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POTENTIAL USE OF CHEMICAL CUES FOR COLONY-MATE RECOGNITION IN THE BIG BROWN BAT, *Eptesicus fuscus*

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Abstract—Bats should benefit from recognition of their roost-mates when colonies form stable social units that persist over time. We used Y-maze experiments and gas chromatography–olfactometry (GC-O) to evaluate whether female big brown bats *Eptesicus fuscus* (Chiroptera: Vespertilionidae) use chemical cues to distinguish among conspecifics. In dual-choice Y-maze experiments, females chose the scent of another female from their own roost over a conspecific female from a different roost in a majority of trials. Analysis of total body odors using GC-O suggests that individuals from a given colony may share a more common odor signature with roost-mates than with non-roost-mate conspecifics. Using four principle components derived from 15 odor variables, discriminant function analysis correctly assigned most individuals to the correct colony.

Key Words—Big brown bat, Chiroptera, colony recognition, *Eptesicus fuscus*, female philopatry, gas chromatography–olfactometry, olfaction.

INTRODUCTION

Chemicals potentially provide discrete information about an animal's physical and social environment (Albone, 1984; Dusenbery, 1992). Olfactory cues may function at the individual and species level for the detection of predators and prey,

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for advertisement of social and reproductive status, as territorial markers, and for kin-recognition (e.g., Eisenberg and Kleiman, 1972; Halpin, 1986; Loughry and McCracken, 1991; Ylönen, 1994; Ferkin and Johnston, 1995).

Because many bats are highly gregarious and form stable social units (Kunz, 1982; McCracken and Wilkinson, 2000), olfactory cues may convey important information about individual or group identity (Bloss, 1999). Olfaction is important in mother–infant recognition in *Tadarida brasiliensis* (Gustin and McCracken, 1987; Loughry and McCracken, 1991), *Plecotus auritus* (De Fanis and Jones, 1995a), and *Pipistrellus pipistrellus* (De Fanis and Jones, 1996). Harem-males in *Phyllostomus discolor* respond to odors from other males (Höller and Schmidt, 1993), and *Noctilio leporinus* (Brooke and Decker, 1993, 1996), *Pipistrellus pipistrellus* (De Fanis and Jones, 1995b), *Mops condylurus*, and *Chaerephon pumilus* (Bouchard, 1998) appear to use olfactory cues to recognize roost-mates.

Recent advances in the genomics of the olfactory receptor protein (OR) have revealed a large homology among mammals (Glusman et al., 2001). Given the structural and biochemical similarities in the olfactory systems of animals as diverse as humans and insects (Acree and Bloss, 1996), and the number of odors perceived by humans that induce biological activity in other organisms (Acree and Bloss, 1996), human olfaction potentially offers a valuable tool for investigating the ecology of animals that rely on chemical cues. Because humans share the same transduction system with other mammals and are likely to detect similar odors, chemicals detected by humans should include volatiles that are biologically important to both.

Olfactory-induced behavior in mammals is elicited when mixtures of chemicals reach the olfactory epithelium (Dusenbury, 1992). The present model for humans is that all chemicals in mixtures that contribute to behavior cause some perception when experienced separately (Lawless and Heymann, 1999). Although many of the chemicals detected by humans do not influence the behavior of animals, chemicals that do affect their behavior are likely to be among those detected by humans. Using gas chromatography–olfactometry (GC-O), humans can be asked about perceptions of chemicals derived from animals, although perceptions of animals cannot be measured directly without employing invasive procedures.

This study was designed to use behavioral assays to evaluate whether female big brown bats (*Eptesicus fuscus*) respond to the odors from colony-mates over non-roostmate conspecifics. If females respond to olfactory cues from colony members, we predicted that these individuals should respond to the odors to which they are most familiar. We used GC-O to test for colony-specific odors. GC to separates overall body odor into its component chemicals and employes human olfaction to identify volatiles with possible odor-induced activity.

METHODS AND MATERIALS

The big brown bat, *Eptesicus fuscus* (Pallisot de Beauvois, 1796) is one of the most common and widely distributed bat species in North America (Kurta and Baker, 1990). Females form relatively stable maternity colonies that reestablish annually following hibernation and generally persist throughout the warm months. Maternity colonies are comprised primarily of females and their young and vary in size from a few dozen to several hundred individuals. Males are generally solitary and roost separate from females. In eastern North America, females usually roost in buildings and give birth to twins in mid-June. In western North America, females more commonly roost in tree cavities and give birth to singletons (Kurta, 1999).

Maternity colonies were located in southern New Hampshire (Milford) and central and eastern Massachusetts (Sherborn, Harvard, Sterling) and sampled during June and July from 1996 to 1998. Banding records were used to assess roost fidelity during the three-year study. Colonies were located in barns and consisted of approximately 30–75 adult females and their pups. Adult females were captured using either a hand net or harp trap (Kunz and Kurta, 1988) as individuals returned from their predawn foraging bouts between 04:00 and 07:00 hr. Nonvolant young were captured by hand from the maternity roost. Body mass and length of forearm were measured for each bat, and a numbered, lipped alloy band (Lambournes, Ltd., Leominster, England) was attached to the forearm of each bat for identification. Males were banded on the right forearm and females were banded on the left.

Odor samples were collected from several adult females from five different maternity roosts. Upon capture, a sterile cotton swab was rubbed 10 times over the head, chin, chest, genital area, and wings of individual females to collect odor samples. Swabs were placed in 3.5-ml amber septum vials with Teflon-lined screw caps. The tips of all swabs were bathed in 2 ml of ethyl acetate to prevent odor degradation. Four blank swabs (no odor) were treated in the same manner and used in the chemical analysis to verify that all odors were from the individual bats and not from the cotton swabs. All vials were concentrated to a volume of 0.2 ml before analysis.

We conducted behavioral tests to evaluate whether a female preferred the odor from a member of her own colony to that of a conspecific from a different colony. In a Plexiglas Y-maze (36.5-cm-long base with 31- × 6- × 6-cm arms; Figure 1), the odor swab from a colony member was placed in one arm and a swab from a noncolony member was placed in the other arm. A coin toss determined swab placement. Females to be tested were introduced individually into the base of the Y-maze, and the amount of time that each subject spent in each arm of the maze was recorded during a 5-min trial. Because only the odor swabs were presented to the females, their preferences could not be based on auditory or visual cues. Females were never tested with their own scent or tested more than twice. Between

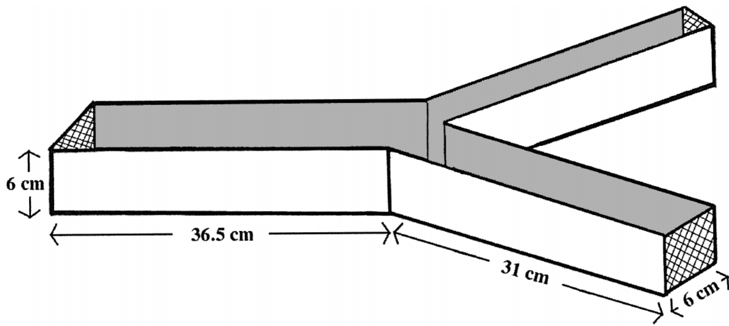

FIG. 1. Schematic diagram of the Plexiglas Y-maze used in behavioral assays to test responses of adult female *Eptesicus fuscus* to the odors of conspecifics collected from different colonies. Metal screens at each end of the Y-maze facilitated airflow through the maze.

trials, the maze was washed with ethyl alcohol to remove potentially confounding odors from previous trials.

Results of the behavioral assays were analyzed using a one-tailed binomial test (Zar, 1984; see Loughry and McCracken, 1991). When the difference in time spent in each arm of the maze was less than 30 sec, or the female did not move from the third arm of the maze, the choice was treated as a tie and excluded from subsequent analysis (Loughry and McCracken, 1991).

Each odor sample was analyzed using gas chromatography–olfactometry (GC-O) (Acree, 1997). With GC-O, gas chromatography was used to separate the components of a chemical mixture before being diluted in the humidified air of an olfactometer and blown into the face of a human detector or "sniffer." The sniffer assigned a descriptor to the perceived odor and recorded a chromatographic retention time. With the exception of a few diastereoisomers, the retention time is distinctive for each chemical. Because the odor of most chemicals is unique, when two chemicals share the same odor as well as the same retention time, they are assumed to be the same (Acree, 1997). This made it possible for us to compare the presence or absence of like chemicals across samples. GC-O involves little sample preparation time and is often more sensitive than standard methods of chemical detection such as gas chromatography–mass spectometry (GC-MS) (Pollien et al., 1999). Because a human sniffer was used, only chemicals that have odors sensed by humans were detected using GC-O. Thus, a large group of odorless chemicals was ignored, simplifying analysis and comparison across samples.

All samples were analyzed using a GC-O system designed by DATU, Inc. (Geneva, New York). One of us (J.M.B) served as the sniffer, so that descriptors assigned to each odor could be consistent across all samples. Each sample was analyzed by GC-O in duplicate, and only those responses that occurred in both runs

were used for making colony comparisons. Samples were coded so that colony affiliation was unknown at the time of analysis.

Samples were injected (splitless mode) on a 0.25-mm × 10-m OV-101 (HP1) column held at 35°C for 5 min and then programmed at 6°C/min to 225°C in a HP 5890 modified by DATU, Inc. The effluent was sniffed in a 5-liter/min air flow at approximately 75% relative humidity, and all responses were standardized in Kovats retention indices using C_7–C_{18} normal parafins (Acree, 1997).

The presence and character of each odor was recorded as the mean retention index for the response with a single descriptor. Fifteen odors were detected and measured for each sample. In addition, the duration of odor detection was recorded by the sniffer and used as a semiquantitative measure for each odor. The duration of time that a sniffer detects an odor corresponds to the range of retention indices (peak width) over which a odor is eluted. This peak width is roughly associated with the amount of the chemical that produced the odor in the sample. Factors that may have biased these measurements include: (1) overestimation of the most polar odor caused by peak broadening on methyl silicon, (2) differences in the odor thresholds of bats and humans, and (3) selective loss of volatiles in the sampling procedure. Nevertheless, using presence or absence, an odor description and a semiquantitative measure of the amount of odor were recorded for each of 15 chemicals.

Principle component analysis was first used to collapse the 15 odor variables, followed by discriminant function analysis of the first four principle components. Cross-validation was used to estimate error rates and to control for the potential bias associated with using the same set of observations to classify as well as to generate the discriminant function (Johnson and Wichern, 1992). Principle component analysis and discriminant function analysis were conducted using the SAS statistical package (SAS Institute Inc., 1990).

The average retention index and odor descriptor for each of the 15 chemicals analyzed in this study were then compared to published OV-101 retention indices and descriptors for known compounds found on the Flavornet database (Acree and Arn, 1997). When the retention index and descriptor were identical or similar, a possible odor match was indicated. A literature search for these chemicals was conducted to determine if there was published evidence that would suggest biological activity in other systems.

RESULTS

Female big brown bats (*Eptesicus fuscus*) selected one arm of the Y-maze (difference in time spent between arms >30 sec) during 127 trials using 117 different individuals. Individual females spent more time in the arm of the maze that contained the odor from their own colony-mate in a majority (77%; $N = 98$) of trials (one-tailed binomial test; $P < 0.001$).

Our banding records indicate that many adult females and female young-of-the-year return each summer to the same maternity roost. Of 108 adult females banded in 1996, 70 were recaptured as adults at the same colony in 1997 and, among these, 34 were recaptured in 1998. Twenty-two of the 182 female pups banded in 1996 returned to their natal colony as adults in 1997. Among these 22 females, 17 females showed signs of lactation. The following year, 10 of these females were recaptured and showed signs of lactation or postlactation. Fourteen of the 230 female pups banded in 1997 were recaptured as adults in 1998, and 10 were reproductively active. Only four pups were recaptured at a colony other than the colony of initial capture. Two were individuals who moved from the Milford A to Milford B roost. The other two were adult females banded at Harvard in 1996. One was captured at Milford A, and the other was captured in Milford B in 1997. Switching between roosts was extremely rare, and those recorded may represent errors in the recording of band numbers.

Because the extracts of swabs that contained odor samples were well below the sensitivity of GC-MS, we used human GC-O to detect and semiquantify these odors. The presence and quantity of 15 odors from 20 individual samples were used in principle component analysis. Chromatograms were generated for each sample and indicated the presence and quantity (duration of elution time/peak width) for odors shared among individuals. For example, one female from the Harvard colony and another from the Sterling colony shared seven common odors that differed in quantity (Figure 2). None of the four control swabs indicated a detectable odor when analyzed using GC-O. The first four principle components accounted for 67.9% of the variation and were subsequently used in discriminant function analysis (Table 1). Examination of character loadings for the first four principle components revealed that the first principle component was most heavily loaded with the peak widths of the chemicals eluting at retention times 848 and 1007. The second principle component was most heavily loaded with the chemical at retention time 1187 and most negatively associated with the odor emitted at 1302. Odors detected at 1076 and 1149 both loaded most strongly on the third principle component, and those detected at 772 and 1145 loaded most heavily on the fourth

TABLE 1. PRINCIPAL COMPONENT ANALYSIS OF VOLATILE ODORS IN *E. fuscus*[a]

Component	Eigenvalue	Difference	Proportion	Cumulative variation
Prin 1	3.21536	0.329315	0.214357	0.21436
Prin 2	2.88604	0.643554	0.192403	0.40676
Prin 3	2.24249	0.402898	0.149499	0.55626
Prin 4	1.83959	0.557045	0.122639	0.67890

[a] Measurements of 15 volatile odors from female *Eptesicus fuscus* were collapsed to four principle components. The first four principle components describe 67.9% of the variation and were used in discriminant function analysis.

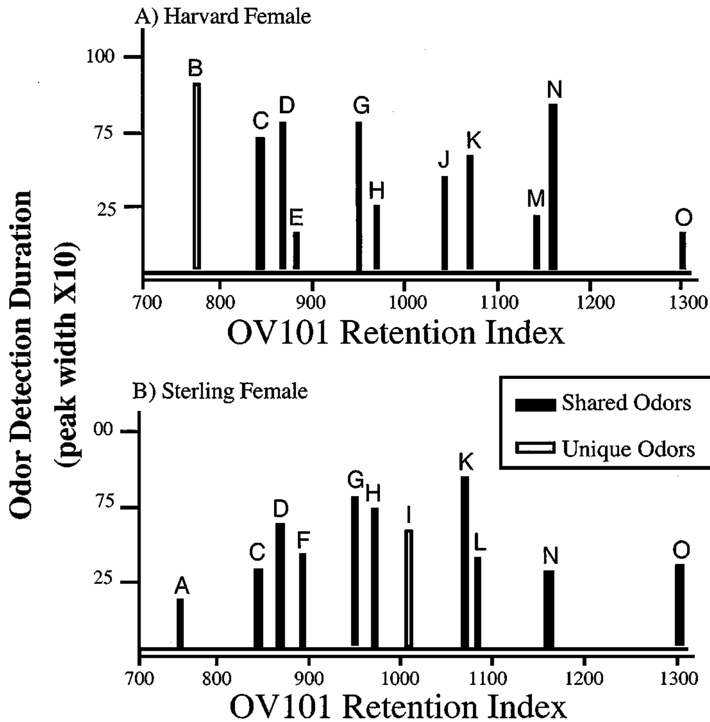

FIG. 2. GC-O chromatograms from two adult female *Eptesicus fuscus* collected from two different maternity colonies in central Massachusetts. These two females shared seven common chemicals (as determined by retention time and descriptions of odors) and quantity (peak width). Letters refer to variables listed in Table 2.

principle component (Table 2). While individuals varied in the relative contribution of each odor component (Figure 2), principle component analysis clearly revealed a separation of odor signature by colony (Figure 3).

Using the first four principle components, discriminant function analysis with cross-validation correctly classified 100% of the bats analyzed from Sherborn ($N = 6$), Harvard ($N = 4$), Milford A ($N = 3$), and Milford B ($N = 3$). For the Sterling colony ($N = 4$), two individuals were incorrectly classified as belonging to the Milford A colony.

From similarities in odor descriptors and OV-101 retention indices, likely chemical matches were found for 14 of the 15 odors used in the colony analysis. A literature search of these compounds shows that many of the chemicals identified as being potential matches were important in other biological systems (Table 3).

TABLE 2. RETENTION TIMES, DESCRIPTORS, AND PCA LOADINGS FOR THE 15 VARIABLES[a]

			Component loadings			
Variable	Retention time	Descriptor	Prin 1	Prin 2	Prin 3	Prin 4
A	772	Fruity	0.2136	−0.2632	0.0982	<u>−0.4592</u>
B	788	Sweet	−0.2365	−0.2106	−0.3235	0.0664
C	848	Musty	<u>0.4586</u>	0.2158	0.0577	−0.8761
D	870	Sweet/Plastic	0.2603	−0.1714	0.2758	−0.1721
E	884	Musty	0.2457	−0.1505	−0.0369	0.3529
F	896	Corn Meal	−0.2775	0.0870	0.1954	0.1029
G	949	Musty/Plastic	0.2096	0.0906	0.1698	0.2319
H	960	Eggplant	0.2690	0.2090	0.1629	0.3615
I	1007	Musty	<u>0.4170</u>	−0.0484	0.0900	0.2673
J	1049	Burning	−0.3587	0.2396	0.2781	0.2739
K	1076	Maple	0.0626	0.3243	<u>−0.5374</u>	−0.0478
L	1187	Fruity	−0.1191	<u>−0.5144</u>	−0.1803	0.1395
M	1145	Burning	0.0349	0.0759	0.2376	<u>−0.4938</u>
N	1149	Plastic	0.0507	0.3192	<u>−0.5415</u>	−0.0670
O	1302	Urine	0.2055	<u>0.4692</u>	0.2420	−0.1063

[a] The 15 variables were used in the principle component analysis of odors detected in female *Eptesicus fuscus*. The variables that load most heavily on the first four principle components are underlined. The first principle component is most heavily loaded for variables C and I; the second principle component for variables L and O; the third for K and N and the fourth for A and M.

DISCUSSION

It has become increasingly evident that olfactory cues are used by bats to identify individual colony members (Schmidt, 1988; Brooke and Decker, 1993), as territorial markers (Höller and Schmidt, 1993; Haffner, 1995; French and Lollar, 1998), and in mother–infant recognition (e.g., Watkins and Shump, 1981; Gustin and McCracken, 1987; Esser and Schmidt, 1989; De Fanis and Jones, 1995a; see Bloss, 1999 for a review). Olfactory cues may also be used by bats to complement auditory cues associated with mother–infant recognition (e.g., Thomson et al., 1985; Balcombe, 1990; Jones et al., 1991; Balcombe and McCracken, 1992; De Fanis and Jones, 1996).

This study provides the first evidence that female big brown bats, recognize the scent from members of their own colony. De Fanis and Jones (1995b) found similar results in the common pipistrelle, *Pipistrellus pipistrellus*. As in the study of De Fanis and Jones (1995b), we were not able to determine whether bats responded to the odor of familiar individuals or whether a group-distinct odor was recognized. Notwithstanding, the similarity among odors produced by members of the same colony of *Eptesicus fuscus* is supported by our chemical analysis. Discriminant function analysis was successful in grouping odor signatures by

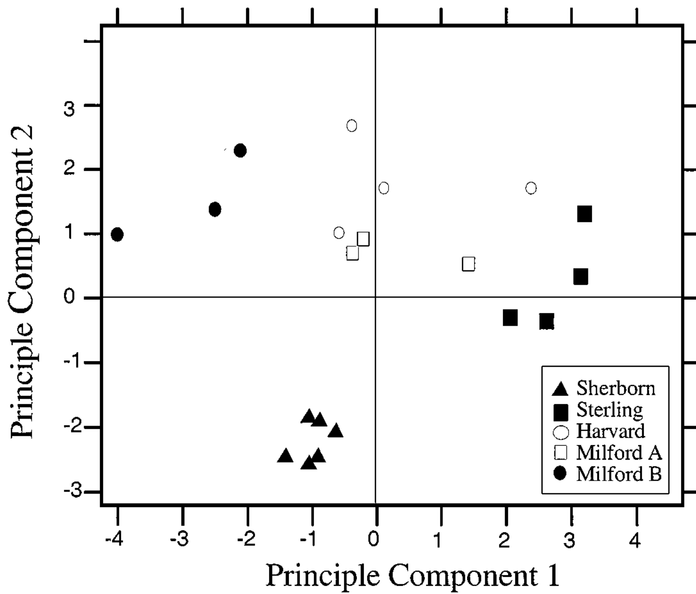

FIG. 3. Principle component analysis of the overall body odor female *Eptesicus fuscus* was used to collapse 15 measured odor variables into four components (see Table 1). This analysis reveals a separation of colonies by the first and second principle components.

the correct colony in a majority of cases, providing evidence for colony-specific odor signatures.

If colony members share similar odor signatures with one another more than they do with conspecifics from other colonies, the logical question becomes what factors produce these similarities? The consistency of odors among colony mates may be due to similarity in microbial cultures, shared food sources, identical roosting substrate, genetic relatedness, or a combination of any or all of the above.

Odors may be derived from several sources. Secretions from specialized glands, urine, or feces or by-products of microbial metabolism may all contribute to the overall odor of an individual. For example, glandular pockets of the fish-eating bat, *Noctilio leporinus*, contain pungent odors (Brooke and Decker, 1993, 1996). When bacteria from these pockets have been cultured, the odors produced were similar to those produced by the bats that were sampled (Studier and Lavoie, 1984). Thus, group-specific odors may reflect unique bacterial cultures shared by grooming or roosting in proximity to members of the same colony (Dapson et al., 1977; Studier and Lavoie, 1984). Members of the genus *Eptesicus* possess well-developed facial and pararhinal glands (Quay, 1970), which may contribute to odor production.

TABLE 3. POSSIBLE CHEMICALS ASSOCIATED WITH ODORS DETECTED FROM GC-O ANALYSIS OF *Eptesicus fuscus*[a]

RI	Possible compound(s)	Biological activity in other systems
772	Methyl-2-methylbutanoate	?
788	Ethyl butanoate	Dung beetle attractant (Burger et al., 1995)
848	2-Methyl-1-butanol	Sap beetle attractant (Nout and Bartelt, 1998; Bartelt and Wicklow, 1999)
870	(*E*)-2-Hexene-1-ol	Green capsid bug attractant (Groot et al., 1999); spined soldier bug attractant (Sant'Ana et al., 1999)
884	Ethyl valerate	?
896	[A]2-Furfurylthiol or [B]2-acetyl-1-pyrroline	[A]Corn volatile (Buttery and Ling, 1998); [B]mousy aroma in wines (Herderich et al., 1995); maize volatile (Bredie et al., 1998)
949	Heptanol	?
960	[A]6-Methyl-5-hepten-2-one or [B]1-octen-3-ol	[A]Volatile involved in attracting aphid-tending ants (Cordova-Yamauchi et al., 1998); ant pheromone (Do Nascimento et al., 1998); aphid parasitoid attractant (Du et al., 1998); aphid spacing chemical (Gonzales et al., 1999); [B]vertebrate volatile attractant to mosquitos (Takken, 1999) and ticks (Osterkamp et al., 1999); green capsid bug attractant (Groot et al., 1999)
1007	Phenylacetaldehyde	Sternal gland secretion in male koalas (Salamon and Davies, 1998); fall armyworm attractant (Meagher and Mitchell, 1998); floral scent attracting Lepidoptera (Omura et al., 1999a,b) and Hymenoptera (Meagher and Mitchell, 1999)
1049	(*E*)-Ocimene	Volatile released by damaged cotton plant (Rose et al., 1998) or broad bean (Du et al., 1998) which attracts herbivore enemies
1076	2-Phenylethanol	Sheep blowfly attractant to host odor (Park and Cork, 1999); pineapple beetle (Zilkowski et al., 1999), house fly (Chapman et al., 1998), twelve-spotted lady beetle and green lacewing attractant (Zhu et al., 1999)
1087	Linalool	Volatile released by damaged plants is an attractant for spined soldier bugs (Sant'Ana et al., 1999); lavender compound used in aromatherapy (Lis-Balchin and Hart, 1999); green capsid bug attractant (Groot et al., 1999); volatile used by Egyptian cotton leaf worm to decriminate between damaged and undamaged plants (Jonsson and Anderson, 1999); moth-pollinated flower volatile attractant to hawkmoths (Raguso et al., 1996; Raguso and Light, 1998)
1145	Benzyl acetate	Moth-pollinated flower volatile attractant to hawkmoths (Raguso et al., 1996; Raguso and Light, 1998)
1149	?	?
1292	*O*-Aminoacetophenone	Component of Japanese weasel anal sac (Acree et al., 1990); mountain beaver (Nolte et al., 1993) and bird repellent (Clark, 1998)

[a] The potential compounds were determined using the descriptors and OV-101 retention times listed on the Flavornet (Acree and Arn, 1997). Many of these chemicals have been shown to be biologically active odorants in other systems. The retention index (RI) refers to the average RI used in this study and corresponds to the retention times listed in Table 2. A question mark denotes that the RI and odor desciptors did not match any listed on the Flavornet or the compound was not found in recent literature.

By a similar mechanism, members of a colony that forage in the same general area may share a similar diet. Diets of different big brown bat colonies are known to differ in insect composition (Whitaker, 1995). The metabolism of related food items, especially by related individuals could produce similar volatile by-products and, thus, affect overall body odor (Schellinck et al., 1997). In our study, two members of the Sterling colony were misclassified into the Milford A colony. These colonies are separated by approximately 45 km. Individuals from the two Milford colonies, separated by only 2 km were not misidentified as belonging to the other Milford maternity roost. This evidence suggests that shared diet may not be an important factor in determining colony odor. However, further investigations using dietary controls are needed to assess this possible source of odor production, since even bats from the same roost may forage in different locations and consume varied diets. In addition, roosting substrate should be evaluated, as individuals in our study may have responded to the familiar odor of their roost in behavioral tests.

Female *Eptesicus fuscus*, especially those from maternity colonies in human-made structures, tend to show high site fidelity during pregnancy and lactation (Brigham and Fenton, 1986). After disturbance, colony members often relocate as a group, indicating that roosting assemblages may function as social groups rather than random aggregations of individuals (Brigham and Fenton, 1986). Since our banding records support female philopatry, inhabitants of these maternity roosts are likely to be genetically related. Colony-specific scents could be the result of genetically related individuals that produce similar odors.

The major histocompatibility complex (MHC) has been shown to affect the odor of an individual (Eggert et al., 1996, 1998). MHC is a highly polymorphic group of genes that controls the immune system's recognition of self and nonself (Eggert et al., 1998; Penn and Potts, 1999). Although the precise mechanism for how MHC loci influence odor production is not entirely clear, based on olfactory cues, some rodents and humans are able to distinguish individuals who vary in this gene complex (Eggert et al., 1998; Penn and Potts, 1998). A similar mechanism might exist in bats if chemosensory recognition of kin occurs among individuals that share similar MHC regions. Further studies are needed to elucidate such a recognition system in bats, and a first step would be to evaluate the degree of genetic relatedness among colony members.

Group recognition via olfactory or other cues could facilitate the stability of social groups. Familiar individuals are more likely to cooperate and avoid agonistic interactions than those with infrequent contact or that are unrecognized (Lewis, 1995). Roosting groups are important for bats, especially in maternity colonies where stable temperatures are vital for proper development of the young (Kunz and Hood, 2000). The cost of thermoregulation is decreased by clustering behavior (Herreid, 1967; Trune and Slobodchikoff, 1976; Bonaccorso et al., 1992; Lewis, 1995). The recognition of colony mates may also facilitate winter aggregations in hibernacula.

When the possible chemical identities of the 15 odors used in the study were investigated, 11 of the 14 potentially identified compounds were found to be biologically active in other taxa (Table 3). For example, the odor described as musty, with an average retention index of 1007 (Table 2), is probably phenylacetaldehyde with a retention index of 1004 (Acree and Arn, 1997). This compound was identified as a heavily loaded variable in the first principle component (Table 2) and may be important in colony recognition. Phenylacetaldehyde is present in the secretion from the sternal gland of male koalas (Salamon and Davies, 1998). It is also an attractant for many lepidopteran (Omura et al., 1999a,b) and hymenopteran species (Meagher and Mitchell, 1999). The biological importance of an odor among diverse taxa is not surprizing given the homology that exists in the olfactory detection systems of a wide diversity of organisms (Acree and Bloss, 1996).

Most of what is understood about pheromones comes from the study of insects. This group accounts for about 70% of all described species, and many of these are agricultural crop pests (Agosta, 1992). Since considerable research is devoted to understanding insect attractants and repellents, most compounds identified as potential chemicals in big brown bat colony odor also were considered to be important in insect systems (Table 3). In fact, many of the insect families represented in Table 3 have been reported in the diet of *E. fuscus* (Whitaker, 1995; W. R. Hood, unpublished data). As the study of chemical ecology expands, many insect pheromones have been shown to be biologically important in vertebrates (Acree and Bloss, 1996).

Our study does not unequivocally demonstrate that colony members recognize each other using olfactory cues or that group-distinct colony odors are present. However, it does suggest that female big brown bats prefer the odor of a roost-mate to that of a conspecific from another colony. Such a preference may promote colony stability and, thus, have implications for foraging, kin recognition, and mating. Olfactory cues, like the well-studied auditory cues of bats may be important in establishing and maintaining social interactions within a species.

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