scores were calculated. AIC statistically quantifies the quality of each model by considering the optimum log likelihood (l) and the number of parameters (p) ($AIC = -2l + 2p$), enabling model comparison.

TABLE 2 Branch-Sites with evidence of positive selection in *lep* exon 3

Branch	TR	Sites	EBF	Posterior probability
Node 3	— ^a	75	210.2	1
Node 8	— ^a	7	15.3	0.56
Node 10	— ^a	5	>1,000	1
		6	>1,000	1
		7	11.8	0.5
		75	15	0.86
108	46.2	0.39		
Node 13	— ^a	75	209	1
Node 26	— ^a	78	319.6	0.99
		86	42.1	1
Node 57	— ^a	86	436.3	1
<i>Eidolon helvum</i>	Homeothermic	86	>1,000	1
<i>Hipposideros armiger</i>	Hibernation	7	23.8	0.67
		75	48.4	0.95
		78	3.6	0.61
		108	529.1	0.88
<i>Homo sapiens</i>	Homeothermic	91	>1,000	1
<i>Myotis lucifugus</i>	Hibernation	6	>1,000	1
<i>Pteronotus parnellii</i>	Homeothermic	4	>1,000	1
		56	>1,000	1
		86	23.2	1
<i>Rhinolophus ferrumequinum</i>	Hibernation	7	17.3	0.59
		78	3.6	0.61
		108	206.5	0.74
<i>Rhinopoma microphyllum</i>	Hibernation	4	>1,000	1
		7	36.2	0.75
		78	234	0.99
<i>Syconycteris australis</i>	Daily Torpor	86	>1,000	1
<i>Taphozous melanopogon</i>	Hibernation	6	>1,000	1
		75	>1,000	1

Note. EBF: empirical Bayes factor for having $\omega > 1$; Posterior: posterior probability; TR: thermoregulatory regime.

^aNot Applicable.

2.3 | Correlation between positive selection and phenotypic traits

To determine whether evolution of *lep* exon 3 is correlated with evolution of thermoregulatory regimes, we ran a threshold model (Felsenstein, 2012). Here, a discrete trait is presumed to change state when an underlying variable, the liability, crosses a certain threshold (Felsenstein, 2005, 2012). This liability is assumed to have a multivariate normal distribution and to evolve under Brownian motion (Felsenstein, 2005, 2012). Contrasting the commonly used continuous-time Markov (Mkn) model of discrete character evolution (Lewis, 2001; Pagel, 1994), character states under the threshold model are inherently ordered and the evolution of discrete character states is not memoryless (Felsenstein, 2005, 2012); the character state at one node is influenced by the character state at previous nodes. We categorized thermoregulatory regimes into three states: hibernation, daily torpor, and homeothermy. The presence (1) or absence (0) of positive selection in *lep* exon 3 served as the liability. Species were considered to have undergone positive selection if *lep* exon 3 was determined to be under positive selection per the results from MEME and BUSTED. Only data for the terminal branches were considered, as character states at internal nodes are necessarily unknown.

Parameters for the model were estimated with a Bayesian Markov chain Monte Carlo (MCMC) approach using the *threshBayes* function in R, with default priors and liabilities (Revell, 2012). We ran the chain for 3 million generations, thinned to 1,000 samples per chain, to account for autocorrelation, and discarded the first 500,000 as burn-in. To quantify the relationship between *lep* evolution and TR, a correlation coefficient was calculated between the liability and the thermoregulatory regime. Finally, the highest posterior densities (HPDs) were estimated from the correlation coefficients to determine whether the correlation between traits statistically differed from 0, indicating a statistically significant relationship between the two variables. To test the alternative hypothesis that *lep*

exon 3 evolution is correlated with diet, we used the same methods as above but used diet as the discrete trait evolving under the liability.

2.4 | Ancestral state reconstruction of thermoregulatory regimes

To model the ancestral thermoregulatory regimes of bats, we first used the Mkn model of discrete character evolution (Lewis, 2001; Pagel, 1994) with the states hibernation, daily torpor, and homeothermy. It was important to test which transition rate matrix best described the data. Transition matrices describe the rate of transitioning from state *i* to state *j*. Here, we tested the data to fit one of three transition matrices: (a) equal transitions between all states (equal rates), (b) different transition rates between, but not among, pairs of states (symmetric), and (c) different transition rates between and among pairs of states (all rates different). We also tested each transition matrix under different transformations to determine whether transition rates varied overtime. We ran these tests for 100 iterations within the *fitDiscrete* function in the *geiger* package in R (Harmon, Weir, & Brock, 2008). The model with the lowest weighted AIC score was subsequently chosen to run the ancestral state reconstruction. The symmetric model under a kappa transformation had the best fit to these data. This suggests that, given our data, transition rates vary over time depending on the number of speciation events between two species and that the transition rate between one pair of character states is identical in the forward and reverse but pairs of states can have different transition rates. No transformations were indicated by the data, indicating that the transition rate does not vary over time.

Given these parameters, we ran an ancestral state reconstruction using the *Ace* function from the *ape* package in R (Figure 2) with maximum likelihood estimation to obtain probabilities of states at interior nodes (Paradis et al., 2004). Stochastic character mapping (Bollback, 2006; Huelsenbeck, Nielsen, & Bollback, 2003) was also used to estimate states at interior nodes and to determine how well the chosen parameters matched the real data. Stochastic character maps were built using the *make.simmap* function in the *phytools* package in R (Revell, 2012). Fifty thousand simulations were run using the parameters described previously. A Q-Q plot was generated to compare the Mkn model to the stochastic character map to evaluate the goodness of fit. The stochastic character map was then used to quantify the number of transitions between states across simulations.

TABLE 3 *lep* exon 3 codons with evidence of positive selection in bats

Codon	α	Unconstrained $\beta+$	ω	p-Value
4	0	7.21	∞	0.03
5	2.04	48.00	23.53	0.04
6	0	5.64	∞	0.03
7	0.56	43.45	77.59	0.05
56	0	207.15	∞	0.01
75	0	5.50	∞	0.02
78	0	5.84	∞	0.07
86	0	1.37	∞	0.08
91	0.66	11.68	17.70	0.06
108	0	38.31	∞	<0.001

Note. α : maximum likelihood estimation (MLE) of synonymous rate; $\beta+$: unconstrained MLE of non-synonymous rate.

3 | RESULTS

3.1 | Data collection

We obtained new *lep* sequences for four bats, *Pteropus hypomelanus* (484 bp), *Nyctimene major* (487 bp), *Macroglossus minimus* (788 bp), and *Syconyteris australis* (489 bp). These were aligned to

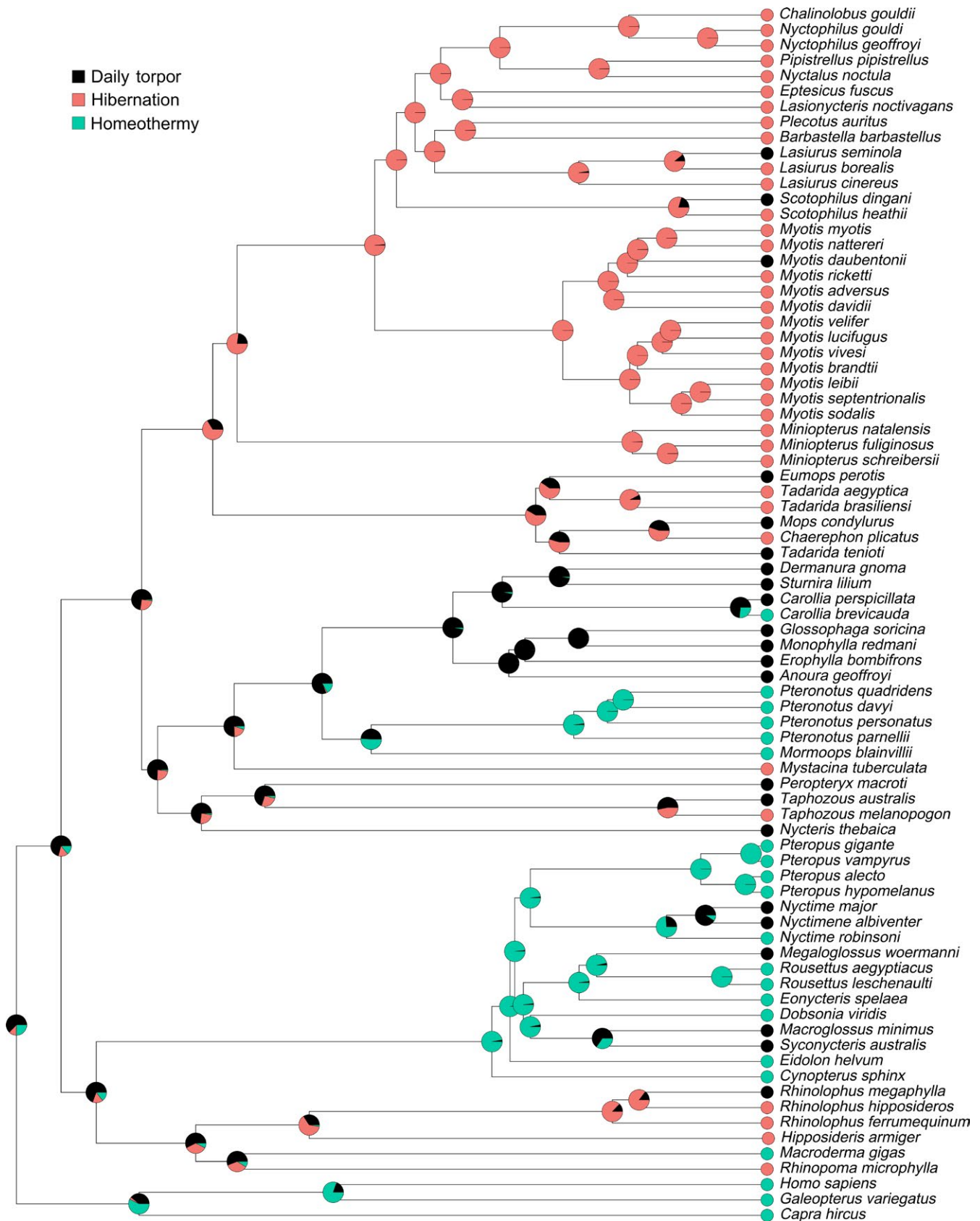


FIGURE 3 Ancestral state reconstruction from stochastic character mapping. Topology of tree generated from Amador et al. (2016). Pie charts represent the posterior probability of each thermoregulatory regime at a given node

the other sequences in the dataset. Once aligned, sequences were trimmed to the coding region of *lep* exon 3 at 357 bp across 34 taxa.

3.2 | Measuring positive selection

From the MEME results, evidence for positive selection was detected in 15 branches (Table 2) over a total of 10 codons (Table 3). When these lineages were selected as the foreground branches in the BUSTED analyses, evidence for episodic positive selection was detected ($p = 0.009$; Figure 1b). Positive selection was not detected when the foreground branches were selected according to those previously found by Yuan et al. (2011) to be under positive selection ($p = 0.220$).

3.3 | Correlation between positive selection and phenotypic traits

No relationship was found between thermoregulatory regimes and lineages with positive selection of *lep* exon 3 (95% HPD = -0.271 to 0.600), or between positively selected lineages and diet (95% HPD = -0.222 to 0.692).

3.4 | Ancestral state reconstruction

The scaled likelihoods from the Mkn model (Supporting Information Table S1) and the posterior probabilities from stochastic character mapping were highly correlated ($\rho = 0.985$, $p = 2.2 \times 10^{-16}$; Figures 2 and 3). The state of the most recent common ancestor of bats could not be fully resolved in either model (Daily Torpor, $\log L = 0.660$, posterior = 0.714 ; Hibernation, $\log L = 0.005$, posterior = 0.154 ; Homeothermy, $\log L = 0.289$ posterior = 0.131). In the Mkn model (Figure 2), the transition rate between daily torpor and hibernation was similar to that between daily torpor and homeothermy (MLE = 0.054 ± 0.012 and MLE = 0.052 ± 0.014 , respectively), while no transitions were found between hibernation and homeothermy. Across the 50,000 stochastic simulations, an average of 33 state changes occurred per tree (summarized in Figure 3). Over each tree, an average of eight transitions occurred from daily torpor to hibernation, and 10 transitions occurred from hibernation to daily torpor. An average of eight transitions occurred from daily torpor to homeothermy, and seven transitions from homeothermy to daily torpor. Transitions between hibernation and homeothermy, in either direction, never occurred across all simulations. Across all regimes and simulations, the mean proportion of time spent in each state was 0.308 , 0.436 , and 0.256 for daily torpor, hibernation, and homeothermy, respectively. Here, time was measured by the proportion of branch lengths for which lineages are predicted to use a specific regime in the phylogeny averaged over all simulations.

Based on the ancestral state reconstruction, hibernation appears to have evolved four times in bats. Vespertilionidae and Molossidae both have taxa that use hibernation. Their most recent common

ancestor may have used hibernation ($\log L = 0.533$). The MRCA of Vespertilionids likely hibernated ($\log L = 0.850$), and multiple internal nodes in Molossidae suggest a heterothermic ancestor. Our results suggest that hibernation evolved at least once in this group. Our data suggest that hibernation evolved independently at least three other times, in the species *Taphozous melanopogon* (Family Emballonuridae) and *Mystacina tuberculata* (Family Mystacinidae), and again in the clade comprised of Rhinopomatidae and Rhinolophidae. However, the node(s) at which hibernation arose in these groups remains ambiguous.

4 | DISCUSSION

We found that the common ancestor of bats most likely used daily heterothermy and is very unlikely to have used hibernation. We also found that leptin evolution is not associated with the evolution of thermoregulatory regimes in bats. Twente and Twente (1964) hypothesized that the most recent common ancestor of bats was homeothermic and that heterothermy evolved secondarily as an adaptation to survive cold climates (Bieber & Ruf, 2009; Geiser & Turbill, 2009; Kortner & Geiser, 2000). However, daily heterothermy remains adaptive even at warmer temperatures because it increases energy savings and long-term survival (Geiser & Stawski, 2011; Stawski & Geiser, 2012). Flexible use of torpor may have enhanced the ability of some bats to survive in warm and/or tropical climates, such as the environments likely encountered throughout the Eocene when bats likely originated (Amador et al., 2016; Czenze & Dunbar, 2017; Czenze, Brigham, Hickey, & Parsons, 2017b; Meredith et al., 2011; O'Leary et al., 2013; Simmons, 2005; Simmons & Geisler, 1998; Simmons, Seymour, Habersetzer, & Gunnell, 2008; Teeling, 2005).

Supporting our results, recent work estimating the ancestral thermoregulatory regimes of bats (Yuan et al., 2011) and evidence for the commonality of heterothermy in bats (Geiser & Stawski, 2011) suggests that heterothermy was the ancestral state for Chiroptera and that homeothermy was secondarily derived (Geiser & Stawski, 2011; Yuan et al., 2011). This scenario mirrors the hypothesis that the common ancestor of all mammals was heterothermic (Grigg et al., 2004; Lovegrove, 2012). However, until now, the question of which heterothermic regime was used by the ancestor of bats—hibernation or daily heterothermy—was unresolved. Here, we show evidence that the ancestor of bats was likely a daily heterotherm.

Consistent with the lack of evolutionary advantages that a hibernator would be expected to accrue during the early Eocene, a relatively warm time period characterized by widespread tropical and subtropical conditions, our results suggest that the common ancestor of bats did not hibernate (Humphries, Thomas, & Speakman, 2002). Although our results suggested a marginal likelihood that the ancestor of bats was a homeotherm, this seems unlikely based on previous studies (e.g., Geiser & Stawski, 2011; Yuan et al., 2011). Taken together with the high likelihood of daily heterothermy at this node, we argue that the most recent common ancestor of bats

was a daily heterotherm. Our reconstruction therefore also indicates several reversals back to daily heterothermy. *Pteronotus davyi*, *Nyctimene major*, *Macroglossus minimus*, and *Syconycteris australis* all represent reversals back to daily heterothermy after their lineages evolved homeothermy. However, due to inconsistent methods for measuring T_b and the setting of arbitrary thresholds to determine a torpid state (Levesque et al., 2016), some of the bats categorized as homeothermic in our study may in fact be heterothermic. This may alter the interpretation of reversals back to heterothermy. Increased data collection following consistent operationalized definitions of torpor should be performed for more species and recollected for species currently identified as homeothermic. Our suspicion is that many bats thought to be endothermic are actually facultative homeotherms under some conditions (e.g., see Czenze & Dunbar, 2017). Future work should also focus on other species across the mammal phylogeny in order to reconstruct the ancestral states at deeper nodes.

Our analyses suggest that hibernation evolved approximately four times in Chiroptera—at the base of Vespertilionidae and Molossidae, in the species *Taphozous melanopogon* and *Mystacina tuberculata* from the families Emballonuridae and Mystacinidae, and in the clade comprised of Rhinolophidae and Rhinopomatidae. Our analyses also suggest that the MRCA of Rhinolophidae used hibernation; however, it is unclear if this is derived or ancestral. Conservatively, we suggest that hibernation arose at least once in this group. Similarly, we suggest that hibernation arose at least once in the clade comprised of Vespertilionids and Molossids. We found no evidence for reversals in the hibernation phenotype—no lineages that lost and subsequently regained the ability to hibernate.

Significant evidence for positive selection of *lep* was detected in some lineages of Chiroptera, but this had no correlation with the thermoregulatory regimes of those lineages (Figure 1b).



FIGURE 4 *Pteronotus parnellii* photograph taken by Brock and Sherri Fenton in a cave in the western end of Cuba

Compared to results from Yuan et al. (2011), our dataset included 11 additional bat taxa, and the thermoregulatory regimes of *Carollia brevicauda* and *Pteronotus parnellii* (Figure 4) were reclassified to be consistent with the literature (Avila-Flores & Medellín, 2004; Bonaccorso, Arends, & Genoud, 1992). Leptin evolution is impacted by pleiotropic effects on other physiological and developmental processes beyond thermoregulation. Leptin can effect thyroid function (Ghamari-Langroudi et al., 2010) and bone development (Crespi & Denver, 2016), induction of mitosis (Gat-Yablonski & Phillip, 2008), and immune and stress responses (Ahima & Osei, 2004; Procaccini, Lourenco, Matarese, & La, 2009). The potential for positive selection of leptin for alternative traits (Carey, Andrews, & Martin, 2003; Jastroch et al., 2016; Yang et al., 2008) makes it difficult to find correlations between positive selection of *lep* and a singular function. Therefore, in retrospect it is perhaps not surprising that we found no evidence for a tight correlation between leptin and chiropteran thermoregulatory regimes, and also found no correlation between leptin selection and diet despite the known influence of high-fat diets on leptin functioning (Frederich et al., 1995; Koch et al., 2014).

Complex genomic mechanisms and associated physiological alterations to organ systems and physiological functions across species with different thermoregulatory regimes suggest that the evolution of thermoregulatory regimes may intrinsically have no correlation with positive selection of a single gene (Andrews, 2004; Grabek et al., 2011; Hindle, Grabek, & Epperson, 2014; Morin & Storey, 2009; Villanueva-Cañas, Faherty, Yoder, & Albà, 2014). A non-synonymous substitution at codon site 91 in exon 3, found here to be under positive selection, was previously inferred to cause a functional difference in hibernating bats compared to homeothermic bats (He et al., 2010). However, we found that this substitution in the sequence of the hibernating bat is shared with *Homo sapiens*, a homeothermic species. Therefore, a direct relationship to thermoregulatory regime and sequence variation cannot be made for this site. Recent evidence suggests that thermoregulatory regimes are mostly influenced by the regulation of gene expression, rather than the sequence specificity of protein-coding genes (Geiser & Stawski, 2011; Grabek, Martin, & Hindle, 2015; Morin & Storey, 2009; Schwartz, Hampton, & Andrews, 2013; Yan, Barnes, Kohl, & Marr, 2008).

In our ancestral state reconstruction, zero direct transitions occurred between hibernation and homeothermy. In this model, we assumed a priori that character states are not ordered. Therefore, the lack of transitions between hibernation and homeothermy is not an artifact of the model. This suggests that thermoregulatory regimes may be better represented as an ordered trait (with daily torpor as a necessary intermediate between homeothermy and hibernation) rather than as an unordered trait. Recent evidence suggests that heterothermy exists along a continuum (Boyles et al., 2013; Dunbar & Brigham, 2010; Lovegrove, 2012; Wilz & Heldmaier, 2000). If true, this suggests that there is an inherent order to the evolution of thermoregulatory regimes, which our results indicate. Our results suggest that future research on mammalian thermoregulation should

treat thermoregulatory regimes as an ordered and possibly continuous trait.

The genomics underlying thermoregulation in mammals remains largely unclear. Future research should aim to sequence whole genomes of mammals that vary in thermoregulatory regime. Comparing these data in a phylogenetic framework would enable a more complete understanding of the genomic components involved in the evolution of thermoregulatory regimes. Our results revealed that leptin does not appear to be directly involved in the evolution of thermoregulatory regimes but many candidate genes including LEPR (Rezai-Zadeh et al., 2014), MEF2 (Tessier & Storey, 2010), and GOS2 (Jessen et al., 2016) have yet to be examined in a similar framework. Such studies will reveal the importance these candidate genes have in the evolution of thermoregulation across diverse taxa.

ACKNOWLEDGMENTS

This research was supported through the Theodore Roosevelt Memorial Grant, the Ecology Evolution and Environmental Biology Department's MA Student Travel Grant from Columbia University, and the Earth Institute Travel Grant. Thanks also Rebecca Hersch in the Sackler Institute for Comparative Genomics for helping with laboratory work, and Angelo Soto-Centeno for the many discussions that inspired this project.

CONFLICT OF INTEREST

None declared.

AUTHOR CONTRIBUTIONS

M.E.L., F.T.B., and N.B.S. contributed to the concept and design of project. M.E.L. performed laboratory work, data collection, and analyses, and drafted the manuscript. All authors contributed to manuscript preparation and approval of final draft.

DATA ACCESSIBILITY

Sequence data are available on GENBANK (<https://www.ncbi.nlm.nih.gov/genbank/>) with accession numbers listed in Table 1. Log likelihoods for the thermoregulatory regime at each internal node in the phylogeny under the Mk model are available in Supporting Information Table S1. All other data can be accessed through Dryad or by contacting Margaret Lazzeroni.

ORCID

Margaret E. Lazzeroni  <http://orcid.org/0000-0002-6660-7599>

REFERENCES

Agosta, S. J. (2002). Habitat use, diet and roost selection by the big brown bat (*Eptesicus fuscus*) in North America: A case for conserving

- an abundant species. *Mammal Review*, 32, 179–198. <https://doi.org/10.1046/j.1365-2907.2002.00103.x>
- Ahima, R. S., & Osei, S. Y. (2004). Leptin signaling. *Physiology & Behavior*, 81, 223–241. <https://doi.org/10.1016/j.physbeh.2004.02.014>
- Altringham, J. (1996). *Bats: Biology and behaviour*. Oxford, UK: Oxford University Press.
- Amador, L. I., Arévalo, R. L. M., & Almeida, F. C. (2016). Bat systematics in the light of unconstrained analyses of a comprehensive molecular supermatrix. *Journal of Mammalian Evolution*, 25(1), 37–70. <https://doi.org/10.1007/s10914-016-9363-8>
- Andrews, M. T. (2004). Genes controlling the metabolic switch in hibernating mammals. *Biochemical Society Transactions*, 32, 1021–1024. <https://doi.org/10.1042/BST0321021>
- Arlettaz, R., Ruchet, C., Aeschmann, J., Brun, E., Genoud, M., & Vogel, P. (2000). Physiological traits affecting the distribution and wintering strategy of the bat *Tadarida teniotis*. *Ecology*, 81, 1004–1014. <https://doi.org/10.2307/177174>
- Audet, D., & Thomas, D. W. (1997). Facultative hypothermia as a thermoregulatory strategy in the phyllostomid bats, *Carollia perspicillata* and *Sturnira lilium*. *Journal of Comparative Physiology B: Biochemical, Systemic, and Environmental Physiology*, 167, 146–152. <https://doi.org/10.1007/s003600050058>
- Avila-Flores, R., & Medellín, R. A. (2004). Ecological, taxonomic, and physiological correlates of cave use by Mexican bats. *Journal of Mammalogy*, 85, 675–687. <https://doi.org/10.1644/BOS-127>
- Baker, R. J., Jones, J. K., & Carter, D. C. (1976). *Biology of bats of the New World family Phyllostomatidae*. Special publications the Museum Texas Tech University.
- Banerjee, A., Meenakumari, K. J., & Krishna, A. (2007). Relationship between delayed embryonic development and metabolic factors and fat deposition in fruit bat *Cynopterus sphinx*. *Reproduction, Fertility, and Development*, 19(5), 626–633.
- Banerjee, A., Udin, S., & Krishna, A. (2011). Regulation of leptin synthesis in white adipose tissue of the female fruit bat, *Cynopterus sphinx*: Role of melatonin with or without insulin. *Experimental Physiology*, 96, 216–225. <https://doi.org/10.1113/expphysiol.2010.055129>
- Bartels, W., Law, B. S., & Geiser, F. (1998). Daily torpor and energetics in a tropical mammal, the northern blossom-bat *Macroglossus minimus* (Megachiroptera). *Journal of Comparative Physiology B: Biochemical, Systemic, and Environmental Physiology*, 168, 233–239. <https://doi.org/10.1007/s003600050141>
- Bartholomew, G. A., Dawson, W. R., & Lasiewski, R. C. (1970). Thermoregulation and heterothermy in some of the smaller flying foxes (Megachiroptera) of New Guinea. *Zeitschrift Für Vergleichende Physiologie*, 70, 196–209. <https://doi.org/10.1007/BF00297716>
- Belwood, J. J., & Fenton, M. B. (1976). Variation in the diet of *Myotis lucifugus* (Chiroptera: Vespertilionidae). *Canadian Journal of Zoology*, 54, 1674–1678. <https://doi.org/10.1139/z76-194>
- Best, T. L., & Jennings, J. B. (1997). *Myotis leibii*. *American Society of Mammalogists*, 547, 1–6.
- Bieber, C., & Ruf, T. (2009). Summer dormancy in edible dormice (*Glis glis*) without energetic constraints. *Naturwissenschaften*, 96, 165–171. <https://doi.org/10.1007/s00114-008-0471-z>
- Biomatters (2017). *Geneious 10.1 Manual* (pp. 1–264).
- Bohmann, K., Monadjem, A., Lehmkuhl Noer, C., Rasmussen, M., Zeale, M. R. K., Clare, E., ... Gilbert, M. T. P. (2011). Molecular diet analysis of two African free-tailed bats (molossididae) using high throughput sequencing. *PLoS ONE*, 6(6), e21441. <https://doi.org/10.1371/journal.pone.0021441>
- Bollback, J. P. (2006). SIMMAP: Stochastic character mapping of discrete traits on phylogenies. *BMC Bioinformatics*, 7, 88. <https://doi.org/10.1186/1471-2105-7-88>
- Bonaccorso, F. J., Arends, A., Genoud, M., Cantoni, D., & Morton, T. (1992). Thermal ecology of moustached and ghost-faced bats

- (Mormoopidae) in Venezuela. *Journal of Mammalogy*, 73, 365–378. <https://doi.org/10.2307/1382071>
- Bonaccorso, F. J., Winkelmann, J. R., Dumont, E. R., & Bat, F. F. (2002). Home range of *Dobsonia minor* (Pteropodidae): A solitary, foliage-roosting fruit bat in Papua New Guinea. *Biotropica*, 34, 127–135. <https://doi.org/10.1111/j.1744-7429.2002.tb00248.x>
- Boyer, B. B., Ormseth, O. A., Buck, L., Nicolson, M., Pellemounter, M. A., & Barnes, B. M. (1997). Leptin prevents posthibernation weight gain but does not reduce energy expenditure in Arctic ground squirrels. *Comparative Biochemistry and Physiology Part C: Pharmacology, Toxicology and Endocrinology*, 118, 405–412. [https://doi.org/10.1016/S0742-8413\(97\)00172-2](https://doi.org/10.1016/S0742-8413(97)00172-2)
- Boyles, J. G., Bennett, N. C., Mohammed, O. B., & Alagaili, A. N. (2017). Torpor patterns in Desert Hedgehogs (*Paraechinus aethiopicus*) represent another new point along a thermoregulatory continuum. *Physiological and Biochemical Zoology*, 90, 445–452. <https://doi.org/10.1086/691542>
- Boyles, J. G., Thompson, A. B., McKechnie, A. E., Malan, E., Humphries, M. M., & Careau, V. (2013). A global heterothermic continuum in mammals. *Global Ecology and Biogeography*, 22, 1029–1039. <https://doi.org/10.1111/geb.12077>
- Brack, V. (2007). Temperatures and locations used by hibernating bats, including *Myotis sodalis* (Indiana bat), in a limestone mine: Implications for conservation and management. *Environmental Management*, 40, 739–746. <https://doi.org/10.1007/s00267-006-0274-y>
- Brigham, R. M. (1991). Prey detection, dietary niche breadth, and body size in bats: Why are aerial insectivorous bats so small? *American Naturalist*, 137, 693–703.
- Bumrungsri, S., Lang, D., Harrower, C., Sripaoraya, E., Kitpipit, K., & Racey, P. A. (2013). The dawn bat, *Eonycteris spelaea* Dobson (Chiroptera: Pteropodidae) feeds mainly on pollen of economically important food plants in Thailand. *Acta Chiropterologica*, 15, 95–104. <https://doi.org/10.3161/150811013X667894>
- Canale, C. I., Levesque, D. L., & Lovegrove, B. G. (2012). Tropical heterothermy: Does the exception prove the rule or force a re-definition. In T. Ruf, C. Bieber, W. Arnold, & E. Milleli (Eds.), *Living in a seasonal world* (pp. 29–40). Berlin, Germany: Springer-Verlag.
- Carey, H. V., Andrews, M. T., & Martin, S. L. (2003). Mammalian hibernation: Cellular and molecular responses to depressed metabolism and low temperature. *Physiological Reviews*, 83, 1153–1181. <https://doi.org/10.1152/physrev.00008.2003>
- Chen, J., Yuan, L., Sun, M., Zhang, L., & Zhang, S. (2008). Screening of hibernation-related genes in the brain of *Rhinolophus ferrumequinum* during hibernation. *Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology*, 149, 388–393. <https://doi.org/10.1016/j.cbpb.2007.10.011>
- Clarke, A., & Connor, M. I. O. (2014). Diet and body temperature in mammals and birds. *Global Ecology and Biogeography*, 23, 1000–1008. <https://doi.org/10.1111/geb.12185>
- Concannon, P., Levac, K., Rawson, R., Tennant, B., & Bensadoun, A. (2001). Seasonal changes in serum leptin, food intake, and body weight in photoentrained woodchucks. *American Journal of Physiology: Regulatory, Integrative and Comparative Physiology*, 281, R951–R959. <https://doi.org/10.1152/ajpregu.2001.281.3.R951>
- Cory Toussaint, D., & McKechnie, A. E. (2012). Interspecific variation in thermoregulation among three sympatric bats inhabiting a hot, semi-arid environment. *Journal of Comparative Physiology B: Biochemical, Systemic, and Environmental Physiology*, 182, 1129–1140. <https://doi.org/10.1007/s00360-012-0683-6>
- Cory Toussaint, D., McKechnie, A. E., & van der Merwe, M. (2010). Heterothermy in free-ranging male Egyptian Free-tailed bats (*Tadarida aegyptiaca*) in a subtropical climate. *Mammalian Biology*, 75, 466–470. <https://doi.org/10.1016/j.mambio.2009.06.001>
- Courts, S. E. (1998). Dietary strategies of Old World Fruit Bats (Megachiroptera, Pteropodidae): How do they obtain sufficient protein? *Mammal Review*, 28, 185–194. <https://doi.org/10.1046/j.1365-2907.1998.00033.x>
- Crespi, E. J., & Denver, R. J. (2016). Leptin (ob gene) of the South African clawed frog *Xenopus laevis*. *Proceedings of the National Academy of Sciences of the United States of America*, 103, 10092–10097. <https://doi.org/10.1073/pnas.0507519103>
- Crompton, A. W., Taylor, C. R., & Jagger, J. A. (1978). Evolution of homeothermy in mammals. *Nature*, 272, 333–336. <https://doi.org/10.1038/272333a0>
- Cryan, P. M. (2003). Sex differences in the thermoregulation and evaporative water loss of a heterothermic bat, *Lasiurus cinereus*, during its spring migration. *Journal of Experimental Biology*, 206, 3381–3390. <https://doi.org/10.1242/jeb.00574>
- Czenze, Z. J., Brigham, R. M., Hickey, A. J. R., & Parsons, S. (2017a). Winter climate affects torpor patterns and roost choice in New Zealand lesser short-tailed bats. *Journal of Zoology*, 303, 236–243. <https://doi.org/10.1111/jzo.12486>
- Czenze, Z. J., Brigham, R. M., Hickey, A. J. R., & Parsons, S. (2017b). Stressful summers? Torpor expression differs between high- and low-latitude populations of bats. *Journal of Mammalogy*, 98, 1249–1255. <https://doi.org/10.1093/jmammal/gyx071>
- Czenze, Z. J., & Dunbar, M. B. (2017). Hot bats go cold: Heterothermy in neotropical bats. *Canadian Journal of Zoology*, 95, 909–912.
- Delport, W., Poon, A. F. Y., Frost, S. D. W., & Kosakovsky Pond, S. L. (2010). Datamonkey 2010: A suite of phylogenetic analysis tools for evolutionary biology. *Bioinformatics*, 26, 2455–2457. <https://doi.org/10.1093/bioinformatics/btq429>
- Diekmann, Y., & Pereira-Leal, J. B. (2016). Gene tree affects inference of sites under selection by the branch-site test of positive selection. *Evolutionary Bioinformatics*, 11, 11–17. <https://doi.org/10.4137/EBO.S30902>
- Dietz, M., & Kalko, E. K. V. (2006). Seasonal changes in daily torpor patterns of free-ranging female and male Daubenton's bats (*Myotis daubentonii*). *Journal of Comparative Physiology B: Biochemical, Systemic, and Environmental Physiology*, 176, 223–231. <https://doi.org/10.1007/s00360-005-0043-x>
- Dodd, G. T., Worth, A. A., Nunn, N., Korpala, A. K., Bechtold, D. A., Allison, M. B., ... Luckman, S. M. (2014). The thermogenic effect of leptin is dependent on a distinct population of prolactin-releasing peptide neurons in the dorsomedial hypothalamus. *Cell Metabolism*, 20, 639–649. <https://doi.org/10.1016/j.cmet.2014.07.022>
- Dunbar, M. B., & Brigham, R. M. (2010). Thermoregulatory variation among populations of bats along a latitudinal gradient. *Journal of Comparative Physiology B: Biochemical, Systemic, and Environmental Physiology*, 180, 885–893. <https://doi.org/10.1007/s00360-010-0457-y>
- Dunbar, M. B., & Tomasi, T. E. (2006). Arousal patterns, metabolic rate, and an energy budget of eastern red bats (*Lasiurus borealis*) in winter. *Journal of Mammalogy*, 87, 1096–1102. <https://doi.org/10.1644/05-MAMM-A-254R3.1>
- Dzulhelmi, M. N., & Abdullah, M. T. (2009). Foraging ecology of the sunda colugo (*Galeopterus variegatus*) in bako national park, Sarawak, Malaysia. *Malayan Nature Journal*, 61, 285–294.
- Eisentraut, M. (1956). *Der Winterschlaf mit seinen ökologischen und physiologischen Begleiterscheinungen*.
- Enriori, P. J., Sinnayah, P., Simonds, S. E., Garcia Rudaz, C., & Cowley, M. A. (2011). Leptin action in the dorsomedial hypothalamus increases sympathetic tone to brown adipose tissue in spite of systemic leptin resistance. *Journal of Neuroscience*, 31, 12189–12197. <https://doi.org/10.1523/JNEUROSCI.2336-11.2011>
- Felsenstein, J. (2005). Using the quantitative genetic threshold model for inferences between and within species. *Philosophical Transactions of*

- the Royal Society of London. Series B, Biological Sciences, 360, 1427–1434. <https://doi.org/10.1098/rstb.2005.1669>
- Felsenstein, J. (2012). A comparative method for both discrete and continuous characters using the threshold model. *American Naturalist*, 179, 145–156. <https://doi.org/10.1086/663681>
- Fenton, M. B. (1990). The foraging behaviour and ecology of animal-eating bats. *Canadian Journal of Zoology*, 68, 411–422. <https://doi.org/10.1139/z90-061>
- Fenton, B. M., & Simmons, N. B. (2015). *Bats: A world of science and mystery*. Chicago, IL: University of Chicago Press.
- Fleming, T. H. (1991). The relationship between body size, diet, and habitat use in frugivorous bats, genus *Carollia* (Phyllostomidae). *Journal of Mammalogy*, 72, 493–501. <https://doi.org/10.2307/1382132>
- Frederich, R. C., Hamann, A., Anderson, S., Löllmann, B., Lowell, B. B., & Flier, J. S. (1995). Leptin levels reflect body lipid-content in mice – Evidence for diet-induced resistance to leptin action. *Nature Medicine*, 1, 1311–1314. <https://doi.org/10.1038/nm1295-1311>
- Freeman, P. W. (1979). Beetle-eating and moth-eating molossid bats. *Journal of Mammalogy*, 60, 467–479.
- Freeman, P. W. (1995). Nectarivorous feeding mechanisms in bats. *Biological Journal of the Linnean Society*, 56(3), 439–463. <https://doi.org/10.1111/j.1095-8312.1995.tb01104.x>
- Freeman, D. A., Lewis, D. A., Kauffman, A. S., Blum, R. M., & Dark, J. (2004). Reduced leptin concentrations are permissive for display of torpor in Siberian hamsters. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology*, 1650, 97–103. <https://doi.org/10.1152/ajpregu.00716.2003>
- Fristoe, T. S., Burger, J. R., Balk, M. A., Khaliq, I., Hof, C., & Brown, J. H. (2015). Metabolic heat production and thermal conductance are mass-independent adaptations to thermal environment in birds and mammals. *Proceedings of the National Academy of Sciences of the United States of America*, 112, 15934–15939. <https://doi.org/10.1073/pnas.1521662112>
- Gat-Yablonski, G., & Phillip, M. (2008). Leptin and regulation of linear growth. *Current Opinion in Clinical Nutrition and Metabolic Care*, 11, 303–308. <https://doi.org/10.1097/MCO.0b013e3282f795cf>
- Geiser, F. (2004). Metabolic rate and body temperature reduction during hibernation and daily torpor. *Annual Review of Physiology*, 66, 239–274. <https://doi.org/10.1146/annurev.physiol.66.032102.115105>
- Geiser, F. (2006). Energetic, thermal biology and torpor in Australian bats. In A. Zubaid, G. F. McCracken, & T. Kunz (Eds.), *Function and evolutionary ecology of bats* (pp. 1–22). Oxford, UK: Oxford University Press.
- Geiser, F., & Ruf, T. (1995). Hibernation versus daily torpor in mammals and birds: Physiological variables and classification of torpor patterns. *Physiological Zoology*, 68, 935–966. <https://doi.org/10.1086/physzool.68.6.30163788>
- Geiser, F., & Stawski, C. (2011). Hibernation and torpor in tropical and subtropical bats in relations to energetics, extinctions and the evolution of endothermy. *PLoS ONE*, 51, 337–348. <https://doi.org/10.1093/icb/1093/042>
- Geiser, F., & Turbill, C. (2009). Hibernation and daily torpor minimize mammalian extinctions. *Naturwissenschaften*, 96, 1235–1240. <https://doi.org/10.1007/s00114-009-0583-0>
- Genin, D., & Pijoan, A. P. (1993). Seasonality of goat diet and plant acceptabilities in the coastal scrub of Baja California, Mexico. *Small Ruminant Research*, 10, 1–11. [https://doi.org/10.1016/0921-4488\(93\)90102-N](https://doi.org/10.1016/0921-4488(93)90102-N)
- Genoud, M. (1993). Temperature regulation in subtropical tree bats. *Comparative Biochemistry and Physiology Part A: Physiology*, 104, 321–331. [https://doi.org/10.1016/0300-9629\(93\)90324-W](https://doi.org/10.1016/0300-9629(93)90324-W)
- Genoud, M., Bonaccorso, F. J., & Anends, A. (1990). Rate of metabolism and temperature regulation in two small tropical insectivorous bats (*Peropteryx macrotis* and *Natalus tumidirostris*). *Comparative Biochemistry and Physiology Part A: Physiology*, 97, 229–234. [https://doi.org/10.1016/0300-9629\(90\)90177-t](https://doi.org/10.1016/0300-9629(90)90177-t)
- Ghamari-Langroudi, M., Vella, K. R., Srisai, D., Sugrue, M. L., Hollenberg, A. N., & Cone, R. D. (2010). Regulation of thyrotropin-releasing hormone-expressing neurons in paraventricular nucleus of the hypothalamus by signals of adiposity. *Molecular Endocrinology*, 24, 2366–2381. <https://doi.org/10.1210/me.2010-0203>
- Grabek, K. R., Karimpour-Fard, A., Epperson, L. E., Hindle, A., Hunter, L. E., & Martin, S. L. (2011). Multistate proteomics analysis reveals novel strategies used by a hibernator to precondition the heart and conserve ATP for winter heterothermy. *Physiological Genomics*, 43, 1263–1275. <https://doi.org/10.1152/physiolgenomics.00125.2011>
- Grabek, K. R., Martin, S. L., & Hindle, A. G. (2015). Proteomics approaches shed new light on hibernation physiology. *Journal of Comparative Physiology B: Biochemical, Systemic, and Environmental Physiology*, 185, 607–627. <https://doi.org/10.1007/s00360-015-0905-9>
- Grigg, G. C., Beard, L. A., & Augee, M. L. (2004). The evolution of endothermy and its diversity in Mammals and Birds. *Physiological and Biochemical Zoology*, 77, 982–997. <https://doi.org/10.1086/425188>
- Harmata, W. (1987). The frequency of winter sleep interruptions in two species of bats hibernating in limestone tunnels. *Acta Theriologica (Warszawa)*, 32, 21–31. <https://doi.org/10.4098/AT.arch.87-23>
- Harmon, L. J., Weir, J. T., Brock, C. D., Glor, R. E., & Challenger, W. (2008). GEIGER: Investigating evolutionary radiations. *Bioinformatics*, 24, 129–131. <https://doi.org/10.1093/bioinformatics/btm538>
- Harvey, J., McKay, N. G., Walker, K. S., Van derKaay, J., Downes, C. P., & Ashford, M. L. J. (2000). Essential role of phosphoinositide 3-kinase in leptin-induced K ATP channel activation in the rat CRI-G1 insulinoma cell line. *Journal of Biological Chemistry*, 275, 4660–4669. <https://doi.org/10.1074/jbc.275.7.4660>
- He, L., Pan, Y., He, G., Lin, B., Liao, C.-C., Zuo, X., & Yuan, L. (2010). Structural and functional studies of leptins from hibernating and non-hibernating bats. *General and Comparative Endocrinology*, 168, 29–35. <https://doi.org/10.1016/j.ygcen.2010.04.001>
- Heard, D. J., & Whittier, D. A. (1997). Hematologic and plasma biochemical reference values for three flying fox species (*Pteropus* sp.). *American Association of Zoo Veterinarians*, 28, 464–470.
- Herreid, C. F. I. (1963). Temperature regulation of Mexican free-tailed bats in cave habitats. *Journal of Mammalogy*, 44, 560–573. <https://doi.org/10.2307/1377140>
- Herreid, C. F. II, & Schmidt-Nielsen, K. (1966). Oxygen consumption temperature and water loss in bats from different environments. *American Journal of Physiology*, 211, 1108–1112. <https://doi.org/10.1152/ajplegacy.1966.211.5.1108>
- Hill, J. E., & Smith, J. D. (1984). *Bats: A natural history*. Austin, TX: University of Texas Press.
- Hillenius, W. J., & Ruben, J. A. (2004). The evolution of endothermy in terrestrial vertebrates: Who? When? Why? *Physiological and Biochemical Zoology*, 77, 1019–1042. <https://doi.org/10.1086/425185>
- Hindle, A. G., Grabek, K. R., Epperson, L. E., Karimpour-Fard, A., & Martin, S. L. (2014). Metabolic changes associated with the long winter fast dominate the liver proteome in 13-lined ground squirrels. *Physiological Genomics*, 46, 348–361. <https://doi.org/10.1152/physiolgenomics.00190.2013>
- Hollar, L. J., & Springer, M. S. (1997). Old World fruitbat phylogeny: Evidence for convergent evolution and an endemic African clade. *Proceedings of the National Academy of Sciences of the United States of America*, 94, 5716–5721. <https://doi.org/10.1073/pnas.94.11.5716>
- Hope, P. R., & Jones, G. (2012). Warming up for dinner: Torpor and arousal in hibernating Natterer's bats (*Myotis nattereri*) studied by radio telemetry. *Journal of Comparative Physiology B: Biochemical, Systemic, and Environmental Physiology*, 182, 569–578. <https://doi.org/10.1007/s00360-011-0631-x>
- Hu, K. L., Wei, L., Zhu, T. T., Wang, X. Z., & Zhang, L. B. (2011). Dietary composition, echolocation pulses, and morphological measurements

- of the long-fingered bat *Miniopterus fuliginosus*. *Zoological Research*, 32, 163–167.
- Huelsenbeck, J. P., Nielsen, R., & Bollback, J. P. (2003). Stochastic mapping of morphological characters. *Systematic Biology*, 52, 131–158. <https://doi.org/10.1080/10635150390192780>
- Humphries, M. M., Thomas, D. W., & Speakman, J. R. (2002). Climate-mediated energetic constraints on the distribution of hibernating mammals. *Nature*, 418, 313–316. <https://doi.org/10.1038/nature00903.1>
- Ivanov, K. P. (2006). The development of the concepts of homeothermy and thermoregulation. *Journal of Thermal Biology*, 31, 24–29. <https://doi.org/10.1016/j.jtherbio.2005.12.005>
- Izoz, R. J. (1979). Winter range of the silver-haired bat. *Journal of Mammalogy*, 60, 641–643. <https://doi.org/10.2307/1380114>
- Jacobs, D. S., Eick, G. N., Schoeman, C., & Matthee, C. A. (2006). Cryptic species in an insectivorous bat, *Scotophilus dinganii*. *Journal of Mammalogy*, 87, 161–170. <https://doi.org/10.1644/04-MAMM-A-132R2.1>
- Jacobs, D. S., Kelly, E. J., Mason, M., & Stoffberg, S. (2007). Thermoregulation in two free-ranging subtropical insectivorous bat species: *Scotophilus* species (Vespertilionidae). *Canadian Journal of Zoology*, 85, 883–890. <https://doi.org/10.1139/Z07-067>
- Jastroch, M., Giroud, S., Barrett, P., Geiser, F., Heldmaier, G., & Herwig, A. (2016). Seasonal control of mammalian energy balance: Recent advances in the understanding of daily torpor and hibernation. *Journal of Neuroendocrinology*, 28, 1–6. <https://doi.org/10.1111/jne.12437>
- Jessen, N., Nielsen, T. S., Vendelbo, M. H., Viggers, R., Støen, O.-G., Evans, A., & Frøbert, O. (2016). Pronounced expression of the lipolytic inhibitor G0/G1 Switch Gene 2 (GOS2) in adipose tissue from brown bears (*Ursus arctos*) prior to hibernation. *Physiological Reports*, 4, e12781. <https://doi.org/10.14814/phy2.12781>
- Kaciuba-Usciz, H., Jessen, C., Feistkorn, G., & Brzezinska, Z. (1987). Work performance, thermoregulation and muscle metabolism in thyroidectomized goats (*Capra hircus*). *Comparative Biochemistry and Physiology Part A: Physiology*, 87, 915–921. [https://doi.org/10.1016/0300-9629\(87\)90015-6](https://doi.org/10.1016/0300-9629(87)90015-6)
- Kaiyala, K. J., Ogimoto, K., Nelson, J. T., Schwartz, M. W., & Morton, G. J. (2015). Leptin signaling is required for adaptive changes in food intake, but not energy expenditure, in response to different thermal conditions. *PLoS ONE*, 10, 1–19. <https://doi.org/10.1371/journal.pone.0119391>
- Kass, R. E., & Raftery, A. E. (1995). Bayes factors. *Journal of American Statistical Association*, 90, 773–795. <https://doi.org/10.1080/01621459.1995.10476572>
- Kayser, C. H. (1964). La dépense d'énergie des mammifères en hibernation. *Archives Des Sciences Physiologiques*, 18, 137–150.
- Kearse, M., Moir, R., Wilson, A., Stones-Havas, S., Cheung, M., Sturrock, S., ... Drummond, A. (2012). Geneious Basic: An integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics*, 28, 1647–1649. <https://doi.org/10.1093/bioinformatics/bts199>
- Kimura, K., & Uchida, T. A. (1983). Ultrastructural observations of delayed implantation in the Japanese long-fingered bat, *Miniopterus schreibersii-fuliginosus*. *Journal of Reproduction and Fertility*, 69, 187–193. <https://doi.org/10.1530/jrf.0.0690187>
- Koch, C. E., Lowe, C., Pretz, D., Steger, J., Williams, L. M., & Tups, A. (2014). High-fat diet induces leptin resistance in leptin-deficient mice. *Journal of Neuroendocrinology*, 26, 58–67. <https://doi.org/10.1111/jne.12131>
- Korine, C., Izhaki, I., & Arad, Z. (1999). Is the Egyptian fruit-bat *Rousettus aegyptiacus* a pest in Israel? An analysis of the bat's diet and implications for its conservation. *Biological Conservation*, 88, 301–306. [https://doi.org/10.1016/S0006-3207\(98\)00126-8](https://doi.org/10.1016/S0006-3207(98)00126-8)
- Kortner, G., & Geiser, F. (2000). Torpor and activity patterns in free-ranging sugar gliders *Petaurus breviceps* (Marsupialia). *Oecologia*, 123, 350–357. <https://doi.org/10.1007/s004420051021>
- Kosakovsky Pond, S. L., Murrell, B., Fourment, M., Frost, S. D. W., Delport, W., & Scheffler, K. (2011). A random effects branch-site model for detecting episodic diversifying selection. *Molecular Biology and Evolution*, 28, 3033–3043. <https://doi.org/10.1093/molbev/msr125>
- Koteja, P., Jurczyszyn, M., & Woloszyn, B. W. (2001). Energy balance of hibernating mouse-eared bat *Myotis myotis*: A study with a TOBEC instrument. *Acta Theriologica (Warszawa)*, 46, 1–12. <https://doi.org/10.1007/BF03192411>
- Kronfeld-Schor, N., Richardson, C., Silvia, B. A., Kunz, T. H., & Widmaier, E. P. (2000). Dissociation of leptin secretion and adiposity during prehibernatory fattening in little brown bats. *American Journal of Physiology: Regulatory, Integrative and Comparative Physiology*, 279, R1277–R1281. <https://doi.org/10.1159/000088358>
- Krutzsch, P. H. (1979). Male reproductive patterns in nonhibernating bats. *Journal of Reproduction and Fertility*, 56, 333–344. <https://doi.org/10.1530/jrf.0.0560369>
- Kulzer, E., Nelson, J. E., McKean, J. L., & Möhres, F. P. (1970). Untersuchungen über die Temperaturregulation australischer Fledermäuse (Microchiroptera). *Zeitschrift Für Vergleichende Physiologie*, 69, 426–451. <https://doi.org/10.1007/BF00333769>
- Kulzer, E., & Storf, R. (1980). Schlaf-Lethargie bei dem afrikanischen Langzungenflughund *Megaloglossus woermanni* Pagenstecher. *Zeitschrift Für Säugetierkunde*, 45, 23–29.
- Kulzer, E. (1965). *Temperaturregulation bei fledermäusen (Chiroptera) aus verschiedenen klimazonen*.
- Kusuminda, T., & Yapa, W. B. (2017). First record of wrinkle-lipped free-tailed bat *Chaerephon plicatus* Buchannan, 1800 (Mammalia: Chiroptera: Molossidae) colony in Sri Lanka, with notes on echolocation calls and taxonomy. *Journal of Threatened Taxa*, 9, 10115–10120.
- Leitner, P. (1966). Body temperature, oxygen consumption, heart rate and shivering in the California mastiff bat, *Eumops perotis*. *Comparative Biochemistry and Physiology*, 19, 431–443. [https://doi.org/10.1016/0010-406X\(66\)90152-6](https://doi.org/10.1016/0010-406X(66)90152-6)
- Levesque, D. L., Nowack, J., & Stawski, C. (2016). Modelling mammalian energetics: The heterothermy problem. *Climate Change Responses*, 3, 7. <https://doi.org/10.1186/s40665-016-0022-3>
- Levin, E., & Kronfeld-schor, N. (2012). Summer torpor and sexual segregation in the subtropical bat *Rhinopom*. In S. Giroud, C. Turbill, & T. Ruf (Eds.), *Living in a seasonal world* (pp. 481–491). Berlin, Germany: Springer-Verlag.
- Lewis, P. O. (2001). A likelihood approach to estimating phylogeny from discrete morphological character data. *Systematic Biology*, 50, 913–925. <https://doi.org/10.1080/106351501753462876>
- Liu, J. N., & Karasov, W. H. (2011). Hibernation in warm hibernacula by free-ranging Formosan leaf-nosed bats, *Hipposideros terasensis*, in subtropical Taiwan. *Journal of Comparative Physiology B: Biochemical, Systemic, and Environmental Physiology*, 181, 125–135. <https://doi.org/10.1007/s00360-010-0509-3>
- Lovegrove, B. G. (2012). The evolution of endothermy in Cenozoic mammals: A plesiomorphic-apomorphic continuum. *Biological Reviews*, 87(1), 128–162. <https://doi.org/10.1111/j.1469-185X.2011.00188.x>
- Lyman, C. P. (1970). Thermoregulation and metabolism in bats. In W. A. Wimsatt (Ed.), *Biology of bats* (pp. 301–330). Cambridge, MA: Academic Press.
- Ma, J., Jones, G., Zhang, S., Shen, J., Metzner, W., Zhang, L., & Liang, B. (2003). Dietary analysis confirms that Rickett's big-footed bat (*Myotis ricketti*) is a piscivore. *Journal of Zoology*, 261, 245–248. <https://doi.org/10.1017/S095283690300414x>
- Maloney, S. K., Bronner, G. N., & Buffenstein, R. (1999). Thermoregulation in the Angolan Free-Tailed Bat *Mops condylurus*: A Small Mammal That Uses Hot Roosts. *Physiological and Biochemical Zoology*, 72, 385–396. <https://doi.org/10.1086/316677>

- McNab, B. K., & Armstrong, M. I. (2001). Sexual dimorphism and scaling of energetics in flying foxes of the genus *Pteropus*. *Journal of Mammalogy*, 82, 709. [https://doi.org/10.1644/1545-1542\(2001\)082<0709:SDASOE>2.0.CO;2](https://doi.org/10.1644/1545-1542(2001)082<0709:SDASOE>2.0.CO;2)
- McNab, B. K., & Bonaccorso, F. J. (2001). The metabolism of New Guinean pteropodid bats. *Journal of Comparative Physiology B: Biochemical, Systemic, and Environmental Physiology*, 171, 201–214. <https://doi.org/10.1007/s003600000163>
- Mekjavic, I. B., & Eiken, O. (2006). Contribution of thermal and nonthermal factors to the regulation of body temperature in humans. *Journal of Applied Physiology*, 100, 2065–2072. <https://doi.org/10.1152/jappphysiol.01118.2005>
- Meredith, R. W., Janecka, J. E., Gatesy, J., Ryder, O. A., Fisher, C. A., Teeling, E. C., ... Murphy, W. J. (2011). Impacts of the cretaceous terrestrial revolution and KPg extinction on mammal diversification. *Science*, 334, 521–524. <https://doi.org/10.1126/science.1211028>
- Morin, P., & Storey, K. B. (2009). Mammalian hibernation: Differential gene expression and novel application of epigenetic controls. *International Journal of Developmental Biology*, 53, 433–442. <https://doi.org/10.1387/ijdb.082643pm>
- Murrell, B., Weaver, S., Smith, M. D., Wertheim, J. O., Murrell, S., Aylward, A., ... Kosakovsky Pond, S. L. (2015). Gene-wide identification of episodic selection. *Molecular Biology and Evolution*, 32, 1365–1371. <https://doi.org/10.1093/molbev/msv035>
- Murrell, B., Wertheim, J. O., Moola, S., Weighill, T., Scheffler, K., & Kosakovsky Pond, S. L. (2012). Detecting individual sites subject to episodic diversifying selection. *PLoS Genetics*, 8(7), e1002764. <https://doi.org/10.1371/journal.pgen.1002764>
- Naidoo, S., Mackey, R. L., & Schoeman, M. C. (2011). Foraging ecology of insectivorous bats (Chiroptera) at polluted and an unpolluted river in an urban landscape. *Durban Natural Science Museum Novitates*, 34, 21–28. <https://doi.org/10.1017/CBO9781107415324.004>
- Nielsen, R., & Yang, Z. (1998). Likelihood models for detecting positively selected amino acid sites and applications to the HIV-1 envelope gene. *Genetics*, 148, 929–936.
- Noll, U. G. (1979). Body temperature, oxygen consumption, noradrenaline response and cardiovascular adaptations in the flying fox, *Rousettus aegyptiacus*. *Comparative Biochemistry and Physiology Part A: Physiology*, 63, 79–88. [https://doi.org/10.1016/0300-9629\(79\)90631-5](https://doi.org/10.1016/0300-9629(79)90631-5)
- Nowak, R. M. (1994). *Walker's bats of the world*. Baltimore, MD: JHU Press.
- Ochoa-Acuña, H., & Kunz, T. H. (1999). Thermoregulatory behavior in the small island flying fox, *Pteropus hypomelanus* (Chiroptera: Pteropodidae). *Journal of Thermal Biology*, 24, 15–20. [https://doi.org/10.1016/S0306-4565\(98\)00033-3](https://doi.org/10.1016/S0306-4565(98)00033-3)
- O'Leary, M. A., Bloch, J. I., Flynn, J. J., Gaudin, T. J., Giallombardo, A., Giannini, N. P., ... Cirranello, A. I. (2013). The placental mammal ancestor and the post-K-Pg radiation of placentals. *Science*, 339, 662–667. <https://doi.org/10.1126/science.1229237>
- Ortega, J., & Alarcón-D, I. (2008). *Anoura geoffroyi* (Chiroptera: Phyllostomidae). *Mammalian Species*, 818, 1–7. <https://doi.org/10.1644/818.1.Key>
- Pagel, M. (1994). Detecting correlated evolution on phylogenies: a general method for the comparative analysis of discrete characters. *Proceedings of the Royal Society of London*, 255, 37–45. <https://doi.org/10.1098/rsta.1892.0001>
- Paradis, E., Claude, J., & Strimmer, K. (2004). APE: Analyses of phylogenetics and evolution in R language. *Bioinformatics*, 20, 289–290. <https://doi.org/10.1093/bioinformatics/btg412>
- Park, K. J., Jones, G., & Ransome, R. D. (1999). Winter activity of a population of greater horseshoe bats (*Rhinolophus ferrumequinum*). *Journal of Zoology*, 248, 419–427. <https://doi.org/10.1111/j.1469-7998.1999.tb01041.x>
- Pohl, H. (1961). *Temperaturregulation und Tagesperiodik des Stoffwechsels bei Winterschläfern*.
- Procaccini, C., Lourenco, E. V., Matarese, G., & La, C. A. (2009). Leptin signaling: A key pathway in immune responses. *NIH Public Access*, 4, 22–30. <https://doi.org/10.2174/157436209787048711>
- Raghuram, H., Thangadurai, C., Gopukumar, N., Nathar, K., & Sripathi, K. (2009). The role of olfaction and vision in the foraging behaviour of an echolocating megachiropteran fruit bat, *Rousettus leschenaulti* (Pteropodidae). *Mammalian Biology*, 74, 9–14. <https://doi.org/10.1016/j.mambio.2008.02.008>
- Ransome, R. (1990). *Natural History of Hibernating Bats*.
- Rashid, N., Irfan, M., Nadeem, M. S., & Shabbir, A. (2016). Comparative seasonal haematology of two bat species, *Scotophilus heathii* and *Pipistrellus pipistrellus*, in a Subtropical Area of Pakistan. *Pakistan Journal of Zoology*, 48, 1503–1510.
- Rasweiler, J. J. I. (1973). Care and management of the long-tongued bat, *Glossophaga soricina* (Chiroptera: Phyllostomatidae), in the laboratory, with observations on estivation induced by food deprivation. *Journal of Mammalogy*, 54, 391–404.
- Revell, L. J. (2012). phytools: An R package for phylogenetic comparative biology (and other things). *Methods in Ecology and Evolution*, 3, 217–223. <https://doi.org/10.1111/j.2041-210X.2011.00169.x>
- Rezai-Zadeh, K., Yu, S., Jiang, Y., Laque, A., Schwartzburg, C., Morrison, C. D., ... Münzberg, H. (2014). Leptin receptor neurons in the dorso-medial hypothalamus are key regulators of energy expenditure and body weight, but not food intake. *Molecular Metabolism*, 3, 681–693. <https://doi.org/10.1016/j.molmet.2014.07.008>
- Richter, H. V., & Cumming, G. S. (2006). Food availability and annual migration of the straw-colored fruit bat (*Eidolon helvum*). *Journal of Zoology*, 268, 35–44. <https://doi.org/10.1111/j.1469-7998.2005.00020.x>
- Riedesel, M. L., & Williams, B. A. (1976). Continuous 24-hour oxygen consumption studies of myotis velifer. *Biochem Physiol*, 54A, 95–99.
- Rodriguez-Duran, A. (1995). Metabolic rates and thermal conductance in four species of neotropical bats roosting in hot caves. *Comparative Biochemistry and Physiology*, 110, 347–355. [https://doi.org/10.1016/0300-9629\(94\)00174-R](https://doi.org/10.1016/0300-9629(94)00174-R)
- Rojas, D., Vale, Á., Ferrero, V., & Navarro, L. (2011). When did plants become important to leaf-nosed bats? Diversification of feeding habits in the family Phyllostomidae. *Molecular Ecology*, 20, 2217–2228. <https://doi.org/10.1111/j.1365-294X.2011.05082.x>
- Ruby, J., Nathan, P. T., Balasingh, J., & Kunz, T. H. (2000). *Chemical composition of fruits and leaves eaten by short-nosed fruit bat, Cynopterus sphinx*.
- Ruf, T., & Geiser, F. (2015). Daily torpor and hibernation in birds and mammals. *Biological Reviews*, 90, 1–70. <https://doi.org/10.1111/brv.12137>
- Russo, D., Cistrone, L., Budinski, I., Console, G., Della Corte, M., Milighetti, C., ... Ancillotto, L. (2017). Sociality influences thermoregulation and roost switching in a forest bat using ephemeral roosts. *Ecology and Evolution*, 7, 5310–5321. <https://doi.org/10.1002/ece3.3111>
- Rydell, J., & Arlettaz, R. (1994). Low-frequency echolocation enables the bat *Tadarida teniotis* to feed on tympanate insects. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, 257, 175–178.
- Salinas, R. V. B., Herrera, M. L. G., Flores-Martínez, J. J., & Johnston, D. S. (2014). Winter and summer torpor in a free-ranging subtropical desert bat: The fishing myotis (*Myotis vivesi*). *Acta Chiropterologica*, 16, 327–336. <https://doi.org/10.3161/150811014X687288>
- Scholander, P. F., Hock, R., Walters, V., & Johnson, F. (1950). Heat regulation in some arctic and tropical mammals and birds. *Biological Bulletin*, 99, 237–258. <https://doi.org/10.2307/1538741>
- Schwartz, C., Hampton, M., & Andrews, M. T. (2013). Seasonal and regional differences in gene expression in the brain of a hibernating mammal. *PLoS ONE*, 8(3), e58427. <https://doi.org/10.1371/journal.pone.0058427>
- Sharifi, M., & Hemmati, Z. (2002). *Variation in the diet of the Greater Mouse-tailed Bat, Rhinopoma microphyllum* (Chiroptera: Rhinopomatidae) in south-western Iran In dry habitats, large and seasonally rich insect patches may support large colonies of bats, 7140.

- Simmons, N. B. (2005). An eocene big bang for bats. *Evolution (New York)*, 307, 527–529.
- Simmons, N. B., & Geisler, J. H. (1998). Phylogenetic relationships of Icaronycteris, Archaeonycteris, Hassianycteris, and Palaeochiropteryx to extant bat lineages, with comments on the evolution of echolocation and foraging strategies in Microchiroptera. *Bulletin of the American Museum of Natural History*, 55, 4–182.
- Simmons, N. B., Seymour, K. L., Habersetzer, J., & Gunnell, G. F. (2008). Primitive Early Eocene bat from Wyoming and the evolution of flight and echolocation. *Nature*, 451, 818–821. <https://doi.org/10.1038/nature06549>
- Smith, L., & Hood, L. (1987). Mapping and sequencing the human genome: How to proceed. *Nature Biotechnology*, 5, 933–939. <https://doi.org/10.1038/nbt0987-933>
- Solari, S., Hooper, S. R., Larsen, P. A., Brown, A. D., Bull, R. J., Guerrero, J. A., ... Baker, R. J. (2009). Operational criteria for genetically defined species: analysis of the diversification of the small fruit-eating bats, Dermanura (Phyllostomidae: Stenodermatinae). *Acta Chiropterologica*, 11, 279–288. <https://doi.org/10.3161/150811009X485521>
- Srivastava, R. K., & Krishna, A. (2008). Seasonal adiposity, correlative changes in metabolic factors and unique reproductive activity in a vespertilionid bat, *Scotophilus heathi*. *Journal of Experimental Zoology Part A: Ecological Genetics and Physiology*, 309, 94–110. <https://doi.org/10.1002/jez.440>
- Stawski, C., & Currie, S. E. (2016). Effect of roost choice on winter torpor patterns of a free-ranging insectivorous bat. *Australian Journal of Zoology*, 64, 132–137. <https://doi.org/10.1071/ZO16030>
- Stawski, C., & Geiser, F. (2012). Will temperature effects or phenotypic plasticity determine the thermal response of a heterothermic tropical bat to climate change? *PLoS ONE*, 7, e40278. <https://doi.org/10.1371/journal.pone.0040278>
- Stawski, C., Turbill, C., & Geiser, F. (2009). Hibernation by a free-ranging subtropical bat (*Nyctophilus bifax*). *Journal of Comparative Physiology B: Biochemical, Systemic, and Environmental Physiology*, 179, 433–441. <https://doi.org/10.1007/s00360-008-0328-y>
- Stawski, C., Willis, C. K. R., & Geiser, F. (2014). The importance of temporal heterothermy in bats. *Journal of Zoology*, 292, 86–100. <https://doi.org/10.1111/jzo.12105>
- Stehling, O., Doring, H., Ertl, J., Preibisch, G., & Schmidt, I. (1996). Leptin reduces juvenile fat stores by altering the circadian cycle of energy expenditure. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology*, 40, R1770–R1774. <https://doi.org/10.1152/ajpregu.1996.271.6.R1770>
- Stier, S. C., & Mildenstein, T. L. (2005). Dietary habits of the world's largest bats: The Philippine Flying Foxes, *Acerodon jubatus* and *Pteropus vampyrus lanensis*. *Journal of Mammalogy*, 86, 719–728.
- Swoap, S. J. (2008). The pharmacology and molecular mechanisms underlying temperature regulation and torpor. *Biochemical Pharmacology*, 76, 817–824. <https://doi.org/10.1016/j.bcp.2008.06.017>
- Tamura, K., & Nei, M. (1993). Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. *Molecular Biology and Evolution*, 10, 512–526. <https://doi.org/10.1093/molbev/msl149>
- Tattersall, G. J., Sinclair, B. J., Withers, P. C., Fields, P. A., Seebacher, F., Cooper, C. E., & Maloney, S. K. (2012). Coping with thermal challenges: Physiological adaptations to environmental temperatures. *Comprehensive Physiology*, 2, 2151–2202. <https://doi.org/10.1002/cphy.c110055>
- Tavaré, S. (1986). Some probabilistic and statistical problems in the analysis of DNA sequences. *American Mathematical Society Lectures on Mathematics in the Life Sciences*, 17, 57–86.
- Teeling, E. C. (2005). A molecular phylogeny of bats illuminates biogeography and the fossil record. *Science*, 307, 580–584.
- Tessier, S. N., & Storey, K. B. (2010). Expression of myocyte enhancer factor-2 and downstream genes in ground squirrel skeletal muscle during hibernation. *Molecular and Cellular Biochemistry*, 344, 151–162. <https://doi.org/10.1007/s11010-010-0538-y>
- Tinkle, D. W., & Patterson, I. G. (1965). A study of hibernating populations of *Myotis velifer* in Northwestern Texas. *Journal of Mammalogy*, 46, 612–633. <https://doi.org/10.2307/1377932>
- Turbill, C., Law, B. S., & Geiser, F. (2003). Summer torpor in a free-ranging bat from subtropical Australia. *Journal of Thermal Biology*, 28, 223–226. [https://doi.org/10.1016/S0306-4565\(02\)00067-0](https://doi.org/10.1016/S0306-4565(02)00067-0)
- Twente, J. W., & Twente, J. A. (1964). *An hypothesis concerning the evolution of heterothermy in bats*.
- van Breukelen, F., & Martin, S. L. (2015). The hibernation continuum: Physiological and molecular aspects of metabolic plasticity in mammals. *Physiology (Bethesda)*, 30, 273–281. <https://doi.org/10.1152/physiol.00010.2015>
- Van Der Merwe, M. (1973). Aspects of temperature and humidity in preferred hibernation sites of the natal clinging bat *Miniopterus Schreibersi Natalensis* (A. Smith, 1834). *Zoologica Africana*, 8, 121–134. <https://doi.org/10.1080/00445096.1973.11447471>
- Vaughan, N. (1997). The diets of British bats (Chiroptera). *Mammal Review*, 27, 77–94. <https://doi.org/10.1111/j.1365-2907.1997.tb00373.x>
- Villanueva-Cañas, J. L., Faherty, S. L., Yoder, A. D., & Albà, M. M. (2014). Comparative genomics of mammalian hibernators using gene networks. *Integrative and Comparative Biology*, 54, 452–462. <https://doi.org/10.1093/icb/048>
- Vivier, L., & Van Der Merwe, M. (2007). The incidence of torpor in winter and summer in the Angolan free-tailed bat, *Mops condylurus* (Microchiroptera: Molossidae), in a subtropical environment, Mpumulanga, South Africa. *African Zoology*, 42, 50–58. [https://doi.org/10.3377/1562-7020\(2007\)42\[50:TIIOTIW\]2.0.CO;2](https://doi.org/10.3377/1562-7020(2007)42[50:TIIOTIW]2.0.CO;2)
- Weigle, D. S., Breen, P. A., Matthys, C. C., Callahan, H. S., Meeuws, K. E., Burden, V. R., & Purnell, J. Q. (2005). A high-protein diet induces sustained reductions in appetite, ad libitum caloric intake, and body weight despite compensatory changes in diurnal plasma leptin and ghrelin concentrations. *American Journal of Clinical Nutrition*, 82, 41–48. <https://doi.org/10.1093/ajcn/82.1.41>
- Weterings, R., Wardenaar, J., Dunn, S., & Umponstira, C. (2015). Dietary analysis of five insectivorous bat species from Kamphaeng Phet, Thailand. *Raffles Bulletin of Zoology*, 63, 91–96. <https://doi.org/10.3161/150811010X537927>
- Whitaker, J. O., & Karataş, A. (2009). Food and feeding habits of some bats from Turkey. *Acta Chiropterologica*, 11, 393–403. <https://doi.org/10.3161/150811009X485611>
- Willis, C. K. R., Brigham, R. M., & Geiser, F. (2006). Deep, prolonged torpor by pregnant, free-ranging bats. *Naturwissenschaften*, 93(2), 80–83. <https://doi.org/10.1007/s00114-005-0063-0>
- Wilz, M., & Heldmaier, G. (2000). Comparison of hibernation, estivation and daily torpor in the edible dormouse, *Glis glis*. *Journal of Comparative Physiology B*, 170, 511–521. <https://doi.org/10.1007/s003600000129>
- Yan, J., Barnes, B. M., Kohl, F., & Marr, T. G. (2008). Modulation of gene expression in hibernating arctic ground squirrels. *Physiological Genomics*, 32, 170–181. <https://doi.org/10.1152/physiolgenomics.00075.2007>
- Yang, Z., & Nielsen, R. (2002). Codon-substitution models for detecting molecular adaptation at individual sites along specific lineages. *Molecular Biology and Evolution*, 19, 908–917. <https://doi.org/10.1093/oxfordjournals.molbev.a004148>
- Yang, J., Wang, Z. L., Zhao, X. Q., Wang, D. P., Qi, D. L., Xu, B. H., ... Tian, H. F. (2008). Natural selection and adaptive evolution of leptin in the *Ochotona* family driven by the cold environmental stress. *PLoS ONE*, 3(1), e1472. <https://doi.org/10.1371/journal.pone.0001472>
- Young, R. A. (2001). The eastern horseshoe bat, *Rhinolophus megaphyllus*, in south-east Queensland, Australia: Colony demography and dynamics, activity levels, seasonal weight changes, and capture-recapture analyses. *Wildlife Research*, 28(4), 425–434.

- Yuan, L., Zhao, X., Lin, B., Rossiter, S. J., He, L., Zuo, X., ... Zhang, S. (2011). Adaptive evolution of leptin in heterothermic bats. *PLoS ONE*, 6(11), e27189. <https://doi.org/10.1371/journal.pone.0027189>
- Zaidan, N. K. K. S. (1980). *Aging phenomena: Relationships among different levels of organization*. New York, NY: Plenum Press.
- Zhang, G., Cowled, C., Shi, Z., Huang, Z., Bishop-Lilly, K. A., Fang, X., ... Wang, J. (2013) Comparative analysis of bat genomes provides insight into the evolution of flight and immunity. *Science*, 339(6118), 456–460. <https://doi.org/10.1126/science.1230835>
- Zhang, Y., Kerman, I. A., Laque, A., Nguyen, P., Faouzi, M., Louis, G. W., ... Munzberg, H. (2011). Leptin-receptor-expressing neurons in the dorsomedial hypothalamus and median preoptic area regulate sympathetic brown adipose tissue circuits. *Journal of Neuroscience*, 31, 1873–1884. <https://doi.org/10.1523/JNEUROSCI.3223-10.2011>
- Zhang, Y., Pan, Y.-H., Yin, Q., Yang, T., Dong, D., Liao, C.-C., & Zhang, S. (2014). Critical roles of mitochondria in brain activities of torpid *Myotis ricketti* bats revealed by a proteomic approach. *Journal of Proteomics*, 105, 266–284. <https://doi.org/10.1016/j.jprot.2014.01.006>
- Zhu, T., Yuan, L., Jones, G., Hua, P., He, G., Chen, J., & Zhang, S. (2014). OB-RL silencing inhibits the thermoregulatory ability of Great Roundleaf

Bats (*Hipposideros armiger*). *General and Comparative Endocrinology*, 204, 80–87. <https://doi.org/10.1016/j.ygcen.2014.04.028>

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

How to cite this article: Lazzeroni ME, Burbrink FT, Simmons NB. Hibernation in bats (Mammalia: Chiroptera) did not evolve through positive selection of leptin. *Ecol Evol*. 2018;8:12576–12596. <https://doi.org/10.1002/ece3.4674>