Lawrence T. Nielsen, David K. Eaton, Donald W. Wright, and Barbara Schmidt-French – Characteristic odors of *Tadarida brasiliensis mexicana* Chiroptera: Molossidae. *Journal of Cave and Karst Studies*, v. 68, no. 1, p. 27–31.

CHARACTERISTIC ODORS OF *TADARIDA BRASILIENSIS MEXICANA* CHIROPTERA: MOLOSSIDAE

LAWRENCE T. NIELSEN
Microanalytics, 2011A Lamar Drive, Round Rock, TX 78664, larry.nielsen@mdgc.com
DAVID K. EATON
Microanalytics, 2011A Lamar Drive, Round Rock, TX 78664, david.eaton@mdgc.com
DONALD W. WRIGHT
Microanalytics, 2011A Lamar Drive, Round Rock, TX 78664, don.wright@mdgc.com
BARBARA SCHMIDT-FRENCH
Bat Conservation International, PO Box 162603, Austin, TX 78716, french@batcon.org

*The odors in a central Texas cave with a large roosting population of Mexican free-tailed bats (*Tadarida brasiliensis mexicana*) were identified and related to captive individual bats. Solid phase microextraction (SPME) was used to sample and concentrate the volatile organics from the cave and individual bats. Odors were detected organoleptically and simultaneously quantified and identified. The characteristic odor for* T. b. mexicana *is due principally to 2'-aminoacetophenone.*

INTRODUCTION

Olfactory cues play many roles in the lives of bats, from feeding to social communication, kin recognition and group identification (Suthers, 1970; Gustin and McCracken, 1987; Loughry and McCracken, 1991; De Fanis and Jones, 1995; Bloss, 1999; Bouchard, 2001). Some bats prefer odors of roost mates, and both sex discrimination and roostmate recognition have been associated with the use of olfactory cues (De Fanis and Jones, 1995; Bouchard, 2001; Bloss *et al*., 2002). Male quality is associated with olfactory cues in *Saccopteryx bilineata* (Voight and von Helversen, 1999; Voight, 2002).

As with many other mammals, body odors derive from a variety of sources on bat's bodies. Urine, feces, glandular products and fermentation products all have been associated with typical odors (Voight and von Helversen, 1999; Scully *et al*., 2000; Voight, 2002).

Female bats use chemical cues to identify their young among millions of pups, and males can discriminate their own odors from those of other males (Gustin and McCracken, 1987). The roosts of bats often assume the odors of the residents, and Mexican free-tailed bats (*Tadarida brasiliensis mexicana*) are a good example because many bat biologists readily use the characteristic odor to recognize roosts. Human observers can sense the characteristic roost odor at considerable distances from roosts. The distinctive "corn tortilla" or "taco shell" aroma is a sure indicator of a *T. brasiliensis* roost. Closer to the roost, the overall odor is stronger and at the same time more complex. Here the single taco shell descriptor is no longer adequate to describe the roost (Wright *et al*., 2005).

The goals of our study were first to use GC-MS to identify the compound in the colony odor responsible for an aroma similar to taco shells, and second, by sampling known roosts and bats' bodies, to determine where the odor originates. We collected data from a known cave roost and from captive bats and their roosts.

METHODS AND MATERIALS

We sampled organic compounds in the Bracken Cave environment via an artificial ventilating shaft that had a continuous draft of air from the interior. Five SPME fibers (Carboxen/PDMS, 85 µm, 2 cm length, 23 gauge, on Stableflex™ Supelco, Supelco Park, Bellefonte, PA, 16823-0048) each were suspended in the airflow from the cave for 120 minutes on June 30, 2001. We made four additional collections on August 31, 2001. After sampling, the fibers were wrapped in conditioned aluminum foil and analyzed within 1–2 days after collection.

In 2001, we sampled fabric roosting pouches of five captive *T. brasiliensis* originating from central Texas on September 7 (2 roosts), September 24 (1 roost) and October 12 (2 roosts). Samples were collected by inserting an SPME fiber into each cloth roosting pouch for various lengths of time. The cloth pouches were used by only one individual but were open to ambient air. Unused pouches also were sampled and analyzed as blanks.

We collected urine samples from captive *T. brasiliensis* bats originating from central Texas on September 16, 2001 (3 specimens) and on September 30, 2001 (5 specimens). For comparison, we also collected urine samples from a female *Lasiurus cinereus* on October 30, a female *Lasiurus intermedius* on October 31, a male *Nycticeius humeralis* on October 30, and a male *Myotis velifer* on October 30. The bats' urine was collected in glass pipettes and the samples were placed in 40 ml Eagle-Picher EPA vials. We sampled the gular glands of two captive male *T. brasiliensis* and the anus of one captive male *T. brasiliensis* on September 16, 2001. These samples also were placed in EPA vials. We inserted SPME fibers into the vials through the vial septa and exposed them to the urine and glandular volatiles for various lengths of time.

Table 1. Selected volatile organic compounds and principal odors of Bracken Cave.

Retention Time (min)	Identification (odor)	Retention Time (min)	Identification (odor)
1.74	acetaldehyde (fermented)	17.13	acetylpyrazine (roasted)
1.76	methyl mercaptan (skunky)	17.21	decanal
2.02	Not identified (foul)	17.42	isovaleric acid (foul, rancid)
2.16	carbon disulfide	17.83	acetophenone
3.89	2 & 3-methylbutanal (foul, aldehydic)	18.27	methionol
4.13	benzene	18.28	3-methylfuranone
6.71	dimethydisulfide	18.39	1-chloro-4-methoxybenzene
6.73	1-aza-1,3-butadiene	18.43	geraniol
7.01	isoxazole	18.69	2,6,6-trimethylcyclohex-2-en-1,4-dione
7.34	isobutanenitrile	18.86	acetamide
7.41	hexanal	19.58	2-methylpropanamide
8.77	pyrazine	19.80	4-ethyl-3-methyl-2H-pyran-2-one
9.07	2,3-dihydro-4-methylfuran (sweet, phenolic)	20.51	2-chlorophenol
9.35	an amine	20.52	ethyl decanoate
9.98	an amine	20.61	hexanoic acid
10.24	methylpyrazine	20.85	guaiacol
10.71	2-propanone oxime	21.05	butamide
10.75	N-nitrosodimethylamine	21.52	thyjopsene (musty)
11.06	beta-myrcene	21.61	phenylethyl alcohol
12.08	dimethylpyrazine isomers (roasted, nutty)	21.63	methylcumate
12.15	limonene	22.17	benzoacetonitrile
12.19	1-octen-3-one (earthy)	22.66	not identified (moldy)
12.38	octanal (sweet, aldehydic)	23.12	phenol
12.61	cumene	23.72	p-anisaldehyde
13.22	acetic acid (sour)	23.73	1,2,3,4-tetrahydro-1,6-dimethyl-4-(1-methylethyl) naphthalene (grainy, floral)
13.26	Dimethyltrisulfide (skunky, foul)		
13.75	trimethylpyrazine	23.92	5-methyl-2-pyrazinylmethanol
14.58	1H-pyrrole (musty, burnt)	24.03	4-(2,6,6-trimethyl-1-cyclohexenyl)-3-buten-2-one (floral, herbaceous)
14.59	2-nonanone		
14.95	nonanal	24.02	m-cresol
15.00	2-methyl-6-vinyl-pyrazine	24.61	p-cresol (musty)
15.06	propionic acid	25.03	2,4-dimethylquinazoline
15.25	benzaldehyde	25.62	2,4-dichlorophenol
15.83	isobutyric acid	25.65	2,6-dimethylphenol
16.29	2-pentylthiophene	26.22	2'-aminoacetophenone (taco shell)
16.64	benzonitrile	27.95	cedrol
16.66	dihydro-5-methyl-2(3H)-furanone	27.45	6-methyl-2H-1-benzopyran-2-one
16.79	camphor	28.85	indole
16.81	butyrolactone	28.91	benzoic acid
16.98	trans-2-nonenal	31.42	1-(2-aminophenyl)-1-butanone

Table 2. Roosting Odors *(Tadarida brasiliensis)*

No.	Retention Time (min)	Male A	Male B	Male C	Female A	Female B	Identification
1	8.60	Not described				Foul	
2	12.18			Roasted	Meaty	Nutty, roasted	
3	12.46		Sweet, aldehydic		Sweet		
4	12.59			Roasted		Roasted, savory	
5	13.16		Not described		Foul, sour	Acidic	
6	14.89				Sweet	roasted	
7	16.29		Foul, musty		Not described		
8	16.53		Soapy, aldehydic	Sweet, floral	Sweet, aldehydic	Sweet, floral	
9	16.74				Foul, soapy	Foul	
10	17.30		Foul acidic	Stale	Acidic	Acidic	
11	17.84		Foul			Musty	
12	18.64				Sweet	Foul	
13	19.26		Foul			Floral	
14	20.32		Meaty		Animal	Resiny	
15	22.26		Not described	Herbaceous		Herbaceous	
16	23.89		Musty	Sweet			
17	24.01		Aldehydic		Sweet, aldehydic		
18	26.22	Taco shell	Taco shell	Taco shell	Taco shell	Taco shell	2'-aminoacetophenone
19	31.40				Sweet		1-(2-aminophenyl)-1-butanone

We performed odor analysis on a standard configuration AromaTrax™ instrument (Microanalytics, Round Rock, TX). The inlet for the thermal desorption of the SPME fibers was equipped with a Merlin Microseal™ septum. Odor volatiles were separated on the AromaTrax™ system using the standard arrangement of tandem BP1 and BP20 columns and detected simultaneously with photoionization (PID), mass spectral (MS) and olfactory detectors. We recorded the sniff port olfactory response using AromaTrax™ odor tracking software.

To identify the hundreds of volatiles in the Bracken cave samples, we used the multidimensional gas chromatography (MDGC) capability of the AromaTrax™ system to enhance separation and identification of individual odor compounds. Identification of odor compounds was made by use of Benchtop/PBM Software Library Search program (Palisade Corp., N. Y.). Simultaneous detection of the resolved odors was done using PID, MS and olfactory detection.

Results

During the time when we obtained our samples, Bracken Cave was occupied by an estimated 20 million Mexican free-tailed bats. Samples from both dates gave essentially the same odor compositional results. We detected hundreds of volatile compounds and present data for the principal odors detected (Table 1). In the samples, 2'-aminoacetophenone was the most concentrated compound in the air exhausting from the roost. This also was the most intense odor sensed at the sniff port during GC-O analysis and the odor most characteristic of the cave roost. The next most intense odors are the earthy odor of 1-octen-3-one, the phenolic odor of 2-chlorophenol and the floral or herbaceous aroma of the tentatively identified 4-(2,6,6-trimethyl-1-cyclohexen-1-yl)-3-buten-2-one.

Roost pouches of five captive *T. brasiliensis* corrected for odors common to unused pouches indicated the dominant presence of 2'-aminoacetophenone (taco shell) for all five individuals (Table 2). One male had two detectable odors while others had seven to 12 odors. Five of 19 odors from individual profiles were among the major odors from Bracken Cave including octanal, acetic acid, isovaleric acid, 4-(2,66-trimethyl-1-cyclohexenyl)-3-buten-2-one and 2'-aminoacetophonone (Table 1).

All seven *T. brasiliensis* had the characteristic taco shell odor of 2'-aminoacetophonone in their urine (Table 3). Except for acetic acid and butyric acid detected in most samples, there was considerable variation in other odor compounds among the seven bats' urine. Ten of the odors found in urine samples also were found in roosting pouches.

We did not find the odor of 2'-aminoacetophenone in the urine of *Lasiurus cinereus*, *Lasiurus intermedius*, *Nycticeius humeralis* or *Myotis velifer* (Table 4). *Lasiurus cinereus* had a strong characteristic amine odor identified as trimethylamine, but no single strong characteristic odor was detected from *Lasiurus intermedius*, *Nycticeius humeralis* or *Myotis velifer*.

We found only acetic acid and another somewhat sour odor in the sample from the gular gland of a male *T. brasiliensis* while gular gland extract from a second male *T. brasiliensis* had sour acetic acid propionic acids, a nutty pyrazine odor and 2'-aminoacetophenone. The other odors we detected also were present in the unused roosting pouch material.

Table 3. Urine Odors (*Tadarida brasiliensis*).

Retention Time (min)	Female A	Female C	Female D	Male A	Male A (anus)	Male B	Male D	*Identification*
6.62	Foul							Trimethylamine
7.01						Foul		
7.40						Not described		
8.51	Not described							
8.96		Savory Pyrazine						
10.02			Sweet			Not described		
10.61			Sweet					
11.80				Savory				2, 5-dimethylpyrazine
12.17	Sour							
12.28		Savory	Earthy	not described	Earthy	Earthy, foul	Musty, foul	
12.36		Foul				Foul		
12.60		Sweet						
13.28	Sour	Sweet	Acidic	Acidic	Acidic	Sour		Acetic acid
14.58							Sweet	Dichlorobenzene
15.10	Not described	Not described		Not described				
15.35		Foul			Foul	Musty, foul		
15.47						Sweet		
16.12	Foul							
16.56	Sour, acidic			Acidic	Acidic	Foul, acidic	Sweet	Butyric acid
17.05		Aldehydic						
17.30						Sour, acidic		
17.55		Not described						
19.15	Foul							
19.87	Sour							
21.32		Aldehydic						
21.65					Floral	Sweet		Phenylethyl alcohol
23.70		Not described				Animal	Not described	
23.90		Not described			Not described			
26.01		Not described						
26.26	Taco shell	Taco shell	Taco shell	Taco shell	Taco shell	Taco shell	Taco shell	2'-aminoacetophenone
31.53	No odor	No odor	No odor	No odor	Not described	No odor	Slight odor	1-(2-aminophenyl)-1-butanone

Discussion

Our data indicate that 2'-aminoacetophenone is the principal odorant responsible for the characterisitic taco shell odor of *Tadarida brasiliensis mexicana* roosts. This odor carries in the air for a considerable distance from the roost and is readily recognized by humans because of its unique character. It also may be used by the bats to identify their roosts. The fact that 2'-aminoacetophenone is a polar molecule that is strongly absorbed on solid surfaces and dust particles (Wright *et al*., 2005) means that it accumulates in the roost and, over time, also is concentrated on surfaces around the roost. The odor can be quite intense when the ambient temperature is high and when local surfaces are wet with rain or other moisture, leading to displacement of the compound into the air (Wright *et al*., 2005).

There are many other odorants present that contribute to the roost odor. One of these is the polar odorant p-cresol. P-cresol acts in a similar way to 2'-aminoacetophenone in terms of its absorption and desorption properties. Most of the odors, however, have less polarity than 2'-aminoacetophenone or p-cresol and do not accumulate on surfaces to the same degree. They generally dissipate after traveling a short distance from the roost. Near the roost, the combination of all the odors is very intense and not well tolerated by humans. Further from the roost, only a few polar odorants dominant.

A significant source of 2'-aminoacetophenone is *T. brasiliensis* urine. In our study, four other species of bats (*Lasiurus cinereus*, *L. intermedius*, *Nycticeius humeralis*, and *Myotis velifer*) did not have detectable levels of 2'-aminoacetophenone and therefore had no taco shell odor.

One of several metabolites of skatole (3-methylindole), 2'-aminoacetophenone, is a metabolite of tryptophan and is produced in the gut of many animals by microbial action (Diaz, *et al*., 1999). Skatole is known to be a pneumotoxin in domestic animals (Diaz, *et al*., 1999), and this property may be important for understanding the chemical makeup of the roost environment. If skatole is toxic to *Tadarida brasiliensis mexicana*, then the accumulation of this compound from 20 million bats in a restricted area could cause health problems for that population. The fact that skatole is not detected under the conditions of analysis in the Bracken Cave roost may mean it is effectively metabolized by microbial action somewhere in the environment or within the bats themselves, thus reducing this potential health hazard for the bats.

Table 4. Urine Odors (select species).

Retention Time (min)	*Lasiurus cinereus* (female)	*Lasiurus intermedius* (female)	*Nycteceius humeralis* (male)	*Myotis velifer* (male)	*Identification*
1.67	Amine				Trimethylamine
2.17	Amine				
3.39		Not described			
4.83	Must				
5.08	Musty				
6.40		Not described		Not described	
6.68				Not described	
7.54	Foul				
8.81			Foul		
12.10			Not described	Musty	
12.24	Musty				
13.28	Acidic	Foul	Acidic	Acidic	
15.27	Not described	Not described	Not described	Not described	Acetic acid
16.55	Acidic	Acidic, rancid			
20.81		Aldehydic		Floral	
21.47	Not described			Floral	
22.35				Sweet	
26.26	Not detected	Not described	Not detected	Not detected	2'-aminoacetophenone

Considering the high concentration of 2'-aminoacetophenone in the Bracken Cave roost and the apparent good health of the 20 million bats in the colony, 2'-aminoacetophenone does not appear to pose a health risk to *T. brasiliensis*. Subsequent work may lead to answers to the larger question of what factors contribute to creating and maintaining the chemical composition of ambient air in long established confined animal areas such as this cave, which could have commercial application in domestic animal production. In addition, the odor collection technique used in this study has implications for the identification of otherwise inaccessible bat roosts.

REFERENCES

Bloss, J., 1999, Olfaction and the use of chemical signals in bats: Acta Chiropterologica, v. 1, p. 31–45.

Bloss, J., Acree, T.E., Bloss, J.M., Hood, W.R. and Kunz, T.H., 2002, Potential use of chemical cues for colony-mate recognition in the big brown bat, *Eptesicus fuscus,* using olfactory cues-behavioral and chemical analysis: Journal of Chemical Ecology, v. 28, p. 799–814.

Bouchard, S., 2001, Sex discrimination and roostmate recognition by olfactory cues in the bats, *Mops condylurus* and *Chaerephon pumilus*: Journal of Zoology (London), v. 254, p. 109–117.

De Fanis E., and Jones, G., 1995, The role of odour in the discrimination of conspecifics by pipistrelle bats: Animal Behavior, v. 49, p. 835–839.

Diaz, G.J., Skordos, K.W., Yost, G.S., and Squires, E.J., 1999, Identification of phase I metabolites of 3-methylindole produced by pig liver microsomes: Drug Metabolism and Disposition, v. 27, p. 1150–1156.

Gustin, M.K., and McCracken, G.F., 1987, Scent recognition between females and pups in the bat *Tadarida brasiliensis mexicana*: Animal Behaviour, v. 35, p. 1–13.

Loughry, W., and McCracken, G., 1991, Factors influencing female-pup recognition in Mexican free-tailed bats: Journal of Mammalogy, v. 72, p. 624–626.

Scully, W.R., Fenton, M.B., and Saleuddin, A.S.M., 2000, A historical examination of the holding sacs and glandular scent organs of some bat species (Emballonuridae, Hyperspherical, Phyllostomidae, Vespertilionidae, and Molossidae): Canadian Journal of Zoology, v. 78, p. 613–623.

Suthers, R.A. 1970, Vision, olfaction, taste, *in* Wimsatt, W.A., ed., Biology of Bats, Volume II. Academic Press, New York, p. 265–309.

Voight, C.C., 2002, Individual variation in perfume blending in male greater sac-winged bats: Animal Behaviour, v. 63, p. 907–913.

Voigt, C.C, and von Helversen, O., 1999, Storage and display of odour by male *Saccopteryx bilineata* Chiroptera, Emballonuridae: Behavioral Ecology and Sociobiology, v. 47, p. 29–40.

Wright, D.W., Eaton, D.K., Nielsen, L.T., Kuhrt, F.W., Koziel, J.A., Spinhirne, J.P., Parker, D.B., 2005, In Review. Multidimensional gas chromatography-olfactometry for identification and prioritization of malodors from confined animal feeding operations: Journal of Agricultural and Food Chemistry.