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GC-MS analysis of glandular secretions of the South Indian bat, *Taphozous melanopogon*

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Abstract
The male neck gland of *Taphozous melanopogon* during breeding season showed nine compounds, four of them are odoriferous. During non-breeding season, the secretions exhibited four different compounds with two aromatic compounds. The anal glandular secretions of male during breeding season showed nine compounds with four odoriferous compounds. Among the odoriferous compounds in the anal region, of both sexes possess cholesterol derivatives. The male possesses Cholestan-3-one and female had 25-Ethyl-24-hydroxy-3-beta-methoxy-4, 4, 14, alphatrimethyl cholest-9 (11)-ene. The throat gland secretions of male showed acids with ester during breeding ((Bromomethyl) methyl bis 5, 6-hydroxy imino heptyl ester of Propanedioic acid) and non-breeding (3-Phenyl-methyl ester of 4-Isothiazole carboxylic acid) seasons.

Keywords: *Taphozous melanopogon*, glandular secretions, compound, GC-MS

Introduction
In recent years the behaviour of animals has attracted much more attention. Environmentalists were very much interested in monitoring behavior of bats. Tinbergen (1951) [11] earlier defined ethology as the study of behavior. Behavior can be defined as the activity of any animal in relation to its particular environment. Behavior is almost unique in some cases. Social behavior mainly depends on the modes of communication that organisms have evolved. Communication mainly involves an exchange of various signals between conspecifics, resulting in changes in adaptation in the recipient's behavior and physiology (Wilson, 1973) [14]. Communication is an important essence of almost all behavior. The integration and co-ordination of most of the social activities including reproduction mainly depends upon effective exchange of information between individuals. Many mammals use highly specialized glandular secretions for territorial function, marking partners, mating and groom selection, and rearing. Biochemical signals from scent glands significantly convey information among the members of the same species and also with other species. Like other animals, bats possess a variety of communication systems including, auditory, visual, tactile and olfactory. Among these communication system, olfactory signals seem to be very successful in breeding season (Scully *et al.*, 2000) [8]. The odoriferous compounds are mainly secreted through highly specialised skin glands of bats. Some of these effective odoriferous compounds are highly useful in the dark, which is useful for the nocturnal habit of bats. Odours act as a stimulant and even trigger of various hormonal functions and thus mainly facilitate long lasting responses in most of the vertebrates.
Odours from males critically stimulate females to attain oestrous in seasonal breeders, and help synchronise sexual activity in bats (Ville *et al.*, 1985) [12]. Scent marking is an important strategy for marking territories in the roosting sites, selection of mates (Sun and Schwarze, 1998) [10] and also useful in group recognition and individual (Kamran and Kerth, 2003) [6]. In bats, olfactory communication greatly varies depends on roosting environment, ecological pressure, the nature of the social organization of the species (Bloss, 1999) [3]. In bats, glandular secretions and scents mainly convey various types of messages. The mode of transformation of message between individuals depends on the habitat on which organism live and nature of the environment (Balakrishnan, 1975) [2]. In territorial mammal's active marking or direct rubbing of specialized Scent glands on objects in the organism and leaving the glandular secretions as mark is a common phenomenon. French and Lollar (1998) [5] reported that male Mexican free tailed bat, *Tadarida brasiliensis inexicana*, scent mark their roost with various secretions from the gular gland and actively defend the marked territory during breeding season. In bat species dispersal of odour is highly frequent in males. In another report, males of the bat, *Saccopteryx bilineata* bend their head towards the ventral side to anoint their genital region and belly body with glandular secretions (Voigt and Helversen, 1999) [13].

Materials and Methods
Experimental animal
The present study was carried out in Tirunelveli District, Tamilnadu, India. The common South Indian bat *Taphozous melanopogon* was used for observation. The climatic conditions of the area is highly tropic. The common Indian bat, *Taphozous melanopogon* was collected and glandular secretions were used for GC-MS analysis.

GC-MS analysis of glandular secretions
In the present study, the glandular secretion was collected during breeding and non-breeding season by using cotton swabs and immediately dissolved in Dichloromethane. The

Glandular extracts were stored in airtight containers at 20 ºC until they were chemically ananlysed under GC-MS. A fused silica capillary column (25 m X 0.25 mm i.d) on Schimadzu 17 A equipped with mass spectrometer (Schimadzu GP 5000) was used to separate and identify the volatiles in the glandular secretions of bats. The initial column temperature was set to 70 ºC for 2 min increased to 250 ºC by 30 ºC per minute and held for 30 min. Helium was used as a carrier gas at a flow rate of 0.6 ml per minute. The transfer line temperature and electron ionisation was set at 300 ºC and 70 ev respectively. The mass spectrometer was operated in scan mode over a mass range of 25 to 700 amu.

Results and Discussion

Identification of compounds using GC-MS analysis

The extracts collected from the glandular regions (facial, neck and anal) of *T. melanopogon* during breeding and non-breeding seasons were subjected to GC-MS analysis. The GC-MS chromatogram exhibits the composition of the chemical components. The peaks indicated the presence of the compounds with highest volatile composition. The fractionations of peaks confirm the individual chemical compounds with their linkage groups. These chemical compounds differ within the sex and also in the same individual from region to region depending on the glandular position. In addition, the compounds vary between breeding and non-breeding seasons. The various types of the major components of the glandular secretions the species during breeding season, the odour producing components of the glandular secretions with the organic nature of the compounds in the species are given in Table 1 -3.

During non-breeding season, the male *T. melanopogon* possesses an aromatic compound and there was no remarkable peak in the female. The male neck gland of *T. melanopogon* during breeding season showed nine compounds, four of them are odoriferous. During non-breeding season, the secretions exhibited four different compounds with two aromatic compounds. There was no neck gland in females. The anal glandular secretions of male during breeding season showed nine compounds including four odoriferous compounds. In females, the anal secretions showed four peaks with three aromatic compounds. There was no remarkable peak in the anal glandular secretions of both sexes during non-breeding season. Among the odoriferous compounds in the anal region, of both sexes possess similar cholesterol derivatives, (male: Cholestan-3-one, female: 25-Ethyl-24-hydroxy-3-beta-methoxy-4, 4, 14, alphatrimethyl cholest-9 (11)-ene). The throat gland secretions of male showed acids with ester during breeding ((Bromomethyl) methyl bis 5, 6-hydroxy imino heptyl ester of Propanedioic acid) and non-breeding (3-Phenyl-methyl ester of 4-Isothiazole carboxylic acid) seasons. The integumentary glands empty their products outside the animal's body to expel the odour. Variations in the chemical products are responsible for the species-specific odour. Fatty acids like octanoic, decanoic, tetradecanoic and octadacanoic acid serve to scent the habitat (Sugiyama *et al*., 1981) [9]. GC-MS profile of glandular secretions of the species in the present study confirms they have a specific fatty acid composition (octadecanoic, tetradecanoic acid) in association with odoriferous compounds (alkane, alkene and steroid derivatives). These secretary compositions differ in Accordance to the nature of gland also with that of the sex and species. These specific compositions in the glandular secretions are the major contributors that bring species-specific odour to the roosting site of bats. This specific pungent smell is more intense and noticeable during breeding seasons. The present study has confirmed the glandular secretion mixed with feces and urine spread on the floor of the roost and together with bat body odour dispersed the 'pungent' smell to the surroundings of the roost. The fatty acid odorant compounds of the secretions are used as sex attractant in many animals. C_9 fatty acid acts as the queen substance, which is broadcast by the queen bee, *Apis mellifera* during spawning (Maisonnasse 2010a) [7]. In the present study *T. melanopogon*, the facial region of male and the female possess the sex attracting secretions during breeding season with C_{30} decanoic acid and C_{28} octadecanoic acid in male and C_{20} octadecanoic acid in female. The neck glandular secretions of the male are more pronounced during breeding and enhanced with C_{12} and C_{30} octadecanoic acid whereas female has no such scent glands in the neck. This confirms that the bat species use these compounds as sex attractants. When compared to females, the males possess these attractants in all the glandular secretions.

In the present study males of *T. melanopogon* produced chemical signals during breeding season. Some of these fatty acids with high carbon number present in male were also noticed in the glandular region of the females during breeding seasons. This may be due to the rubbing of these secretary compounds on the selected females. Such remarkable similarity in possession of fatty acid compounds are noticed in the facial glandular secretions of *T. melanopogon* (decanoic acid). This has confirmed the males of all the species use decanoic acid compounds as sex attractants and the markers of their selected females. The biochemical report of no such result during non-breeding season also confirms this finding. Such type of sex attractants with mixed fatty acid compounds and more number of carbon atoms were observed in the following animals, C_{15}-C_{25} fatty acids in the occipital secretion of camel, *Camelus bactrianus* (Ayorinde *et al*., 1982) [1], C_{14}-C_{18} in the interdigital secretions of bontebok, *Damaliscuss dorcas dorcas* and C_{20} fatty acid in the blesbok, *Damalicus dorcas phillipsi* (Burger *et al*., 1999a) [4].

In addition, some other chemical compounds like acids with ester, alkanes, alkenes and specific compounds with sulphur and nitrogen composition also proved to be odour-producing substances. Earlier reports suggest that these compounds specially help in group recognition in a colony and also aids in sex identity in a social system (Kamran and Kerth, 2003) [6]. Compounds like acid ester with phenols and ethanol compounds are present in the male *T. melanopogon* neck glandular secretions. They are (2-[(Oxododecyl) oxy]-1, 3-propanediyl ester of Octadecanoic acid; 3-(1-Oxohexadecyl) oxy-2- (1-Oxotetradecyl) oxy propyl ester of Octadecanoic acid and (Bromomethyl) methyl bis 5, 6 (hydroxyimino) heptyl ester of Propanedioic acid. There are also specific compounds with sulphur present in the male frontal and anal secretions who does the similar functions like esters, (male

frontal: (2-Iodothiophene 1, 3-bis (1, 1, Demethyl ethyl) 5-methyl benzene and female anal: 5-Heptadecyl-methyl ester of 2-thiophene carboxylic acid).

Table 1: GC-MS analysis of facial gland of *Taphozous melanopogon*

Type of gland	Male				Female			
	Breeding	**Nature**	**Non breeding**	**Nature**	**Breeding**	**Nature**	**Non breeding**	**Nature**
Facial gland	Decanoic acid	Acid	Cholesta 7,9(11)-dien-3-ol *	Steroid	Octadecyl ester of Octadecanoic acid *	Ester containing acid	No compounds	
	3-[(1-Oxohexa decyl) oxy] propyl ester of octa decanoic acid	Ester containing acid	-		Decyl ester of Acetic acid *	Ester containing acid		
	Bis (2-ethyl hexyl ester of 1, 2 Benzene dicarboxylic acid	Ester containing acid			1, 1-Di (2-Me thoxy phenyl azo Benzene*)	Aromatic		
	2-Bromo-cyclic 1,2-ethane diyl Cholestan –3-one	Steroid			-	-		

*odoriferous compounds.

Table 2: GC-MS analysis of male neck gland of *Taphozous melanopogon*

Type of gland	Male				Female			
	Breeding	**Nature**	**Non breeding**	**Nature**	**Breeding**	**Nature**	**Non breeding**	**Nature**
Neck gland	2-[(Oxo dodecyl) oxy] - 1, 3-propanediyl ester of Octadecanoic acid *	Acid with ester	2, Butyl, 1-octanol	Aliphatic	No Specialised glands in the neck region of female			
	2-(1-Methyl ethylidene)- cyclic 1,2-ethanediyl acetal) Cholestan-3-one *	Steroid	3-Phenyl- methyl ester of 4-Isothiazole carboxylic acid	Acid with ester				
	3-(1- Oxohexa decyl) oxy-2-(1 oxotetradecyl) oxy propyl ester of Octadecanoic acid *	Acid with ester	-	-				
	(Bromomethyl) methyl bis (hydroxyl imino) heptyl ester of Propanedioic acid *	Acid with ester	-	-				

*odoriferous compound

Table 3: GC-MS analysis of male and female anal gland of *Taphozous melanopogon*

Type of gland	Male				Female			
	Breeding	**Nature**	**Non breeding**	**Nature**	**Breeding**	**Nature**	**Non breeding**	**Nature**
Anal Gland	-1-Hexadecyl ester of acetate*	Aliphatic ester	No compounds		1, 3, 5, 2, 4, 6-Triazatriphos- phorine	Phosphorus compound	No compounds	
	Decyl ester of Acetic acid.	Aliphatic ester			Estra-1,3, 5 (10)-trien-17- one *	Steriod		
	1- [3-(Octa decyloxy) propoxyl] - 9 Octadecane*	Aliphatic			Prostaglandin *	Steriod		
	Cholestan-3-one *	Steriod			25 Ethyl-24-hydroxy-3-beta-methoxy-4, 4, 14, alpha-Tri methyl * cholest-9(11)-ene	Steriod		

*odoriferous compound.

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