

Chemical Signaling and Scent Gland Secretions in Bats: Understanding Olfactory Communication and Its Role in Breeding Behavior and Reproductive Success

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Abstract

Bats communicate among themselves by visual, acoustic and olfactory signals. They possess modified skin glands at distinct regions to secrete odoriferous compounds. Breeding behavioural studies reveal chemical signals from scent glands are very much involved in communication. The secretions from the scent glands were chemically identified on the basis of mass spectra identified by GC-MS. The results confirmed that these secretions differ in chemical composition with respect to sex, vicinity of the glands and the season. These signals during breeding season provide sexual identity, information about reproductive status and related communications among the individuals which facilitate to bring reproductive success. Bats are one of the key stone species in the ecosystem. They need to be protected to ensure a sustainable ecosystem.

Keywords: Communication, Odoriferous compounds, chemical signals, ecosystem.

Introduction

Bats have a wide variety of odour producing organs which include specialized integumentary glands (Quay, 1970). These glands produce chemical substances in the form of a liquid or sebum (oily secretion) which serves as an important odour source (Gustin and McCracken, 1987; French and Lollar, 1998; Haffner, 1998). Bat's scent glands are generally located on regions like frontal in *Hipposideros gigas, Hipposideros commersoni* and throat in *Craseonycteris thanglangyal* (Hill, 1974) ; shoulder as in the case of *Pteropus giganteues* (Neiweiler, 1969) and the surroundings of the anal opening in *Taphozous melanopogon* and *Hipposideros spearis* (Kunz, 1990 ; Bates and Harrison, 1997 ; Kannan, 1998 ; Lily, 2005). The glands are of two types namely apocrine sudoriferous and holocrine sebaceous. The differences in gland type and their secretions among and between the species of bat strongly suggest that the olfactory cues are highly variable among bat species (Schmidt, 1985).

The nocturnal and social life style of bats suggest that besides acoustic signals, scent plays a key role in communication to achieve reproductive sources (Kannan and Kerth, 2003). Chemical cues are likely to be an important mode of gathering information, advertisement of social and reproductive status, territory marking and kin recognition (Gustin and McCracken, 1987 ; Brooke and Decker, 1993, 1996). Chemical signals from integumentary glands and mate recognition in big brown bat, *Eptesicus fuscus* (Blosset *al.*, 2002), mate choice in sac winged bat, *Saccopteryx bilineata* (Voigt and Helversen, 1999) and colony recognition in fishing bat, *Noctilio leporinus* (Brook and Decker, 1996). These signals honestly inform females even about the genetic compatibility of potential males (Wedekind *et al.*, 1995 ; Rich and Hurst, 1998).

In mammals, scent production is strong and act as a secondary sexual signal during mating season. This is one of the man's causes for the reproductive success. Brooke and Decker (1993) reported the dominant male of *Noctilio leporinus* during breeding season roost with a harem of females and produce more odorous and voluminous secretions through subaxial glands. Lily (2005), Lily and Vanitharani (2005a, b) have documented that during mating season in *Hipposideros speoris* (frontal), *Taphozous melanopogon* (neck) and *Cynopterus sphinx* (anal) the integumentary glandular secretions were enormous and acted as sexual attractants. With these secretions, they make territory markings, self-anointment, body rubbing, wind flapping and much more specific courtship behaviours.

Materials and methods

The bats were caught by the mist nets from the specific roost. The mature adult males and females were selected and different integumentary tissues from various parts, frontal region, surrounding of eye, lip region, neck and surrounding of the anal regions were removed both during breeding and non-breeding seasons. The tissues were fixed in Bouin's fluid and stored in 70% ethyl alcohol for histological study. After routine processing, these tissues were embedded in paraffin wax for sectioning. Microsections of 10 μ thickness were cut and stained with haematoxylin and eosin. The nature and type of glands of each region were identified using Nikon Microscope under 10x, 40x and 100x magnification.

Fractionation of Glandular Secretions

The glandular secretions collected from three bat species (*Hipposideros speoris*, *Taphozous melanopogon* and *Rousettus leschenaulti*) during breeding season were collected by cotton swabs and immediately dissolved in Dichloromethane. The glandular extracts were stored in air tight containers at -20oC until they were analysed under GC-MS (Rosell and Sundsdal, 2001). A fused silica capillary column 125 m x 0.25 mm id.) on Shimadzu 17A equipped with mass spectrometer (Shimadzu QP 5000) was used to separate and identify the volatiles of the glandular secretions. The initial column temperature was set to 70°C for 2 minutes, increased to 250oC by 30oC per minute and held for 30 minutes. Helium was used as a carrier gas at a flow rate of 0.6 ml per minute. The transfer line temperature and electron ionization were set at 300oC and 70oC respectively. The Mass Spectrometer was operated in scan mode over a mass range of 25 to 700 amu.

Behavioural studies

Bat species have been selected for observing the behavior during breeding and non - breeding season Video recording was made to study the behavioural patterns. The infrared sensitive, night vision 120x digital (3 mega pixel) Sony handycam was used to record the scent marking activity. The behavioural movements were recorded. The recordings were processed and still images were stored as jpg files.

Results

The histological study reveals that the frontal glandular secretions were observed in between the eyes. It is more prominent in males during mating season. The sebaceous glandular layer produces new cells continuously. The thick secretory product is called 'sebum'. The smell of this product is similar to rotten degraded fruit odour in their roosting site.

The glandular secretions collected from three different species during breeding and non-breeding season were subjected to GC-MS analysis. The GC-MS chromatogram results exhibit the chemical compounds of the secretions. These chemical compounds differ even within the sex and also in individuals from region to region depending upon the glandular position and nature. The odour producing components of the glandular secretions with their organic nature are given in Table 1, 2, 3.

In the males of *H. speoris* there is an obvious specialized pouch filled with sudoriferous glandular secretions in the frontal region. During mating season, a conspicuous yellow oily sebaceous secretions released from the anal gland of males, soaks the surroundings of the anal area. This secretion is deposited along with urine in the day roosts to mark the territory. During mating period, male *Taphozous melanopoqon* m has a prominent beard below the chin. The small glandular openings of the sudoriferous glands produce a sticky secretion which wets the hairs of the beard. Males of *Rousettus leschenaulti* produces transparent, sticky and liquid like secretion from the skin of neck region which wets that surrounding area.

Discussion

Generally, in mammals, glandular secretions contribute much for the individual odour and also in its habitat where the animal lives. The integumentary glands empty their products outside the animal's body to expel the odour. Such types of glands are prominent more in the head (Kunz, 1990) and the surroundings of the anal opening (Bates and Harrison, 1997 ;Kannan, 1998).

The frontal gland secretions of black tailed deer, *Odocoileus hemionus* (Thiessen and Rice, 1976), *Odocoileus virginianus* (Atkesan and Marchinton, 1982) and ferret, *Mustela fura* (Clapperton, 1989) were used by these mammals to mark their territories and select their mates. Similarly, the male frontal glandular secretions rubbed over the selected female indicater that these are the chemical substance used to attract and mark the females. The anal secretion mixed along with the urine disperses the scent in their roosting sites. The fishing bat, *Noctilloleporinus* produces a musky sweet odour from sub-axial glands and also a bitter scent from the inguinal pockets found on either side of the testes (Gudger, 1945). Such specific pungent smell was observed in the roost of *Rousettus leschenaulti.*

Odorous secretions as sex attractants

GC-MS profile of glandular secretions of bat species confirm that they have specific fatty acid composition like octadecanoic acid, hexadecanoic acid, steroid derivatives, aromatic and heterocyclic compounds. These major constituents bring species specific odour to the bats and their roosting site.

In bat species, the males possess fatty acids with higher number of carbon atoms in the frontal glandular secretions $C_{16} - C_{17}$dexadecanoic acid and C14 decanoic acid. These fatty acid secretions were not noticed in both sexes during non-breeding season. Similar possession of fatty acid compounds was reported in other animals, $C_{15} - C_{25}$ fatty acids from the occipital secretions of camel (Ayorinde*et al.*, 1982); $C_{26} - C_{18}$ fatty acid secretions from preorbital secretions of Ourebiaorebi (Mo *et al.*, 1995) and $C_{20} - C_{25}$ fatty acids from precloacal secretions of Liolaemus lizard (Carlos *et al.*, 2001) are used as sex attractants to select their mate.

During breeding season, the frontal grandular secretions wet the hair of the face (Genherd, 1977) and the bats show peculiar rubbing and sniffing behavior with the selected mates. Clapperton (1989) reported that the male ferret sniffs the anal region of the female to identify the resident females. Schaller (1967) observed the tiger sniffs the genital region of females to identify the partner. Similar reports have been identified in Kangaroo (Brown and MacDonald, 1985). In the present study, the presence of ester compounds in three bat species. This type of peculiar rubbing and sniffing behavior by the males with the selected females also observed in bat species. Anal secretions of females convey the oestrous status to the males (Hurst, 1987, 1990).

Odorous secretions as markers

Chemical odorants show a great deal of variation in the composition by the possession of various functional groups. Such groups with oxygen atoms play a vital role in the production of strong odour for a prolonged time. In bat species; these chemical compounds are helpful to mark the territory.

Odorous secretions for individual recognition

The chemical compounds like alkanes, steroid, heterocyclic compounds and specific compounds with sulphur and nitrogen composition proved to be odour producing substances. These compounds specially helps individual and group recognition in a colony and also aids in sex identify in a social system (Scully *et al.*, 2000 ; Kamran and Kerth, 2003 ; Lily, 2005 ; Lily and Vanitharani, 2005).

The same biochemical composition is present in three bat species. Reports about similar biochemical compounds playing a vital role in individual recognition are known from the preorbital secretion of Ox, *Ovibas maschatus* (Flood *et al.*, 1989) and blesbok, *Damaliscus dorcus phillipsi* (Burger *et al.*, 1999).

Odorous secretions for self-anointment

Behavioural studies specifically mentioned about the territory marking and self-anointment to advertise the social status and reproductive fitness of the individuals in a colony. Similarly in Beaver, *Castor fiber* (Rosell and Sundsdal, 2001 ; Collins *et al.*, 2001) and rabbit, *Oryhctologus cuniculus* (Hayes *et al.*, 2002) the chemical signals help the individuals to recognize the age and the maturity status of the partners during breeding season. Observations in the present study reveal the sticky secretions from the facial glandular secretions of bat species spread all over the body during breeding season. This self-anointment can be seen obviously on the fur if the bats are captured during breeding season. Self-anointment of glandular secretions not only creates odour but also help the animal to look more attractive to their conspecifics (Bowyer and Kitchen, 1987).

In addition, bats use a special way of dispersing body fragrance by way of wing flapping and fanning behavior. In all the three bat species, males fan the odour by fluttering fast to disperse the scent. Most of the time one wing is folded and the other wing is fluttered over the responding female. This type of behavior was observed by Voigt and Helversen (1999) in *Saccopoteryx bilineata* and in *Taphozous melanopogon* by Lily (2005). Dispersal of odour by self-anointment has been noticed in several mammals, black tailed deer, *Odocolleus hemionus* (Muller-Schwarze, 1971); *Genetta tigrina* (Wemmer, 1972); ground squirrel *Spermophilus columbianus* (Steiner, 1973) and fallow deer, *Dama dama* (Chapman and Chapman, 1975).

Bat pollination is an integral process in tropical communities with 500 tropical plant species completely or partially, dependent on bats for pollination (Heithaus, 1974). Plants pollinated by bats have large amount of nectar and emit a smell that attracts bats, such as a strong fruity or musky odour (Gibson, 2001). Bats are attracted to odours that contain esters, alcohols, aldehydes and aliphatic acids (Gibson, 2001). This study on chemical communication in temple bat species is certainly an important contribution to protect temple bat species and thereby the ecosystem.

Chemical cues during breeding season in bat species play an important role in mate choice, individual identity and also to propagate the reproductive status to colony mates. The chemical signals during non-breeding season mainly help the colony to maintain the social status and roost fidelity. This study strongly confirms chemical signals through glandular secretions are important factor and immensely responsible for bringing reproductive success as well as the continuity of the race. The glandular hairs are thick with larger shaft and darkly pigmented. The space between the scales of the hair helps to hold and conduct the glandular secretions. This helps to hold pollen and aids in cross pollination. This suggested that the bats are important for pollination, seed dispersal and pest control.

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Table 1. GC-MS analysis of male and female frontal gland of facial gland of *Rousettus leschenaultia*(*indicates the Odoriferous compounds)

Types of gland	Male				Female			
	Breeding	**Nature**	**Non-Breeding**	**Nature**	**Breeding**	**Nature**	**Non-Breeding**	**Nature**
Frontal gland	Hexadecyl ester of octadecanoic acid*	Ester	13-Decyl-13-heptyl Tritriacontane	Aliphatic	Methyl ester of 15-Tetracosenoic acid*	Ester with acid	1, 1-Bi*Phenyl, 4, 4-dimethyl	Aromatic
	2-(Octadecyl oxy) ethyl ester of 9-Octadecanoic acid*	Aliphatic	2methyl ester of 9-Octadecanoic acid*	Acid with ester	Cyano acetate	Aliphatic		
	2-Hydroxy methyl ester of Hexadecanoic acid	Ester	1-Penta bromo 2-Benzene	Aromatic	3-Chloro-17 beta Hydroxy-acetate androsta-2, 4, 6-triene-2-carboxaldehyde*	Steroid		
	2-Iodo Thiophene	Heterocyclic	Cholestan-3-*One	Steroid	3, 20-Diamine pregnane*	Steroid		
	1, 3 Bis (1, 1-dimethyl ethyl)-5-methyl Benzene*	Aromatic						
	5-Undecen-4-one	Aliphatic						
	Dimethyl ester of Dodecaneioic acid	Ester						

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Table 2. GC-MS analysis of neck gland of *Rousettus leschenaultia* (*indicates the Odoriferous compounds)

Types of gland	Male				Female			
	Breeding	**Nature**	**Non-Breeding**	**Nature**	**Breeding**	**Nature**	**Non-Breeding**	**Nature**
Neck gland			1, 2, 3-Propane triyl ester of Octadecanoic acid*	Ester containing acid	1-[(Dodecyl) methylethane] ester of Octadecanoic acid*	Acid	No Compounds	
	Tetradecyl ester of Octadecanoic acid*	Ester containing acid	Triacontane	Aliphatic	Dioctyl ester of 1, 2 benzene dicarboxylic acid*	Ester containing acid		
	6-Methyl Octadeeane	Aliphatic	-		-			
	2-Methyl Pentadecane	Aliphatic						
	Disodecyl ester of 1, 2-Benzene dicarboxylic acid*	Ester containing acid						

Table 3. GC-MS analysis of male and female anal gland of *Rousettus leschenaultia* (*indicates the Odoriferous compounds)

Types of gland	Male				Female			
	Breeding	**Nature**	**Non-Breeding**	**Nature**	**Breeding**	**Nature**	**Non-Breeding**	**Nature**
Anal gland	Dilsooctyl ester of 1, 2-benzene carboxylic acid*	Ester containing acid	2-Cyano-3-(3-bromo phenyl)-ethyl ester of -2-Propenoic acid*	Ester containing acid	-2-ketone derivative of Anopteryl alcohol*	Aromatic	No compounds	
	2(Dimethyl amino)-1-phenyl-5- nonanone	Aromatic	1-(2, 4, 6-Trimethyl Phenyl)-3 methyl-1-butene	Aromatic	Cholesta-2, 8-dien-6-01	Steroid		
	1, 1-(1-Fluoro, 1, 2-ethene diyl)* bisbenzene	Aromatic	-	-	N-Octyl-1-Octananmine	Aliphatic		
	-				5-Heptadecyl-methyl ester of -2-Thiophene carboxylic acid*	Ester containing acid		