Chapter II

Reproduction and olfaction

INTRODUCTION

Reproduction is a crucial phase for all living organisms. The timing and frequency of reproduction are major determinants of life history strategies. Bats have distinct life history strategies among mammals of their size. Most small animals have evolved as “live fast – die young” strategy (Promislow and Harvey, 1990) characterised by rapid reproduction and high mortality. Assessment of reproductive status and observation of reproductive behaviour in bats are fundamental to most field and laboratory studies. The cost of reproduction is related to its social contact (Clutton-Brock et al., 1989). Bats show the benefits of recognition from their roost mates when the colonies form stable units that persist over time. Although bats are the most gregarious animals, most research have documented the complexity of their social interaction in the past few decades. The concept of chemical communication during reproduction and their challenges during maternal care brings reproductive success. This become a crown in the ethology of an organism.

Chemical communication performs a lot of wonders in the world of nocturnal animals leading a good social organization. Chemicals potentially provide discrete information about an animal’s physical and social environment.
(Dusenbery, 1992). Olfactory cues may function at individual and species level (Dominic, 1991). Most mammals gather information about their environment using a combination of auditory and olfactory cues. Several studies have explored the role of acoustic communication in Chiroptera but little attention has been focused on specific chemical cues and their behavioural significance (Johanna et al., 1996). Animals communicate information regarding reproduction to conspecifics in order to co-ordinate the reproductive activities by eliciting endocrine response. Biological odours produced from urine, faeces, saliva, vaginal mucus, sweat and scent glands are known to be important in a variety of behavioural interactions in many mammalian species. Such chemical cues are seen in microorganisms, insects, rodents, farmyard and domestic animals and primates. In humans sweat plays a significant role in sexual behaviour (Archunan, 2003). Olfactory ability of bats is highly valuable and bats rely on them for foraging, breeding and recognition to interact with their roost mates (Baron et al., 1996).

Of all types of communication, the olfactory signal seems to be successful during breeding seasons (Scully et al., 2000). Specific odoriferous secretory compounds from skin glands are effective in dark and very helpful for nocturnal life of bats. The mode of message transformation between individuals depends on the nature of environment and the habitat in which they live (Balakrishnan, 1975). The potential males in many mammalian species make specific territory marking with urine (Buesching and Mac Donald, 2001) and settle on the marked territory to show-off breeding behaviour to attract the
females. In bats the mating partners rub the glandular secretions on their mates. Such rubbing behaviour has been noticed in velvet free tailed bat *Molossus molossus* (Haussler, 1987), grey headed flying foxes *Pteropus poliocephalus* (Ratcliffe, 1932 and Nelson, 1965), Maxican free tailed bats *Tadarida brasiliensis mexicana* (Davis *et al.*, 1962), *phyllostomid* bats (Valdivieso and Tamsitt, 1964) and in *H. speoris* (Lily and Vanitharani, 2005 a). In some species of bats the odour dispersal is through self-grooming which helps to maintain social bond between the pair and reduce tension (Voigt and Helversen, 1999). Potential males show wing-flapping behaviour to fan the anointed odour towards the responding females. Such wing-flapping behaviour has been noticed in many bats species like *Saccopteryx, Cyttarops, Peropteryx* and *Cormura* by Robbins and Sarich (1988), in *Cynopterus sp.* by Nowak (1994) and in *T. melanopogon, R. lechenaulti* by Lily (2005).

Even before the colourful emergence of research in the field of ethology, people were interested in observing the behaviour of animals. Any form of parental behaviour appears likely to increase the fitness of parents and offspring (Clutton-Brooke, 1991). Meticulous care of young is a norm in microchiropterans (Bradbury, 1977). Parental care strengthens the reproductive success of bats which are in constant stress due to various threats in the habitat.

In bats as most other animals, mother provides continuous protection to their offspring from gestation to weaning. The nutritional and non-nutritional care of mother may be associated with several factors including phylogeny, diet, age of offspring, gender, litter size, climate, habitat, and risk
of predation (Kunz and Hood, 2000). Mammals are unique in their ability to produce milk from specialized mammary glands. The milk composition and output are generally closely related to suckling behaviour (Ofstedal, 1984). One of the vital roles of maternal behaviour is its nutritional investment for her offspring which leads to an intimacy towards flightless pup with its mother. Acoustic and olfactory responses are important for promoting mother-infant recognition, association and reunion. Acoustic communication in mother-pup relation has received moderate attention but few studies have investigated the role of olfactory cues in these contexts (Kunz and Hood, 2000).

Bats that roost in large aggregation appear to rely on a combination of spatial memory, acoustic, olfactory, tactile and visual cues to identity the young or to communicate with the conspecifics. Early observations on bats suggested that females which form large aggregations, suckled the young indiscriminately (Brosset, 1962). However, more recent studies on this aspect have shown that nearly all female bats selectively suckle their own infants (Mac Cracken, 1984 and Bishop et al., 1992). Several investigators have suggested that bats recognize their own young ones using olfaction, but few studies have been conducted to test this hypothesis (Kunz and Hood, 2000). The presence of odoriferous glands in females, apparent scent marking and maternal sniffing have been reported in many bats species (Gustin and Mac Cracken, 1987 and Brooke, 1994). Although olfaction is extremely acute in bats, few studies have examined the role of odours in recognition (Wilkinson, 1985). One study has unambiguously demonstrated that mothers
recognize their young ones using olfaction in *T. brasiliensis* (Gustin and Mac Cracken, 1987).

Jeyaprakash and Alexander (1993) observed *P. giganteus* marking their pups with their integumentary secretion for easy recognition. Such pup marking by mothers are also seen in *M. lucifugus* (Thomson *et al.*, 1985), *T. brasiliensis* (Balcombe, 1990) and *P. pipistrellus* (Jones *et al.*, 1991).

The present study is an innovation to understand the olfactory behavioural activities expressed by *H. ater* during reproduction.

**MATERIALS AND METHODS**

**Biochemical fragmentation of glandular secretions**

*H. ater* colony shows a sharply defined bimodal reproductive cycle. Observations on the reproductive and olfactory signals during reproduction were done continuously during four reproductive seasons of *H. ater* colony. The colony with smaller size located in Perumalpuram (Elev; 250ft N: 80° 42.67’ E: 077° 198’) has been selected for these observations. Tagging of individuals helped in sharp identification of male and female.

To study the scent marking activity three tagged individuals under each sex of *H. ater* colony were selected. Samples of urine, saliva, anal and integument secretions of different glandular regions of male and female of *H. ater* during breeding and non-breeding seasons along with pup were subjected to GC-MS for analysis. The secretions, saliva and urine were swabbed with the help of cotton and dissolved in dichloromethane. The
dissolved samples were stored in airtight containers at 20°C till they were chemically analysed under GC-MS. A fused silica capillary column (25m x 0.25mm i.d.) on Schimadzu 17A equipped with mass spectrometer (Schimadzu GP 5000) was used to separate and identify the volatiles. The initial column temperature was set into 70°C for 2 minutes increased to 250°C by 30°C per minute and held for 30 minutes. Helium was used as a carrier gas at a flow rate of 0.6ml/min. The transfer line temperature and electron ionisation was set at 300°C and 70 ev respectively. The mass spectrometer was operated in scan mode over a mass range of 25-700 amu.

The reproductive behaviour like marking the territory, wing-flapping, dragging and mother-pup association were observed for one hour once in five days to avoid disturbance to the colony members. Video recordings were made to observe mating behaviour. Single mating pair was observed each time through infrared sensitive night vision video camera Sony 120x digital zoom (3.0 mega pixels). The recordings were processed and marking activities were converted as JPG files for documentation.

During breeding season male and female show peculiar behaviour like wing-flapping, dragging, creeping over etc. Such behaviour help to disperse and recognise the odours in the surroundings and also to mark or attract the partners. As *H. ater* showed a sharply defined bimodal reproductive cycle, the risk of observation of mating behaviour and mother-pup association in the concerned roost is minimized. Tagging of individuals also helped in the
sharp identification of them. As the colony size was small and the area of roost was bigger the observation became still easier.

For four continuous reproductive seasons, observations were made right from mating, birth of newborn till they were weaned. All the mothers of the maternity colony were marked by coloured bands. Daytime observations were made with binocular (Zenith) at a distance of 10m. The diffused sunlight inside the roost facilitated a clear observation even without any artificial illumination. Stop clocks were used to note the duration of mother and pup association. The observations were tabulated to study the correlation between the age and duration of lactation and mother-pup association.

RESULTS

The GC-MS chromatogram of the analysis exhibits the composition of the chemical compounds and the peaks indicate the presence of the compounds with high volatile composition. The fractions of peaks confirm the individual chemical compounds with their linkage groups. These chemical compounds differ within sex and also in the same individual from region to region depending on the glandular position. The compounds also vary between the breeding and non-breeding seasons (Tables 7, 8 and 9). The various types of major components of glandular secretions are given in figure 6 and 7 for male breeding and non-breeding seasons and that of the female are given in figure 8, 9 and 10.
<table>
<thead>
<tr>
<th>Breeding Season</th>
<th>Name of the compound</th>
<th>Nature</th>
<th>Non-Breeding Season</th>
<th>Name of the compound</th>
<th>Nature</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urine</td>
<td>2,2,5 - Trimethyl hexane - 3,4-diene</td>
<td>Alkane</td>
<td>4,5-Dimethyl octane</td>
<td>Fatty acid</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2,2-Dimethyl butane</td>
<td>Ketone</td>
<td>Decanoic acid</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>5-Hexen-2-one</td>
<td>Ketone</td>
<td>Eicosytrichlorosilane</td>
<td>Orangosilicon compound</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3-Methyl heptyl acetate</td>
<td>Ester</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saliva</td>
<td>2,3-Epoxy hexanol</td>
<td>Alcohol</td>
<td>2-Methyl-nonane</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Decanoic acid</td>
<td>Fatty acid</td>
<td>2-Hydroxy-2-pentanone</td>
<td>Alkane</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Eicosytrichlorosilane</td>
<td>Ketone</td>
<td>2,2-Dimethyl butane</td>
<td>Ketone</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Methyl-2-hydroxypentadecane</td>
<td>Ester</td>
<td>2,3,3,5-Trimethyl hexane</td>
<td>Alkane</td>
<td></td>
</tr>
<tr>
<td>Ear</td>
<td>2,2,5 - Trimethyl hexane - 3,4-diene</td>
<td>Alkane</td>
<td>1,3-Epoxy-4-Methyl pentane</td>
<td>Alcohol</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1-Hexanal</td>
<td>Ketone</td>
<td>2,2-Dimethyl butane</td>
<td>Ketone</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3-Octan-2-one</td>
<td>Ketone</td>
<td>2,2,5 - Trimethyl hexane - 3,4-diene</td>
<td>Alkane</td>
<td></td>
</tr>
<tr>
<td>Neck</td>
<td>3-Methyl-3-butanol</td>
<td>Alcohol</td>
<td>2,2,5 - Trimethyl hexane - 3,4-diene</td>
<td>Alcohol</td>
<td></td>
</tr>
<tr>
<td></td>
<td>5-methyl-5-hexen-2-one</td>
<td>Ketone</td>
<td>2,3,3,5-Trimethyl pentane</td>
<td>Alcohol</td>
<td></td>
</tr>
<tr>
<td></td>
<td>N-Hexanal</td>
<td>Ketone</td>
<td>5-Hexen-2-one</td>
<td>Ketone</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2,2,5,5,5-pentanone</td>
<td>Ketone</td>
<td>3-Methyl-5-hexen-2-one</td>
<td>Ketone</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2,2,5-Trimethyl pentane</td>
<td>Alcohol</td>
<td>3-Methyl-5-hexen-2-one</td>
<td>Ketone</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3-Butyl propyl ether</td>
<td>Ether</td>
<td>2,3,5-Trimethyl pentane</td>
<td>Alcohol</td>
<td></td>
</tr>
</tbody>
</table>

Table 7: GC-MS analysis indicating odoriferous compound exhibits of male H. ater during breeding and non-breeding seasons.
<table>
<thead>
<tr>
<th>Sample</th>
<th>Name of the compound</th>
<th>Nature</th>
<th>Name of the compound</th>
<th>Nature</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urine</td>
<td>3-Methyl - 3 - butanol</td>
<td>Alcohol</td>
<td>Urine</td>
<td>Alkane</td>
</tr>
<tr>
<td></td>
<td>5-Methyl 1-5-hexen - 2-one</td>
<td>Ketone</td>
<td>4,6,8 - Trimethyl nonene</td>
<td>Alkane</td>
</tr>
<tr>
<td></td>
<td>4-Methyl-1-Octene</td>
<td>Ketone</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saliva</td>
<td>2,4 Dimethyl decane</td>
<td>Alkane</td>
<td>Saliva</td>
<td>Alkane</td>
</tr>
<tr>
<td></td>
<td>Decanoic acid</td>
<td>Fatty acid</td>
<td></td>
<td>Aldehyde</td>
</tr>
<tr>
<td></td>
<td>2,4,6 Trimethyl decane</td>
<td>Alkane</td>
<td>7-Methyl -4-decane</td>
<td>Fatty acid</td>
</tr>
<tr>
<td></td>
<td>4,6,8 Trimethyl nonane</td>
<td>Alkane</td>
<td>Palmitic acid</td>
<td></td>
</tr>
<tr>
<td>Ear</td>
<td>Iso butenyl carbinol</td>
<td>Alcohol</td>
<td>Ear</td>
<td>Aliphatic amine</td>
</tr>
<tr>
<td></td>
<td>2,2,5 -Trimethyl hexane - 3,4-dione</td>
<td>Ketone</td>
<td>5-Methyl-2-heptanamine</td>
<td>Compound (foul smell)</td>
</tr>
<tr>
<td></td>
<td>1-3 Epoxy 4- methyl pentane</td>
<td>Alkane</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Face</td>
<td>2,3,3, Trimethyl pentane</td>
<td>Alkane</td>
<td>Face</td>
<td>Acid derived compound (milky odour)</td>
</tr>
<tr>
<td></td>
<td>2-Chloro octane</td>
<td>Alkane</td>
<td>E-3-Dodecyl acetate</td>
<td>Acid derived compound (pleasant odour)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Ethyl-3-butenoate</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Alpha D-Arabinopyranose</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>2-Propanamine</td>
<td>Aliphatic amine</td>
</tr>
<tr>
<td>Neck</td>
<td>3-Hexane -2,5-diol</td>
<td>Alcohol</td>
<td>Abdomen</td>
<td>Alkane</td>
</tr>
<tr>
<td></td>
<td>2,3,4-Trimethyl heptane</td>
<td>Alkane</td>
<td>3-Ethyl octane</td>
<td>Alkane</td>
</tr>
<tr>
<td></td>
<td>2,4,6,8 - Tetramethyl undecane</td>
<td>Alkane</td>
<td>Octacosane</td>
<td>Alkane</td>
</tr>
<tr>
<td></td>
<td>1,6 - Anhydro 3,4 dideoxy Beta - D - gluco hexopyranose</td>
<td>Aliphatic compound</td>
<td>Hexa decane</td>
<td>Alkane</td>
</tr>
<tr>
<td>Anal</td>
<td>Neoheptanol</td>
<td>Alcohol</td>
<td>Anal</td>
<td>Alkane</td>
</tr>
<tr>
<td></td>
<td>2,3,3- Trimethyl pentane</td>
<td>Ketone</td>
<td>n- Tetradecane</td>
<td>Alkane</td>
</tr>
<tr>
<td></td>
<td>2,2,5 - Trimethyl hexane 3,4-dione</td>
<td>Ketone</td>
<td>n-Penta decane</td>
<td>Alkane</td>
</tr>
<tr>
<td></td>
<td>2-Ethyl 1-butanol</td>
<td>Ketone</td>
<td>Decanoic acid</td>
<td>Fatty acid</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1-Iodo - 2-methyl undecane</td>
<td>Haloalkane</td>
</tr>
</tbody>
</table>

Table: GC-MS analysis indicating odoriferous compound exhibits of female *H. ater* during breeding and non-breeding seasons.
<table>
<thead>
<tr>
<th>Sample</th>
<th>Name of the Compound</th>
<th>Nature</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dorsal</td>
<td>n-Heptanal</td>
<td>Aldehyde</td>
</tr>
<tr>
<td></td>
<td>Isonononal</td>
<td>Aldehyde</td>
</tr>
<tr>
<td></td>
<td>5-Methyl 2-hepatamine</td>
<td>Aliphatic amine (foul smell)</td>
</tr>
<tr>
<td></td>
<td>Imidazole</td>
<td>Heterocyclic compound with foul smell</td>
</tr>
<tr>
<td>Ear</td>
<td>Tetradecane</td>
<td>Alkane</td>
</tr>
<tr>
<td></td>
<td>Hexa decane</td>
<td>Alkane</td>
</tr>
<tr>
<td>Face</td>
<td>3-Hexane –2, 5-diol</td>
<td>Alcohol</td>
</tr>
<tr>
<td></td>
<td>Isonononal</td>
<td>Aldehyde</td>
</tr>
<tr>
<td>Ventral</td>
<td>Pyrrolidine</td>
<td>N₂ compound</td>
</tr>
<tr>
<td></td>
<td>3-Hydroxy cyclohexanone</td>
<td>Ketone</td>
</tr>
<tr>
<td></td>
<td>Decanoic acid</td>
<td>Fatty acid</td>
</tr>
<tr>
<td></td>
<td>Isooctyl vinyl ether</td>
<td>Ether</td>
</tr>
</tbody>
</table>

Table 9: GC-MS analysis indicating odoriferous compounds present in different regions of pup of *H. ater*
Urine

Fragmentation of

Peak No.2: Compound Name: 2,2,5-Trimethyl hexane-3,4-dione
Mol.wt: 156

Peak No.3: Compound Name: 2,2-Dimethyl butane
Mol.wt: 86

Peak No.4: Compound Name: 5-Hexen-2-one
Mol.wt: 98

Peak No.6: Compound Name: 3-Methyl heptyl acetate
Mol.wt: 172

Peak No.8: Compound Name: 2,3-Epoxy hexanol
Mol.wt: 116

Saliva

Fragmentation of

Peak No.3: Compound Name: Decanoic acid
Mol.wt: 172

Peak No.4: Compound Name: Eicosyl trichlorosilane
Mol.wt: 414

Peak No.5: Compound Name: Methyl-2-hydroxydodecanonate
Mol.wt: 230

Peak No.4: Compound Name: 3-Octen-2-one
Mol.wt: 126

Ear

Fragmentation of

Peak No.1: Compound Name: 1,3-Epoxy-4-methyl pentane
Mol.wt: 100

Peak No.2: Compound Name: N-Hexanal
Mol.wt: 100

Peak No.3: Compound Name: 2,2-Dimethyl butane
Mol.wt: 86

Peak No.6: Compound Name: 2,2,5-Trimethyl hexane-3,4-dione
Mol.wt: 86

Neck

Fragmentation of

Peak No.1&5: Compound Name: 3-Methyl-3-butanol
Mol.wt: 86

Peak No.2: Compound Name: 5-Methyl-5-hexen-2-one
Mol.wt: 112

Peak No.4: Compound Name: 2,2,5-Trimethyl hexane-3,4-dione
Mol.wt: 226

Peak No.6: Compound Name: N-Hexanal
Mol.wt: 100

Peak No.7: Compound Name: 2,3,3-Trimethyl pentane
Mol.wt: 114

Peak No.10: Compound Name: 2-Methyl-1-pentanol
Mol.wt: 102

Anal

Fragmentation of

Peak No: Compound Name: Hexyn-3-ol
Mol.wt: 98

Peak No: Compound Name: 5-Methyl 5-hexen-2-one
Mol.wt: 112

Peak No: Compound Name: 3-Butenyl propyl ether
Mol.wt: 114

Figure: GC-MS analysis indicating odoriferous compound exhibits of male H.ater during breeding seasons
Figure 7 GC-MS analysis indicating odoriferous compound exhibits of male *H. ater* during non-breeding seasons
Figure: 8 GC-MS analysis indicating odoriferous compound exhibits of female *H. ater* during breeding seasons
Urine

Fragmentation of
Peak No.3: Compound Name: 4,6,8-Trimethyl nonene
 Mol. wt : 168

Saliva

Fragmentation of
Peak No.2: Compound Name: 7-Methyl-4-decane
 Mol. wt : 154
Peak No.3: Compound Name: Palmitic acid
 Mol. wt : 256

Ear

Fragmentation of
Peak No.2: Compound Name: 5-Methyl-2-heptanamine
 Mol. wt : 129

Face

Fragmentation of
Peak No.1: Compound Name: E3-Dodecenyl acetate
 Mol. wt : 226
Peak No.2, 4 & 8: Compound Name: Ethyl-3-butenoate
 Mol. wt : 114
Peak No.12: Compound Name: Alpha D-Arabinopyranose
 Mol. wt : 278
Peak No.16: Compound Name: 2-Propanamine
 Mol. wt : 113

Abdoman

Fragmentation of
Peak No.1: Compound Name: 3-Ethyl octane
 Mol. wt : 142
Peak No.3: Compound Name: Octacosane
 Mol. wt : 394
Peak No.2, 4, 6 & 1: Compound Name: Hexadecane
 Mol. wt : 226

Anal

Fragmentation of
Peak No.1: Compound Name: n-Tetradecane
 Mol. wt : 198
Peak No.2: Compound Name:n-Pentadecane
 Mol. wt : 212
Peak No.5: Compound Name: Decanoic acid
 Mol. wt : 172
Peak No.13: Compound Name: 1-Ido-2-methyl undecane
 Mol. wt : 1296

Figure: 9 GC-MS analysis indicating odoriferous compound exhibits of female *H. ater* during non-breeding seasons
Figure: 10 GC-MS analysis indicating odoriferous compounds collected from different regions of pup of *H.ater*
2,2,5-Trimethyl hexane 3,4-dione is present in the urine, ear and neck region of male and in the anal secretion of female during breeding seasons. Both the sexes have decanoic acid in their saliva during the breeding seasons. The urine of the breeding female has 3-Methyl-3-butanol, an alcoholic compound which is also present in the neck region of the breeding male. The aliphatic hydrocarbon 1,3-Epoxy -4-methyl pentane is identified in the ear of the mating partners of *H.ater* during their breeding seasons. The neck of breeding male and the face of breeding female have 2,3,3-Trimethyl pentane compound with kerosene smell.

The lactating mother alone has some odoriferous compounds like deconoic acid (foul smell), E-3-Dodecenyl acetate (milky odour) and 2-Propanamine (fishy odour) (Table 8). Alkanes like hexadecane are present in mother's abdomen and pup's ears. Another alkane, tetradecane is present in the anal region of mother and in the ear region of its pup. Nitrogen compound, 5-Methyl -2-heptamine (foul smell) is present in the ear region of the mother and the dorsal region of pup. Other odoriferous compounds like imidazole (heterocyclic compound with foul smell) and pyrrolidine (a nitrogen containing compound) are seen in the dorsal and ventral region of the pup (Table 9).

Plate 4 shows the series of reproductive activities like male settling in marked territory, flapping the wings to spread the odour, dragging the partner and other mating behaviour.

Observation of the mother and pup association of *H.ater* during their lactation period in their day roost is tabulated. Table 10 relates the age of
Plate: 4 Marking behaviour of *H. ater* during reproduction

1. Members of the colony settled in marked territory with inter individual space.

2. Potent male flap its wings to spread its odour to attract the potent female.

3. Potent male marks the responding female with saliva.

4. The engaged pairs settled in the marked territory.

5. Potent male sniff and mark the responding female.

6 and 7. Male embraces and rubs its secretions all over the responding female.
Plate 4 Marking behaviour of H. ater during reproduction
<table>
<thead>
<tr>
<th>Age of pups in days</th>
<th>Gap between adjacent visit by pup to mother (in minutes)</th>
<th>Duration of association with mother (in sec)</th>
<th>Number of observation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>25-30</td>
<td></td>
<td></td>
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<tr>
<th>Age of pups in days</th>
<th>Grand average of gap between adjacent visits (in sec.)</th>
<th>Grand average duration of association (in sec.)</th>
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Table: 10  Mother – pup association of *H. ater* during their lactation period
pup in days and their adjacent visit to their mother for suckling (in /seconds). A positive correlation exists between these two variables 0.874 (P < 0.05).

Figure 11 shows that as the age of pup increases, they try to become independent and they approach their mother for suckling at intervals. This gap between each visit seems to increase with increase of its age. As the age of pup increases, their duration of association decreases and thus shows a negative correlation of − 0.986 (P < 0.05). Plate 5 shows the mother pup association.

DISCUSSION

Mammalian chemical signals are characteristically complex. The multiple scent sources such as urine, anal, saliva and faeces are widely involved in recognition of sex, individuality, reproductive condition and social status (Mac Donald et al., 1985). They also reported that role of glandular secretions play a vital role in olfactory communication and persists for a longer period. Individual recognition is ultimately based on some form of communications such as visual, olfactory, auditory, tactile or gustatory stimuli that provide the cues for identification. With nocturnal bats one might expect auditory or olfactory stimuli to take precedence over visual cues. Odoriferous compounds of male and female integumentary secretions involve in individual recognition, mate attraction and also responsible for characteristic odour for their roosting site. The females of mammalian species except human are receptive to their males only during oestrous period. This readiness is informed through chemical signals. In the present study H.ater possess special secretions
Figure: 11  Correlation in mother-pup relationship of *H.ater* based on the age of the pup and duration of association with the mother
Plate: 5 Picture depicting the mother - pup association of *H. ater*

1. Inseparable association of mother and pup

2. Mother carrying pup during her foraging flight.

3. The female nurse their pup through their lactating nipples.

4. The pup attaches to its mother by sucking the false teeds.

5. The weaned mother showing the prominent false teeds, the holdfast for the infant.
Plate: 5  Picture depicting the mother-pup association of *H. ater*
in their integumentary glands of the anal regions. In addition urine, saliva and integumentary secretions of neck and ear regions also were used as chemical markers. The chemical compositions of the secretion and markers differ between the breeding and non-breeding seasons. According to Johnston (2005) in mammals the compounds affecting the physiology and behaviour are present mainly in urine. Normally, the biological fluids like urine, vaginal discharge, anal glandular secretion and saliva have mammalian pheromones binding proteins and odorant binding proteins. These chemicals are involved in social and sexual behaviour (Guiraudie et al., 2003).

The importance of chemical communication system in an animal’s reproductive and social behaviour has gained attention in recent decades because chemicals provide discrete information about an animal’s physical and social environment (Mykytowycz, 1970 and Dusenbery, 1992). Secretions of skin glands would facilitate the evaluation of olfactory signals of the species concerned and also helpful for effective conservation management. The olfactory signals emitted by bat species from the secretory glands and other markers are effective in the maintenance of social environment in a colony and also it brings reproductive success (Dominic, 1991; Brooke and Decker, 1993, 1996; Bloss, 1999; Bloss et al., 2002; Lily, 2005 and Lily and Vanitharani, 2005 a). In the present study H.ater also possess similar chemical compounds which emit olfactory signals. These olfactory signals are air borne volatile odorants. Volatility is primarily dependant on the molecular size and weight of the compounds (Dominic, 1991).

In mammals the main functional groups of glandular secretion are alcohol, aldehyde, and ketone derivatives. Such compounds with sulphur and nitrogen composition produce odour (Kannan, 1998 and Burger et al., 1999). These odoriferous compounds are effective in dark (Ville et al., 1985). They are helpful to the nocturnal and social life styles of bats in additional to their acoustic signals for communication (Kamran and Kerth, 2003). The chemical analysis of secretions and markers of H.ater possess alcohol and ketone derivatives which are the major chemical signals mainly in the urine, saliva, neck and anal secretions.
Individual recognition among birds through various behavioural activities has received a great deal of attention (Thrope, 1968 and Heinz and Gysel, 1970). While among mammals, the behavioural activities are species specific and especially about the chiropterans the literature on this subject is relatively sparse. The present study has recorded *Hater* showed specific mating behaviour using chemical signals as indicators. Similarly the females of *Hater* used specific markers to show mother - infant relationship. Similar mother-infant recognition in Alaskan fur seals was noted by Bartholomew (1959).

Chemical cues clearly inform the females about the genetic compatibility of the potential males and the males to attract and mark the corresponding females (Wedekind *et al.*, 1995). Both the sexes can discriminate individuals with deleterious disease (Lingington, 1983). Variations in the chemical products of skin glands are responsible for the species specific odour (Kunz and Fenton, 2003). Within the species secretory compositions differ in accordance with the nature of gland and also with that of sex and species. In *Hater* the chemical compositions of urine, saliva and secretory glands located in the ear, neck and anal are totally different. There is a remarkable difference in the nature of compound between the sexes than between the individuals. The chemical compositions with the characteristic odour alone are taken for this comparative note.
Chemical cues specific to the day roost

The roosts of Mexican free tailed bats *T. brasiliensis mexicana* are known for their characteristic odour “taco shell” aroma (Gustin and Mac Cracken, 1987). The rabbity odour of *Oryctolagus cuniculus* is due to lipid extract of anal glands (Mykytowycz, 1968). Specific odour of hamster *Phodopus sungoreus sungoreus* is due to peculiar fatty acid of buccal secretion and characteristic odour of male goat *Trichosorus velpecula kerr* is because of the fatty acid components of their cloacal gland (Woolhouse *et al.*, 1993). Similarly Gudger (1945) remarked musky sweet odour from the roosting site of *N.leporinus*, Voigt and Helverson (1999) has noticed sweet smell from the roost of *S.bilineata*, Scully (2000) noticed spicy odour from most of the phyllostomid bats *Sturnira lilium*, Lily and Vanitharani (2005 b) has remarked about the specific pungent smell from the roost of *H.speoris*. Earlier chiropteralogist also suggested specific fatty acid compound like octadecanoic acid, tetradecanoic acid, alkene and steroid derivative of integumentary anal glandular secretions are species specific (Sugiyama *et al.*, 1981; Brooke and Deckar 1993 and Bloss *et al.*, 2002). The colony of *H.later* has a characteristic pungent odour. During the non-breeding season they maintain maternity colony during which the odour is intensified and spreads into the environment as a mixture of fishy and milky odour along with a pungent smell. This is due to pyrrolidine a nitrogen-containing compound and fatty acids like palmitic acid and decanoic acids produced by anal and salivary secretion of the sub adult males and lactating mothers.
Chemical signals as sex attractant and markers

Fatty acid odorant compounds of integumentary secretions are used as sex attractants in many animals like bee *Apis mellifera* (Temphare, 2005), civet cat *Civettictis civetta* (Xavier, 1994), cockroach *Blatella germanica* (Nojima *et al.*, 2002). The compounds like glycolipids, non-polar lipids and phospholipids are used to mark the partners during pairing in fishing bat *N. leporinus* (Brooke and Decker, 1993). In the present investigation decanoic acid in the saliva and 1,3-Epoxy-4-methyl pentane in the ear were used by *H. ater* as sex attractants between breeding pairs. Before mating, the potential male selects the mating territory on the roosting sites and marks the territory with saliva and urine. According to Lily (2005) fatty acids like decanoic acids are enormously used in *H. speoris, T. melanopogon, R. leschenaulti* the temple bats species as territory markers. Similarly in *H. ater*, decanoic acid and esters like 3-Methyl heptyl acetate, Methyl 2-hydroxy dodeconate of urine and saliva were used as territory markers.

Functional groups like aldehyde, alcohol and ketone derivatives give information about the male dominance and individual recognition in mammals (Martin and Lopenz, 2000). GC-MS analysis of *H. ater* also shows some of such derivatives like ketones, 2,2,5-Trimethyl hexane-3,4-dione in the neck and ear of potential males and in the urine of competent females of the mating pair. Similarly an alcohol, 3-Methyl-3-butenol is found in the neck region of potent males and urine of females. This may be due to rubbing behaviour of male to select and mark the responding competent female partner.
The presence of 2,3,3-Trimethyl pentane in the neck of potent males and on the face is due to rubbing and marking behaviour between the partners during the mating. Such similarity in chemical compounds at different regions of mating pairs may be due to their different types of sexual behaviour like grooming, sniffing, biting and rubbing behaviour. The potent male show wing-flapping behaviour to attract the responding females. The repeated self-grooming by potent males aid in self-anointment and disperse the odourous fragrant to attract the partner. Sex and individual recognition by glandular secretions have been reported in bats by earlier chiropteralogists (Brooke and Decker, 1993; Voigt and Helverson, 1999; Bloss et al., 2002; Lily and Vanitharani, 2005 a, b and Lily, 2005).

Another major composition of the volatile substance is nitrogen containing compounds. Such compounds may be due to their protein rich diet (Wood et al., 2002). Occurrence of such compounds has been recorded in several animals like seven carnivorous species under the genus Mustela. Similar nitrogenous compounds also provide possibility of inter sexual communication in Steppe polecat and Siberian weasel (Zhang et al., 2002). The behaviour elicited by these compounds keep the individuals occupy very close to each other and to maintain social behaviour which is another crucial concept for the maintenance of social package of life. H.aeter shows specific pairing behaviour during breeding season and segregation behaviour with inter individual space during non-breeding season. Adult males of the colony form separate. Females and sub adult stay back in the native colony. The nitrogen
containing compounds are present only during non-breeding season in both male and female integumentary glandular secretions. The presence of pyrrolidine compound in the anal glandular secretions of adult males might have been the chemical substance to maintain segregated colonies. Similarly adult females possess foul smelling 5-Methyl-2-heptamine in the ear glandular secretions and 2-Propylamine which produce fishy odour from the facial glandular secretions during the non-breeding season alone. These compound might have helped the mothers to maintain the maternity colony. At the same time the pups of the maternity colony possess 5-Methyl-2-heptamine on the dorsal surface of the body and pyrrolidine on the ventral surface of the body. The presence of the 5-Methyl-2-heptamine on the dorsal surface of body might be due to the marking of facial secretion of the mother. Pyrrolidine might be helpful in the maintenance of social behaviour by the pups in the nursery colony. The integuments of new born are free from secretory glands like adult bats. The secretions present on the body of pups are mainly through the marking behaviour of the mother.

Mating behaviour

The courtship behaviour among bats is exhibited by a combination of activities like sniffing the genitalia, licking, grooming and huddling as a regular pre and post copulatory behaviour (Walkenshaw, 1999). Similar mating behaviour have been observed in *H. speoris* (Lily and Vanitharani, 2005 a) and *T. melanopogon* and *R. teschenaullti* by Lily (2005). During the courtship behaviour in breeding season both sexes of *H. ater* show
series of sexual behaviour. When at rest, they undergo self-grooming and wing-flapping to disperse their odours. The potential males of *H. ater* settle in their own marked territory of the roost and flap their wings to disperse their odour and thereby attract the females to respond them (Plate 4). Then the males drag the selected females and rub to mark the females with their secretions. Such settlement behaviour continues for a few days. After the partner’s bondage is confirmed, the females allow the males to creep over it. Embraced mating occurs roughly for 80-95 seconds. Body rubbing behaviour might be the reason for both the partners to have similar compounds on their body surface like 2,2,5-Trimethly hexane-3,4-dione, 3-Methyl-3-butenol, 2,3,3-Trimethly pentane, decanoic acid and 1,3-Epoxy-4-methyl pentane. In mammals chemical signals are characteristically complex with multiple components. The chemical analysis approaches are particularly desirable and offer the first step in deciphering a complex chemical signal (Sun and Schwarze, 1998). Production of bats’ sex attractant analogues and their deciphering will definitely pave way for species specific conservation of these bats in their roosts as well as in bat houses.

**Parturition**

The breeding cycles of most of the bats are seasonal and the young ones are produced at a time of the year which is most favourable for their survival in nature (Isaac, 1993). Colonies of *H. ater* produce young ones twice in correspondence to the monsoon prevailing in their roosting area. Like other mammals the pregnant female bats of *H. ater* are found restless
throughout the process of parturition and chew the placenta. The over all process of labour during parturition of *H.ater* takes 25-32 minutes and for *H. speoris* it is 32-36 minutes (Doss, 2004). The birth occurs in three phases. Like any other mammal first delivery of the head second the remaining of the body and third the placenta. The female supports her neonate with her wings and moves its pup towards her teat. The mother vigorously licks the infant i.e. “infant groom” to remove the birthing fluid and presumably to stimulate it. It is the first afflictive interaction of mother with her pup. Neonates of *H.ater* are found to have a body mass of 37.13% of mother’s body mass (Number-5). The physical contact remains uninterrupted for the first few days. In many species of bats, the rate of mortality is high between the onset of flight and the end of the first year of life (Humphrey and Cope, 1970). In the present study *H.ater* possess an overall increase in death rate of $\mu_1 = +0.39$ during the entire study period. The bats show 80% of observed death rate during pre volant stage and sex ratio analysis shows death rate of male pups is high. Certain aspects of social organization undoubtedly serve to minimize the mortality and hence a careful examination of mother-pup relationship is crucial for the understanding of social behaviour of *H.ater* during reproduction.

**Maternal nourishment**

Production of milk is generally considered as the most costly aspect of mammalian reproduction (Oftedal, 1984). Bats provide exceptional opportunity for maternal investment and proximal cost of reproduction. They are of special interest energetically since females of most species of bats suckle

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their pups until they approach their adult size almost. By contrast the young ones of other Eutherian mammals are weaned and begin to feed independently well before they attain 40% of adult body size. Since milk is the only source of nourishment and exogenous water before the young bats fly, the energy investment of the mother on pup accounts for 60% of its daily budget (Kunz and Stern, 1995). *H.ater* pups are altricial. The mothers’ lavish meticulous care is needed for the survival of the young ones. This reproductive period need extra conservation and legislative protection. The females nurse their offspring through a pair of lactating nipples located in the anterolateral position. In addition, the females have a pair of false nipple on either side of the anal region which primarily act as ‘hold fasts’ for infants (Plate 5). According to Kunz (1987) the successful assignment of female’s lactation period is based on the relative lactating nipple size, presence or absence of tuft of hair on nipple, degree of nipple cornification, the assignment of female’s relative stage of lactation. Duration of lactation appears to vary among chiropteran species. Lactation in insectivorous bats is relatively short and most species are being weaned between 4 and 8 weeks of age (Kunz and Stern, 1995). However, Brosset (1962) reported in *H. commersoni* the lactation lasts for more than 21 weeks and in *M. lyra* the lactation lasts for 10 weeks (Balasingh, 1990). In the present study *H. ater* seems to exhibit lactation for a maximum of 8 weeks (55-60 days). Isaac (1993) reported that it is less than 5 weeks in *P. minus*.

During the first 25-30 days of age, the infants of *H.ater* are inseparable from the mother. Similar uninterrupted physical contact between
mothers and infants during the first 4 weeks after birth appears typical of megachiropterans. The newborn of *Pteropus sp.* remains attached to the females even when the mother is in flight and accompany them on their nocturnal foraging (Puddicombe, 1990). Similarly *H. ater* also carries its pup while foraging during infant stage (Plate 5). In majority of bat species, flight is acquired before weaning (Tuttle and Stevensen, 1982). The record of late weaning in chiropteran appears in *H. commersoni* after 20 weeks (Brosset, 1962). Zortea and Rodrigues (1998) reported that during the first 20 days, offsprings stayed full time with their mother in *Rhynchonycteris narco* and after 35 days they were no longer observed clinging to their mothers. Early activity such as wing-flapping of pup was noticed around 25 days of age in *H. ater* which might initiate the development of flight muscles. In this observation after 35 days infants of *H. ater* showed a tendency of intermittent flight. From fourth week onwards, duration of suckling decreases with increasing intervals of adjacent approach for suckling. Around 50-55 days the mother performs a peculiar behaviour to push away or reject its own pups after a brief sniffing in the neck region of the pups. Similar pushing and rejection of pups during prevalant period was reported by Nueweiler (1966) in *P. giganteus*. After the rejection behaviour mother of *H. ater* never allows her pup for suckling either by chasing it or by leaving from its place in the roost. During their rejection behaviour of the mother, the pup have repeated tendency to approach the mother for suckling than to stay back for independent mode of life. This behaviour is more obvious in male pups than the female pups. The 80% death
rate of male pups results in the neonatal stage also confirms the inability of males to start the independent life style.

Mother pup recognition

Recognition of the mother is one of major importance for the survival of mammalian neonates. Among the bat species when the mother returns to its nursery colony after her daily feeding excursion, she must locate her own infant. Such selective feeding of her own pup was reported among bats (Nelson, 1965). In *H. ater* for the first 30 days of its age, the mother and the pup are inseparable even during foraging and when the baby becomes volant its mother retrieves it at the maternity roost while foraging. After return the mother nuzzles her young ones before its reunion for suckling at the same time the mother rejects the approach of other pups of the colony which indicates the role of olfactory cues in its pup recognition. Such recognition is based on the detection of odours that have been learnt by the foetus in utero (Capraz et al., 2005). Odours in turn help in recognition and communication which immensely regulate social behaviour and emotional reactions (Johnston, 2005). One day old rabbits were able to detect and discriminate abdominal odours emitted by adult conspecifics (Coureand and Schaal, 2000). The search of nipple by the new born with closed eyes is based on olfactory information produced mainly by fatty acids (Hurpka et al., 2000).

The appeasing behaviour of pre-pubertal pigs appears to result from the perception of maternal odours produced by fatty acids (Guiraudie et al., 2003). Such maternal pheromones involve in the regulation of
nursing pig behaviour (Morrow-Tesh and Mc Glong, 1990) and reduced agonistic behaviour in piglets. These pheromones include fatty acids like palmitic acid, oleic acid, linoleic acid, lauric acid, myristic acid and decanoic acid (Pageat, 2001). The presence of E-3-Dodecyl acetate with milky odour in the abdomen region of mother during the lactation is an indication for the female pups to be attached and hold fast the nipple which help in their recognition. In addition hexadecane from the neck of mother and pentadecane from the anal secretion of female present in the of pups ear deposits may be the mother's identification mark done during their association. *H. ater* also uses decanoic acid and palmitic acid secretion from the anal glands and saliva to tame the pre-pubertal behaviour of the newborn. In addition the 5-Methyl-2-heptamine secretion of mother's ear gland is helpful for (foul smell) marking on the dorsal region of the pup which helps in their recognition. This deposition might be done possibly while the mother often licks and nuzzles its pup. Juvenile bats generally produce no secretion (Brooke and Decker, 1993).

High pitched trilling calls were also noticed during this formal observation which is a kind of acoustic communication among the members of the colony particularly between the mother and the older pup. The infants emit faintly audible vocalization by which mothers are attracted presumably a olfactory means that aids the final confirmation.