SCENT GLANDS AND CHEMICAL SIGNALING IN *HIPPOSIDEROS SPEORIS* (LEAF NOSED BAT) DURING BREEDING SEASON

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Abstract

Bats communicate among themselves by visual, acoustic and olfactory signals. Hipposideros speoris, possesses 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 obtained 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.

Keywords : *Communication, odoriferous compounds, chemical signals, scent glands.*

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Introduction

Chemical communication through scent gland is universal. 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 Lillar, 1998 ; Haffner, 1998). Bat's scent glands are generally located on regions like frontal in Hipposideros gigas, Hipposideros commersoni and throat in Craseonycteris thonglongyai (Hill, 1974); shoulder as in the case of Pteropus giganteus (Neiweiler, 1969) and the surroundings of the anal opening in Taphozous melanopogon, Hipposideros speoris and Rousettus leschenaulti (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 the between the species of bat strongly suggest that the olfactory cues are highly variable among bat species (Schmidt, 1985).

The nocturnal and social lifestyle of bats suggests that besides acoustic signals, scent plays a key role in communication to achieve reproductive success (Kamran 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 aid mate recognition in big brown bat, *Eptesicus fuscus* (Bloss et al., 2002), mate choice in sac winged bat, *Saccopteryx bilineata* (Voigt and Helversen, 1999) and colony recognition in fishing bat, Noctilio leporinus (Brooke 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 main 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 odours 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), Rousettus leschenaulti (anal) the integumentary glandular secretions were enormous and acted as sexual attractants. With these secretions, they make territory markings, self anointment, body rubbing, wing flapping and much more specific courtship behaviours.

Materials and methods

Scent gland histology

Mist nets were placed near the Bat Research Laboratory (Ele : 436ft, N 8°21.59', E 77°30.75') and collelction of Cynopterus sphinx was made to study the integumentary glands. Different integumentary tissues from various parts, frontal region, surrounding the eyes, lip region, neck, shoulder, ear, wing and the surroundings of the anal region were removed from the adult male and female bats, fixed in Bouins fluid and stored in 70% ethyl alcohol for histological studies. After routine processing, these tissues were embedded in paraffin wax for sectioning. Micro sections 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 (Nikon Trinocular Microscope model Eclipse 50I, Tokyo) under 10x, 40x and 100x magnifications. The glandular type has been microphotographed using Nikon Cool Pix 5400 digital camera.

Fractionation of Glandular secretions

The glandular secretions from the specialized glands of male and female C. sphinx during breeding season were collected by cotton swabs and immediately dissolved in Dichloromethane. The glandular extracts were stored in airtight containers at -20ºC until they were analysedundr GC-MS (Rosell and Sundsdal, 2001). A fused silica capillary column 125 m x 0.25 mm i.d.)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 70ºC 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 was 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

A colony of Hipposideros speoris roosting in has been selected for observing the behavior during breeding and non-breeding seasons.

Video recording was made to study the behavioural patterns. The infrared sensitive, night vision 120x digital (3 mega pixels) Sony handycam was used to record the scent marking activity. The behavioural movements were recorded between 6.30 to 8.30 p.m. during mating season in May 2005. The recordings were processed and still images were stored as JPG files.

Result

The histological study reveals that the scent glands are located in the frontal, neck, ear and anal regions of both the sexes (Fig. 1 : a-e). All glandular regions possess sebaceous glandular layers. The number of the glandular cells was enormous and they produce thick, yellowish brown secretory product called sebum during breeding season. The potential males make specific territory marking in the roosting tree with anal glandular secretions and settle in the marked territory. They rub the neck gland secretions all over the body and fragrance the environment by wing flapping. This self-anointment induces the females to respond and settle around the marked territory. The selected female is dragged by the male repeatedly and made to study in the marked territory. Glandular secretions facilitated courtship behavior, roost selection and territory marking (Fig. 2 : a-f).

Fractionation of chemical compounds

The glandular secretions collected from both sexes of Hipposideros Speoris 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.

Discussion

Generally, in mammals, glandular secretions contribute much for the individual odour and also to 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). Table 1 explains in Hipposideros Speoris the frontal, neck, ear and anal secretions of both the sexes remarkable and produce specific odours.

The frontal gland secretions of black tailed deer, Odocoileushemionus (Thiessen and Rice, 1976), Odocoileusvirginianus (Atkesan and Marchinton, 1982) and ferret, Mustelafuro (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 indicate that they 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, Noctilio leporinus 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 from the body and roost of Taphozous melanopogon and Rousettus leschenaulti (Lily, 2005).

Odourous secretions as sex attractant

GC-MS profile of frontal and anal glandular secretions of Hipposideros Speoris confirms that they have specific fatty acid composition like octadecanoic acid, alkene, heterocyclic compounds and steroid derivatives. These major constituents bring species – specific odour to the bats and their roosting site. The facial region of male and the female possess these specific sex attracting secretions during breeding season with C_{18}octadecanoic acid and C_{10}decanoic acid respectively.

In Hipposideros Speoris the female anal glandular secretions consist of C10 – C15 and C18 – C20 fatty acid composition. These fatty acid secretions were not noticed in both sexes during non-breeding season. Similar possession of fatty acid compounds were reported in other animals, C15 – C25 fatty acids from the occipital secretions of camel (Ayorinde et al., 1982); C16 – C18 fatty acids secretions from preorbital secretions of oribi, Ourebiaorebi (Mo et al., 1995) and C10 – C25 fatty acids from precloacal secretions of Liolaemus lizard (Carlos et al., 2001) are used as sex attractant to select their mate.

During breeding season, the frontal glandular secretions wet the hair of the face (Genherd, 1997 ; Lily, 2005) and the bats show peculiar rubbing and sniffing behavior with the selected mates. Lily (2005) has reported that male R. leschenaulti licks the vaginal opening of the female to confirm its response. 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 octadecanoic acid in the male frontal secretion of C. sphinx has been identified from the surroundings of female anal region [(2, 2-Dimethyl-1, 2-dioxolan-4yl). This type of peculiar rubbing and sniffing behavior by the males with the selected females also observed in C. sphinx. Anal secretions of females convey the oestrous status to the males (Hurst, 1987, 1990).

Odourous 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. Male facial and anal glandular secretion of C. sphinx possess such compounds 2-Methyl-2-isobutyl-1, 3-benzoxathiole; and the anal gland, Tetraphenyl-1, 3-oxathiol and octahydrobenezopyrene. Earlier reports confirm animals generally use such glandular secretion to mark their mates and territory (Burger et al., 2001).

Secretions for individual recognition

The earlier studies by Martin and Lopez (2000) revealed that functional groups such as alcohol, aldehyde and ketone derivatives give information about the male dominance and individual recognition. The same biochemical compsotion is present in the facial region of male Hipposideros Speoris ketone, 17-Hydroxy-androstan-3-one and alcohol group, cholan-3-ol. The male ear region consists of Dihydroxycholestenone and anal region possess 1-Benzopyran-4-one. Reports about similar biochemical compounds playing vital role in individual recognition are known from the preorbital secretion of musk Ox, Ovibos moschatus (Flood et al., 1989) and blesbok, Damaliscus dorcas phillipsi (Burger et al., 1999).

The chemical compounds like alkenes, steroid, heterotrophic compounds and specific compounds with sulphur and nitrogen composition proved to be odour producing substances. These compounds specially helps in 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).

Secretions for self-anointment

Breeding behavioural studies of H. speoris (Lily and Vanitharani, 2005) specifically mentioned about the territory marking and self-anointment behavior. These chemical cues are important to advertise the social stuatus and reproductive fitness of the individuals in a colony. Similarly in Beaver, Castor fiber (Rosell and Sundsdal, 2001), mice, Mus musculus (Collins et al., 2001) and rabbit, Oryctolaguscuniculus (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 facial, neck and anal gland of Hipposideros Speoris spread all over the body during breeding season. This self-anointment can be seen obviously on the fur and can be felt if the bats are captured during breeding season. Self-anointment of glandular secretions not only creates odour but also helps the animal to look more attractive to their conspecifics (Bowyer and Kitchen, 1987) and also fragrant to attract partners (Bloss et al., 2002) and settle in pairs prior to mating (Lily, 2005). Females also recognize such signals from potential males and respond to them.

In addition, bats use a special way of dispersing body fragrance by way of wing flapping and fanning behavior. In C. sphinx 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 S. bilineata and in T. melanopogon and R. leschenaulti by Lily (2005). Dispersal of odour by self-anointment has been noticed in several mammals, black tailed deer, O. hemionus (Muller-Schwarze, 1971); Genetta tigrina (Wemmer, 1972); ground squirrel Spermophilus columbianus (Steiner, 1973) and fallow deer, Dama dama (Chapman and Chapman, 1975).

Chemical cues during breeding season in Hipposideros Speoris play an important role in mate choice, individual identify 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 grandular secretions are important factor and immensely responsible for bringing reproductive success as well as the continuity of the race.

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Table 1. GC-MS analysis indicating the odoriferous compounds of male and female integumentary glandular secretions of C. sphinx during breeding and non-breeding season

Types of	**Male**	**Female**

gland	**Breeding**	**Nature**	**Non-Breeding**	**Nature**	**Breeding**	**Nature**	**Non-Breeding**	**Nature**
Face	2-Phenyl selenophene	Aromatic	7-Methyl-4-Octyl acetate	Aliphatic ester	1, 2, 3 – Triphenyl naphthalene	Aromatic	3-Methyl-2-pentanone	Alkanone
	2-Thiozolamine	Hetrocyclic	3-oxy-4-octane	Alkanone	Tetramethylundecene	Alkene		
	2-Methyl-2-isobutyl-1, 3-benzoxathiole	Hetrocyclic	3-Decyn-2-ol	Alcohol	Decanoic acid	Aliphatic acid		
	Octadecanoic acid	Aliphatic acid	-	-				
Neck	Cholan-3-oII H-1, 4-Benzodiazepine	Alcohol	1-Hexyn-3-ol	Alcohol	5□-Androstan-12-one	Aromatic	4-Methyloctane	Alkane
		Aromatic			Ether derivative of prostaglandin	Steroid	Trimethyl pentane	Alkane
	Pregnane-3, 11, 17, 20-tetrol	Steroid			4, 5-Epoxy cholstane	Aromatic	3, 3-Dimethyl-2-hexanone	Alkanone
	17-Hydroxy-indrostan-3-one	Steroid			Bis(4, 5-Dimethoxy-2-nitrophenyl) sulphide	Aromatic sulphur compound		
	1, 2, 3, 4-Tetrahydro-11-butylehrysene	Alkene						
Ear	Norcocaine	Aromatic			Trimethyl pentane	Alkane		
	Dihydroxy-Cholest-8(14)-en-15-one	Aromatic			Isopropyloxetan	Alkane		
	Tri-m-Cresyl phosphate	Aromatic			3-Methyl-4-ethyl hexane	Alkane		
	Dihydroxycrinane	Alkane			2, 4-dimethyl decane	Alkane		
Anal	1-Benzopyran-4-one	Aromatic	2-Oxopropanal	Aliphatic aldehyde	II-(5-pentyl-3, 4-dimethyl-2-furyl) undecanoic acid	Aliphatic acid	2, 3, 3-Trimethyl pentane	Alkane
	Tetraphenyl-1,3-Oxathiol	Aromatic			2, 4-Dinitro-N-[(1, 2-trimethylsilysoxy) 2-methyl]-3-Benzenamine	Aromatic amine	3-Methyl-3-buten-1-ol	Aliphatic alcohol
	Hydroxy methyl cholesterol	Steroid			N, N-Didodecyl-trifluoroacetami	Aliphatic amide		

					de			
	Octahydrobenzopyrene	Aromatic			2,2-Dimethyl-1,3-dioxolan-4yl) methyl Octadecanoic acid	Aliphatic acid		