



Paleontology and paleoecology of guano deposits in Mammoth Cave, Kentucky, USA



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ABSTRACT

Bat guano deposits are common in the Mammoth Cave system (Kentucky, USA). Paleontological remains associated with these deposits are important records of local landscape changes. Recent excavations in the cave suggest that vertebrate remains in most of these deposits are dominated by Chiroptera. Although no extinct fauna were identified, the presence of a large roost of *Tadarida brasiliensis* in the Chief City section is beyond the northern extent of its current range suggesting that this deposit dates to an undetermined interglacial period. Stable isotope analyses of *Tadarida*-associated guano indicate a C3 prey signature characteristic of forested habitat. This was unexpected since this species is typically associated with open environments. Further ecomorphological analysis of wing shape trends in interglacial, Holocene, and historic-aged assemblages indicate that interglacial faunas are dominated by fast-flying, open-space taxa (*T. brasiliensis*) while late Holocene and Historic assemblages contain more taxa that utilized closed forest or forest gaps.

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Introduction

Bat guano is an important component of the Mammoth Cave paleontological record. Guano derived from roosting bats provides a nutrient-rich substrate for life in deep cave environments. In addition to the remains of bats that created the guano, arthropod, microbe, and non-aerial vertebrate communities rely on guano nutrients (Fletcher, 1982; Fenolio et al., 2006). In Mammoth Cave, all of these guano communities have the potential to be recovered from guano deposits.

The guano of insectivorous bats is a valuable archive of paleo-environmental information. Osteological remains of guano-producers and associated fauna are often preserved in cave guano deposits (Frank and Benson, 1998; McFarlane et al., 2002; Gaudin et al., 2011) providing specific clues to the ecological context of guano communities. Recent work has explored the use of stable isotopes and pollen in bat guano as climate proxies (Maher, 2006; Wurster et al., 2007, 2008, 2010a,b; Batina and Reese, 2011). The balance of research on cave guano deposits in the last few decades has demonstrated the importance of these contexts for preserving materials that are useful for reconstructing local climate and ecological conditions.

Paleontological remains within Mammoth Cave have been recognized since the 1950s. Although extensively mined in the early 19th century for the production of salt-peter, significant deposits of guano remain, most notably in the Chief City section. Jegla and Hall (1962) reported on the fossil *Tadarida* from this area, indicating an age for the guano deposits

>38,000 ¹⁴C yr BP. Investigations carried out nearly 40 yr later found guano in this area to be >54,000 ¹⁴C yr BP (Colburn, 2005).

Mammoth Cave National Park was formally established in 1941. Located in the central Kentucky counties of Barren, Edmonson, and Hart, the principal feature of the Park is Mammoth Cave, the world's longest mapped cave system (~644 km, mapped length). The portion of the Park south of the Green River lies within the Central Kentucky Karst portion of the Pennyroyal Plateau, while the northern portion lies within the Chester Upland of the Western Coal Field (Palmer, 1981). The cave lies entirely within the Interior Low Plateaus of the Interior Plains physiographic province.

The primary cave strata are Mississippian limestones including the St. Louis, Ste. Genevieve, and Girkin formations. In the uplands near the Green River, these strata are capped by the Big Clifty Formation, a sandstone layer roughly 15 m thick (Palmer, 1981:48).

This study focuses on the identification of materials recovered during the Mammoth Cave Trail Rehabilitation Project (2007–2008). After a discussion of the geochronological context of paleontological samples, we shift to our main analytical focus, the identification of vertebrate remains (MC) and the geochemical analysis of guano-bearing sediments (CW).

Materials and methods

Field sampling and excavation

Coordinated excavations with the University of Kentucky, Program for Archaeological Research were undertaken in three field sessions between

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Table 1
Size categories in identified bat remains.

Small bat
<i>Myotis leibii</i>
<i>Perimyotis subflavus</i>
Medium bat
<i>Corynorhinus rafinesquii</i>
<i>Corynorhinus townsendii</i>
<i>Lasionycteris noctivagans</i>
<i>Myotis lucifugus</i>
<i>Myotis septentrionalis</i>
<i>Myotis austroriparius</i>
<i>Myotis sodalis</i>
<i>Lasiurus borealis</i>
<i>Myotis grisescens</i>
<i>Nycticeius humeralis</i>
<i>Tadarida brasiliensis</i>
Large bat
<i>Eptesicus fuscus</i>
<i>Lasiurus cinereus</i>

December 2007 and July 2008 (Ahler, 2012). Areas of archaeological and paleontological importance were examined via test excavations and spot sediment sampling (Table S1; Fig. S1). A total of 43 m² was excavated throughout the Historic Section, Historic Lantern Tour, Gothic Avenue, Cleveland Avenue, and Frozen Niagara. Additional spot collecting for paleontological remains was undertaken in Great Onyx Cave. This total encompasses all of the excavation undertaken for this project, including areas of both archaeological and paleontological importance. Test units targeting archaeological contexts were dry-screened through 6.5 mm mesh in the cave. Intact paleontological deposits were typically removed from the cave as bulk samples. In addition to a small number of bones that were removed directly from excavation units, ~1233 L (~280 gal) of sediment and guano samples were transported to the Illinois State Museum to be processed and analyzed for paleontological remains. To our knowledge, these are the first stratigraphically controlled paleontological excavations to take place within the cave.

Paleontological remains and trace organic materials

Bulk sediment samples were set aside for off-site processing and paleontological analyses based on their potential to preserve small bones or organic materials (i.e., hair, insect remains). Large bulk samples (>1 gal) were wet screened through nested 6.4 mm, 2.0 mm, and 0.8 mm screens. Small samples (<4.3 L or <1 gal) were dry screened through the same mesh sizes and the residue retained in case it was needed for further analyses. All samples were subsequently sorted by hand and a 1× hand-lens. Identifiable bones were removed from all screened samples, however, some unidentifiable, fragmentary remains were left in the smallest sized matrix (<2.0 mm) because we could not justify the labor of sorting very small materials that were undiagnostic below family level. All extracted bones were subsequently examined, and the number of identified specimens (NISP) calculated. Portions of the remaining bulk samples were examined for trace organic materials (e.g., hair, insect cuticle) and processed for biogeochemical analyses (¹⁴C and stable isotopes). We identified osteological remains to the finest taxonomic category possible, which in many cases meant only identification to higher taxonomic levels encompassing multiple species or even genera. Sometimes remains could not be identified any more precisely than simply “Bat” with reference to relative size (Table 1). Taxonomic identification of specimens is tabulated by excavation unit and cave section (Tables S1, S2).

Stable isotope and ¹⁴C analyses

Wet chemistry pretreatment of bulk guano samples followed protocols modified from Mizutani et al. (1992). Bulk samples were dry-sieved and the 1 mm to 220 μm fraction retained for analyses. These samples were de-carbonated with 2M HCl, for 3–4 h, until the reaction was complete. The resulting slurry was screened through 10 μm Nitex mesh to remove clay-sized particles, rinsed to neutrality with DI water and lyophilized. The remaining sample consists of highly comminuted organic material and silicate minerals. Examination under low- and high-power microscopy indicate the organic fraction of these samples is predominantly insect fragments, with varying amounts of detrital charcoal and trace amounts of degraded chiropterid hair. Although these protocols are similar to previous work on bat guano deposits from the West Indies (McFarlane et al., 2002), they are not as aggressive as the guano pretreatment technique of Wurster et al. (2010b) in their study of *Tadarida* guano deposits in Arizona. Trials on MACA guano samples utilizing these methods yielded very low sample weights, primarily due to the chemical removal of the highly comminuted insect fragments.

Stable carbon isotope analyses of biological tissues have the potential to quantitatively measure contributions of C3 and C4 vegetation to herbivore diets (DeNiro and Epstein, 1978; Schimmelmann and DeNiro, 1985). The source of guano deposits in Mammoth Cave is insectivorous bats. The guano produced by these animals contains abundant, albeit fragmentary, remains of their insect prey. In eastern North America, bats are relatively flexible feeders, optimally utilizing seasonal “booms” in the populations of certain insect taxa (Lacki et al., 1995; Lee and McCracken, 2005).

Stable isotope analyses were performed on 27 bulk guano samples. After de-carbonation, organic preservation was evaluated through C:N (Carbon:Nitrogen ratio) and loss-on-ignition analyses. C:N and stable isotope ($\delta^{13}\text{C}$, $\delta^{15}\text{N}$) analyses took place on a ThermoFinnigan MAT 253 continuous flow system with an attached thermal combustion/elemental analyzer device at the Keck Paleoenvironmental and Environmental Stable Isotope Laboratory at the University of Kansas, Lawrence. Montana soil (NIST Ref Mat. 2711) and a peach leaves standard (NIST Ref Mat. 1547) were used to determine quality control. Results compiled over 2 yr show the $\delta^{15}\text{N}$ to be measured better than $\pm 0.42\%$, and the $\delta^{13}\text{C}$ to be better than $\pm 0.22\%$. Loss-on-ignition (LOI) analyses follow internal lab protocols used in sediment analyses (following Dean, 1974). Small (~0.5 mL) samples were dried overnight at 100 °C, weighed and ignited at 500 °C for 1 h, then weighed. They were ignited again at 900 °C for 1 h, and weighed a second time. Prior to weighing, samples were cooled in a desiccator. Complete combustion of organic matter was expected to occur at 500 °C, and the disassociation of CaCO₃ at 900 °C.

Decarbonated bulk guano samples were submitted to the W. M. Keck Carbon Cycle AMS facility at the University of California, Irvine and Aeon Laboratories in Tucson, AZ for ¹⁴C dating. All finite ¹⁴C dates were calibrated using Calib 7.0 (Stuiver and Reimer, 1993) using the intCal13 dataset (Reimer et al., 2013). All reported calibrated age ranges are 2-sigma (Table 2). It is important to note that all ¹⁴C dates are on bulk guano, therefore they provide only minimum constraints on the age of cave deposits in this study (Wurster et al., 2009).

Results

Chronology of paleontological localities

Four chronological periods are recognized in the Mammoth Cave paleontological record based on stratigraphic context, geochronological data, and biogeographic information. The earliest contexts are those associated with abundant *T. brasiliensis* remains dating to an undetermined interglacial period. A second group of assemblages from Gothic Avenue dates to the late Wisconsinan Farmdalian interstadial. Finally,

Table 2
Results of C:N, LOI, stable isotope, and ^{14}C analyses.

Age	Cave section	ISM #	Unit-lvl	LOI org (%)	$\delta^{15}\text{N}$ (Air)	$\delta^{13}\text{C}$ (PDB)	N%	C%	C/N	C/N quality ^c	Radiocarbon age ^{14}C yr BP (Lab number)	Calibrated age cal yr BP
Late Holocene	Gothic Avenue	09_53	P2-2B	5.18	6.55	-27.25	0.12	1.2	9.5	3		
	Chief City	09_64	T2-3	8.7	17.94	-26.07	0.34	1.4	4.1	2		
Farmdalian interstadial	Gothic Avenue	09_69	P1-3a	5.19	7.36	-26.96	0.11	0.5	4.7	1		
	Gothic Avenue	09_78	P1-3b	5.24	6.15	-26.62	0.14	0.7	4.9	1		
	Gothic Avenue	09_70	P2-3a		7.65	-26.2	0.07	0.3	4.2	2		
Interglacial	Gothic Avenue	09_68	P2-3b	3.95	7.78	-25.01	0.11	0.5	4.3	2	23,910 ± 490 (UCIAMS76221)	27,200–29,000
	Chief City	09_79	T7-2	19.75	23.27	-26.6	7.49	35.9	4.8	1		
	Chief City	09_75	T7-3	25.21	23.82	-26.62	10.27	44	4.3	2	33,090 ± 360 (UCIAMS76223)	36,300–38,300
	Chief City	09_76 ^a	T7-3	38.84	23.07	-26.63	11.66	48.1	4.1	2		
	Chief City	09_77	T7-5	40.48	22.82	-26.68	10.3	48.5	4.7	1	>48,000 (Aeon 826)	>48,000
	Chief City	09_42	U2-1	18.73	23.76	-26.45	8.41	37.6	4.5	1		
	Chief City	09_43	U2-1	12.69	24.27	-26.54	10.38	47.5	4.6	1		
	Chief City	09_41 ^a	U2-1	25.22	22.26	-26.26	11.43	44.8	3.9	3		
	Chief City	09_47	U2-3	8.31	23.23	-25.88	1.07	4.3	4.1	2	32,420 ± 1090 (UCIAMS76222)	34,300–39,000
	Chief City	09_46	U2-4	7.25	23.6	-25.96	0.42	1.7	4.1	2		
	Chief City	09_65	U2-4	24.74	23.02	-26.64	4.47	20.1	4.5	1		
	Chief City	09_55	U2-4	2.01	20.24	-3.37	0.11	2.7	23.4	3		
	Chief City	09_36	U2-4	18.42	23.74	-26.34	4.91	21.6	4.4	1		
	Chief City	09_40	U2-4	13.78	24.01	-26.51	3.94	17.3	4.4	1		
	Chief City	09_38	U2-4	21.85	23.12	-26.47	5.24	24.6	4.7	1		
	Chief City	09_44	U2-4	16.96	23.77	-26.4	4.49	20	4.5	1		
	Chief City	09_39 ^a	U2-4	40.63	23.05	-26.43	10.19	50.2	4.9	1		
Chief City	09_50 ^b	W1-4	19.26	22.26	-26.62	5.27	27.1	5.1	2	30,580 ± 300 (UCIAMS76220)	34,000–35,100	
Chief City	09_51	W1-5	10.36	21.77	-26.82	0.84	4.2	5	1			
Unknown age	Haines Dome	09_48	X1-2	9.13	21.16	-25.81	1.08	6.4	6	3	18,560 ± 210 (UCIAMS76219)	21,900–22,900
	Chief City	09_54	T5-3	5.93	23.04	-25.76	1.26	4.9	3.9	3		
	Chief City	09_49	U3-3	13.04	25.53	-26.21	2.79	12.7	4.5	1		

^a Compressed guano.

^b Acid-Base-Acid pretreatment yielded a ^{14}C date of $13,735 \pm 45$ ^{14}C yr BP (UCIAMS76212), 16,300–16,800 cal yr BP.

^c 1) Fresh guano, C:N = 4.4–5.0; 2) Recent guano exhibiting limited diagenesis, C:N = 4.1–5.4; and 3) Diagenetically altered guano. C:N ranges from Wurster et al., 2007.

late Holocene and Historic assemblages are identified by associated cultural debris (Table S3).

Tadarida-bearing deposits (unspecified interglacial period)

Consideration of bat zoogeography is key to constraining the age of these deposits. Guano in the lower excavation levels of Chief City, adjacent areas of Settle's Gorge and Mummy's Ledge, and Haines Dome, are massive and the result of large maternity colonies roosting in the cave for many years. Mammoth Cave is 300 km north of the modern *Tadarida* range in the eastern U.S., which is primarily distributed along the Gulf and Atlantic coastal plains (Fig. 1). Although *Tadarida* vagrants have rarely been recorded in Kentucky (Barbour and Davis, 1974) they occur only as individuals and do not congregate in large roosts. Notably, the nearest *Tadarida* roosts of appreciable size are located in Florida or Arkansas (Brown, 1997; Saughey et al., 2001; Whitaker and Hamilton, 1998; Wilkins, 1989). In the eastern US, colonies typically occupy human structures and are much smaller than those that would have produced the massive guano deposits in Mammoth Cave. Modern *Tadarida* in the southeastern US avoids natural features such as caves for major roost sites (Whitaker and Hamilton, 1998).

By this logic, the presence of large *Tadarida* maternity colonies in Mammoth Cave suggest much warmer conditions than present, probably an interglacial period. The most recent interglacial is the Sangamon period (Marine Oxygen Isotope Stage [MIS] 5e) represented locally in the speleothem record from Davis Hall by an abrupt increase in $\delta^{18}\text{O}$ at ~110–121 ka (Harmon et al., 1978). A second, possibly more extended warm period is evident in the Mammoth Cave speleothem record between ~170 and 220 ka which is roughly co-eval with globally warmer conditions during MIS 7a/e (~180–220 ka). Massive guano deposits from *Tadarida*-associated assemblages may be attributed to these, or possibly an earlier interglacial age.

Bulk guano and bone samples from excavation units were also submitted for ^{14}C dating (Table 3). Unlike previous attempts to date the

Chief City guano deposits (Jegla and Hall, 1962; Colburn, 2005), these dates are not infinite. All but one of the Chief City samples were collected from high in the guano deposits and have been contaminated by Holocene-era charcoal from prehistoric/historic use of the cave by human explorers in the last ~5000 yr. Microscopic examination of guano samples (up to 400×) indicates the presence of micro-charcoal. Possible diagenesis of the lipid fraction in insect chitin (Wurster et al., 2009) may also contribute to anomalously young ^{14}C ages. A basal guano sample from the Chief City section dates to >48,000 ^{14}C yr BP (Aeon 826). This sample lacks intrusive micro-charcoal, constrains chronology of early stages of guano deposition, and is expected given the zoogeographic reasoning for an interglacial age estimate.

Late Wisconsin interstadial deposits (28.1 ka)

A guano sample from Gothic Avenue lacking *Tadarida* remains was also submitted for dating. Organic preservation in this sample was poor but experienced minimal diagenesis (LOI = 4.0%; C:N = 4.3). This sample contains no evidence of contamination by intrusive micro-charcoal. A date of $23,910 \pm 490$ ^{14}C yr BP (UCIAMS76221; 27,200–29,000 cal yr BP) suggests that this deposit dates to the Farmdalian interstadial (MIS 3).

Late Holocene assemblages (5.5 ka to present)

Late Holocene contexts investigated throughout the course of this project can be defined as those assemblages having associated dates on archaeological torch debris and/or associated diagnostic archaeological remains. Holocene-aged faunal assemblages were found stratigraphically above older deposits in the Chief City section and Post Oak Pillar excavations. Deposits from Kinney's Arena, Star Chamber, Snow Room, Lover's Leap, Giant's Coffin, the Wooden Bowl Room, Vanderbilt Hall, Audubon Avenue, and Cyclops Gateway sections were also assigned a late Holocene age based on association with dated archaeological remains. The earliest human exploration of the cave occurred between 5500 and

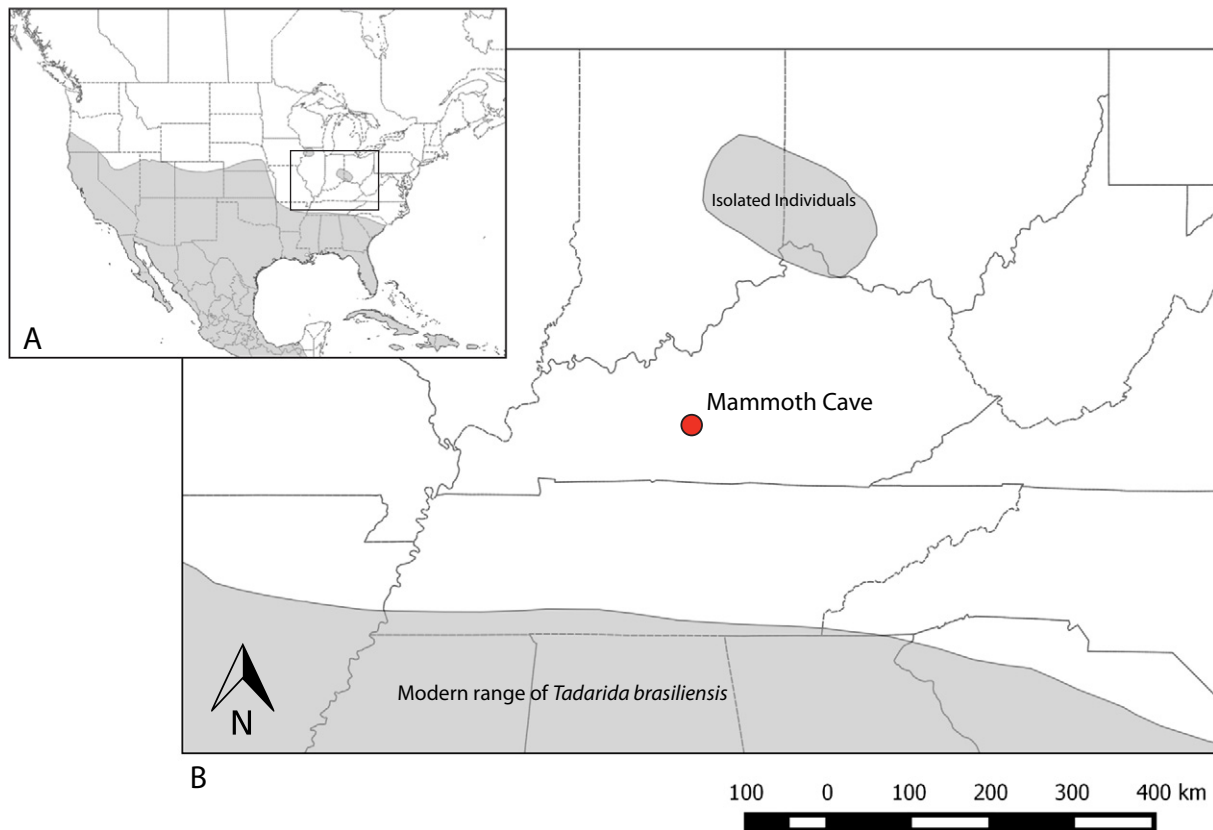


Figure 1. Modern distribution of *T. brasiliensis* (from Barquez et al., 2008). A) North American *Tadarida* distribution, and B) regional *Tadarida* distribution. The entrance to Mammoth Cave is at 37°11'14.8"N, 86°06'12.4"W.

4500 cal yr BP (Watson, 1997) although regular entry into the cave occurred much later, 3000–2200 cal yr BP (Crothers et al., 2002). Contexts that were a mix of late Holocene and Historic remains were included in the more inclusive late Holocene chronological category for purposes of analysis.

Historic assemblages (AD 1800 to present)

These faunal assemblages were associated with 19th and 20th century archaeological debris, including the remains of domesticated animals (e.g., chicken), ticket stubs, and flash bulbs. These assemblages include near-surface deposits in the Audubon Avenue, Giants Coffin, Star Chamber, and Chief City sections. They also include deposits in Pensacola

Avenue, Grand Central Station, near Jenny Lind's Table (Cleveland Avenue) and near a stone structure used to house tuberculosis patients in the 1840s.

Systematic description of paleontological remains

Although vertebrates from guano deposits are dominated by bat taxa, they also include small numbers of rodents, carnivores, and reptiles (Tables 4, S1). When insect cuticle was present, it was highly comminuted and too degraded to identify. Occasionally, hair was preserved. It was also too degraded to identify to species.

Table 3
Comparison of ^{14}C pretreatment protocols.

Cave section	Age	ISM lab #	Excavation unit	Treatment	Yield (%)	Radiocarbon age (^{14}C yr BP)	Microscopic evaluation of sample
Gothic Avenue	Farmdalian	09_68	P2-3b	Acid only	47.85	23,910 ± 490 (UCIAMS76221)	No micro-charcoal visible
Haines Dome	Interglacial	09_68	P2-3b	Acid–base–acid	9.03	No date	–
		09_48	X1-2	Acid only		18,560 ± 210 (UCIAMS76219)	Small sample, micro-charcoal dominated
Chief City	Interglacial	09_48	X1-2	Acid–base–acid	2.50	No date	–
		09_47	U2-3	Acid only	37.48	32,420 ± 1090 (UCIAMS76222)	Small sample, micro-charcoal dominated
Chief City	Interglacial	09_47	U2-3	Acid–base–acid	2.62	No date	–
		09_75	T7-3 (N)	Acid only	17.07	33,090 ± 360 (UCIAMS76223)	No observable organics remain
Chief City	Interglacial	09_75	T7-3 (N)	Acid–base–acid	0.00	No date	–
		09_50	W1-4	Acid only	19.51	30,580 ± 300 (UCIAMS76220)	Equal parts insect cuticle and micro-charcoal
Chief City	Interglacial	09_50a	W1-4	Acid–base–acid	21.12	13,735 ± 45 (UCIAMS76212)	–
		09_77	T7-5	Acid only	28.41	>48,000 (Aeon 826)	No micro-charcoal visible

Table 4
Identified taxa from Historic, Late Holocene, glacial and interglacial contexts from Mammoth Cave.

Taxon	Historic	Late Holocene	Farmdalian interstadial	Interglacial
<i>Meleagris gallopavo</i>	1			
<i>Gallus gallus</i> (cf.)	20	5		
Aves usp.	1	13		
<i>Tadarida brasiliensis</i>		1		261
<i>Eptesicus fuscus</i>	1	9	1	21
<i>Lasiurus borealis</i>		2		3
<i>Lasiurus cinereus</i>				2
<i>Lasiurus</i> sp.				1
<i>Corynorhinus</i> sp.				1
<i>Perimyotis subflavus</i>	6	5		7
<i>Myotis grisescens</i>		1	1	9
<i>Myotis leibii</i>				70
<i>Myotis sodalis</i>	1			
<i>Myotis</i> spp.	5	50	1	172
Chiroptera usp.	40	890	163	1204
<i>Mustela</i> sp.				2
<i>Odocoileus virginianus</i>	2			
<i>Peromyscus</i> sp.				13
<i>Neotoma</i> sp.		1		7
Rodentia usp.	2	57		80
Mammalia usp.		7		10
Turtle usp.		1		
Squamata usp.		1		
Total NISP	52	958	166	1751

Overall, the Gothic Avenue section exhibits the densest fossil bearing deposits examined in this project (Table S2, Fig. 2). A total of 1237 bones was recovered from excavation units in this section. Excavated units in the Chief City section also had a high number of osteological materials (N = 1134) followed by the lower Historic Loop (N = 414). Fewer bones were found in units located in Haines Dome (N = 62) and the upper Historic Loop (N = 47). Systematic description of major taxonomic groups is presented below.

Class MAMMALIA

Order CHIROPTERA

Family Molossidae

Tadarida brasiliensis

T. brasiliensis (Mexican Free-tailed bat) remains are concentrated in interglacial guano deposits in the Chief City section (Units T7, T8, U2, W1) and Haines Dome (Unit X1) (Fig. 3A). *Tadarida* remains are also present in lower densities in Gothic Avenue. A single *Tadarida* dentary from Giant's Coffin may or may not be in primary context. The archaeological materials associated with this specimen indicate a late Holocene age for the deposit, and the growth of selenite crystals on the bone itself indicates

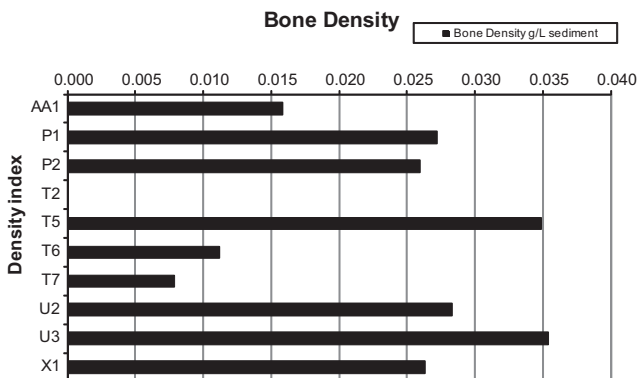


Figure 2. Bone density by excavation context. Unit W1 not shown due to extremely high density of faunal materials (>70 g/L).

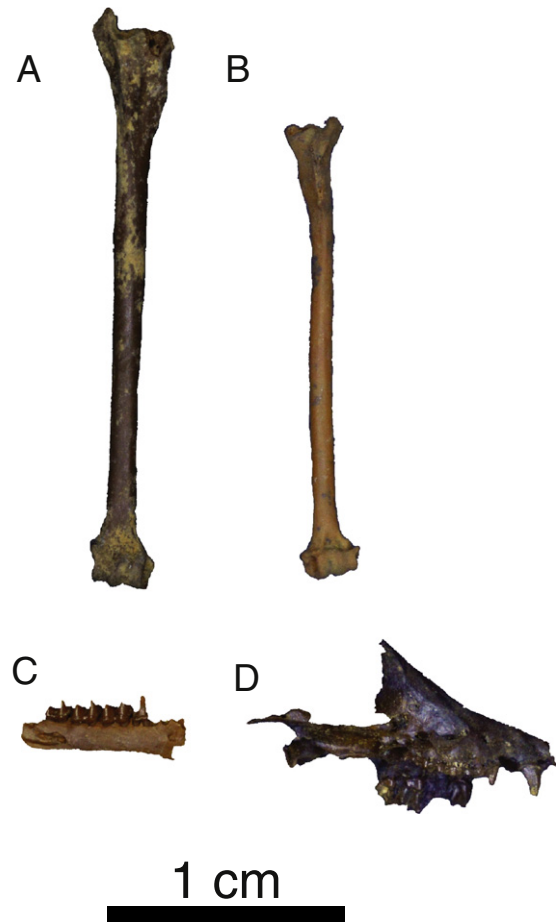


Figure 3. Chiroptera remains from interglacial assemblages in the Chief City section. A) *T. brasiliensis* humerus, B) *M. leibii* humerus, C) *E. fuscus* dentary, and D) partial *M. grisescens* cranium.

a taphonomic history for this specimen that is more complex than other bat remains from this unit.

T. brasiliensis no longer maintains a breeding population in Kentucky. *T. b. cynocephala*, prefers human structures for major roost sites, all of which are located in the southern part of its range (e.g., Florida, Arkansas, Louisiana; Whitaker and Hamilton, 1998). Modern populations in the eastern US do not migrate, although summer and winter roosts may be in different places (Choate et al., 1994). Morphological analyses of fossil *Tadarida* from the Chief City section of Mammoth Cave suggest that fossil individuals are slightly larger than modern *T. b. cynocephala* (Jegla and Hall, 1962). The southern distribution of modern and historic populations of *T. brasiliensis* suggests that the extensive remains of these bats in Mammoth Cave date to a period when the local environment was much warmer. The occasional remains of juvenile individuals indicate that the extensive guano deposits in Chief City are the product of summer maternity roosts.

Class MAMMALIA

Order CHIROPTERA

Family Vespertilionidae

Eptesicus fuscus

Although not common, remains of *Eptesicus fuscus* (Big Brown Bat) are found in late Holocene, Farmdalian interstadial, and interglacial deposits throughout the cave. This species was recorded from Giant's Coffin, Star Chamber, Kinney's Arena, Chief City, and Gothic Avenue. No more than eight specimens are identified from any single excavation context (Fig. 3C).

This species is common throughout much of North America but is usually non-migratory in the eastern US. Its use of cave environments is limited to winter hibernacula where they often roost near entrances in small groups (Barbour and Davis, 1974; Harvey et al., 2011). In the summer, these bats avoid roosting in areas of high temperatures (33–35 °C) (Barbour and Davis, 1974). *E. fuscus* diet consists of beetles (their primary prey according to Barbour and Davis (1974)), wasps, flies, moths, true bugs, and leaf hoppers (Choate et al., 1994).

Class MAMMALIA
Order CHIROPTERA
Family Vespertilionidae
Lasiurus borealis

Lasiurus borealis (Eastern Red Bat) was found in late Holocene and interglacial deposits in Gothic Avenue. No more than two specimens were found in any single excavation context. Previous work in Mammoth Cave recovered *L. borealis* from late Holocene and historic assemblages in the Corkscrew (~1500 ¹⁴C yr BP), Lookout Mountain, Gothic Avenue (1195 ± 50 ¹⁴C yr BP; CAMS 83262) and recent mummies from Gothic Avenue and the Waldach Dome area (Colburn, 2005).

L. borealis is common throughout its range, which covers much of North America east of the Rocky Mountains (Harvey et al., 2011). Although not typically found in caves, *L. borealis* has been known to swarm entrances and will, at times, venture deep into caves. In areas where small passages lead to large rooms they may get lost and come to rest on the ceiling (Barbour and Davis, 1974). These migratory bats forage at low altitude among trees, above meadows, and along water-courses (Brown, 1997) eating a variety of insects, including those usually found on the ground (e.g., crickets).

Class MAMMALIA
Order CHIROPTERA
Family Vespertilionidae
Lasiurus cinereus

An articulating front limb (humerus, radius) of *Lasiurus cinereus* (Hoary Bat) was found in interglacial guano deposits in the Chief City section. These specimens are stained red brown, matching the surrounding matrix.

L. cinereus is a strong, high flying, migratory bat that is distributed throughout eastern North America (Choate et al., 1994). In the southern part of its range, it forages on large insects (primarily moths) in pine woodlands, hardwood forests, and mixed pine–hardwood areas (Brown, 1997). *L. cinereus* generally roosts in trees, however they have been known to utilize caves in late summer during migration (Bailey, 1933).

Class MAMMALIA
Order CHIROPTERA
Family Vespertilionidae
Myotis grisescens

Myotis grisescens (Gray Bat) was found in late Holocene and Farmdalian interstadial deposits in Gothic Avenue and interglacial deposits in the Chief City section (Fig. 3D). Colburn (2005) also noted an association of *M. grisescens* with guano dating to 8460 ± 40 ¹⁴C yr BP (CAMS 63100; 9375–9520 cal yr BP) in Wright's Rotunda, possibly indicating an early Holocene presence of this species in the cave.

M. grisescens populations are distributed in the cave areas of the Midwest and southeastern US although they may have been more widely distributed prior to historic landscape changes. They are true cave bats in that they hibernate and bear young in caves. These bats prefer relatively inaccessible deep pits (i.e., vertical shaft entrances) as winter hibernacula. Almost the entire known population of Gray bats hibernates in only three southeastern caves in Tennessee and Alabama (Whitaker and Hamilton, 1998). A small summer colony is also known from nearby Bat Cave (Colburn et al., 2015). Maternity colonies prefer

“warm” cave areas near water. Aquatic species are important components of the diet but are rarely preserved in guano (Whitaker and Hamilton, 1998).

Class MAMMALIA
Order CHIROPTERA
Family Vespertilionidae
Myotis leibii

Myotis leibii (eastern small-footed *Myotis*) was recovered exclusively from interglacial deposits in the Chief City section (Fig. 3B).

M. leibii is distributed from Tennessee and Kentucky northwards through New England (Harvey et al., 2011). This species is not expected to occur in high densities in deep caves. However, a relatively large number of *M. leibii* specimens were recovered from Unit T7 in the Chief City section in association with the remains of *T. brasiliensis*. These remains include both cranial and post-cranial elements that have been stained the same color of the surrounding matrix.

M. leibii is currently only a summer resident of Mammoth Cave National Park, which is one of the largest concentrations of this species in the eastern US (Barbour and Davis, 1974). This species roosts on the ground or among rocks, even in cave environments. It is a slow, low-altitude flyer (<6 m) that forages over streams, ponds, and near cliffs, ledges, and wooded areas (Choate et al., 1994).

Class MAMMALIA
Order CHIROPTERA
Family Vespertilionidae
Myotis sodalis

A partial skeleton of *Myotis sodalis* (Indiana myotis) was recovered from historic deposits in the Giant's Coffin area.

M. sodalis may have limited distribution in Kentucky, (Barbour and Davis, 1974). There was a major wintering population in Mammoth Cave National Park in the 1970s (~100,000 in several caves), this number has declined in recent decades. *M. sodalis* prefers large, cool, moist limestone caves, usually with streams or ponds nearby, for hibernation (Brown, 1997). They hibernate in tightly packed clusters. Although maternity colonies are usually located in forests, *M. sodalis* also uses cave roosts during the summer months. Moths from riparian or floodplain habitats are primary components of the diet (Choate et al., 1994).

Class MAMMALIA
Order CHIROPTERA
Family Vespertilionidae
Corynorhinus (Plecotus) sp.

A dentary of *Corynorhinus* sp. (Big Eared Bat) was recovered from interglacial deposits in Chief City. This is the only *Corynorhinus* specimen found during this project.

C. rafinesquii (Rafinesque's Big Eared Bat) and *C. townsendii* (Townsend's Big Eared Bat) are found in the southeastern US and lower Midwest. Both species hibernate in caves (Harvey et al., 2011). *C. townsendii* is considered a western species with a relict population in Kentucky (Barbour and Davis, 1974). *C. rafinesquii* is more widely distributed, and is found in Mammoth Cave National Park. The two species often roost together in limestone caves. Both bats prefer cold hibernacula and form tight clusters on the walls or ceilings of a cave near entrances (Choate et al., 1994). Moths are the primary prey of both species (Brown, 1997).

Class MAMMALIA
Order CHIROPTERA
Family Vespertilionidae
Perimyotis (Pipistrellus) subflavus

Perimyotis subflavus (Tri-colored Bat, formerly Eastern Pipistrelle) was found in historic (Little Bat Avenue), late Holocene (Gothic Avenue,

Star Chamber), and interglacial (Haines Dome, Chief City) deposits. In most cases, the remains of this bat are found in very low densities in these localities. Little Bat Avenue is the exception, where fur and bones were found, along with the remains of medium-sized *Myotis* spp.

P. subflavus is one of the most abundant bats in Kentucky (Barbour and Davis, 1974), and is distributed throughout eastern North America, commonly hibernating in cave environments (Harvey et al., 2011). *P. subflavus* is a forest-edge species that is not found in closed forests or open fields unless there are large trees nearby. It also forages above ponds and streams; aquatic insects are particularly common in the diet. Although they roost in trees during the summer months, they hibernate singly in caves during the winter (Barbour and Davis, 1974).

Class MAMMALIA
Order RODENTIA
Family Cricetidae
Neotoma sp.

Woodrat remains were found in interglacial deposits of the Chief City section. Most of these remains are post-cranial elements, including vertebrae ($N = 5$), a rib, and a possible scat concentration consisting of three fecal pellets cemented together by CaCO_3 . These remains are stained the color of the surrounding matrix, suggesting at least rough contemporaneity with the period of deposition.

Until recent genomic studies, *Neotoma* in this region was considered to be almost exclusively *N. floridana*, the Eastern Woodrat. However, mtDNA and morphological analyses on historical museum specimens indicate that modern woodrats in the Mammoth Cave region are *N. magister*, the Allegheny woodrat (Wright, 2008). These rodents are common in the caves and crevices of Mammoth Cave National Park, and are found as far as two miles back into cave passages (Bailey, 1933:424).

Class MAMMALIA
Order RODENTIA
Family Cricetidae
Peromyscus sp.

A minimum of four individuals (13 specimens) were recovered from interglacial levels in the Chief City section. With the exception of a single humerus, most of these specimens are unstained and do not show the red brown coloring that would be expected if they were deposited at the same time as the surrounding matrix.

Peromyscus leucopus (White-footed mouse) is recorded historically for Mammoth Cave National Park (Bailey, 1933:426). Although it shows no particular affinity for underground habitats it has been known to build nests in cliffs, crevices, and in cave openings. *P. maniculatus* (Deer Mouse) is also recorded for this area. *P. maniculatus* has been known to prey on bats (Fenton, 1970).

Class MAMMALIA
Order CARNIVORA
Family Mustelidae
Mustela sp.

A radius and canine from *Mustela* sp. was recovered from interglacial levels of the Chief City section. Both specimens are stained black to brown–black. These specimens are intermediate in size between modern *M. nivalis* and *M. frenata*. Both species are highly sexually dimorphic and exhibit possible geographic clines in body size (Ralls and Harvey, 1985). Mustelid predation on bats has been documented in Kentucky caves (Goodpaster and Hoffmeister, 1950) and *M. frenata* has been known to prey upon roosting Big Brown Bats (*E. fuscus*, Mumford, 1969). Ecological studies of large *Tadarida* colonies in Texas note that surfaces below major roosts are littered with bat remains which make them attractive to carnivores (Eads et al., 1955).

Stable isotope analyses of bat guano

Processed guano samples were relatively heterogenous, consisting of insect remains, bat hair and proteins, and sulfate minerals (i.e., gypsum). Diagenesis and mineralization affecting guano deposits can be problematic for accurate analyses of $\delta^{13}\text{C}$, $\delta^{15}\text{N}$, and ^{14}C . Pieces of insect cuticle dominate Mammoth Cave guano samples under magnification. However, an insect exoskeleton consists of four primary layers: epicuticle, procuticle, epidermis, and basement membrane. Although there is the potential for all parts of this exoskeleton to be preserved in the fossil record, chitin microfibrils in the procuticle are the most resistant to decay due to cross-linking with proteins. Insect chitin sensu stricto is a complex biopolymer primarily made up of polycondensed N-acetyl-glucosamine. It also contains proteinaceous, carbohydrate, and lipidic components (Schimmelmann, 2011). These components are differentially affected by decay processes. Although chitin and some proteins are relatively robust (Stankiewicz et al., 1998) to taphonomic processes, some lipid fractions are known to be less resistant to diagenesis (Wurster et al., 2009).

Sample heterogeneity and the potential for unique diagenetic pathways for different fractions of bulk guano mean that we must independently assess the quality of each sample. LOI results indicate that there is a relatively wide range of organic preservation in Mammoth Cave guano deposits, ranging from 3.95% in the lower levels of Gothic Avenue (Unit P2) to >40% in the Chief City section (Unit U2). The mean LOI for Gothic Avenue is 4.89%. *Tadarida* guano from Chief City exhibits significantly higher LOI values ($\bar{x} = 20.64\%$).

C:N analyses can be used to further evaluate the preservation of organic materials in guano deposits. Fresh guano from *Tadarida* roosts in the southwestern US exhibit a range of C:N values between 4.4 and 5.0. The range of C:N values in a profile of guano spanning multiple decades from Eagle Creek Cave (Greenlee Co., AZ) is slightly larger, between 4.1 and 5.4 (Wurster et al., 2007). These values provide a baseline for assessing the extent of diagenesis in ancient guano deposits. C:N values of 85% of the samples ($N = 23$) on Mammoth Cave guano fall within the range of values from Eagle Creek Cave (Table 2; Fig. 4). These samples have experienced limited diagenesis and stable isotope values should reflect the dominant component of the sample, the prey of insectivorous bats.

Stable isotope values of the processed guano samples were highly uniform (Table 2). The mean $\delta^{13}\text{C}$ value of -26.4% ($\sigma = 0.4$) is consistent with insects that foraged within deciduous forests like those that are found in the modern floodplain. The low variability in $\delta^{13}\text{C}$ samples suggests a consistent foraging pattern throughout the time of formation

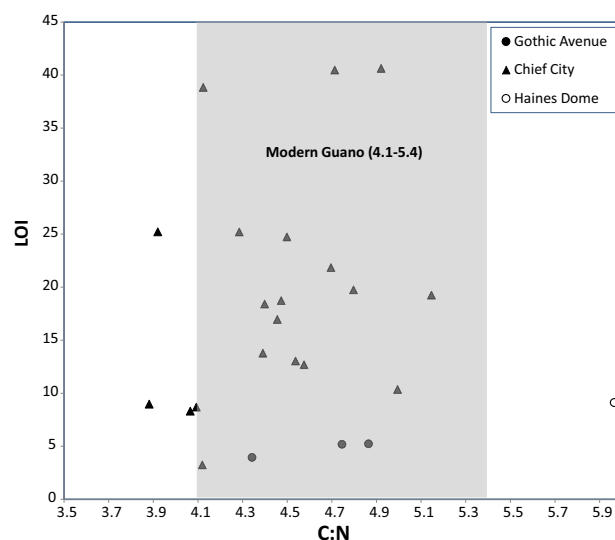


Figure 4. LOI and C:N values of Mammoth Cave guano.

of guano deposits in Chief City and Gothic Avenue. It is possible that bat populations, when present in numbers, relied heavily on emergent insects (Lee and McCracken, 2005). A reliance on seasonally emerging soft-bodied taxa such as moths and flies would explain the minimal variability in $\delta^{13}\text{C}$ values and the relatively poor preservation of insect cuticle structures.

Although $\delta^{15}\text{N}$ values for these samples are more variable, it is unclear how $\delta^{15}\text{N}$ is related to environmental factors (Wurster et al., 2007; Schimmelmann, 2011). The $\delta^{15}\text{N}$ values for guano samples from the Gothic Avenue section are low, ~ 6 to 7‰ . The Chief City section, on the other hand, has higher $\delta^{15}\text{N}$ values (18 – 25‰). Mineralization of ancient guano deposits may alter the original $\delta^{15}\text{N}$ value, as would ammonification or ammonia volatilization (McFarlane et al., 1995). It is unclear at this time what factors control $\delta^{15}\text{N}$ in insectivorous bat guano (Wurster et al., 2007).

Discussion

Paleontology and ecomorphology

Taxa associated with interglacial deposits (Table 4) include the chiropteran taxa: *T. brasiliensis*, *E. fuscus*, *L. borealis*, *L. cinereus*, *Lasiurus* sp., *Corynorhinus* sp., *P. subflavus*, *M. grisescens*, *M. leibii*, and *Myotis* spp. *Mustela* sp., *Peromyscus* sp., and *Neotoma* sp. are also present. These bat taxa prefer a range of habitats suggesting a mosaic landscape surrounding Mammoth Cave at this time. This environment included upland grasslands or savanna habitat as well as relatively closed riparian forests.

The guano dating program undertaken as part of this project identified a Farmdalian interstadial ($\sim 28,000$ cal yr BP) assemblage near Post Oak Pillar (Gothic Avenue). Unfortunately, most of the 166 bones that are part of this assemblage can only be identified to unspecific bat (Table 4). Single elements of *E. fuscus*, *M. grisescens*, and *Myotis* spp. were identified from this context. These species are currently extant in the Mammoth Cave area.

Taxa from late Holocene deposits in the cave include the bat taxa: *E. fuscus*, *L. borealis*, *P. subflavus*, *M. grisescens*, and *Myotis* spp. *Gallus gallus*, unidentified bird remains, *Neotoma* sp., turtle, and lizard remains are also present. All of these taxa are currently extant in the Mammoth Cave area. These species occupy forest-edge and closed-forest niches. Aquatic insects and moths are common components of diets of bat taxa in these assemblages.

Finally, the presence of historic debris or domesticated animal bones (e.g., chicken) indicate a historic age for some contexts. Although the historic exploration of Mammoth Cave extends over the last two centuries, diagnostic materials indicate the majority of these contexts date to the early to mid-20th century (Table 4). Like the late Holocene assemblages, all taxa present in deposits of historic age are currently extant in the Mammoth Cave area. Taxa present during the historic period include: *G. gallus*, *E. fuscus*, *P. subflavus*, *Myotis sodalis*, *Myotis* spp., and *Odocoileus virginianus*. Like the late Holocene assemblages, the historic-aged assemblages are dominated by species that prefer forest-edge or closed forest habitats.

Broadly speaking, bat anatomy is a function of an evolutionary history directed toward exploiting a specific foraging niche, that of aerial prey (Norberg, 1994). Although modern bats utilize a diverse array of prey resources, niche relationships within the Chiroptera are strongly related to patterns in body and wing morphology. Bats were already relatively specialized to certain habitats early in the Cenozoic, so it follows that morphological adaptations to these environments may appear in the fossil record.

Recent eco-morphological research focused on Chiroptera has identified the environmental correlates of body and wing variables, specifically wing loading and shape (Norberg and Raynor, 1987; Norberg, 1994). These patterns have been used to infer landscape conditions in ancient bat assemblages (Stimpson, 2012). In many ways, the deep

cave origins of Mammoth Cave paleontological assemblages are ideally suited to these types of analyses, being minimally affected by non-cave fauna.

To better understand the relationship between bat diversity and landscape conditions, we explored trends in average wing loading and wing aspect ratio of bat taxa excavated from Mammoth Cave paleontological contexts. Eco-morphological research in the Chiroptera distinguishes between different bat foraging strategies (Fig. 5). Norberg and Raynor (1987) describe five different foraging strategies in extant bat taxa. The earliest bats were generalized foragers who utilized aerial prey in the forest understory (Strategy I). These early bats and subsequent taxa who utilized this niche have low to moderate wing loading values and aspect ratios. They fly relatively slowly over short distances. This wing morphology is also maneuverable for navigating through closed canopy environments. Bats utilizing a second strategy (Strategy II) are somewhat faster, foraging in semi-open environments within a more closed landscape. These bats forage in gaps (e.g., meadows, ponds, streams) within forested landscapes. Strategy III bats are fast-flying, open-space foragers. Bats such as these, including *T. brasiliensis*, lack the maneuverability to navigate closed, forested environments. Instead they forage at high altitudes, above vegetation, or in open areas. Fruit and nectar-eating species occupy both the cluttered spaces beneath the tree canopy (Strategy IV) and open, un-obstructed spaces (Strategy V). Taxa utilizing the last two strategies were not identified in Mammoth Cave; therefore, the following discussion focuses on the differences between strategies I and III.

In order to compare overall changes in the foraging strategies of fossil bats, wing loading and wing aspect ratio values for Mammoth Cave bat taxa were weighted by NISP. The sum of these weighted values was calculated for each chronological sample, and then divided by the total NISP for the sample (Table 5). The result is a single value that represents the average wing loading and wing aspect ratios, weighted by taxa that are more common during a particular period. This method provides a coarse way of identifying large-scale changes in the foraging strategies of bats recovered from buried contexts, but also removes the effects of more subtle environmental changes (e.g., cave microenvironment).

Comparison of these values (Fig. 5) suggests a range of landscapes surrounding Mammoth Cave during late Quaternary. Interglacial faunas are dominated by fast-flying, open-space taxa (*T. brasiliensis*) while late Holocene and Historic assemblages contain more taxa that utilize closed forest or forest gaps.

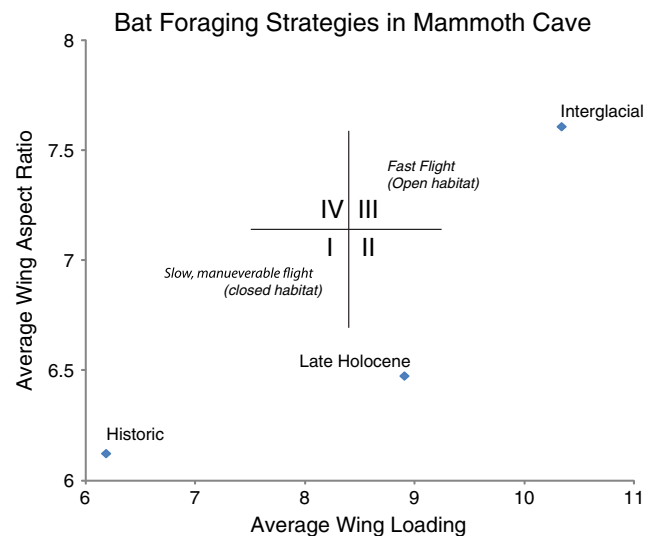


Figure 5. Foraging strategies in Mammoth Cave Chiroptera. Strategy I: generalized foragers, forest understory. Strategy II: fast, foraging in semi-open patches within a closed landscape. Strategy III: fast-flying open-space foragers. Strategy IV: cluttered understory, fruit/nectar feeders.

Table 5
Ecomorphology of Mammoth Cave Chiroptera.

Taxon	Average wing loading			Average aspect ratio		
	Historic	Late Holocene	Interglacial	Historic	Late Holocene	Interglacial
<i>Tadarida brasiliensis</i>	0.0	11.5	3001.5	0.0	8.2	2140.2
<i>Eptesicus fuscus</i>	9.4	84.6	197.4	6.4	57.6	134.4
<i>Lasiurus borealis</i>	0.0	28.0	42.0	0.0	13.4	20.1
<i>Lasiurus cinereus</i>	0.0	0.0	33.0	0.0	0.0	16.2
<i>Perimyotis subflavus</i>	33.6	28.0	39.2	37.2	31.0	43.4
<i>Myotis grisescens</i>	0.0	8.2	73.8	0.0	6.4	57.6
<i>Myotis leibii</i>	0.0	0.0	469.0	0.0	0.0	427.0
<i>Myotis sodalis</i>	6.5	0.0	0.0	5.4	0.0	0.0
Average	6.19	8.91	10.34	6.13	6.48	7.61

For the interglacial fauna, these trends contrast with $\delta^{13}\text{C}$ results of *T. brasiliensis* guano which suggests foraging in a C3 dominated environment. This may indicate that either: 1) ancient *T. brasiliensis* in the eastern U.S. occupied a forest niche, in contrast to modern representatives of the species; and/or 2) the landscape has no modern analogue and contains large numbers of bat prey with C3 diets, while at the same time being open-enough to support large *T. brasiliensis* populations.

Late Holocene trends in bat foraging strategies suggest a relatively closed forest, possibly with extensive gaps or open areas. This is likely representative of local foraging areas available to bats, not broad regional trends, where upland prairies made up a larger portion of the landscape.

Bat foraging strategies during the Historic period were, overall, oriented toward more closed, below-canopy, niches. This is consistent with many accounts of Historic period “rewilding” that occurred throughout much of eastern North America (e.g., Denevan, 1992). Early successional vegetative types were very common during this period due to large-scale human population movements and targeted exploitation of specific economic species.

Stable isotope ecology

The modern landscape surrounding Mammoth Cave is diverse, including barrens, upland forests, and river-bottom growth. At a regional scale, it is located in a transitional zone between open grasslands and drier oak-hickory forests to the west, and relatively moist, mixed mesophytic forest to the east. The local landscape provides a mosaic of habitats for animal populations, including: dry upland ridges, wooded ravines, and alluvial bottomlands along the Green River supporting wetlands and forests (Transeau, 1935; cf. Baskin et al., 1994). Isotopically, the native prairies in this part of Kentucky, consisting predominantly of tallgrass taxa such as big bluestem (*Andropogon* sp.), is a C4 dominant community (i.e., $\delta^{13}\text{C}$ values from -15% to -10%). Oak-hickory forests provide C3 habitats and exhibited $\delta^{13}\text{C}$ values between -33% and -22% . Finally, the mixed, relatively closed bottomland forests have the potential for negative excursions in $\delta^{13}\text{C}$ values due to ground-level CO_2 recycling (i.e., the canopy effect; van der Merwe and Medina, 1991). Significant C4 landscape elements were also present 230–100 ka. Speleothem CaCO_3 from Davis Hall was strongly C4 through glacial and interglacial periods at this time (Harmon et al., 1978).

The $\delta^{13}\text{C}$ of guano is determined by the diets of insects digested by bats. Given the complete absence of coleopteran fragments in guano samples, flying lepidopteran (moths) and dipteran (flies) prey make up the bulk of the diet as in modern *T. b. cynocephala* (Whitaker and Hamilton, 1998). The $\delta^{13}\text{C}$ value of insect chitin is fractionated from dietary input by -0.5% to -1.5% (Ostrom et al., 1997; Hobson et al., 1999).

This study of 27 guano samples is a pilot study illustrating the potential of stable isotope analyses on Mammoth Cave ancient guano deposits. $\delta^{13}\text{C}$ values are remarkably uniform, regardless of changes in stratigraphy, preservation, or location within the cave. The absence of a C4 signal in guano samples suggests that bats avoided upland prairie and aquatic habitats. The former would include dominant C4 grasses, such as big

bluestem (*Andropogon* sp.), and relatively open forests where understory $\delta^{13}\text{C}$ values lacked any “canopy effect” (Drucker and Bocherens, 2009). Aquatic insects would take on the $\delta^{13}\text{C}$ values of local waters, which are in equilibrium with local bedrock sources. The dominant bedrock of the region has less negative $\delta^{13}\text{C}$ values (Miller, 2008), mimicking the values of C4 vegetation. The only modern habitats sharing the fully C3 vegetation evident in this study's samples of bat prey would be in closed forests of the Green River bottomlands and tributary valleys. It is possible that these closed, mesic forests could have been more prevalent in the surrounding uplands during the last interglacial (as in central Illinois, Curry and Baker, 2000), so the habitat represented by these isotopic data would have been more widespread. Finally, there is the possibility that guano-producing bats relied heavily on populations of emergent insects. Young insects may retain isotopic values acquired during larval stages (Schimmelmann, 2011). A reliance on emergent insects with a C3 diet during the larval stage would bias guano values in favor of C3 vegetation. At this time, it is difficult to distinguish whether negative $\delta^{13}\text{C}$ values on bat guano are due to characteristics of the landscape surrounding Mammoth Cave or bat prey selection patterns.

Conclusions

- 1) Guano deposits within Mammoth Cave have high potential for the preservation of significant paleobiological materials, including the remains of vertebrates and their prey.
- 2) Mammoth Cave contains extensive guano deposits dating to an unknown interglacial. An abundant and diverse bat assemblage is present within these deposits.
- 3) In these deposits, abundant *Tadarida* remains indicate utilization of open habitats, however, a C3 prey signature suggest the use of relatively closed, arboreal environments. The isotopic values of bat prey may be related to overall landscape structure or the utilization of seasonal booms in emergent insects.
- 4) A small Farmdalian interstadial fauna is present in the Gothic Avenue section. Deposits dating to this time period are rare in Mammoth Cave, and should be further investigated.
- 5) Ecomorphological trends in bat faunas suggest open-area, fast fliers dominated interglacial deposits while slower, more maneuverable understory foragers were present during the late Holocene and Historic periods.

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References

- Ahler, S.R., 2012. Archaeological and paleontological investigations of selected trail segments in Mammoth Cave and Great Onyx Cave, Edmonson County, Kentucky. University of Kentucky Program for Archaeological Research, Technical Report No. 714.
- Bailey, V., 1933. Cave life of Kentucky, mainly in the Mammoth Cave Region. *American Midland Naturalist* 14 (5), 385–635.
- Barbour, R.W., Davis, W.H., 1974. *Mammals of Kentucky*. University of Kentucky Press, Lexington.
- Barquez, R., Diaz, M., Gonzalez, E., Rodriguez, A., Incháustegui, S., Arroyo-Cabrales, J., 2008. *Tadarida brasiliensis*. IUCN 2014. IUCN Red List of Threatened Species (Version 2014.1). <www.iucnredlist.org>. Downloaded on 28 June 2014).
- Baskin, J.M., Baskin, C.C., Chester, E.W., 1994. The Big Barrens Region of Kentucky and Tennessee: further observations and considerations. *Castanea* 59 (3), 226–254.
- Batina, M.C., Reese, C.A., 2011. A Holocene pollen record recovered from a guano deposit: Round Spring Cavern, Missouri, USA. *Boreas* 40 (2), 332–341.
- Brown, L.N., 1997. *A Guide to the Mammals of the Southeastern United States*. University of Tennessee Press, Knoxville.
- Choate, J.R., Knox Jones Jr., J., Jones, C., 1994. *Handbook of Mammals of the South-Central States*. Louisiana State University Press.
- Colburn, M.L., 2005. Paleontological inventory project: vertebrate remains found in select passages and caves at Mammoth Cave National Park, Kentucky. Illinois State Museum Landscape History Program, Technical Report No. 2005-1199-007.
- Colburn, M., Toomey III, R., Widga, C., Olson, R., 2015. Holocene paleontology of Bat Cave, Edmonson County, Kentucky, USA. *Journal of Cave and Karst Studies* (in press).
- Crothers, G.M., Faulkner, C.H., Simek, J.F., Watson, P.J., Willey, P., 2002. Woodland Cave archaeology in Eastern North America. In: Anderson, D.G., Mainfort, R.C. (Eds.), *The Woodland Southeast*. University of Alabama Press, Birmingham, pp. 502–524.
- Curry, B.B., Baker, R.G., 2000. Palaeohydrology, vegetation, and climate since the late Illinois episode (~130 ka) in south-central Illinois. *Palaeogeography, Palaeoclimatology, Palaeoecology* 155, 59–81.
- Dean, W.E., 1974. Determination of carbonate and organic matter in calcareous sediments and sedimentary rocks by loss on ignition: comparison with other methods. *Journal of Sedimentary Petrology* 44, 242–248.
- Denevan, W.M., 1992. The pristine myth: the landscape of the Americas in 1492. *Annals of the Association of American Geographers* 82, 369–385.
- Deniro, M.J., Epstein, S., 1978. Influence of diet on the distribution of carbon isotopes in animals. *Geochimica et Cosmochimica Acta* 42 (5), 495–506.
- Drucker, D.G., Bocherens, H., 2009. Carbon stable isotopes of mammal bones as tracers of canopy development and habitat use in temperate and boreal contexts. In: Creighton, J., Roney, P. (Eds.), *Forest Canopies: Forest Production, Ecosystem Health, and Climate Conditions*. Nova Science Publishers, pp. 103–109.
- Eads, R.B., Wiseman, J.S., Grimes, J.E., Menzies, G.C., 1955. Wildlife rabies in Texas: a preliminary report. *Public Health Reports* 70 (10), 995–1000.
- Fenolio, D.B., Graening, G.O., Collier, B.A., Stout, J.F., 2006. Coprophagy in a cave-adapted salamander: the importance of bat guano examined through nutritional and stable isotope analyses. *Proceedings of the Royal Society, Biological Sciences* 273 (1585), 439–443.
- Fenton, M.B., 1970. Population studies of *Myotis lucifugus* (Chiroptera: Vespertilionidae) in Ontario. *Life Sciences Contributions, Royal Ontario Museum* 77, 1–34.
- Fletcher, M. S., 1982. Microbial ecology of a bat guano community. Unpublished MS Thesis. Southwest Missouri State University, Springfield, MO.
- Frank, E.F., Benson, R., 1998. Vertebrate paleontology of Isla de Mona, Puerto Rico. *Journal of Cave and Karst Studies* 60, 103–106.
- Gaudin, T.J., Miller, A.N., Bramblett, J.L., Wilson, T.P., 2011. Holocene and Late Pleistocene bat fossils (Mammalia: Chiroptera) from Hamilton County, TN, and their ecological implications. *Southeastern Naturalist* 10 (4), 609–628.
- Goodpaster, W., Hoffmeister, D.F., 1950. Bats as prey for mink in Kentucky cave. *Journal of Mammalogy* 31 (4), 457.
- Harmon, R.S., Schwarz, H.P., Ford, D.C., 1978. Stable isotope geochemistry of speleothems and cave waters from the Flint Ridge–Mammoth Cave System, Kentucky: implications for terrestrial climate change during the period 230,000 to 100,000 years B.P. *Journal of Geology* 86, 373–384.
- Harvey, M.J., Altenbach, J.S., Best, T.L., 2011. *Bats of the United States and Canada*. Johns Hopkins Press, Baltimore.
- Hobson, K., Wassenaar, L.I., Taylor, O.R., 1999. Stable isotopes (δD and $\delta^{13}C$) are geographic indicators of natal origins of monarch butterflies in eastern North America. *Oecologia* 120 (3), 397–404.
- Jegla, T.C., Hall, J.S., 1962. A Pleistocene deposit of the free-tailed bat in Mammoth Cave, Kentucky. *Journal of Mammalogy* 43 (4), 477.
- Lacki, M.J., Burford, L.S., Whitaker Jr., J.O., 1995. Food habits of gray bats in Kentucky. *Journal of Mammalogy* 76 (4), 1256–1259.
- Lee, Y.F., McCracken, G.F., 2005. Dietary variation of Brazilian free-tailed bats links to migratory populations of pest insects. *Journal of Mammalogy* 86 (1), 67–76.
- Maher Jr., L.J., 2006. Environmental information from guano palynology of insectivorous bats of the central part of the United States of America. *Palaeogeography, Palaeoclimatology, Palaeoecology* 237 (1), 19–31.
- McFarlane, D.A., Keeler, R.C., Mizutani, H., 1995. Ammonia volatilization in a Mexican bat cave ecosystem. *Biogeochemistry* 30 (1), 1–8.
- McFarlane, D.A., Lundberg, J., Fincham, A.G., 2002. A late quaternary paleoecological record from caves of Southern Jamaica, West Indies. *Journal of Cave and Karst Studies* 64 (2), 117–125.
- Miller, B., 2008. $\delta^{13}C$ of Cave Speleothems Located in Kentucky and Ohio, U.S.A.: Implication for Paleovegetation and Paleoclimate. Unpublished M.S. Thesis, Bowling Green State University, KY.
- Mizutani, H., McFarlane, D.A., Kabaya, Y., 1992. Carbon and nitrogen isotopic signatures of bat guanos as record of past environments. *Mass Spectrometry* 40 (1), 67–82.
- Mumford, R.E., 1969. Long-tailed weasel preys on big brown bats. *Journal of Mammalogy* 50 (2), 360.
- Norberg, U., 1994. Wing design, flight performance, and habitat use in bats. In: Wainwright, Peter C., Reilly, Stephen M. (Eds.), *Ecological Morphology: Integrative Organismal Biology*. University of Chicago Press, pp. 205–239.
- Norberg, U., Raynor, J.M., 1987. Ecological morphology and flight in bats (Mammalia; Chiroptera): wing adaptations, flight performance, foraging strategy and echolocation. *Philosophical Transactions of the Royal Society* 316 (1179), 335–427.
- Ostrom, P.H., Colunga-Garcia, M., Gage, S.H., 1997. Establishing pathways of energy flow for insect predators using stable isotope ratios: field and laboratory evidence. *Oecologia* 109 (1), 108–113.
- Palmer, A.N., 1981. *A Geological Guide to Mammoth Cave National Park*. Zephyrus Press, Teaneck, NJ, p. 210.
- Ralls, K., Harvey, P.H., 1985. Geographic variation in size and sexual dimorphism of North American weasels. *Biological Journal of the Linnean Society* 25, 119–167.
- Reimer, P.J., Bard, E., Bayliss, A., Beck, J.W., Blackwell, P.G., Ramsey, C.B., Buck, C.E., Cheng, H., Edwards, R.L., Friedrich, M., Grootes, P.M., Guilderson, T.P., Hafflidason, H., Hajdas, I., Hatte, C., Heaton, T., Hoffmann, D., Gogg, A., Hughen, K.A., Kaiser, K.F., Kromer, B., Manning, S., Miu, M., Reimer, R.W., Richards, D.A., Scott, E.M., Southon, J.R., Staff, R.A., Turney, C.S.M., van der Plicht, J., 2013. IntCal13 and Marine13 radiocarbon age calibration curves 0–50,000 years cal BP. *Radiocarbon* 55 (4), 1869–1887.
- Saugey, D.A., Sasse, D.B., Wilhide, J.D., Reed, D., Jones, T.R., Heath, D.R., England, D.R., 2001. Distribution of LeConte's free-tailed bat (*Tadarida brasiliensis cyanocephala*) in Arkansas, with notes on reproduction and natural history. *Journal of the Arkansas Academy of Science* 55, 137–147.
- Schimmelmann, A., 2011. Carbon, nitrogen and oxygen stable isotope ratios in chitin. In: Gupta, Neal S. (Ed.), *Chitin. Topics in Geobiology* 34. Springer, The Netherlands, pp. 81–102.
- Schimmelmann, A., Deniro, M.J., 1985. Stable isotopic studies on chitin, measurements on chitin/chitosan isolates and D-glucosamine hydrochloride from chitin. In: Muzzarelli, R., Jeuniaux, C., Gooday, G.W. (Eds.), *Chitin in Nature and Technology*. Plenum Press, New York, pp. 357–364.
- Stankiewicz, A., Mastalerz, B.M., Hof, C.H., Bierstedt, A., Flannery, M.B., Briggs, D.E.G., Evershed, R.P., 1998. Biodegradation of the chitin–protein complex in crustacean cuticle. *Organic Geochemistry* 28 (1), 67–76.
- Stimpson, C.M., 2012. Local scale, proxy evidence for the presence of closed canopy forest in north-western Borneo in the late Pleistocene: bones of Strategy I bats from the archaeological record of the Great Cave of Niah, Sarawak. *Palaeogeography, Palaeoclimatology, Palaeoecology* 331–332, 136–149.
- Stuiver, M., Reimer, P.J., 1993. Extended ^{14}C database and revised CALIB radiocarbon calibration program. *Radiocarbon* 35, 215–230.
- Transeau, E.N., 1935. The Prairie Peninsula. *Ecology* 16 (3), 423–437.
- van der Merwe, N.J., Medina, E., 1991. The canopy effect, carbon isotope ratios and foodwebs in Amazonia. *Journal of Archaeological Science* 18, 249–259.
- Watson, P.J., 1997. Archaeology of the Mammoth Cave Area. Cave Books, St. Louis, MO.
- Whitaker Jr., J.O., Hamilton Jr., W.J., 1998. *Mammals of the Eastern United States*. Cornell University Press, Ithaca.
- Wilkins, K.T., 1989. *Tadarida brasiliensis*. *Mammalian Species* 331, 1–10.
- Wright, J., 2008. History and current status of the allegheny woodrat. In: Peles, J.D., Wright, J. (Eds.), *The Allegheny Woodrat*. Springer, New York, pp. 1–22.
- Wurster, C.M., McFarlane, D.A., Bird, M.I., 2007. Spatial and temporal expression of vegetation and atmospheric variability from stable carbon and nitrogen isotope analysis of bat guano in the southern United States. *Geochimica et Cosmochimica Acta* 71, 3302–3310.
- Wurster, C.M., Patterson, W.P., McFarlane, D.A., Wassenaar, L.I., Hobson, K.A., Athfield, N.B., Bird, M.I., 2008. Stable carbon and hydrogen isotopes from bat guano in the Grand Canyon, USA, reveal Younger Dryas and 8.2 ka events. *Geology* 36 (9), 683–686.
- Wurster, C.M., Bird, M.I., Bull, I.D., Bryant, C., Ascough, P., 2009. A protocol for radiocarbon dating tropical subfossil cave guano. *Radiocarbon* 51 (3), 977–986.
- Wurster, C.M., Bird, M.I., Bull, I.D., Creed, F., Bryant, C., Dungait, J.A., Paz, V., 2010a. Forest contraction in north equatorial Southeast Asia during the Last Glacial Period. *Proceedings of the National Academy of Sciences* 107 (35), 15508–15511.
- Wurster, C.M., McFarlane, D.A., Beavan Athfield, N., 2010b. Stable isotopes of sub-fossil bat guano as a long-term environmental archive: insights from a Grand Canyon Cave deposit. *Journal of Cave and Karst Studies* 72 (2), 111–121.