Chapter

Dispersal Patterns, Mating Strategy and Genetic Diversity in the Short Nosed Fruit Bat *Cynopterus sphinx* (Chiroptera: Pteropodidae) in Southern India

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Abstract

The short-nosed fruit bat, *Cynopterus sphinx* is a common plant-visiting bat that is widely distributed throughout the Indo-Malayan region. In this chapter, we discuss the dispersal patterns, mating strategy and genetic diversity in the short-nosed fruit bat *C. sphinx.* We used a broad-range of techniques, including mark-recapture, radio-telemetry and molecular biology analyses. Our studies uncovered unique aspects of the dispersal, mating system and genetic diversity of these bats. Both the sexes of *C. sphinx* were found to disperse completely from the natal harems before subadult stage and young female *C. sphinx* become members of a harem much earlier than their male counterparts. The nonharem males are reproductively active, gain access to harem females and sire more offspring in July–August breeding season than March–April breeding season and presumably obtain some reproductive success. Our molecular study shows that considerable genetic diversity was observed in this species from different zonal populations, possibly due to complete dispersal of juveniles of both the sexes from their natal groups and gene flow between the zones. All these studies suggest not only a predictive framework for future studies, but also the use of these data in the management and meaningful conservation of this species.

Keywords: *Cynopterus sphinx*, fruit bat, dispersal pattern, mating strategy, genetic diversity

1. Introduction - Study species

The Indian short-nosed fruit bat, *Cynopterus sphinx,* belongs to the Old-World fruit bats (Megachiroptera: Pteropodidae) (**Figure 1a**). It is a common plant-visiting bat that occurs throughout the Indo-Malayan region and roosts solitarily or in small groups in the foliage [1]. It weighs about 45–70 g and lives in small clusters of about 3–30 individuals [2–4]. Dog-shaped head, divergent nostril, large prominent eyes and short ears with white margin are the unique morphological characters by which one can easily identify this species. In males, the chin, anterior part of shoulders, sides of the chest, belly and thighs are characteristically orange tinted

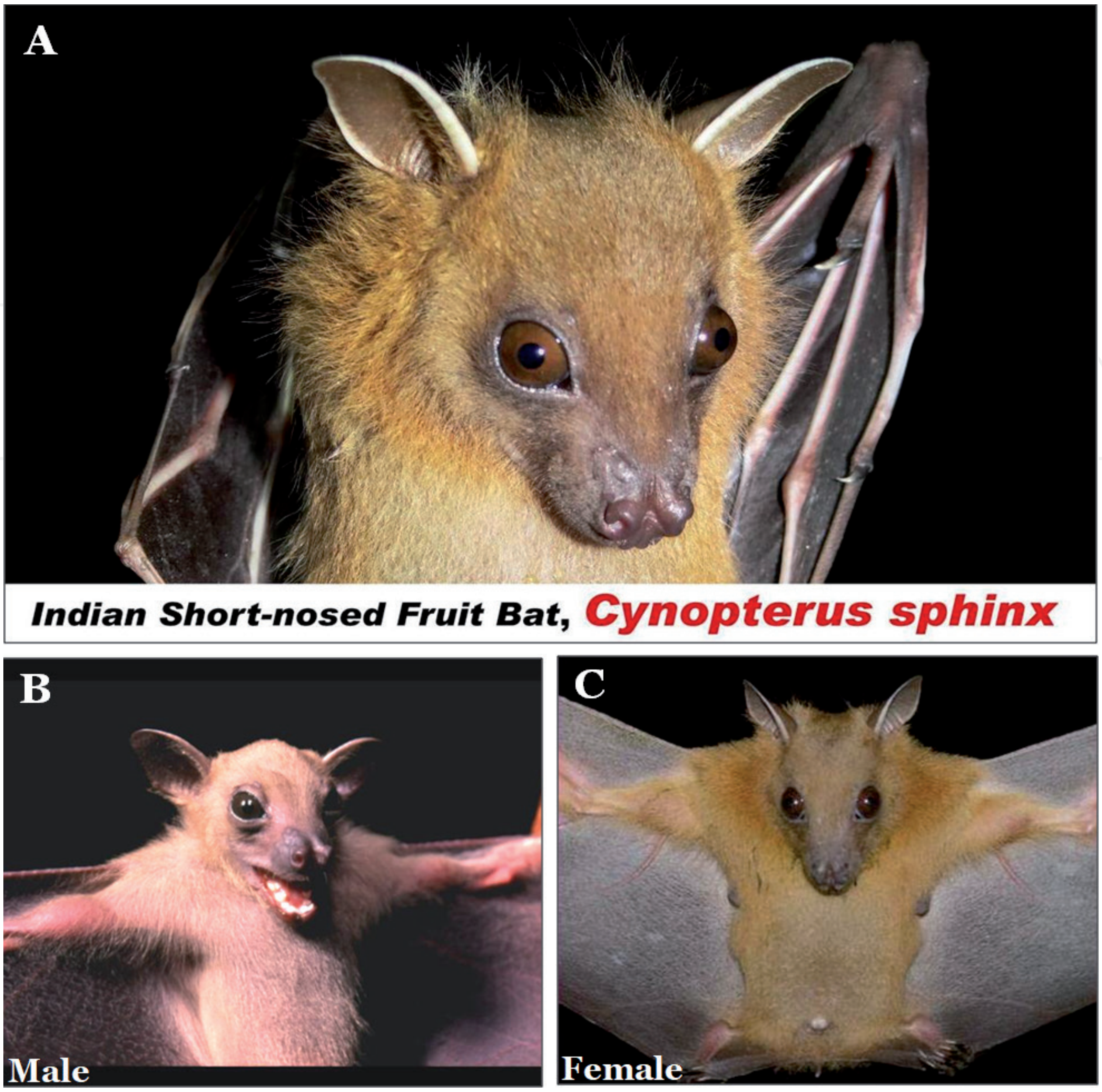

Figure 1.
The study species Indian short-nosed fruit bat (a) Cynopterus sphinx. A close view of (b) male and (c) female.

(**Figure 1b**). In females, the collar is usually tawny brown, the rump is gray brown and the belly is paler gray with slightly lighter hair tips (**Figure 1c**).

These bats use several types of diurnal roosts and are known to alter different types of foliages (palm and mast trees) to construct tents and attract females (**Figure 2a** and **b**) [2, 4–6]. Although different types of altered plant structures are referred to as bat tents [7], the first account of tent making by a male bat came from observations on *C. sphinx* [2]. During the breeding season, most of these bats live in groups called "harems" (**Figure 2c** and **d**) consisting of a single male and more than one female [2–4, 6, 8]. Harem males defend such tents against other males and thereby enabling copulation with a large number of females which is the primary mating strategy adopted by *C. sphinx*. They follow polygynous mating system (prolonged association of one male with more than one female) based on resource, called resource defense polygyny. The recruited females are defended as harem by a single male [2, 6]. However, apart from such successful males, a number of adult males were also observed roosting solitarily [8–10].

C. sphinx is a polygynous-mating bat that has a polyestrous reproductive cycle with two well-defined and highly synchronous parturition periods per year [11, 12]. Typically, in India, parturition takes place during the month of March/April and July/August. Females can reproduce a maximum of two pups in a year [11, 12]. Females endure a postpartum oestrus once the young ones are born during the month of

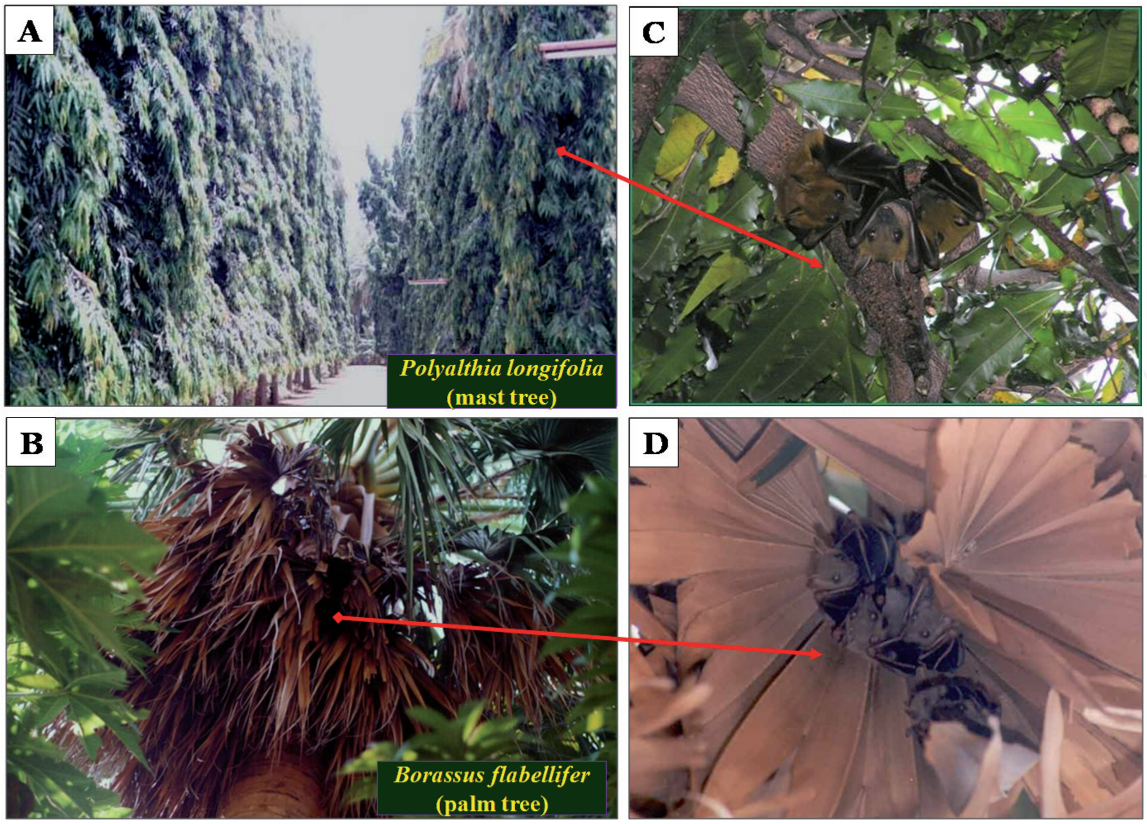

Figure 2.
The major roosting trees (a) Polyalthia longifolia (mast tree), (b) Borassus flabellifer (palm tree) commonly used by the Indian short-nosed fruit bat C. sphinx in South India. A closer view of group of C. sphinx roosting in tents of (c) mast and (d) palm tree. Arrows indicate the bat roosts.

March/April [11, 13]. To wean the cohorts born during March/April, females lactate the young ones while they are pregnant. Females remain anoestrus until October after the birth of July/August cohort [14, 15]. Neonates weigh *ca.* 11 g at birth and begin to fly at approximately 40–50 days of age, although young may continue to suckle from their mothers for 10–15 days after they initiate flight [12, 16]. At weaning, young *C. sphinx* weight about 51% of adult body mass and achieve adult body dimensions at approximately two months of age [1]. In this chapter, we discuss dispersal patterns, mating strategy and genetic diversity in the short-nosed fruit bat *C. sphinx* (Chiroptera: Pteropodidae) in southern India.

2. Dispersal patterns

2.1 Introduction

Displacement of a juvenile from its birth place to the first site of reproduction is termed as natal dispersal [17]. This natal dispersal is one of the factors contributing to the central evolutionary forces that affect the natural populations. Also, it is the key life history trait that is involved in both species persistence and evolution [18]. Understanding the patterns of dispersal is important in population ecology and conservation biology [17, 19]. Bats are known to exhibit varying degrees of dispersal and philopatry based on their social system [20, 21]. The short-nosed fruit bat *C. sphinx* is a group living, frugivorous, megachiropteran bat. In *C. sphinx*, the juveniles of both the sexes are believed to disperse completely from the natal harem [3]. However, their status after dispersion remained unanswered. Do they join established harems immediately or aggregate to form a new harem? Answering this question is the aim of this section.

2.2 Materials and methods

In order to understand the dispersal pattern in *C. sphinx*, the mark-recapture study was carried out in the day-roosting places in and around Palayamkottai, Tamil Nadu, South India (lat: 8° 44′ S; long: 77° 42′ E). We spotted day roosts by citing the accumulation of leaf pellets, rejected fruits, seeds, and leaves under trees especially *Borassus flabellifer, Polyalthia longifolia Washigtonia filifera, Caryota urens,* and *Vernonia scandens.* When any of these accumulations are seen, it is very likely that *C. sphinx* bats roost. Randomly 4–6 day roosts were censused every week. Bats were captured just before emergence with the help of a hoop net attached to an extendable aluminum pole. The entire tree was enveloped with a 6 m x 9 m nylon mist net (Avinet-Dryden, New York, USA) to prevent bats from escaping. The mouth of the hoop net was placed at the entrance of day roosts. A minor disturbance was caused to the trap the bats inside the tent. For each bat, sex, age, forearm length and body mass were recorded [16]. After processing, the bats were held in net cages and were released at their roosts on the evening of the same day they were captured. Only those colonies, which were captured completely, were included in the present study. The proportion of males and females in different age classes viz. pups, juveniles and subadults were estimated and their roosting patterns were recorded.

2.3 Results and discussion

Our results showed that female proportion in harem increases considerably from pups to subadults. Both the sexes are equal in number (1:1) in the pup stage, whereas the sex ratio was female biased in the juvenile (1:1.8) and subadult stage (1:4.2). In mammals, dispersal is usually male biased and this also holds true for most bat species studied to date [17, 22, 23]. However, several studies on tropical species indicate that there may be cases where females also disperse [24]. Our study suggests that the juveniles of both the sexes disperse from their natal group before entering the subadult stage. We mostly captured dispersed juveniles and harems in which post-lactating females were present without the young ones. In many of the day roosts, the number of juvenile bats were disproportionate to the number of post-lactating females, especially when the juvenile bats were predominant.

The capture rate of juvenile females outnumbered the juvenile males, which suggest that the males dispersed early from the natal roost. One could suggest that maternal neglect could be playing a strong role in the altered sex ratio in *C. sphinx*. If mortality is a factor at the pre-weaning stage, both the sexes are expected to suffer equally: whereas if adults eject the males by force, then there may not be any juvenile males in their parental roosts. Contrarily we observed some juvenile males roosting in the natal roost though the number was much less when compared to females. Similar studies in other bats show that young females of short-tailed fruit bat *Carollia perspiscillata* are more likely to disperse away from the natal roosts than the young males [25]. In *Nycticeius humeralis,* all juvenile male bats deserted the roost faster than females and they were neither seen again in the roost nor foraging nearby. Contrary to this, juvenile females remained in the foraging area with their mothers after recruitment. They evidently continued to nurse for about three weeks longer, since milk could be expressed from the mammary glands of captured adult females until mid-July [26]. In a Neotropical bat *Lophostoma silvicolum* both male and female offsprings disperse before maturity and the polygynous mating system may lead to all-offspring dispersal more often than previously assumed in mammals [27].

In accordance with the general mammalian pattern the females of most group-living bats, including some harem-forming species and all temperate zone species, are strongly philopatric which includes evening bat *N. humeralis* [17, 26, 28], vampire bat *Desmodus rotundus* [29], the brown long-eared bat *Plecotus auritus* [30, 31], Bechstein's bat *Myotis bechsteinii* [32–34], greater horseshoe bat *Rhinolophus ferrumequinum* [35], mouse-eared bat *Myotis myotis* [22] and northern long-eared bat *Myotis septentrionalis* [36]. A typical female philopatry was not observed in the group living *C. sphinx*. In this study, juveniles of both the sexes were found to disperse completely from their natal harems. Several reasons for female dispersal have been postulated [37]. It is often assumed that dispersal costs are higher for females than for males, whereas the benefits are thought to be higher for males. Moreover, absence of female philopatry is not uncommon among harem forming Neotropical bats [25, 29].

In this study, the female proportion in harems increases considerably from pups to subadults. Compared to juveniles (1:1.8), sex ratio was highly skewed towards females in the subadult stage (1:4.2). From the total of 52 subadults, only 10 were males. Moreover, not a single subadult male bat was found to be roosting in a harem. This explains that the males disperse from the natal roost before subadult stage. From the 42 captured subadult females, it was observed that four subadult females were pregnant showing that the females matured earlier and were engaged in reproductive activities during the subadult stage itself. Lower rate of juvenile survivorship maybe one of the reasons for the low capture of adult males [12], which remains unclear. The probability of censusing these bats remain low because the male bats preferred to roost in dense, unmodified and previously unoccupied foliages. During the capture, the number of subadult females were larger as they joined established harems, formed a new harem of subadult females with an adult male or they remained alone in the roost.

However, we were not able to find out whether the dispersed juvenile bats return back to their natal harems. The probability of making local migrations even during the breeding season is rare in both *Rhinopoma hardwickei* and *P. auritus* and hence they show greater fidelity towards their roost sites when compared to other bat species [38, 39]. However, the degrees of roost fidelity differ according to the sex and age in different bat species. During the nursing season female *Miniopterus schreibersii* bats strongly exhibit philopatric behavior since they returned to their place of birth to produce young ones [40]. Also, it was observed that the males extended greater level of connection to their birth site and also the juvenile female bats showed higher fidelity to their natal roost than did males and bats of other ages [41]. Moreover a particular population of *C. sphinx* from Pune, there were no recruitment of females to natal harems and also these females did not join other harems in the same or neighboring colonies [3]. Young females joining harems have been documented in bats like *Artibeus jamaicensis* and *Phyllostomus hastatus.* Though, technically the mating system of both these species appears to be a form of polygyny similar to *C. sphinx,* all three species seem to differ in the way young females are recruited in to a harem. In *A. jamaicensis*, it was observed that harems contained females of all ages, which suggests that subadult females join established harems [21], whereas, harem of the greater spear-nosed bat *P. hastatus* forms a new generation of subadult females [20]. However, in *C. sphinx* the harems consist of all age groups of harem females as well as the harems with only subadult females were in common.

2.4 Conclusion

We identify that young female *C. sphinx* become member of a harem group much earlier than the male. The reason for nonharem young females staying near to the established harems is not known. But this roosting pattern may improve the chances of breeding attempts made by young females as they attain sexual maturity earlier (*ca.* 7 months) compared to males (*ca*. 18 months) [12, 42].

3. Mating strategy

Factors responsible for the occurrence of nonharem males and mechanisms used to acquire harem male status.

3.1 Introduction

Polygynous mating is one of the most salient features of mammalian social structure and has potentially far-reaching consequences for a diverse array of evolutionary processes [43]. Male reproductive success in polygynous mammals is largely attributed to the spatial and temporal patterns of female aggregation [43, 44]. Receptive females are reliant on variation in resource distribution, predation pressure, costs of social living and activities of males [43]. One of the major factors that affect the mating success of resource-based polygynous mating animals is the resource distribution. Females choose males indirectly by mating with males that defend the highest quality resource when the males control access to the resources that these females require for reproduction [44]. Males that can make the greatest genetic contribution to the fitness of their offspring are chosen by the females [45].

Bats exhibit various forms of mating behavior ranging from simple monogamy to resource and female defense polygyny, as well as leks [46, 47]. Among these, resource defense polygyny is the most commonly observed mating pattern [48]. Bats establish a harem by defending critical resources such as food, shelter or mates [46]. Males potentially gaining favored access to several females is considered as one of the main benefits in resource defense polygyny and a healthy male inseminates the females [43, 46]. It is observed that several Neotropical fruit bats such as *Uroderma bilobatum* [49, 50], *Vampyressa nymphaea* [51], *Ectophylla alba* [52], *A. jamaicensis* [53–55] and *C. sphinx* [2] follow this type of mating strategy. Most of the bat species often spend the day and a large portion of the night in the roost which shows their attachment to their roosts [7]. However, it was observed that out of 1,300 bat species, only about 20 species are known to make their own roosts [56]. A striking feature of some polygynous bat species is that they often alter different types of foliage to create tents [2, 3, 57].

C. sphinx is known to exhibit polygynous mating system (i.e. prolonged association of one male with more than one female) based on resource defense polygyny [4]. In *C. sphinx,* the adult males are categorized into two groups, harem and nonharem males. Males construct and defend tents (resource) and recruit females to gain mating access and this organization is called a harem. In order to attract females, the harem males defend critical resources during the breeding season and this type of system is called harem-polygynous mating. Though, several studies suggest that the breeding population of *C. sphinx* also consists of nonharem males which dwell in roosts adjacent to harems [3, 4].

If this is true, what is the role of such nonharem males in the population or colony? What are the factors that cause the occurrence of nonharem males in a colony of *C. sphinx*? Are trees and foliage suitable for tent-making a scarce resource? Are solitary males less competitive and so remain isolated from the breeding activities? However, the factors responsible for the occurrence of nonharem males and mechanisms used to acquire harem male status are not understood fully.

3.2 Materials and methods

The study involves understanding the mating status of solitary males, bat captures were confined to day roosting places. Captures at roosting places indicated whether a male was solitary or a harem holder. Every week, we inspected trees and censused day roosts regardless of the number of incumbents (solitary or harem). Roosting groups with a single adult male with one or more adult females were considered as harems [46]. However, apart from such successful males, a number of adult males were also observed roosting solitarily. All the individuals of harems and the solitary males, which roost adjacent to the harems, were captured just before emergence using a hoop net with an extensible aluminum pole.

All the bats of harem groups and nonharem males were tagged with a color-coded bead necklace. We used beads of ten different colors, each color denoting a number from 0 to 9. We loaded each necklace with 1–3 beads. Thus, there were 999 possible sequential arrangements of the color beads. We have used this type of tagging for various studies and have observed no apparent detrimental effects on bats. After marking, all individuals were released at the site of capture. These color coded bead necklace markings allowed us to identify individuals and determine their previous roosting locations. The census, mark-recapture and radio-telemetry studies data were used to assess the reproductive condition, mobility, roosting pattern and status of adult males (harem/nonharem).

3.3 Results and discussion

One of the striking features of tent-making male bats is that they use tents as a resource to recruit large numbers of females and copulate with them [46, 51]. Although experimental evidence supporting causal factors for resource-defense polygyny is lacking, scarcity of resources is thought to be one of the factors for aggregation of females [58]. Solitary roosting existence of some adult males is one of the main consequences of resource-defense polygyny as the males fail to defend a resource. We attempted to study the resource-defense polygyny in *C. sphinx*. We observed that there were no shortage of roosting sites and these solitary males remained reproductively active. Similar, results have been reported in a Jamaican fruit-eating bat *A. jamaicensis* [59, 60].

Our results suggest that the male success in female recruitment was not due to shortage of tents. We found that, nearly 39% of adult males were roosting alone. This observation was based on >90% of nonharem males roosting adjacent to harems and also 50% of nonharem males had scrotal testes. In addition, the mark recapture study showed that the transition status of males from nonharem to harem was possibly due to previously unobserved mode and the female recruitment is associated with resource (roost). It indicates that the solitary males are involved actively in female recruitment to their roosts and also in the process of mating. During our study we observed that many solitary males recruited females within a short period of time. A nonharem male's effort to gain access to adult female cannot be hindered by the solitary nature *per se*, which is found reliable based on expectation. Since it is a choice of every animal to represent itself genetically superior among a population, we believe that the breeding behavior of the animal is not restricted by the resource.

The mode of attaining harem male status differs from species to species. For e.g. young males of *P. hastatus* are known to gain access with harem females if the harem male dies or gets displaced [61]. It was observed that under captive conditions, two adult *C. sphinx* males, competing with each other to take control of a tent resulted in the harem male turnover. However, the turnover of harem male did not have any impact on the cohesion of harem females [62]. In other harem forming bats, males exhibit a typical pattern during their ascendance to the dominant status. A size-based hierarchy for males in the social system was observed in *A. jamaicensis* [59, 63], with some larger harems being occupied by a small sized subordinate male apart from a dominant male. In *S. bilineata*, some individuals are associated in harems over several years and the non-territorial males build up site-specific dominant hierarchies [64] and for coalitions of male manikins (*Chiroxiphia linearis*). In order to attract females, the subordinate males perform costly displays by playing a satellite role. But they do not obtain reward immediately because the dominant male practically fathers all the young bats [65].

However, subordinate manakin males readily take the place of the dominant males in order to obtain a long term benefit from the association. Subordinate males' relationship with dominant males adds an extra benefit to the subordinate males by increasing the inclusive fitness thereby leading to higher reproductive output [66]. Our efforts to identify the morphological differences between harem males and nonharem males were not successful as we did not find substantial differences in the forearm length and body mass. This is surprising because an individual's body condition is often the most important determinant for alternative mating tactics [67, 68]. Larger and heavier males are typically dominant in male–male contests and reproduce more often [69]. In male common shrews *Sorex araneus,* the differences in body weight may vary with age [70]. Body weight of the males that differed in mate-searching behavior varied during the early stage of maturation while no significant difference was observed in the body size of both the types of adult males. In the present study, it was observed that most of the adults with T3 tooth-wear class were harem males, while the ones with T1 tooth-wear were found to be in the group where nonharem males existed. Therefore, among the first breeders, a strong competition for mates takes place. Observation made it evident that approximately 50% of males with T2 tooth-wear class were harem males while the remainders were found to be nonharem males. Also, these results suggest that harem males and nonharem males differ in age slightly.

Individuals with territory and resource, typically have a higher reproductive success than the males without territories due to strong competition for mates in a polygynous mating system. Males with territories usually monopolize and probably fertilize many females [71]. The males which does not possess any territory follow alternative mating strategy either as satellites [72, 73] or as sneakers [74, 75]. Similarly, among polygynous bats such as *Parasenecio hastatus* [20], *D. rotundus* [76] and *Saccopteryx bilineata* [77] it has been observed that the harem males monopolized the females incompletely. Chances of nonharem males fertilizing the harem females increased due to the incomplete monopolization of harem females by harem males. However, the mode of nonharem males gaining access to harem females was not clearly known to determine the "alternative strategy" [58].

The roosting preference of females seems more likely to increase the chances for nonharem males fertilizing some of the females. Apart from the mating success of nonharem males, low paternity for harem males can also occur as a result of female choice. *S. bilineata* are highly mobile and actively select their roosting location. During the course of a day, some females are found to shift their roosting territories while others disperse to other colonies. Importance of female choice specifically in highly mobile animals with harem system was reported [77]. Our radio-telemetry studies suggest that 3-postpartum *C. sphinx* females were found to be visiting a nonharem male especially during the night hours and also were involved in mating. Females were periodically shifting their tents since fluctuations were observed in the harem size on a day-to-day basis [2]. Similarly, movement of females between harems has also been observed among the polygynous bats such as *A. jamaicensis* [60, 78], *P. hastatus* [20, 24], *D. rotundus* [76] and *S. bilineata* [77, 79].

Similarly, pallid bats *Antrozous pallidus* switch roosts without respect to group structure during pregnancy, but invest energy in communication to move as part of a cohesive group during lactation [80]. Storz et al. [4] reported that in *C. sphinx,* new harems are formed when parous females from an established harem join a previously solitary male in a different roost within the same colony. Reproductive success of male *C. sphinx* depends on colony structure during the previous post-partum estrus rather than current parturition [58]. Similar results have been reported in round-eared bat, *L. silvicolum* [47].

In the Bechstein's bat *M. bechsteinii* female bats frequently change between many different roosts [32]. In *M. myotis*, 16% of 435 ringed females appeared at least once in foreign colonies and about 6% switched colonies permanently [81]. Furthermore, in *P. auritus* [39] and in *R. ferrumequinum* [82] females occasionally switch colonies. Moreover, the permanence of roost sites like caves, mines and buildings, in contrast to the ephemeral nature of sites like trees, could account for patterns of roost switching observed in free-ranging bats [80]. It is well known that tree-roosting bats switch roost sites every few days, but the motivation underlying roost switching is not well understood [83]. The roost switching may reflect the maintenance of long-term social relationships between individuals of the larger colony [84]. We observed that the females of *C. sphinx* move between two or more roosts and also, many harems were completely abandoned the tents. In response to changes in access to diurnal roosts with suitable microclimates or the availability of fruit and nectar resources, female *C. sphinx* may alternate between different roosting habitats in the same local area [4], although they do not undergo seasonal migration. A similar pattern has been observed in a breeding population of the Neotropical fruit bat *C. perspicillata* and *Pteropus poliocephalus* [85].

C. sphinx has two well-defined parturition periods per year [11, 12, 86]. A huge difference was found between these two parturition periods while assessing the paternity of harem males [58]. The authors attributed this difference between the parturition periods to the availability of roosting sites associated with seasons. The survey also showed that average size of harem was found to be slightly higher during the dry season than during the wet season. However, we observed little correlation between harem size and availability of roost sites. In the study area, during both wet and dry seasons, bats abundantly roosted in both mast trees and palm trees. Shortage of roosting sites were rarely observed in our year long survey. Throughout the year, more than 90% of the day roosts were occupied by *C. sphinx*. Similarly, food resources in the area were also very vast to influence harem size [87].

During July and August, the frequency of nonharem males were found to be highest. Timing of sexual maturity of young males might be a probable reason, though no reports on timing of sexual maturity of young male bats in southern India. Reports from central India suggest that males born during the June–July parturition and February–March parturition were able to mate during September–October of the following year [88]. The number of nonharem males censused during August to October was relatively high in the study area. This can be attributed to the competition among first time breeding males to establish a day roost to recruit females before securing mating in October–November.

Our radio-telemetry observations suggest that females aggregated with a solitary male. Interestingly, aggregation occurred only after the male occupying a tent which was probably constructed by another male. Our tagging efforts might have probably disturbed the harem but the exciting aspect of this observation is the subsequent female aggregation and the way by which a solitary male succeeded in recruiting females. In a short span of time it may not be possible for a male to succeed in mating, if it followed the primary strategy involving construction and defense of tent leading to female recruitment. In addition, we observed that the solitary male spent less time away from the roost at night after female recruitment by frequently visiting the roost throughout the night and by making several short foraging flights spaced randomly throughout the night [10]. This behavior is consistent with the earlier reports on the activity of harem males in *C. sphinx*, *A. jamaicensis, P. hastatus*, *C. perspicillata* and *Balionycteris maculata* [2, 24, 25, 54, 89]. This suggests that in *C. sphinx,* some type of territoriality is associated with shelter [61]. Situations under natural conditions like displacement or death of harem male can also occur though, roost abandonment of harem appears to be artificial. Among the species, the mode of attaining harem male status differs. In *A. jamaicensis* [59], *S. bilineata* [64] and *P. hastatus* [61], a size based hierarchy was observed. However, in *C. sphinx,* no such pattern has been reported. In order to attract maximum number of females (extreme variation in the group size) and hold them together, some recognizable factors should be considered. But, morphological features of males [90] and the characteristics of tents [2], does not influence in female recruitment.

3.4 Conclusion

Although the high clustering of females in confined roosting places appears to facilitate resource-defense polygyny in *C. sphinx,* recent results showed the failure of harem males in the exclusive defense of harem females and the morphological variables did not differ between harem and solitary males. The present observation suggests that the female recruitment is associated with resource (roost). Taken together with the present results of reproductively active nonharem males, it seems that the solitary nature of some adult males in the population of *C. sphinx* may not be a forced option. However, further investigation is necessary to find whether the solitary adult males adapt any alternative reproductive strategy to usurp mating opportunities of harem males. In order to understand the complex mating strategy of *C. sphinx,* extended molecular genetics techniques to behavioral ecology is required.

4. Molecular genetic analysis of mating strategy

4.1 Introduction

As our understanding on mating systems increases, it becomes obvious that apparently species-specific mating behaviors often vary both between and within population [91]. Reproductive strategies are shaped by natural selection favoring individual with the greatest lifetime reproductive success. However, not all mature individuals adapt to the same reproductive strategies [69]. When competition for access to mates is severe, young reproductive individuals sometimes opt for alternative mating behaviors. Environmental or demographic factors may constrain the number of males that were able to employ the most successful strategy [92]. Alternative tactics in reproductive behavior enable individuals to maximize their fitness in relation to competitors of the same population. Among polygynous mammals, territorial behavior is almost exclusively a male trait believed to function primarily as a reproductive strategy to secure mates. Because mammals are committed to their progeny through gestation and lactation, female reproductive success usually is more readily quantified than male reproductive success. Male reproductive success in polygynous mammals is largely attributed to the spatial and temporal patterns of female aggregation [43, 44, 91].

Most known mating associations in bats are composed of a single male and several females and such organization are usually called harems [46]. *C. sphinx* is known to exhibit polygynous mating system (that is, prolonged association of one male with more than one female) based on resource availability and such behavior is popularly known as resource defense polygyny [4]. Though, several studies have shown that the nonharem males also occupy the roots nearby harems most of the time [4, 8–10, 93]. Although, the role of nonharem males as probable fathers has not been studied well in *C. sphinx* population.

4.2 Materials and methods

Bats were collected from the foliage tents of *P. Longifolia* (mast tree) and *B. labellifer* (palm tree) using a hoop net with an extensible aluminum pole. Bats were sampled over a period of four weeks immediately following each of four annual parturition periods: March–April (dry season) and July–August (wet season). A medical punch will be used for the excision of tissue (4 mm^2) and care will be taken to place it in an area between the blood vessels to avoid injury (wing membranes healed within 3–4 weeks [93, 94]. After each sampling, the punched hole and the punch will be disinfected with 70% ethanol. No negative effects of this treatment on the health of the bats will be observed. It should also be noted that the bats frequently have natural injuries of this type in their wing membranes.

The collected blood samples will be immediately mixed with Anticoagulant Citrate Dextrose (ACD), transferred to microcentrifuge tubes and sealed with parafilm. The blood and tissue samples will be stored in ice, transported to the lab and stored at −20°C until DNA extraction [93, 94]. No bats will be killed or retained as specimens during this project. We will be following the Institutional Ethical and Bio-safety Committee Guidelines of Madurai Kamaraj University. PCR based RAPD strategy was used to study the paternity of harem males and nearby nonharem males to the young born in the harems.

4.3 Results and discussion

During the wet (July–August) season, we captured 27 harem males, 30 nonharem males and 125 offsprings were analyzed to assign the reproductive success of harem and nonharem males. Out of the 125 offsprings the nonharem males sired 73 offsprings (average 58%) and the harem males sired only 52 offsprings (average 42%). During the dry (March–April) season 14 harem males, 18 nonharem males and 142 offsprings were captured and analyzed to assign the reproductive success of harem and nonharem males. Of the 142 offsprings the harem males sired 132 offsprings (average 94%) and the nonharem males sired only 10 offsprings (average 6%). From these results, we identified that the reproductive distribution is unequal between harem and nonharem males. It indicates that the harem males failed to control harem females thereby increasing the chances of nonharem males to fertilize some of the harem females. In addition, in southern India, during the dry season the spatial dispersion of female *C. sphinx* is highly clumped due to limited roosting sites and the harem male sires 96% of offspring conceived during this period [58]. In total contrast during the wet season, more roost sites are available and females are dispersed more widely. In this case, the harem male sired only 40% of offspring, while the other 60% offsprings were sired by other (solitary) males. The possible movement of females between harems was suggested as one of the reasons for this observation. Similarly, among the polygynous bats *A. jamaicensis* [59, 78], *P. hastatus* [20], *D. rotundus* [76] and *S. bilineata* [77, 95], incomplete monopolization of females by harem males has been observed. The harem males failed to control the harem females as result the increases the chances for nonharem males to fertilize some of the females.

The most commonly described mating system in bat species is polygyny, in which males defend a resource to recruit and have exclusive mating access with a large number of females. The resource may be a foraging area or a roosting site or the females themselves. However, several genetic analyses have shown that paternity is biased in polygynous mating systems. For e.g. a paternity study in *S. bilineata* demonstrated that 71% of offspring born into a harem are not sired by the resident harem male, but are instead fathered by non-territorial males [77, 95]. Similarly, in *P. hastatus*, harem male fathered 60–90% offspring [20], while the harem male in *D. rotundus* fathers approximately 45% of young [76] and the estimated paternity for dominant males of *A. jamaicensis* ranged from 33 to 83% [78].

4.4 Conclusion

The molecular genetic analysis of mating strategy assignments based on RAPD results suggest that during July–August breeding season (wet), the nonharem males gained access to females and sired more offspring than March–April breeding season (dry). These results suggest that nonharem males are reproductively active, gain access to harem females and enjoy some reproductive success. To understand the reproduction of nonharem males, further investigations are necessary. Solitary behavior can be an acceptable alternative to territoriality because the reproductive success of some nonharem females were relatively high. Solitary males sired number of juveniles but had no costs for roost defense. Harem males were not able to control the movement of the females in their harems because reproduction by nonharem males is possible [2–4, 77, 93]. Since, harem females provided no parental care, the females were allowed to choose their mating partners. The behavior and reproductive success of nonharem males over their lifetime could clarify whether they potentially compensate lower reproductive success per year with longer persistence in the harem.

5. Genetic diversity within and among populations of *C. sphinx*

5.1 Introduction

Genetic variation is an important factor in determining the ability of a species to adapt to new environmental conditions and therefore may be an important measure of the evolutionary potential and long-term viability of a species. The information on the amount of genetic variation within a species and its distribution within and between populations would aid in bat conservation planning [96, 97]. To understand both the past and current behavioral processes, it is vital to know the population structure of a species. Colonization and/or dispersal events can be inferred by characterization of population structure at the macro-geographical level, while social organization within a population can be used to infer the micro-geographical structure [30]. Both direct (mark-recapture studies) and indirect (genetic) techniques [98] should be used to study the population structure of individuals to understand the degree of spatial variation both in distribution and genetic composition [99].

In general, the high dispersal abilities are associated with a low population structure [100], which has been reported for some mobile species, including birds [101] and bats [102]. Studies describing molecular patterns of intraspecific geographical differentiation in bats have indicated a low level of genetic divergence and a limited geographical structure in species with continental distribution [103]. However, high-intraspecific divergence levels with clearly defined geographical structuring have also been observed. These different results can be attributed to the different molecular markers used in the various studies. Studies on different bat species using the molecular genetics approach have shown genetic diversity among distant populations [102–105].

In *C. sphinx*, the behaviors of tent construction [5], reproduction [11], foraging [106], pollination and seed dispersal [107–110], influence of moonlight [111], sex and reproductive status on the foraging activity [112] are studied in detail. However, the genetic variations within and among populations of *C. sphinx* is not well defined. The lack of genetic information is undoubtedly due, in part, to the difficulties associated with studying them in the wild. The capacity for flight makes bats especially difficult to continuously follow in the wild. In addition, light-tagged animals quickly disappear into dense vegetation making them hard to follow. As a result, data collected by these methods are limited. Direct observation of both sexes are often difficult, therefore genetic analyses may be the only way to obtain reliable data on population structure [31]. An important component required in investigating the population biology of any species is the genetic discrimination of that particular species. This genetic discrimination is the major contributing factor that can help conservation geneticists in evaluating population viability. To provide valuable guidelines for proper conservation and management of *C. sphinx* population, an understanding on genetic diversity is very important.

5.2 Materials and methods

Extensive field trips were carried out to collect *C. sphinx* from different geographical locations in southern Tamil Nadu, India. Bats were captured at the time of emergence from the foliage tents of *P. longifolia* and *B. flabellifer* using a hoop net with an extensible aluminum pole. A small piece of wing membrane from each bat was collected using a sterile biopsy-punch. Tissue samples were obtained from a total of 472 bats from 40 zones. Tissue samples were stored in 70% ethanol at −20°C until DNA extraction [94]. Polymorphism at molecular level was studied by RAPD DNA marker technique. Polymerase chain reaction with 30 arbitrary decamer oligonucleotide primers was applied to the 40 zone samples and to investigate the genetic diversity within and among the populations of *C. sphinx*.

5.3 Results and discussion

Genetic variation is the raw material of evolution and its magnitude is therefore of vital interest in governing the potential of a species to evolve and adapt [96]. The genetic analysis of RAPD markers showed a reasonably high level of diversity. High level of polymorphism was observed in this study which indicates that the genetic base from different zonal population was diverse and extensive. The percentage of polymorphic bands of RAPD was observed to be higher in this species (73.1%). The amount of dispersal and the formation of new social groups are the two factors that strongly affect the genetic structure of the population [113]. Population genetic data from a taxonomically diverse array of social mammals revealed low to moderately high level of genetic differentiation among social groups. This high level of heterozygosity within social groups may be a common feature of mammalian population. The majority of mammalian species exhibit a social system characterized by polygynous-mating and female philopatry [17].

C. *sphinx* is a polygynous-mating bat and both sexes were found to disperse completely from their natal harems [4]. Moreover, it was observed that the young females either became a member of an already established harem group much earlier when compared to their male counterparts or formed a new harem group of subadult females with an adult male. As a result, the colonies were mainly composed of females which are unrelated or distantly related and with diverse age group [4]. This method of group formation by this species enhances genetic variation. Currently, the high level of genetic diversity can be explained using three factors (i) natal dispersal (ii) formation of new groups and (iii) gene flow between the zones. These are a few such probable reasons for some of the zones to be closely related at the genetic level, although geographically they are from distinct zones of highly distinct locations in Tamil Nadu. This situation can arise in natural populations when there is a possibility of free/random mating and this association between genotypes from contiguous zones may be the result of similar geographical habitat conditions. In addition, recent habitat loss and degradation, which may have led to the concentration of the surviving individuals in the remaining areas, the long generation time and lifespan of the species allowed populations to retain diversity for long periods after habitat loss [17].

Genetic differentiation coefficient of *C. sphinx* from RAPD analysis suggests that the species is of a higher genetic diversity among populations than other bat species [94]. For example, the Brazilian free-tailed bat *Tadarida brasiliensis*, southwestern populations that include those occupying distinct migrational groups show low level of genetic differentiation among populations, even though banding and recapture data suggest low exchange among migratory groups and the inter-colony differences in the bat species are even lower [114]. Similarly, the range of genetic mixing during the seasonal migration of the little red flying-fox *Pteropus scapulatus,* exceeded 3.5 million km^2 [103]. Low degree of differentiation among populations and large amount of gene flow between sub-populations was elaborated using allozymes and RAPD data. A similar result has been reported in gray-headed flying fox *P. poliocephalus* [115].

Genetic studies of migratory bats support high level of gene flow among populations even when separated by large geographical distances (up to 4000 km) [102]. Studying the migratory species using mtDNA markers can further confirm the predicted pattern with little or no genetic structure over broad distance. The individuals of lesser long-nosed bats *Leptonycteris curasoae*, shared identical mtDNA haplotypes when sampled at distances up to 1800 km apart [116]. Similar results have been reported in *P. alecto* [117], *T. brasiliensis* [118], *M. myotis* [119], *Hipposideros speoris* and *Megaderma lyra* [105, 109]. The pattern of population structure and gene flow in species that do not undergo seasonal migration is less clearly known although, in general, gene flow among populations appear more restricted than in migratory species. The gene flow mainly occurs through extra-copulation between the colonies without permanent dispersal from the natal colony [105]. But the distance, availability of mating sites or the recently fragmented population might limit the gene flow. Interestingly, no natal dispersal was found to occur in both the sexes, while extra-colony copulation was observed in most animal species [120].

A greater range of genetic differentiation was identified among the migratory species. Also, a significant correlation between geographic and genetic distances is explained in several species. Extraordinarily, in the Australian ghost bat *Macroderma gigas* the degree of structure was found to be high, with significant correlation between geographic and genetic distances studied using both microsatellites and mtDNA markers [121], similar, results have also been reported in *P. auritus* [30], *M. bechsteinii* [33], *Rhinolophus affinis* [122] and the non-migratory island population of *Eidolon helvum* [123], although as the latter two species were located on islands, gene flow may have been also restricted by sea crossing distance. From such studies, it is apparent that whilst individual colonies within a population may show some genetic heterogeneity due to co-ancestry, little genetic subdivision is apparent, possibly due to low reproductive skew or high levels of dispersal [124]. Moreover, differences in social structure are frequently associated with different mating and dispersal behaviors, which also influence the amount of gene flow among groups and populations [125]. However, not all sedentary species show evidence of population subdivision, even at considerable geographic scales. In particular, genetically effective gene flow appears to occur among populations of vampire bats *D. rotundus* distributed from Mexico to Costa Rica [126]. As discussed above, the molecular studies at inter-population level has verified a greater diversity of population genetic structure within the order.

Seasonal movement is expected to be the main influence among the populations of migratory species because the genetic structure generally appears to be low. However, a wide range of factors including dispersal ability, extrinsic barriers to gene flow and historical events determines the degree of genetic partitioning among population of sedentary species [102]. Dispersal and migration do not essentially equate with the gene flow and hence it is important to consider this factor while accessing the impact of migratory behavior on the genetic structure of bat population. In migratory species, the level genetic structure can be low only when the individual's mate during their migration. Patterns of genetic population structure for both migratory and non-migratory species may resemble if mating and conception in migratory species occur prior to their migration [102]. Gene flow may also be greater than the dispersal capability of individuals of a species which might indicate, provided the population distribution is continuous. For example, radio tracking of individual brown long-eared bats *P. auritus* showed that maximum foraging distances from the summer roost were no greater than 2.8 and 2.2 km for males and females, respectively. Furthermore, this non-migratory species is not thought likely to travel much further at other times of the year [30]. A hierarchical analysis of genetic population structure in *P. auritus* across North-east Scotland identified no genetic differentiation among three adjacent regions when data from colonies within each region were combined. This suggests that colonies across the three regions of North-east Scotland form a continuously distributed population, within which genes move *via* a `stepping stone' model [30].

In our study, the maximum similarity was observed as many zones were closer to each other. Therefore, when populations remain closer, the gene flow is expected to be greater. As a result, the nearby populations should remain more similar at neutral loci. This relationship is referred as the method of isolation by distance and serves as the stepping stone model of gene flow [127]. However, the distance between populations and the nature of the surrounding landscape between population are the two factors on which the level of gene flow depends [128]. These findings support that *C. sphinx* is not known to undergo seasonal migrations. Moreover, it is a common plant-visiting bat that occurs throughout India and much of mainland Southeast Asia [1]. Our results showing the high genetic variations in *C. sphinx* population is not surprising because, the distribution of these bats is continuous and the level of gene flow is also high. Similarly, study has been carried out among *C. sphinx* of the Indian subcontinent which suggests high gene flow and equilibrium population dynamics [129]. Thus, for the long-term persistence of *C. sphinx* populations, maintaining the gene flow is considered as a key factor.

5.4 Conclusion

Our study deals with the genetic diversity in natural population of *C. sphinx* at the molecular level. We concluded that *C. sphinx* population maintains high levels of genetic variability despite of increase in fragmentation of their habitat. Though this may be beneficial factor for the conservation of these bat species, some caution should be observed. The results suggest that bats move rather freely between zones and current bat populations may continue to decrease in many of the habitats investigated. Furthermore, *C. sphinx* is still a relatively widespread species; it has suffered dramatic population declines during the past several years. Using coalescent based Bayesian analysis, a significant demographic contraction was found to be evident among a large sample of *C. sphinx* genotypes [129], which were one of the eight localities included in the Indian latitudinal study [130]. These results suggest that Indian *C. sphinx* is strongly associated with open habitat [90]. In addition, our direct observation and mark recapture data show a gradual decline of natural populations of *C. sphinx.* However, this study provides baseline genetic information for future studies. To look at the long-term effects of human induced habitat fragmentation and degradation on genetic diversity and structure, microsatellite and mitochondrial DNA variation should be reassessed among this species. It can be concluded that RAPD analysis revealed high levels of genetic polymorphism and differentiation might play a role in the dynamic evolution of *C. sphinx* in southern India. These results would help in developing an effective and meaningful method in conservation of this species. Future studies of Old-World fruit bats from these areas will be of great bio-geographic and evolutionary interest.

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