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THE ROLES OF MALE CHEMICAL SIGNALING, CONSPECIFIC SCENT PREFERENCE, AND SOCIAL STRUCTURE IN FRINGE-LIPPED BATS

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CHAPTER 1: INTRODUCTION

My work aims at understanding reproductive biology in bats. To explore this topic, I selected fringe-lipped bats, *Trachops cirrhosus*, as a focus species because it presents the unique opportunity to elucidate the role of olfactory communication in bats. In this dissertation I investigate a novel odorous trait in male *T. cirrhosus*. I also test the response of individuals to this odor. Finally, I describe elements of the social structure of this species with the goal of determining whether associations among females influence male behavior. The aim of this chapter is to review current knowledge of sociality, auditory and olfactory communication, and reproduction and mating systems in bats to provide the necessary background that contextualizes my work.

Bats (Chiroptera), the second largest order of mammals with over 1400 described species (Tsang et al. 2015), stem from an Eocene radiation and evolved the key innovations of powered flight and echolocation (Teeling et al. 2005). These adaptations allowed them to exploit different habitats and have ultimately led to their ecological success: they are found on every continent except Antarctica. Leaf-nosed bats, Phyllostomidae, are ecologically diverse, varying in diet from insects, fruits, pollen, nectar, vertebrates, and blood (Nogueira et al. 2009). Additionally, many species vary in roost preferences: while some species can modify leaves to construct tents, others are known to roost in tree cavities and caves (Kunz 1982). This family comprises over 160 species and is restricted to the New World, ranging from southwestern United States to northern Argentina (Wilson and Reeder 2005).

Many bats species are gregarious and roost in large social groups. There are several hypothesis to explain the evolution of sociality in bats. For example, ecological constraints, physiological demands, and demographic traits, have been considered as factors promoting sociality in bats (Kerth 2008). With the exception of 18 species (Kunz et al. 1994), the vast majority of bats rely on pre-existing roosting structures, consequently roost limitation could facilitate sociality. However, for bats that roost in foliage or on vegetation, roosts are often not limiting. Thermoregulation is also considered to impact group size. As bats are small, roosting with other individuals could aid in regulating body temperature and conserving energy. Although this might play a role in determining the social system of temperate species, many bat species in the tropics live in social groups and live in warm, humid roosts. Additionally, in the tropics, some species in the family Emballonuridae, roost on tree trunks and space each other apart and do not engage in mutual warming as they occupy different spaces of a roost (Bradbury and Emmons 1974). Finally, compared to other small mammals, bats have a long lifespan (Foley et al. 2018). In combination with philopatry, this would lead to overlapping generations, which could facilitate sociality. Although a combination of factors likely contributed to the evolution of sociality in bats, the diversity of social groups among Chiroptera remained unexplained.

Many bats are social and live in complex groups, and their ability to communicate with conspecifics has been investigated. Acoustic signals are the best studied sensory modality in bats, often in association with their ability to echolocate. Although echolocation is primarily used in navigation and in detection of prey, it can also function to communicate with nearby bats (Suthers 1965). For example, lesser bulldog bats, *Noctilio albiventris*, respond with a complex suite of social behaviors to the echolocation calls of unfamiliar conspecifics (Voigt-Heucke et al. 2010). However, the communication value of echolocation calls is limited because their high frequency attenuates quickly (Lawrence and Simmons 1982). Additionally, it has been suggested that echolocation calls might result in unintentional cues to eavesdroppers such as predators (Gillam and Fenton 2016). In contrast, social calls are primarily used as signals, since they actively broadcast information (Gillam and Fenton 2016). Social calls are common in mate attraction (see below) and in mediating mother-offspring relationships (Gelfand and McCracken 1986; Scherrer and Wilkinson 1993; De Fanis and Jones 1995a).

Males of several bat species have been reported calling to attract females, including the lekking hammer-headed bat, *Hypsignathus monstrosus* (Wickler and Seibt 1976; Bradbury 1977; Jahelková et al. 2008). Likewise, in the Neotropics, male buffy flower bats, *Erophylla sezekorni*, and Seba's short-tailed bats, *Carollia perspicillata,* perform courtship vocalizations and displays (Murray and Fleming 2008; Knörnschild et al. 2014). The best evidence for the role of vocalizations in courtship in bats comes from long-term studies of greater sac-winged bats, *Saccopteryx bilineata*. Male *S. bilineata* with higher calling rates have higher reproductive success (Behr et al. 2006). Male greater sac-winged bats also tend to produce more courtship vocalizations when they hear the echolocation calls of an approaching female (Knörnschild et al. 2012). Furthermore, males respond to echolocation calls of an approaching male with aggressive vocalizations (Knörnschild et al. 2012). This demonstrates that males can discriminate the sex of the incoming individuals. Recently, Knörnschild et al. (2017) found that the songs of male *S. bilineata* attract dispersing females in search of new colonies.

Although most research has focused on auditory communication, bats also have a highly developed olfactory system (Yohe et al. 2018). Chemical cues can aid mothers in identifying their offspring (Gustin and McCracken 1987) and many frugivorous species rely on olfaction to locate food (Rieger and Jakob 1988; Laska 1990; Elangovan et al. 2006). Odors also play an important role in mediating social interactions, as several bat species are able to discriminate the sex and colony membership of individuals (Bouchard 2001; Safi and Kerth 2003). For example, greater bulldog bats, *Noctilio leporinus*, and Indian short-nosed fruit bats, *Cynopterus sphinx*, apply bodily secretions to roost members (Brooke 1997; Rathinakumar et al. 2017). Brooke (1997) hypothesized that social bonds are reinforced through scent marking. Although this is a plausible inference, it remains to be tested. The studies mentioned have described the use of odors in conspecific interactions however many details remain unknown and further research is needed in this area.

Odorous signals can also play a role in courtship and mate attraction in bats. Several species have specialized odor-producing glands (Quay 1970; Scully et al. 2000). Additionally, there are several accounts of bat species in which males produce strong odors (Gannon et al. 1989; McWilliam 1990; Murray and Fleming 2008). However, in only a few species has the role of male odors been investigated. Odors can be used to mark territories or to attract females (Caspers and Voigt 2009; Muñoz-Romo et al. 2011). Furthermore, odors play a role in species recognition and in mate choice (Caspers et al. 2009; Santos et al. 2016). These studies invite further research into male odorous traits in other bat species. Compared with auditory communication, the role that olfactory signals play in female mate choice and male-male competition is very poorly understood.

Bats vary widely in their reproductive cycles and strategies. Although some species are monoestrus (Heideman et al. 1992; Martino et al. 1998; Duarte and Talamoni 2010) and some species are continuously polyestrus (Wimsatt and Trapido 1952; Godoy et al. 2014), the majority of leaf-nosed bats appear to be bimodally polyestrus (Krutzsch 2000; Durant et al. 2013). Despite living in similar environments, variation in the reproductive cycles and strategies within Phyllostomidae are likely due to differences in diet. For example, although the Jamaican fruit-eating bat, *Artibeus jamaicensis*, is bimodally polyestrus, the second embryo goes through a two-month period of delayed embryonic development (Fleming 1971). This delay results in the offspring being born when fruit is plentiful. In addition to variation in reproductive cycles, species also vary in reproductive strategies. For example, sperm storage, sperm competition, and delayed fertilization also play a role in bat reproduction (Hood and Smith 1989; Wilkinson and McCracken 2003; Orr and Zuk 2013). Some of these different reproductive strategies are correlated with variables such as group size (Hosken 1997). For example, species in which males roost with multiple females, males tend to have larger testes, and testes size is correlated with the risk of sperm competition (Wilkinson and McCracken 2003). Furthermore, sperm competition is thought to be facilitated by delayed fertilization (Orr and Zuk 2013).

Among bats, there is a lot of variation in mating systems (McCracken and Wilkinson 2000), ranging from monogamy (single male/single female, Heller et al. 1993) to leks (Bradbury 1977). Many species of Phyllostomidae have a harem structure (single male/multi-female), but these harems vary in their stability. For example, female greater spear-nosed bats, *Phyllostomus hastatus*, form stable long-term associations. Consequently reproductive males attach to the group and aggressively defend it against intruders. During his tenure, the resident male can sire 53 - 79% of the offspring (McCracken and Bradbury 1977; McCracken and Bradbury 1981). In contrast, groups of female *A. jamaicensis* have more labile compositions. In this species, males defend tree hollows, and females frequently move between roosts (Morrison 1979). In still other species, group composition can vary according to the season, as in the Honduran white bat, *Ectophylla alba*. After females give birth, the group divides into either all-male colonies or maternity colonies containing females, non-volant young, and a single adult male (Brooke 1990). The studies mentioned above illustrate how long-term observations of both males and females can shed light on the temporal dynamics of each sex and elucidate a more accurate representation of the mating system of a species.

Much is known about the predator-prey dynamics and foraging behavior of fringe-lipped bats, *Trachops cirrhosus.* These bats are eavesdropping predators that hunt frogs, particularly túngara frogs, *Physalaemus* (=*Engystomops*) *pustulosus*, by approaching the male frogs' mating calls (Tuttle and Ryan 1981). Female *P. pustulosus* prefer the complex mating call of male *P. pustulosus* (Ryan 1980; Ryan et al. 1982). Fringe-lipped bats are also better at localizing *P. pustulosus* if males produce the complex call (Page and Ryan 2008). In addition to targeting the frogs' sexual advertisement calls, *T. cirrhosus* can detect echolocating frogs by the ripples created by vocal sac inflation as male frogs call from puddles (Halfwerk et al. 2014). These predator-prey dynamics have made fringe-lipped bats a model system for studies investigating cognition with regard to predator perception. For example, *T. cirrhosus* can learn to associate novel cues of prey, suggesting flexibility in their foraging strategies (Page and Ryan 2005). Additionally, these novel cues can spread through the population via social learning (Page and Ryan 2006), and the propensity to approach novel food resources is likely due to the reliability of the cue (Jones et al. 2013). Although these studies have characterized the predator- prey dynamics and foraging behavior of this species, comparatively little is known about its natural history.

My work aims to fill this gap in our knowledge about reproductive behaviors and social system. In Chapter 2, "Novel odorous crust on the forearm of reproductive male fringe-lipped bats (*Trachops cirrhosus*)," I characterize a novel odorous substance found on the forearm of male *T. cirrhosus*, which I refer to as 'forearm crust.' I conducted a long-term study to document whether the forearm crust is present seasonally and whether it is present in all adult males. I also evaluated males to describe whether males with a forearm crust were in better body condition or whether they differed in morphological characteristics such as enlarged testes or enlarged chest glands. Additionally, because males lack specialized glands on their forearm, I conducted behavioral observations to determine how the forearm crust is created. I found that although males with forearm crust are captured throughout the year, there was an increase in the prevalence of males with a forearm crust from September to December. This coincides with the putative mating period; because males with forearm crust had a higher body condition index, this suggests that forearm crust could signal body condition to potential female mates or rivals. After conducting observations in natural roosts and in captivity, I described a novel stereotyped behavior in which males scratch the body dorsally and ventrally, insert a claw into their mouth, and then lick their forearms repeatedly. Males with a forearm crust licked their forearms significantly more than males without a forearm crust. The results from this chapter suggest that this novel odorous forearm crust is a male reproductive trait.

In Chapter 3, "The role of male forearm crust odor in fringe-lipped bats (*Trachops cirrhosus*)," I chemically describe and functionally evaluate the forearm crust. I analyzed the chemical profile of the forearm crust with gas chromatography and mass spectrometry and revealed that, contrary to my prediction, the forearm crust is not created with chest gland secretions or urine. I also investigated the responses of both males and females to the odors of reproductive males with a forearm crust and males without a forearm crust. Results from these preference tests demonstrate that females showed no preference for the odor of males with forearm crust, but reproductive males with a forearm crust avoided the scent of other reproductive males with forearm crust. These findings open the possibility that reproductive male bats could assess competitors via odor and therefore prevent costly confrontations or fighting. This result is the first of its kind for male bats.

Finally, in Chapter 4, “Social structure of the fringe-lipped bat (*Trachops cirrhosus*),” I combine 96 group captures of 172 individuals to characterize the social structure of fringe-lipped bats, and use these associations to determine whether *T. cirrhosus* have preferred roosting partners and whether these are influenced by sex or relatedness. I found that fringe-lipped bats have nonrandom associations and an emergent social structure. Using microsatellite markers, I determined that relatedness strongly predicted association rates, but this was true only for adult females. Understanding the complex social organization of females and group composition can inform how these can influence male behavior.

CHAPTER 2: NOVEL ODOROUS CRUST ON THE FOREARM OF REPRODUCTIVE MALE FRINGE-LIPPED BATS (*TRACHOPS CIRRHOSUS*)*

ABSTRACT

Olfactory cues are especially important for nocturnal mammals, such as bats, and can communicate an individual's condition and facilitate mate choice. Here, we introduce a novel odorous substance found on the forearm of male reproductive fringe-lipped bats (*Trachops cirrhosus*), which we term 'forearm crust'. We continuously captured bats over a three-year period to determine the prevalence and possible seasonal distribution of this forearm crust. We evaluated males to elucidate whether forearm crust was associated with specific morphological characteristics. Males with forearm crust were captured throughout the year, but we found an increase in captures of males with a forearm crust from September to December, prior to peak female pregnancy in March. All males with a forearm crust had enlarged chest glands and testes. Males with a forearm crust had significantly higher body condition indices than males without a forearm crust. We observed males in their natural roosts and in captivity, and describe a novel stereotyped behavior in which males scratch the body dorsally and ventrally, insert a claw into their mouth, and then lick their forearm repeatedly. Males with a forearm crust licked their forearm significantly more than males without a forearm crust. Together, these data suggest that this novel odorous forearm crust is a male reproductive trait. Further investigation is needed to understand its role in reproduction.

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INTRODUCTION

In small mammals, olfactory signals can play a role similar to visually conspicuous sexually selected traits in larger species, such as body size, weapons, or ornaments (Blaustein 1981). Odorous signals tend to be sexually dimorphic, with males producing a wider repertoire of odors and scent marking more frequently than females (Andersson 1994; Gosling and Roberts 2001). Odors can play important roles in sex recognition (Ferkin and Johnston 1995) and mate attraction (Johnston 1974), and are also effective indicators of an individual's health and condition (Penn and Potts 1998; Zala et al. 2004). For example, female mice (*Mus musculus domesticus*) discriminate between parasitized and non-parasitized males based on the odor of urine and associated odorous secretions (Kavaliers and Colwell 1995). Furthermore, olfactory cues are thought to be a crucial part of mammalian mate choice through the major histocompatibility complex, communicating genetic diversity among individuals (Santos et al. 2016). Given their nocturnal habits and social lifestyles, bats are ideal candidates to study the use and function of secondary sexual odorous traits (Dechmann and Safi 2005).

Many bats produce species-specific odors that result from a combination of glandular secretions, diet, and bacterial fermentation (Quay 1970; Schmidt 1985; Scully et al. 2000). For example, some Neotropical bat species perform complex behaviors to create odorous cocktails. Among the family of leaf-nosed bats (Phyllostomidae), male long-nosed bats (*Leptonycteris curasoae*) combine fluids from the mouth, penis, and anus to produce an odorous 'dorsal patch' during the mating season (Muñoz-Romo and Kunz 2009). The dorsal patch is thought to be a sexually selected trait as it is only present in reproductive adult males (Muñoz-Romo and Kunz 2009) and females prefer the odor of males with a dorsal patch than the odor of males without it (Muñoz-Romo et al. 2011). While male long-nosed bats apply their scent mixture to their fur, male greater sac-winged bats (*Saccopteryx bilineata*) transfer genital and chest gland secretions into specialized wing sacs near their forearms to create odorous cocktails, which they then waft towards females in their territory (Voigt and von Helversen 1999; Voigt et al. 2008).

Our study was prompted by the capture of several wild male fringe–lipped bats (*Trachops cirrhosus*) with a conspicuous substance characterized by a pungent odor on the forearm, which we termed 'forearm crust' (Fig. 2.1). Upon a review of the literature we found that this forearm crust had never been described. Since we only found the forearm crust in reproductive adult males, we postulated that it played a role in reproduction. To better understand the function of the forearm crust, we investigated the following questions: First, we asked if the forearm crust signals readiness for mating. If so, we expected the forearm crust to coincide with the female reproductive season and to be correlated with the presence of sexually dimorphic structures (e.g. enlarged chest glands, Fig. 2.2) and other traits involved in reproduction (e.g. enlarged testes). Second, we asked whether the crust reflects male condition. We hypothesized that males would exhibit differences in body condition in relation to crust presence or absence. We predicted that males with a higher body condition index would exhibit a forearm crust. Our third goal was to determine how this crust is produced. Preliminary analyses of four adult *T. cirrhosus* specimens (2 females and 2 males) using a scanning electron microscope showed no evidence of a forearm gland. Given this observation, we predicted that, similar to other bat species, males create this odorous cocktail via complex stereotypical behaviors. Finally, we studied museum specimens to determine the seasonal and geographic distribution of the crust.

Figure 2.1. Image of a reproductive male fringe-lipped bat (*Trachops cirrhosus*) with forearm crust circled.

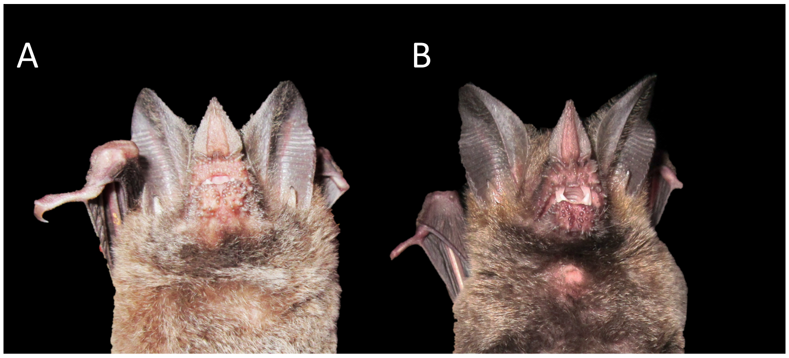


Figure 2.2 Ventral view of adult fringe-lipped bats (*Trachops cirrhosus*). A) Female *T. cirrhosus* with rudimentary chest gland (barely visible). B) Male *T. cirrhosus* with a forearm crust with chest gland enlarged.

METHODS

Study species

Fringe-lipped bats (*Trachops cirrhosus,* Phyllostomidae) range from southern Mexico to Brazil and Bolivia (Cramer et al. 2001) and are found in lowland forest (<500m) where they roost in hollow trees, culverts, buildings, and caves (Kalko et al. 1999). Fringe-lipped bats are considered omnivorous, eating insects, lizards, and frogs (Cramer et al. 2001). The mating system has only been described for ~8% of phyllostomid species (McCracken and Wilkinson 2000) and is not known for *T. cirrhosus.* There is no sexual dimorphism in size (Willig 1983). Females give birth to one offspring at a time and the gestation length is unknown (Cramer et al. 2001).

Study site

We conducted fieldwork in Soberanía National Park (9°07'N, 79°65'W), Panama from February 1, 2014 to May 5, 2017. This tropical lowland forest is characterized by seasonal rainfall (average 2612 mm annually), with a January to April dry season followed by a May to December rainy season (Windsor 1990). Additionally, we conducted behavioral observations in the field and in captivity at the Smithsonian Tropical Research Institute's (STRI) Gamboa field station.

Bat sampling

Bats were captured with 6-m-long 4-shelf 38-mm-mesh mist nets (Avinet, Dryden NY) set along trails and streams and at the exit of culverts and other structures where we found *T. cirrhosus* roosting. We recorded sex, age, and reproductive stage, as well as morphological metrics, such as body mass and forearm length. We identified juveniles by the presence of epiphyseal gaps in the phalanges (Brunet-Rossinni and Wilkinson 2009). Females were classified as non-reproductive or pregnant. Pregnancy was determined by gentle palpation of the abdomen (Racey 2009). Males were determined to be reproductively active by the scrotal position and enlarged size of the testes (Racey 2009). Length of forearm was measured to the nearest 0.1 mm using a dial caliper (Swiss Precision Instruments, Garden Grove, CA). Body mass was recorded using a 100-g scale (Pesola, Schindellegi, Switzerland). We also noted the condition of the chest gland (Fig. 2.2) and classified the gland as: not enlarged (barely visible, not secreting any substance; Fig. 2.2A), semi-enlarged (visible, and secreting substance), or enlarged (prominently visible and secreting substance; Fig. 2.2B). We marked each bat with a passive integrated transponder tag (Biomark, Boise, ID). We released bats at their capture site. All sampling protocols followed guidelines approved by the American Society of Mammalogists for capture, handling, and care of mammals (Sikes et al. 2016) and were conducted in accordance with the standards of the STRI Institutional Animal Care and Use Committee (IACUC# 2014-1001-2017) and the University of Chicago (IACUC# 72356). All research was licensed and approved by the government of Panama (SC/A-45-16; SE/A-89-14; SE/A-9-14; SE/A-86-14; SE/A 69-15; SE/AH-2-16).

Forearm crust and Male Condition

Because portions of the forearm crust flacked off during capture in the mist nets and subsequent handling, we could not accurately measure the total forearm crust area. We thus scored crust as a binary trait: present or absent. Observation of crust application behavior in captivity (see below) reinforced this classification system. Males with crusts frequently reapplied the crust to their forearms; the amount of the forearm covered at any one moment in time varied. We calculated body condition index by dividing body mass (g) by forearm length (mm). This index is commonly used to assess body condition in bats (Reynolds and Korine 2009), and has been validated as the best predictor for the amount of lipid present in big brown bats (*Eptesicus fuscus,* Pearce et al. 2008).

Other bat studies have found a negative relationship between ectoparasite load and bat body condition (the higher the ectoparasite load, the lower the body condition: e.g., Lourenço and Palmeirim 2007). Thus, as another indicator of condition, we quantified ectoparasites on adult males. We categorized an individual bat's ectoparasite load as low (0 – 5), medium (6 – 10), or high (10 +).

Behavioral observations

We recorded the behavior of males at their day roosts in the field with a video camera (DCR-SR45, Sony Corp., Tokyo, Japan) supplemented with infrared lights (CMVision-IR100, Houston, TX). Observations took place in November 2014, July 2015, and September 2015 using an *ad libitum* sampling protocol (Altmann 1974). Additionally, we recorded the behavior of bats in an outdoor flight cage (5 x 5 x 2.5m) under ambient temperature and humidity, illuminated by a 25 W red light bulb. We conducted observations from September to February between 2015 and 2017 using a video recorder (DCR-SR45, Sony Corp., Tokyo, Japan) supplemented with infrared lights (CMVision-IR200, Houston, TX). Each captive bat was observed once between 1700 and 1900 h using an *ad libitum* sampling protocol (Altmann 1974).

Museum Specimens

We examined dry specimens of *T. cirrhosus* at the American Museum of Natural History (New York, NY), the Field Museum of Natural History (Chicago, IL), and the National Museum of Natural History (Washington, DC). We investigated whether specimens had a forearm crust present, whether there was a seasonal distribution of this forearm crust, and whether there was variation in the geographic range of individuals exhibiting this forearm crust.

Statistical analyses

Data were checked for normality using the Shapiro-Wilk test. We tested for a correlation between the percent of males with a crust and the percent of females pregnant with using a Kendall's rank correlation (τ). We analyzed whether males with a forearm crust differed in body condition index from males without a crust using mixed-effect ANOVAs fit in the R packages lme4 and car package (Fox and Weisberg 2011; Bates et al. 2015). We first used a linear mixed model to predict body condition index with forearm crust presence, sampling year, and their interaction as fixed effects. Since we recaptured individuals during different years, we included individual bats as a random effect. Additionally, we analyzed differences in body condition index among the same individuals in different conditions using a paired t-test. We evaluated differences in ectoparasite load between males with a forearm crust and without a forearm crust using a chi-square test. Differences in forearm licking behavior between males with and without a forearm crust were analyzed using t–tests. We performed all statistical tests in R (R Core Team 2013) using $\alpha = 0.05$.

RESULTS

Bat sampling

We captured a total of 473 bats (2014, *n* = 91; 2015, *n* = 126; 2016, *n* = 194; 2017, *n* = 62). After accounting for recaptures (*n* = 237), 236 individual bats (males, *n* = 142; females, *n* = 94) were examined. We analyzed juveniles (*n* = 57) separately. Over 80% of juveniles were captured in June (*n* = 15), July (*n* = 18), and August (*n* = 14). Pregnant females (*n* = 35) were captured from December to May with the majority (74%) captured in March (*n* = 14) and April (*n* = 12, Fig. 2.3). Additionally, we captured four females with pups attached to their nipples in May 2014 (*n* = 1), June 2016 (*n* = 2), May 2017 (*n* = 1).

Forearm crust

We captured a total of 211 adult male *T. cirrhosus* (2014, *n* = 47; 2015, *n* = 47; 2016, *n* = 85; 2017, *n* = 32). After considering recaptures and missing data on forearm crust presence or absence (*n* = 107) we included the first capture of each individual male (*n* = 104) and 46% of adult males captured had a forearm crust present. All males with a forearm crust present had enlarged testes. Although some males with an absent forearm crust had enlarged testes, the majority of males without a forearm crust (77%) did not have enlarged testes. None of the juveniles had enlarged testes or a forearm crust. None of the females examined had a forearm crust present. Although forearm crusts were found throughout the year, there was an increase in the percent of bats captured with a crust from September to December (Fig. 2.3). The percent of males with a forearm crust was not correlated with the percent of pregnant females ($\tau = 0.05$, $P = 0.82$).

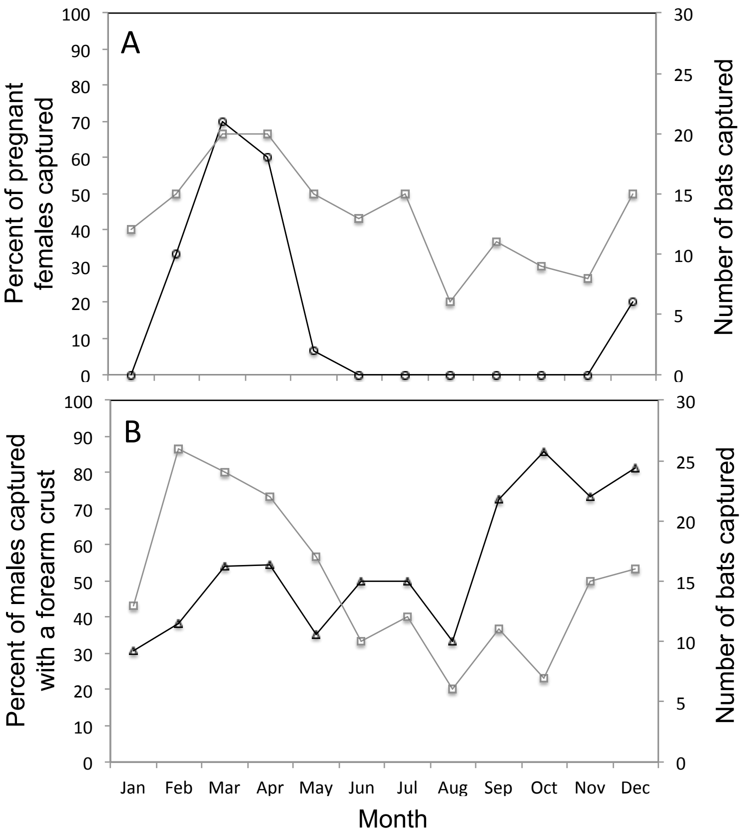


Figure 2.3. Reproductive patterns of fringe-lipped bats (*Trachops cirrhosus*). A) Total number of adult males captured per month (gray line and open square) and percent of males captured with a forearm crust (black line and open triangle). B) Total number of adult females captured per month (gray line and open square) and percent of pregnant females captured per month (black line and open circle).

Chest Gland

We classified chest gland status for 160 individuals. Juveniles had either chest glands that were not enlarged (*n* = 25) or semi-enlarged (*n* = 6). Likewise, adult females that were not reproductive had chest glands that were not enlarged (*n* = 19) or semi-enlarged (*n* = 10). All adult females in reproductive condition had chest glands that were not enlarged (*n* = 24) and barely visible (Fig. 2.2A). The chest glands of adult reproductive females did not produce any secretions. Non-reproductive adult males had chest glands that were enlarged (*n* = 10), semi-enlarged (*n* = 6), and not enlarged glands (*n* = 3). Adult males with enlarged testes had enlarged chest glands, irrespective of forearm crust presence (*n* = 48) or absence (*n* = 9).

Male Condition

After accounting for missing measurements (presence/absence of forearm, mass, or forearm length), 104 individual adult male bats were included in our analyses. Bats with a forearm crust had higher body condition indices than bats without a forearm crust ($F = 34.12$, *d.f.* = 1, $P < 0.0001$, Fig. 2.4). We recaptured eight bats with a forearm crust absent in the first capture and then present in the second capture. In seven of these bats (87.5%), body condition index increased, although not significantly ($t = -1.8807$, *d.f.* = 7, $P = 0.10$).

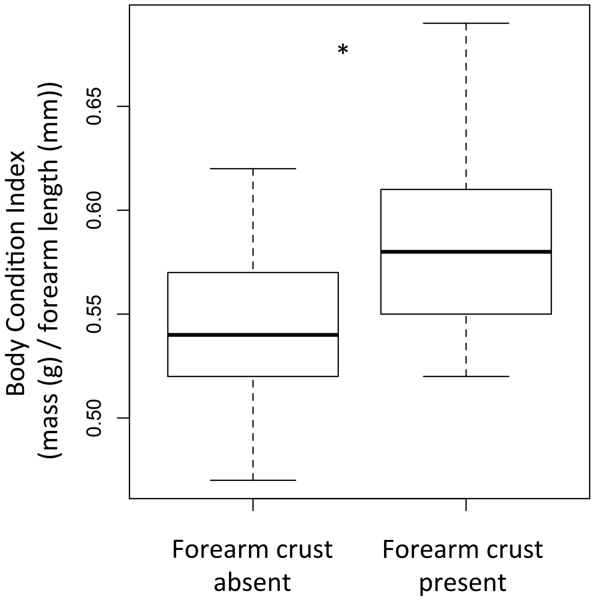


Figure 2.4. Body condition index (body mass (g) / forearm length (mm)) of male fringe-lipped bats (*Trachops cirrhosus*) with and without a forearm crust. Males with a forearm crust present had significantly higher body indices than males with an absent forearm crust.

We classified ectoparasite load for 78 adult males bats (*n* = 46 with forearm crust, *n* = 32 without forearm crust). The main ectoparasites found were bat flies (Diptera), however four bats also had ticks. Although most males with a forearm crust had few ectoparasites, the difference in ectoparasite load between males with and without a forearm crust was not significant ($\chi^2 = 2.65$, *d.f.* = 2, *P* = 0.27, Fig. 2.5).

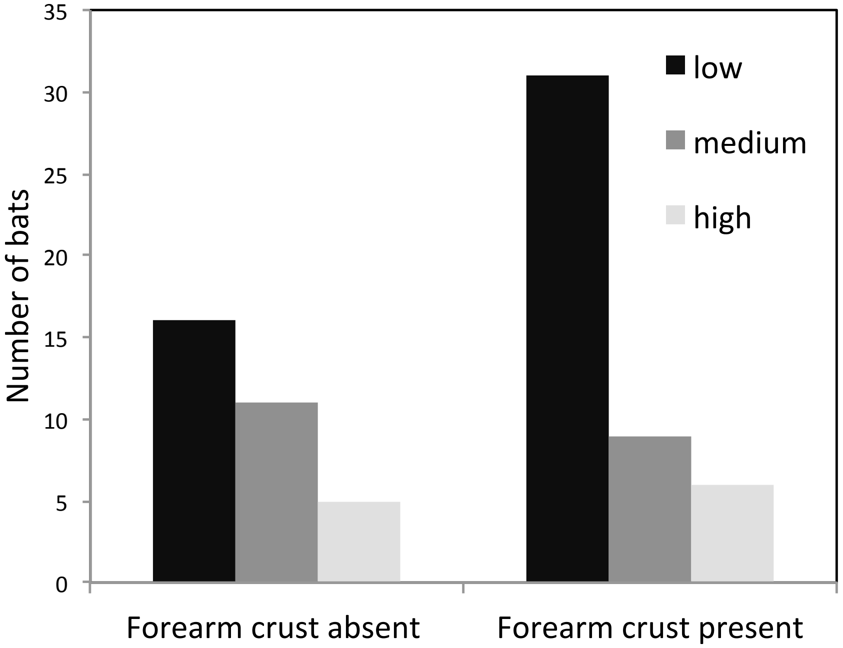


Figure 2.5. Estimated ectoparasite load in adult male fringe-lipped bats (*Trachops cirrhosus*) with and without a forearm crust. Ectoparasite load classified as low (0 – 5), medium (6 – 10), or high (10 +).

Behavioral observations

We recorded three males with a forearm crust in their natural culvert roost for a total of 3.6 hours ($\bar{X}$ = 1.22h ± 0.8h *SD*). Grooming, which consisted of scratching the head and body with one hind claw, licking the body and the wing membranes, then inserting the claw used to groom into the mouth accounted for between 2 – 32% of total observation time. During grooming, we noted a stereotyped behavioral sequence focused on the forearm, which we termed 'forearm licking behavior' (Fig. 2.6). Male bats scratched their body (dorsally and ventrally) with one of the hind claws, inserted this hind claw into the mouth, licked and nibbled the claw, and then licked one of the forearms repeatedly. During this sequence, males also appeared to occasionally scratch the chest gland with the hind claw. While licking the forearm, males continued to scratch the body and insert the claw into their mouth. They also flicked the tongue repeatedly. Each stereotypical forearm licking bout ranged from 21 to 62 seconds ($\bar{X}$ = 46s ± 21.9s *SD*) with 7 to 17 ($\bar{X}$ = 12 ± 5 *SD*) discrete forearm licks per bout. Forearm licking bouts accounted for ~1% of the time each individual was observed.

Forearm licking sequences accounted for 0.59% to 3.23% of total time for males with a forearm crust and for 0.01% to 0.72% for males without a forearm crust. In both males and females, the remainder of the observation period was devoted to other behaviors (urinating, defecating, yawning, and flying), which accounted for less than 2% for all individuals of the observation period.

Several of our observations of males with a forearm crust, in natural roosts (n = 2) and in captivity (n = 4), were with conspecifics. In these, we never observed the male with a forearm crust mark the territory or roost members. Furthermore, males with a crust did not display towards females and we found no evidence that other males prevented each other from forearm licking.

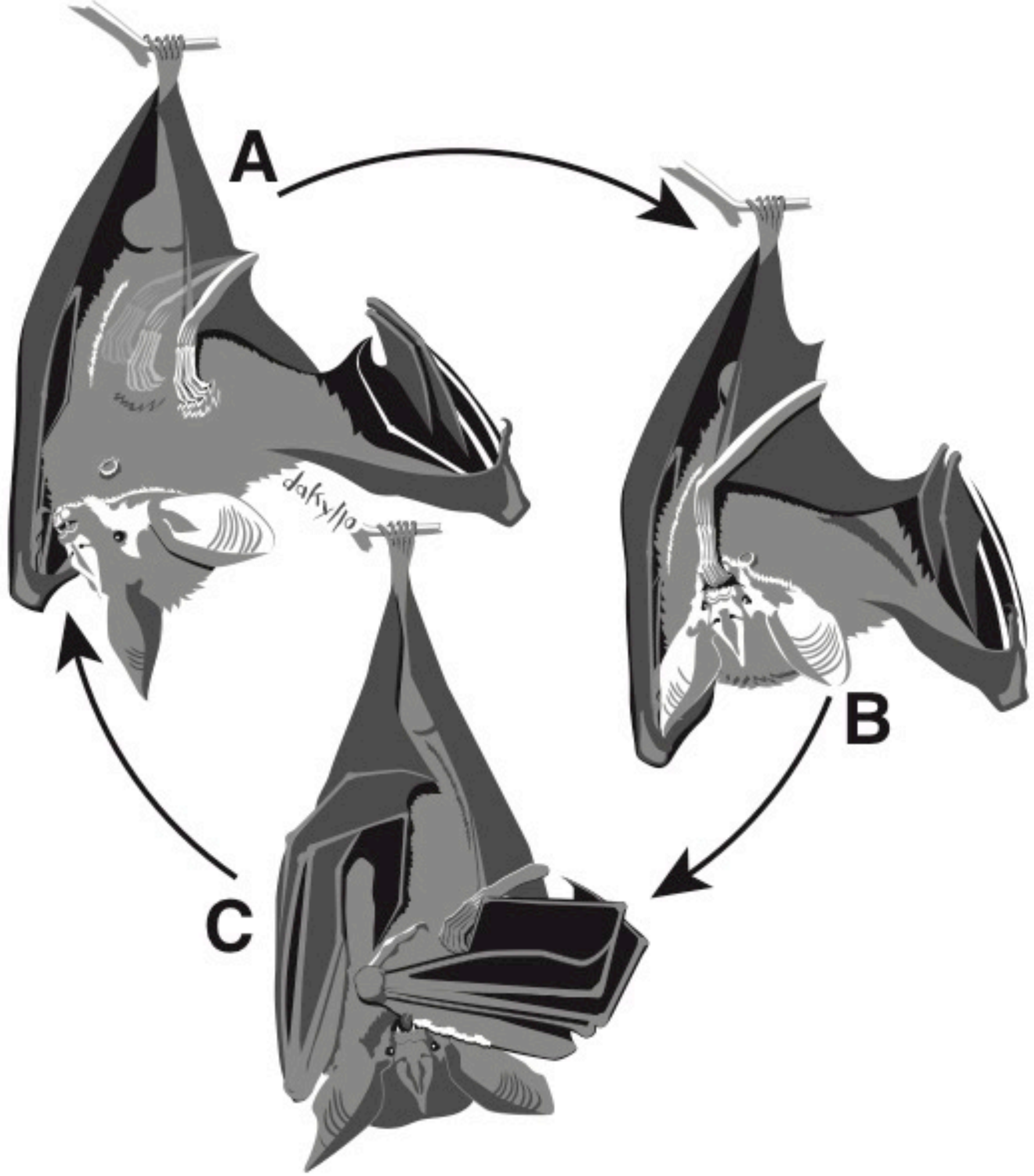


Figure 2.6. Illustration of male fringe-lipped bats (*Trachops cirrhosus*) performing the stereotyped forearm licking behavior. A) Males scratch body (dorsally and ventrally). B) Males insert one claw into mouth. C) Males repeatedly lick forearm. Arrow indicates the cycle which male *T. cirrhosus* repeat several times per bout. Illustration by Damond Kyllo.

Museum Specimens

We examined 70 *T. cirrhosus* dry specimens (25 males and 45 females, specimen age not specified) at the American Museum of Natural History, 60 *T. cirrhosus* dry specimens (22 adult males, 1 juvenile male, and 37 adult females) at the Field Museum of Natural History, and 363 dry specimens (171 adult males, 17 juvenile males, 161 adult females, and 14 juvenile females) at the National Museum of Natural History. In total, we found twelve specimens collected from 1948-1972 with a forearm crust present. These were captured in Panama (*n* = 9), Mexico (*n* = 2), and Guatemala (*n* = 1) in March (*n* = 5), April (*n* = 1), May (*n* = 1), September (*n* = 1), October (*n* = 3), and December (*n* = 1). We did not find forearm crust present in female specimens. Our findings are likely conservative as the forearm crust likely flaked off or was removed during specimen preparation. Additionally, field notes from the 'BCI Bat Project', an extensive mist netting operation spanning nearly a decade on Barro Colorado Island, Panama (Handley et al. 1991; Kalko et al. 1996), document three adult male *T. cirrhosus* that had "sticky substance on both forearms," had a "strong odor," and were "strong smelling" captured January 1980, October 1981, and September 1984, respectively (C.O. Handley, Jr. field notes).

DISCUSSION

Overall, our captures suggest a seasonal pattern to reproduction in *T. cirrhosus*. We found a peak in juvenile captures at the beginning of the wet season (June, July, and August). Our results also show that females were mostly pregnant during the dry season (April and May). Since lactation is the most costly period for females and weaning is the most critical period for pups (Kurta et al. 1989), it appears that pregnancy is timed to coincide with the beginning of the wet season, when prey species, especially frogs, are abundant. Our results are consistent with findings (Durant et al. 2013) that document a single peak in pregnancy during the dry season for gleaning animalivores, such as *T. cirrhosus*.

Although the gestation length of *T. cirrhosus* is unknown, by comparing our observations to reproductive patterns of other leaf-nosed bats (Altringham 1996; Rasweiler and Badwaik 1997), we can infer that gestation is approximately 4 to 6 months in length. If males were creating a forearm crust solely to court females or to compete with other males for access to females, we would expect an increase in captures of males with a forearm crust from October to November. Although males with a forearm crust were found throughout the year, we did find an increase in the percent of males captured with a crust from September to December. Our finding that mature males can display this odorous forearm crust throughout the year (both in our captures and museum observations) is similar to observations in other bats. Male buffy flower bats (*Erophylla sezekorni*) produce a garlic-scented supraorbital secretion and then perform aerial displays to females. This secretion is present in all mature males, regardless of whether they are displaying to females (Murray and Fleming 2008). Similar results have been found in *S. bilineata* where males court females year-round, irrespective of female reproductive state (Knörnschild et al. 2016).

The fact that the forearm crust was only present in adult males suggests that it is involved in reproduction. Additionally, the finding that all males with a forearm crust also had enlarged testes indicates a strong association with sexual maturity and possibly to signal readiness for mating. It is unclear, however, why some males with enlarged testes did not have a forearm crust. Perhaps these males are younger. The condition and size of the epididymides in males with a forearm crust and males without a forearm crust needs to be examined to determine whether both are storing active sperm. Our results are similar to those found in *L. curasoae* (Muñoz-

Romo and Kunz 2009), in which some males have enlarged testes but do not display an odorous dorsal patch, but all males that displayed a dorsal patch had enlarged testes.

Our observation that all males with a forearm crust had enlarged chest glands (Fig. 2.2B) further suggests the forearm crust is involved in reproduction. For the most part the glands that have been described in bats are sexually dimorphic, with adult males having enlarged glands (Quay 1970; Schmidt 1985; Scully et al. 2000). In contrast to other species (Valdivieso and Tamsitt 1965), the chest gland in *T. cirrhosus* is only sexually dimorphic after reproductive maturity. Active chest glands in both female and male *T. cirrhosus* secrete a white oily odorless exudate. However, after maturation, all reproductive females had rudimentary glands that were barely visible and did not produce any secretions. In contrast to other bats, the secretion of the chest gland in *T. cirrhosus* is not odorous (Valdivieso and Tamsitt 1965). The differences found in gland size invite further study into the role that hormones play in determining the size and production of secretions.

In this study we demonstrate that males with a forearm crust had a significantly higher body condition index than males without a forearm crust (Fig. 2.4). These results suggest that the forearm crust could communicate the condition of an individual to conspecifics. Chemical signals are particularly suited to mate choice because they include by-products of everyday life and are thereby honest indicators of an individuals' condition (Voigt 2013). For example, male and female meadow voles (*Microtus pennsylvanicus*) prefer the scents of conspecifics on a higher protein diet (Ferkin et al. 1997). Furthermore, in mammals, odors can honestly convey an individual's condition since the production of chemical signals is linked to hormone levels; once an individual's immunity is suppressed androgens are costly to produce (Zahavi 1975; Wingfield et al. 1990; Wyatt 2014). The fact that males with a forearm crust had significantly higher body condition indices suggests that only males in good condition are able to produce a forearm crust. Finally, odorous signals can play an important role in mate choice because chemical profiles can communicate information about individual heterozygosity and genetic distance (Charpentier et al. 2008; Santos et al. 2016). Further investigation is warranted to elucidate whether this forearm crust is involved in female mate choice.

The forearm licking crust application behavior described here is a novel behavior that, to our knowledge, is exclusively found in *T. cirrhosus*. Preliminary analyses found no glands on the forearm of male fringe-lipped bats, hence, the odorous forearm crust is likely created through a combination of substances from elsewhere. Although several species exhibit self-anointing behaviors through which they spread substances over their body (Brockie 1976; Alfaro et al. 2012), the forearm licking behavior was only observed in males and is therefore sexually dimorphic. Although there are cases in which male mammals rub secretions from glands onto their body to attract females (Gosling 1987), bats appear to be unique in creating odorous cocktails by combining several bodily secretions. Even though we observed bats urinating in many of our observations, this never occurred immediately prior to the crust application behavior. Additionally, we did not observe a urine uptake behavior as described in *S. bilineata* (Voigt 2002). This suggests that urine might not be one of the compounds in the forearm crust. Although male bats did scratch the chest gland area, it was difficult to determine whether they were explicitly collecting secretions from the chest gland to create the forearm crust. Our observations demonstrate that males did flick their tongues repeatedly, which suggests that males are using saliva to create this odorous forearm crust. Several studies indicate that saliva can act as a chemical signal in mammals (Block et al. 1981; Gray et al. 1984). Further studies describing the chemical profile of the forearm crust are needed to determine the exact composition of this odorous forearm crust.

The forearm licking behavior we described is similar to the behavior found in *L. curasoae* where males use their claws to collect bodily fluids and transfer them to their dorsal side (Muñoz-Romo and Kunz 2009) and to the perfume blending behavior in *S. bilineata* (Voigt and von Helversen 1999). However, unlike the restricted afternoon perfume blending *S. bilineata* (Voigt 2013), our roost observations demonstrated that forearm licking occurs throughout the day. Furthermore, each forearm licking bout was shorter in *T. cirrhosus*, lasting only 37 to 93 seconds, in comparison to *S. bilineata*, which perfume blend for an average of seven minutes (Voigt 2002).

Similar to sac-winged bats, the position of this forearm crust odorous patch on the forearm suggests that males might be wafting this odor towards individuals during flight. However, in our observations at the roost and in groups in captivity, we never observed males performing courtship displays to females or scent marking roostmates. It is possible that males are courting females outside of the roost. Females would be able to locate males faster while they are foraging if the forearm crust odors are being spread while in flight. Brooke and Decker (1996) found that male greater fishing bats (*Noctilio leporinus*) were broadcasting their odor during flight. Chemical cues are ideal signals for Neotropical bats because odors have extended ranges than visual signals and can also travel farther through cluttered environments, like tropical forests. Furthermore, it has been suggested that smaller organisms cannot produce the lower frequency sound signals that travel further than higher frequency signal (Dusenbery 1992).

Olfaction is a dominant sense for most mammals and in turn heavily influences their behavior (Wyatt 2014). Odors play an especially important role in the lives of bats. For example, many fruit eating bats (e.g., *Dermanura watsoni*, *Vampyressa pusilla*, *Carollia perspicillata*, *C.*

castanea) use olfactory cues when foraging (Thies et al. 1998; Korine and Kalko 2005). Some bat species also use species-specific scents to mark their territories or colony members (Brooke 1997). Furthermore, bats can discriminate among roost mates and individuals from a different colony based on odor (De Fanis and Jones 1995b; Safi and Kerth 2003). The crucial role that odors play in leaf-nosed bats (Phyllostomidae) was recently highlighted (Yohe et al. 2017). Whereas most families of bats have lost their vomeronasal organ, phyllostomids are a major exception where the vomeronasal organ has persisted. These results suggest that vomeronasal olfaction plays an important role in the lives of phyllostomids.

In conclusion, this study provides new information on an odorous substance found on the forearm of adult male *T. cirrhosus*. We propose that this odor is involved in signaling readiness for mating and communicating male condition. Additionally, we have described a novel behavior performed by males of this species to create this odorous forearm crust. Further studies determining whether the forearm crust is involved in female choice or male-male competition are warranted.

CHAPTER 3: THE ROLE OF MALE FOREARM CRUST ODOR IN FRINGE-LIPPED BATS (*TRACHOPS CIRRHOSUS*)

ABSTRACT

Chemical signals can play important roles in communication, and this is especially true for social mammals such as bats. Male fringe-lipped bats (*Trachops cirrhosus*) produce an odorous substance on their forearm, called a forearm crust. Only adult males with enlarged testes produce forearm crust. This is thus a sexually dimorphic odor, which suggests that it is a sexually selected trait. In this study, we sought to identify the source of the forearm crust and to test female and male preference for this trait. Males lack a specific gland on their forearm to produce the forearm crust. Based on gas chromatography and mass spectrometry of the forearm crust, we tentatively identified several compounds that were exclusive to forearm crust: 2-aminoacetophenone, 4-methylquinazoline, 5,6-dihydro-6-propyl-2H-pyran-2-one, and 2-methylquinoline. Contrary to our prediction, the chemical composition of the forearm crust was not mainly composed of chest gland secretions or urine. We also conducted a y-maze odor preference experiment to test whether adult females and reproductive males preferred the odor of a male with forearm crust to the odor of a male without forearm crust. We found that females did not approach the scent of a male with forearm crust more frequently than the scent of male without forearm crust. Males, however, avoided the odor of other males with forearm crust. Overall our results suggest that in this Neotropical bat species, reproductive males could use odorous signals in the forearm crust to avoid other reproductive males and hence avoid costly competition for mates or aggression.

INTRODUCTION

Odors often modulate social behaviors in mammals. Mammals can use chemicals to mark and defend territories or resources (Gosling and Roberts 2001; Miller et al. 2003), thereby also announcing their competitive ability (Stockley et al. 2013). Through odorous cues, mammals can discriminate between familiar and unfamiliar conspecifics (Kent and Tang-Martínez 2014), allowing individual and kin recognition (Mateo 2003; Thom and Hurst 2004; Brennan and Kendrick 2006). For example, fur seal (*Arctocephalus gazella*) odors can contain information about relatedness, genetic quality, and mother offspring similarity (Stoffel et al. 2015). Furthermore, odors can play a pivotal role in mammalian mate choice (Johansson and Jones 2007).

Odors can convey information about an individual's condition. For example, male and female meadow voles (*Microtus pennsylvanicus*) prefer odors from donors on a higher protein diet (Ferkin et al. 1997). Odor preference tests have also demonstrated that scent can act to communicate information regarding parasitic infections as captive rodents avoid the odors of individuals infected with parasites and bacteria (Kavaliers and Colwell 1995; Zala et al. 2004). Free-living banded mongooses (*Mungos mungo*) scent mark less in response to the odors of opposite-sex conspecifics with high parasite loads (Mitchell et al. 2017). Chemical signals can also inform on the genetic relatedness between individuals (Charpentier et al. 2008; Charpentier et al. 2010). Odorous cues permit females to discriminate other male qualities such as competitive ability (Fisher et al. 2003). Finally, odors can facilitate reproduction by activating sexual motivation and attraction (Swaisgood et al. 2000; Roberts et al. 2010).

The nocturnal and social lifestyle of bats suggests that they could rely on olfactory cues to communicate with conspecifics (Dechmann and Safi 2005). Indeed, in bats, odors have been shown to play a role in species and roost mate recognition (Caspers et al. 2009; Englert and Greene 2009), offer information of individual identity and colony membership (Safi and Kerth 2003), and allow lactating females to discriminate offspring (Gustin and McCracken 1987). Furthermore, the presence of specialized glands (Quay 1970; Schmidt 1985; Scully et al. 2000) and osmetrichia (hairs specialized for scent dispersal) indicate the importance of chemical compounds in bat communication (Hickey and Fenton 1987). In many cases, these are sexually dimorphic with only males possessing specialized glands and hairs, suggesting a role in reproduction (Quay 1970; Hickey and Fenton 1987). Moreover, that many of these structures are seasonal indicates a potential role in mate attraction, male-male competition, or both (Bloss 1999).

Males in several species of bats secrete odors from specialized glands (e.g., *Pteropus giganteus*, *P. pumilus, P. hypomelanus, P. vampyrus*: Wood et al. 2005; *Erophylla sezekorni*: Murray and Fleming 2008), while other species of bats create odorous cocktails through complex behaviors. Greater sac-winged bats (*Saccopteryx bilineata*) perform a ‘perfume blending’ behavior in which reproductive males fill their wing sacs with various bodily fluids and gland secretions (Voigt and von Helversen 1999). This odorous cocktail plays an important role during courtship displays and mate choice (Voigt and von Helversen 1999). Within *S. bilineata*, there are seasonal differences in the relative amount of chemical compounds, suggesting that females could assess the reproductive status of males via odors (Caspers et al. 2008). Additionally, the chemical profiles of reproductive male *S. bilineata* vary between individuals in the relative amount of chemical compounds, implying a potential role for individual identification via wing sac odors (Caspers et al. 2008). Male long-nosed bats (*Leptonycteris curasoae*) do not have specialized wing sacs but similarly create an odorous bouquet through stereotypical behaviors by combining bodily secretions and smearing them to create a 'dorsal patch' (Muñoz-Romo and Kunz 2009). This 'dorsal patch' is likely a male reproductive trait as female *L. curasoae* prefer the odor of a male with a dorsal patch to the odor of a male without a dorsal patch (Muñoz-Romo et al. 2011).

Male fringe-lipped bats (*Trachops cirrhosus*), the focus of our study, produce an odorous substance on their forearm termed 'forearm crust' (Flores and Page 2017). This recently discovered odorous ornament is unique to this species as it is unknown in other bat species. *Trachops cirrhosus* males produce this forearm crust through a series of stereotypical behaviors: while grooming, males scratch their body with one hind claw, insert the same hind claw into the mouth, and then repeatedly lick the forearm (Flores and Page 2017). The forearm crust is produced throughout the year, but there is an increase in the numbers of males captured with forearm crust from September to December, which coincides with the putative mating period of *T. cirrhosus* (Flores and Page 2017). As a further indication that the forearm crust could be involved in reproduction, all males with forearm crust had enlarged testes and enlarged chest glands. Additionally, males with forearm crust had significantly higher body condition indices (body mass (g) / forearm length (mm)) than males without, suggesting that forearm crust could communicate an individual's condition (Flores and Page 2017).

Despite extensive roost observations, there was no evidence of males with forearm crust displaying to females or interacting aggressively toward other males (Flores and Page 2017). Thus, observations did not shed light on the possible function of the forearm crust in social interactions in *T. cirrhosus*. The aim of the present study was to combine chemical analyses and behavioral experiments to elucidate potential roles of this novel forearm crust. Based on previous observations of males' stereotyped crust application behavior (Flores and Page 2017), we predicted that males combine secretions from a prominent mid-ventral chest gland with saliva to produce the forearm crust. We then compared the compounds found in our samples with those thought to elicit behaviors in other animals to provide insight into the potential function of the forearm crust (e.g. Soso and Koziel 2017).

Our second aim was to investigate whether adult females and reproductive males with forearm crust prefer the odor of a male with forearm crust to the odor of a male without this ornament. Although there is evidence for sexually dimorphic chemosignals in bats (Wood et al. 2005; Murray and Fleming 2008), bioassays to test the function of olfactory signals have rarely been conducted (cf. Muñoz-Romo et al. 2011). We predicted that females would prefer the odor of a male with forearm crust to the odor of a male without forearm crust. With respect to males, the response of reproductive males towards odorous sexually dimorphic signals has never been tested in bats, and we considered two alternate outcomes. One possibility was that the forearm crust odor could act as a chemical signal towards competitors (Gosling and Roberts 2001), and, hence, would deter other reproductive males. Alternatively, males would not show a preference because the forearm crust is an odorous cue towards receptive females.

Finally, we tested whether difference in body condition index between the test male and the odor donor were correlated with the amount of time the test male spent in the no crust preference area. We predicted that if reproductive males were avoiding each other based on size, then test males would spend more time in the preference area of a male without a forearm crust if the difference between body condition index between the test male and the odor donor with a forearm crust were larger.

METHODS

Study Species

Fringe-lipped bats (Phyllostomidae) roost in hollow trees, culverts, buildings, and caves (Kalko et al. 1999). They are omnivorous, eating frogs, insects, and lizards (Cramer et al. 2001). The mating system of most (92%) leaf-nosed bats (Phyllostomidae) is not known (McCracken and Wilkinson 2000) and this applies to *T. cirrhosus*. While it has been determined that female fringe-lipped bats give birth to one offspring at a time, the gestation length is unknown (Cramer et al. 2001).

Field methods

We conducted fieldwork in Soberanía National Park in Panamá (Colón province, 9.0743° N, 79.6598° W) from December 2015 to February 2017. This tropical lowland forest is marked by seasonal rainfall (average 2600 mm annually), with a dry season from mid-December to mid-April (Leigh and Wright 1990).

We captured bats using mist nets (Avinet, Dryden, NY) set over streams, over trails, and at the exits of known roosts. We only included adults in our study, which we identified by the absence of epiphyseal gaps in the phalanges (Brunet-Rossinni and Wilkinson 2009). We classified females as pregnant by the presence of enlarged nipples and by gentle palpitation of the abdomen (Racey 2009). We determined if males were reproductively active, by the scrotal position and enlarged size of the testes (Racey 2009). To calculate the body condition index of males, we measured the length of forearm to the nearest 0.1 mm using a dial caliper (Swiss Precision Instruments, Garden Grove, California), and we recorded the body mass using a 100-g scale (Pesola, Schindellegi, Switzerland).

We marked each bat with a passive integrated transponder (Biomark, Boise, ID) to allow for identification of recaptured bats. We released all bats at the site of capture after the preference test or scent collection.

All sampling protocols followed guidelines approved by the American Society of Mammalogists for capture, handling, and care of mammals (Sikes et al. 2016) and were approved by the Smithsonian Tropical Research Institute (STRI) Institutional Animal Care and Use Committee (IACUC# 2014-1001-2017-2-A4) and the University of Chicago (IACUC# 72356). All research was licensed and approved by the government of Panama (SC/A-45-16; SE/A 69-15; SE/AH-2-16).

Gas Chromatography and Mass Spectrometry

We collected samples from the forearms of adult male bats for chemical analyses in November and December 2016. Additionally, we collected samples from the enlarged chest gland of males with a forearm crust in December 2016 and a urine sample from a male with forearm crust in November 2016. Prior to collection, we sterilized cotton swabs and 2ml glass vials (#5182-0715, Agilent, Santa Clara, CA) in dichloromethane (99.99%, Fisher, Fair Lawn, NJ) and dried them at ambient temperature under a fume hood. We collected samples by swabbing forearms of males with sterilized cotton swabs 10 times. For each male, we also sampled the dorsal area surrounding the forearm of the bat to control for compounds that could be present in the overall body. Sterile gloves were worn to prevent contamination. After collection, we transferred samples into vials that were capped with silicone septa (#5182-0717, Agilent, Santa Clara, CA) and added 100 µl of dichloromethane (99.99%, Fisher, Fair Lawn, NJ) as a preservative. All samples were stored at -20^{o} C until chemical analysis.

We analyzed samples with a Gas Chromatography-Mass Spectrometer (Agilent 7890B GC, 5977A MS) equipped with a 30-m column, 250 µm wide. Prior to analyses, we added 1.5ml dichloromethane (99.99%, Fisher, Fair Lawn, NJ) to soak and cover cotton swabs. We sonicated vials for 10 min at room temperature to remove compounds from cotton. With sterilized forceps, we removed cotton swabs and condensed the extract to a 50µl concentration by evaporation under a stream of nitrogen. Extracts were transferred to deactivated glass vial inserts (#5181-8872, Agilent, Santa Clara, CA). We added 104.85ng of 2-tetradecyl acetate (custom made by the Schulz laboratory at the Institute for Organic Chemistry, Technical University Braunschweig) solution as an internal standard.

We injected 2µl of the sample into the GC and collected data under the following conditions: splitless injection, helium as carrier gas, 60° C inlet temperature, 3-min initial time, 5° C min−1 rate, 280° C final temperature, 52-min run time. Blanks of the sampling hardware (vials and cotton swabs) and procedure were run under the same conditions as bat samples. Compounds found in similar quantities in both blanks and samples are not reported. Peaks were matched by the fragmentation patterns of chromatograms using Agilent MassHunter Software (Qualitative Analysis B.07.00, 2015) and by performing mass spectral data base comparisons using the National Institute of Standards and Technology library (2008). We calculated the abundance of the relevant compounds by dividing the peak area of each compounds by the peak area of the internal standard and multiplying this by the concentration of the internal standard.

Odor preference test

The forearm crust of male *T. cirrhosus* exudes a musky scent. This scent permeates the cloth bags used to transport the bats. To collect odor samples we placed individual adult male *T.*

cirrhosus in cloth bags for 1 hour and used these cloth bags as our odor stimuli (similar methodology in Bonadonna and Nevitt 2004). We sterilized bags in bleach and stored them individually in ziplock® bags prior to odor collection. Bags were made for this experiment and had never previously held other bats. Once we used a bag to collect an odor sample the bag was stored again in a ziplock® bag and kept in a -20ºC freezer until used in an experiment. We collected samples from males with a crust (n = 11) and males without a crust (n = 6) from December 2015 to December 2016. None of the bats included in our preference test were tested with the same set of odor samples. Each odor sample was used once per experiment. Given the small population we did have multiple samples from the same male, however these were collected during different capture events.

We tested adult females (n = 13) from October to December 2016 and adult males with forearm crust (n = 12) from October 2016 to February 2017 in a two-choice preference test to examine whether males or females preferred the odor of a male with forearm crust or the odor of a male without forearm crust. We excluded two pregnant and two sub-adult females because we wanted to only include females that were in comparable reproductive stages. All females included in our final analyses were recaptures identified by their transponder number, and hence, we could corroborate they were adults and of reproductive age. Of the 12 reproductive adult males tested, we excluded four males because they failed to investigate either preference zone.

The y-maze consisted of 3 symmetrical arms (cm: L:63; W:12; H:12; angled at 120^0) constructed from plastic garden fence (mesh size 2.54cm) lined with mosquito gauze enabling the bats to crawl and move (Fig. 3.1). The y-maze arena was placed inside a larger outdoor flight cage (5 x 5 x 2.5m) at the STRI Gamboa field station. Thus, we conducted the experiment at ambient levels of temperature and humidity. The 'starting' arm had an opening to another room where we could place bats into the y-maze to avoid leaving our odor traces in the larger flight cage or y-maze (Fig. 3.1). In each odor-choice arm, a small fan (O2Cool Model No. FD05004, Chicago, IL) provided a low-noise, controlled airflow. We cleaned the maze (95% ethanol) between trials to remove residue. We chose the side on which each sample was placed arbitrarily. While wearing sterile gloves, we placed samples at the end of the y-maze arm where bats could come into contact with them.

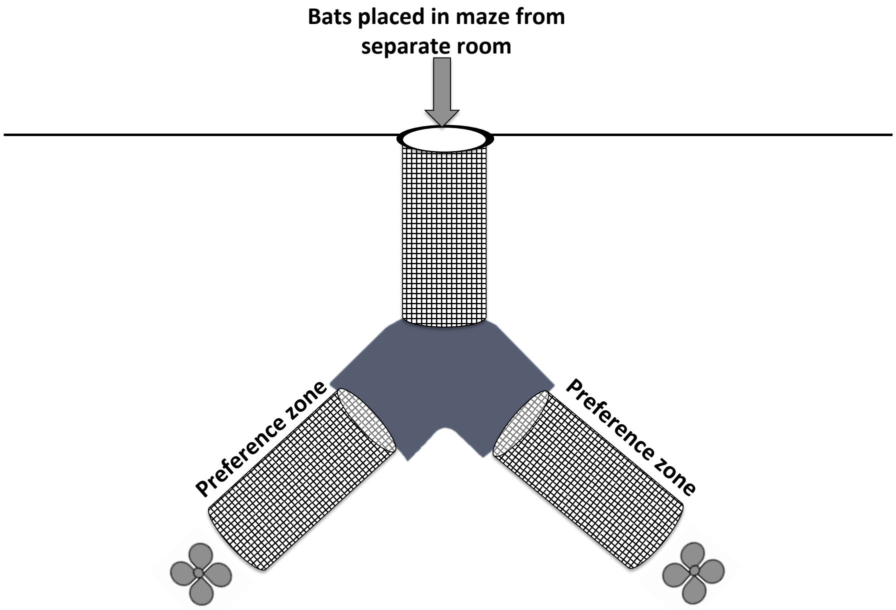


Figure 3.1: Odor preference test y-maze diagram. Each y-maze arm was lined with mosquito gauze (hatched pattern). The stimulus odors were randomly placed at the end of each preference zone. A fan directed odors towards the y-maze opening. Bats were introduced to the arena from a separate room.

We conducted the preference test at night between 18:30h and 1:30h. Each experiment lasted 15 min. We recorded all movements of the bat during this time using an infrared video camera (Sony DCR-TRV 14E, Sony, Tokyo, Japan) supplemented with infrared lights (IRLamp6, Tucson, AZ). To control for prior familiarity between test subjects and odor individuals, we only used odors from male bats captured > 2km distance from the test subject's capture location. Odor sample location ranged from 2.91km to 14.62km from the test subject's capture location. Radio telemetry studies have demonstrated that *T. cirrhosus* have small home ranges, flying an average of 218 m from their day roosts each night to foraging areas averaging 12 ha in size (Jones et al. 2017). To minimize observer bias, all trials were scored by an observer blind to the experimental stimuli.

Statistical Analyses

Data were checked for normality using the Shapiro-Wilk test. We tested for differences in the number of chemical compounds between males with and without forearm crust using *t*–tests. To quantify preference, we defined each arm in the y-maze as a preference zone (Fig. 3.1). We analyzed the amount of time a bat spent in each preference zone in relation to the total time the bats spent in both preference zones. We compared the proportion of successes (>50% of time in a preference zone) with a two-tailed binomial test with an expected probability of $p = 0.5$. We tested for differences between the duration of time spent on the side of the y-maze with the odor of a male with forearm crust versus the odor of a male without forearm crust with a Mann-Whitney *U* test because data were not normally distributed and transformations were not successful. We used the same test to assess possible differences in the body condition index (body mass (g) / forearm length (mm)) of the males chosen by females. We calculated the difference in body condition index between the test subject male with forearm crust and the odor donor male with forearm crust. We then tested whether there was a relationship between the difference in body condition index and the duration of time males spent in the no forearm crust preference zone with a Kendall's *tau* correlation. All statistical tests were performed in R (R

Core Team 2013), and the significance level was set α = 0.05. We report results as the mean ±1 SE.

RESULTS

Chemical Analyses

We collected forearm odor samples from 5 males with forearm crust and 4 males without forearm crust. Of 57 chemical compounds present in forearm samples of males with forearm crust, 25 were tentatively identified. From 29 compounds present in forearm samples without a forearm crust, we tentatively identified 13. Males with forearm crust had more substances on their forearm with an average of 20.6 ± 3.8 (*n* = 5) chemical compounds, whereas males without forearm crust had 13.7 ± 2.4 (*n* = 4) compounds, however, this difference was not significant (t_7 = 1.42, *p* = 0.20).

We found 58 chemical compounds in control samples (swabs of the area surrounding the forearm) of males with forearm crust and we identified 27 compounds. Similarly, we identified 30 compounds of the 57 found in the control samples of males without forearm crust. Males with forearm crust had an average of 21.20 ± 5.98 (*n* = 5) compounds in the area around the forearm. Whereas, males without forearm crust had an average of 23.75 ± 3.33 (*n* = 4) compounds in the area surrounding the forearm. This difference did not differ significantly (t_7= 0.35, *p* = 0.74). The scent profiles of males with forearm crust (Fig. 3.2, Table 3.1) mainly included the following substances (abundance): 2-aminoacetophenone ($\bar{X} = 0.066$ nmol ± 0.017), 4-methylquinazoline ($\bar{X} = 0.048$ nmol ± 0.029), cholesterol ($\bar{X} = 0.045$ nmol ± 0.009), 5,6-dihydro-6-propyl-2H-pyran-2-one ($\bar{X} = 0.042$ nmol ± 0.03), 2-methylquinoline ($\bar{X} = 0.032$ nmol

± 0.018), and squalene ($\bar{X} = 0.015$ nmol ± 0.00). From the 23 compounds found in chest gland samples of males with forearm crust (*n* = 2) we tentatively identified 9 compounds. We found that squalene comprised a large portion of these samples ($\bar{X} = 1.82$ nmol ± 0.32).

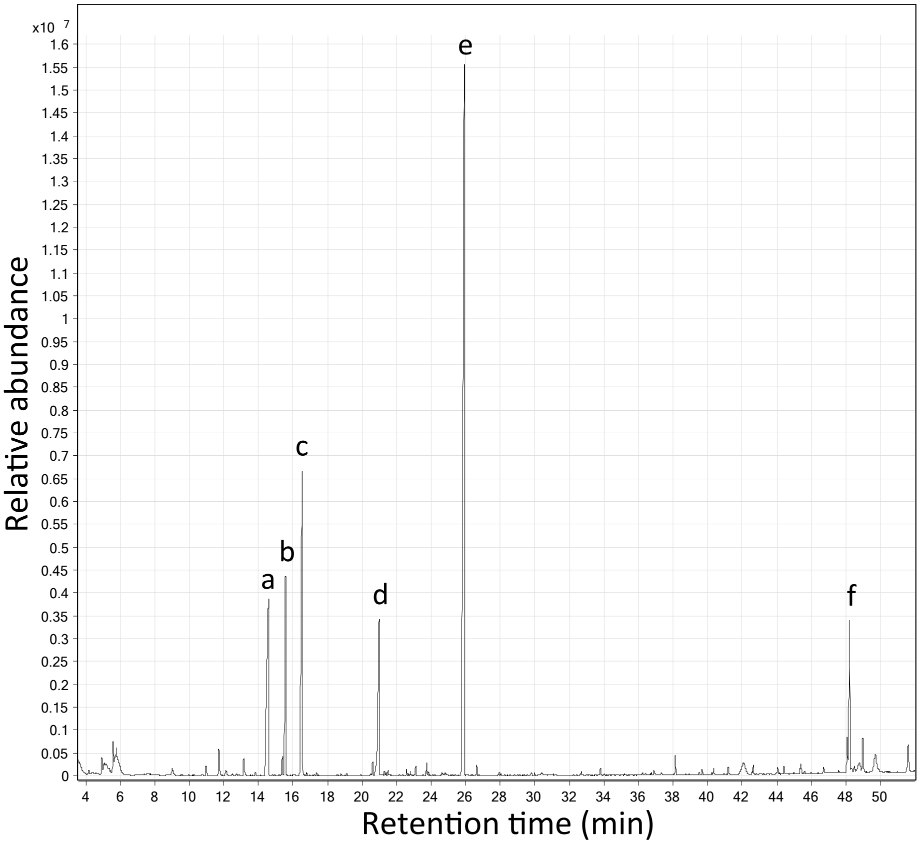


Figure 3.2: Sample chromatogram of forearm crust of an adult male fringe-lipped bat (*Trachops cirrhosus*). a: 5,6-dihydro-6-propyl-2H-pyran-2-one; b: 2-methylquinoline; c: 4-methylquinazoline; d: 2-aminoacetophenone; e: 2-tetradecyl acetate (internal standard); f: Cholesterol.

The scent profile of the urine sample from a male with forearm crust (*n* = 1) contained erucic acid (among other compounds that we could not identify). As the urine and chest gland samples were collected from males with forearm crust we could not compare samples of males with and without forearm crust.

Table 3.1. Chemical compounds tentatively identified in the forearm crust of reproductive male fringe-lipped bats (*Trachops cirrhosus*)

Compounds	Molecular weight	Formula	Retention time	Abundance
5,6-dihydro-6-propyl-2H-pyran-2-one	140.1	$C_8H_{12}O_2$	14.47	0.042 nmol ± 0.03
2-methylquinoline	143.1	$C_{10}H_9N$	15.53	0.032 nmol ± 0.018
4-methylquinazoline	144.1	$C_9H_8N_2$	16.45	0.048 nmol ± 0.029
2'-aminoacetophenone	163.1	C_8H_9NO	20.95	0.066 nmol ± 0.017
Squalene	410.4	$C_{30}H_{50}$	44.44	0.015 nmol ± 0.00
Cholesterol	386.4	$C_{27}H_{46}O$	48.19	0.045 nmol ± 0.009

Preference Test

Of the nine females included in our analyses, three (33.3%) chose the scent of a male with forearm crust (binomial test, N=9, p = 0.25). Females spent an average of 706.7 s ± 64.8 in both preference zones but spent more time in the no forearm crust preference zone ($\bar{X}$= 497.8 s ± 124.2) than they did in the forearm crust side ($\bar{X}$ = 208.9 s ± 104.6, Fig. 3.3a). Females did not differ significantly in the time they spent in the side of the y-maze with the odor of a male with forearm crust versus the odor of a male without forearm crust (Mann-Whitney U = 24, N = 9, P = 0.15). The body condition index of males chosen by females did not differ (Mann-Whitney U = 16, N = 9, P = 0.07). Three out of nine females chose the crust preference zone first (binomial test, N=9, p = 0.25).

Of the eight males analyzed, all but one (87.5 %) spent more time near the odor of a male without forearm crust (binomial test, N=8, p = 0.035). The one male that preferred the scent of a male with forearm crust showed a distinctive change in behavior towards the odor sample, moving back and forth, and ultimately jumping towards the odor cue to the point of knocking the fan. Males spent an average of 582.6 s ± 132.1 in both preference zones, but spent more time in the no forearm crust preference zone ($\bar{X} = 507.4$ s ± 149.6) than they did in the forearm crust preference zone ($\bar{X} = 75.2$ s ± 70.2, Fig. 3.3b). We found that males spent significantly more time in the preference zone with the odor of a male without forearm crust (Mann-Whitney $U = 5$, $N = 8$, $p = 0.004$, Fig. 3.3b). We did not find a significant correlation between the body condition index difference between the male with forearm crust making the choice and the male with forearm crust that we used as an odor donor and the duration of time spent the test subject spent in the no forearm crust preference zone (Kendall's $tau = -0.30$, $N = 8$, $p = 0.31$). Two out of eight males initially chose the crust preference zone (binomial test, N=8, $p = 0.14$).

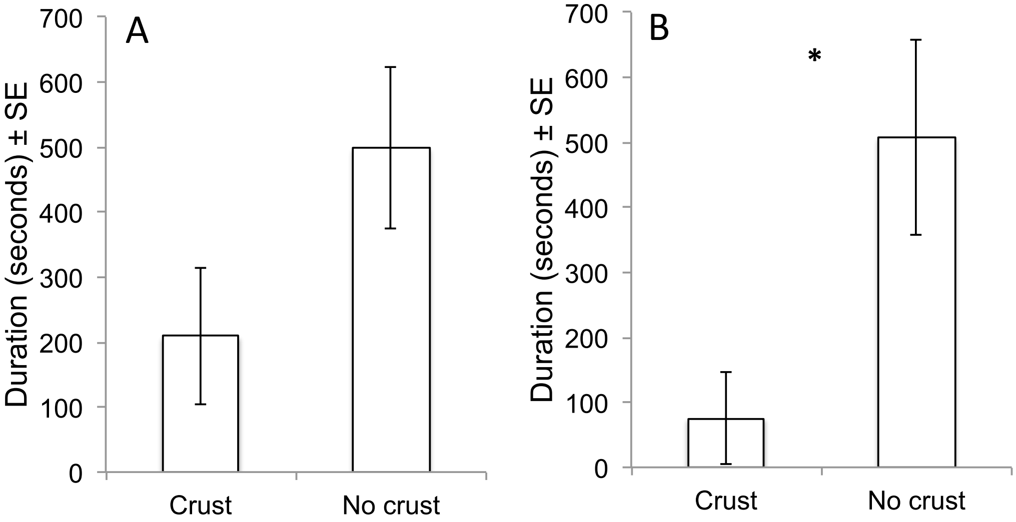


Figure 3.3: A) Female duration and B) Male duration in seconds of time spent in preference zone with odor of male fringe-lipped bat (*Trachops cirrhosus*) with forearm crust or without forearm crust. Significant difference in male preference denoted by asterisk (*).

DISCUSSION

We describe the chemical composition and possible function of a newly described odorous ornament in reproductive male fringe-lipped bats. Our results demonstrate that the forearm crust substance is not created solely with secretions from the chest gland or with urine. Additionally, we found that female fringe-lipped bats did not choose the odor of reproductive male *T. cirrhosus* with forearm crust. However, male *T. cirrhosus* with forearm crust significantly preferred the odor of a male without a forearm crust.

Chemical composition of forearm crust odors

Contrary to our prediction, we did not find congruence between the compounds in the chest gland and urine samples, and the forearm crust samples. Because males create this forearm crust by licking their forearm, saliva may be an important source of compounds. Compelling evidence for this is found in the unique salivary gland in *T. cirrhosus*. Fringe-lipped bats have accessory submandibular salivary glands that are unlike any other described mammalian salivary gland (Phillips et al. 1987). Although this gland is not sexually dimorphic, the previously identified sexually dimorphic forearm licking behavior observed only in males could result in a sexually dimorphic odorous substance (Flores and Page 2017).

Mammalian salivary glands are complex organs that secrete digestive enzymes but can also secrete other products such as hormones (Gröschl 2008). Furthermore, saliva is a likely candidate since it is a known chemical signal in mammals and has varying roles, from eliciting receptivity (Melrose et al. 1971), to impacting mate preference in rodents (Block et al. 1981; Gray et al. 1984; Talley et al. 2001), to increasing aggression in mice (Taha et al. 2009). Likewise, Nagato et al. (1984) suggest that salivary gland secretions in male round-eared bats

(*Lophostoma sylvicolum*) could play a role in species recognition or sexual behavior. Further studies into the chemical composition of the salivary gland secretions of *T. cirrhosus* are necessary to confirm whether these play a role in the forearm crust production.

Cholesterol and its intermediate squalene are ubiquitous in mammals (Albone 1984). Cholesterol is commonly found in mammalian gland secretions (Flood et al. 1989; Burger et al. 2001; Stander et al. 2002; Wood et al. 2005) and was present in our chest gland samples and in all but one of our forearm crust samples. Additionally, we found squalene in several of our samples with particularly high concentrations in the chest gland samples. Squalene has been reported in several flying fox species (*Pteropus hypomelanus* and *P. pumilus*; Wood et al. 2005) as well as other mammals (Scordato et al. 2007; Zhang et al. 2008) and it has been shown to have multiple roles, from increasing the attractiveness of castrated males to females (Zhang et al. 2008), to acting as a male recognition pheromone (Mason et al. 1989). We suggest that similar to other odorous signals (Scordato et al. 2007; Apps et al. 2012), the molecules with lower molecular weights (4-methylquinazoline, 2-methylquinoline, and 2-aminoacetophenone, Table 3.1) could be enveloped in a matrix of cholesterol and squalene, which could act as a fixative or controlled-release carrier material (Burger et al. 2001).

We identified several compounds in the forearm crust that have been reported in other bat species (Nielsen et al. 2006; Caspers et al. 2009; Muñoz-Romo et al. 2012) and in other mammals (Jorgenson et al. 1978; Wood et al. 2002; Zhang et al. 2005). These results provide a tentative chemical characterization that can inform future studies. For example, one of the compounds found in all forearm crust samples, 4-methylquinazoline, has been identified as a component of male sex pheromones in parasitoid wasps (*Nasonia vitripennis*). Although female wasps were not attracted to the odor of this compound alone, 4-methylquinazoline did increase the attractiveness when combined with other sex pheromones (Ruther et al. 2008). Similarly, components of kissing bugs (*Triatoma infestans*) feces are known to elicit aggregation behavior (Schofield and Patterson 1977). Female *T. infestans,* but not males, were attracted to a mixture of 4-methylquinazoline, 2-aminoacetophenone, and other components (Alzogaray et al. 2005). Although most experiments focus on the impact volatile compounds have on insect behavior these results can inform future studies in vertebrates, since these imply that compounds can act as pheromones eliciting behavioral responses.

Odor preference test

Contrary to our predictions, female *T. cirrhosus* showed no preference for the scent of males with forearm crust. Two-thirds of females that made a choice selected the scent of a male without forearm crust. The different responses of female bats to male forearm crust may depend on their sexual receptivity, which we were unable to assess. Voigt and Schwarzenberger (2008) noted that when female *S. bilineata* are not in estrus they refuse copulations and mating attempts by harem males. Additionally, the authors noted that estrus in *S. bilineata* is very short, lasting two to four days from late November to late December with the majority of females in estrus for the first part of December and giving birth in May or June (Voigt and Schwarzenberger 2008). Similar to *S. bilineata,* female *T. cirrhosus* give birth in May or June (Flores and Page 2017). Fringe-lipped bats may also have a short estrous cycle sometime in November and December. Although we tested females in this period, if the window of receptivity is short, it is possible that we might have missed it in some of our test subjects. Future studies should incorporate female receptivity and estrous cycle either through vaginal swabs or hormone analyses.

Although three out of nine females in our experiments did choose the odor of a reproductive male we did not find any noticeable differences in body condition index between the males selected with a forearm crust and without a forearm crust. Odors can convey a wealth of information on an individual's condition. In this study we calculated body condition index because this is commonly used to evaluate the condition in bats since it has been validated as the best predictor for the amount of lipid present (Pearce et al. 2008; Reynolds and Korine 2009). However, we found no significant differences in body condition index between the males selected by females. Alternatively, females might select males based on other qualities. One possibility is that the forearm crust odor could communicate genetic dissimilarity via genes in the major histocompatibility complex (MHC). Some have suggested that odorous signals may provide a more reliable assessment of genotype while evaluating a potential mate than visual or acoustic cues (Johansson and Jones 2007). Studies in both mammals and birds have demonstrated that females can detect MHC-related odors, preferring individuals that are genetically dissimilar (Spehr et al. 2006; Strandh et al. 2012). Recently, Santos et al. (2016) demonstrated that female *S. bilineata* with more variants of olfactory receptors were better at choosing a partner with a complementary MHC. This invites further study into whether *T. cirrhosus* females are choosing males with a dissimilar genotype via the odors of the forearm crust.

We found a significant difference in the choice of males with forearm crust, with seven out of eight males choosing the preference zone with an odor of a male without forearm crust. Furthermore, we had to exclude four males from our analyses because they did not enter either arm of the Y-maze, unlike the females that each entered at least one of the preference zones. This perhaps further indicates a male aversion to the forearm crust odors presented. Previous work suggests males can assess competitors via odors and avoid larger males (Gosling and McKay

1990; Gosling et al. 1996). Furthermore, males will avoid the scent of another male if the male being tested is in poor condition (Amo et al. 2012). Hence, our results suggest that male *T. cirrhosus* may evaluate potential opponents through forearm crust odors and may use odors to avoid intrasexual aggression (Luque-Larena et al. 2001; López and Martín 2011). Although we did not find a significant correlation between the difference in body condition index between males and the duration of time spent in the no forearm crust preference zone, future studies could assess other measures of condition (parasite load and immunocompetence) or androgen levels (testosterone). An interesting future research direction would be to further investigate the relationship between hormone levels and the forearm crust. Testosterone has been correlated with agonistic behaviors and dominance status (Johnston 1981) and with other behavioral states of increased aggression, such as musth in elephants (Poole et al. 1984). Furthermore, odorous signals can produce graded signals that reflect testosterone levels (Ferkin et al. 1994).

Overall our study has tentatively identified the chemical composition of a novel odorous substance in male bats. Previously these compounds were identified as potentially playing a role in mate choice via female choice in other species. However, our results demonstrate the importance of combining chemical analyses and behavior experiments. Unlike previous results in mate choice studies we suggest that these compounds could instead play a role in male-male interactions. Future experiments could test the response of *T. cirrhosus* to individual compound concentrations to determine which compounds are eliciting behavioral preferences.

CHAPTER 4: SOCIAL STRUCTURE OF THE FRINGE-LIPPED BAT (*TRACHOPS CIRRHOSUS*)

ABSTRACT

Social structure emerges either when individuals congregate passively around a shared resource or when they actively seek to associate with particular individuals. Although studies have shown social learning among captive fringe-lipped bats, *Trachops cirrhosus*, their social structure in the wild remains unclear. Using microsatellite marker-based relatedness estimates and 96 roost captures of 172 individually marked adults over 5 years, we examined whether there are preferred associations among *T. cirrhosus* and whether co-roosting associations were influenced by sex and relatedness. Using a permutation-based null model, which controlled for year and population, we found that *T. cirrhosus* often switch roosts but have preferred roosting partners. Relatedness strongly predicted association rates, but only among adult females. These relatedness patterns and recapture records corroborate previous genetic evidence for female philopatry and male dispersal. The general patterns we observed were consistent with other forest-dwelling bat species.

INTRODUCTION

Group-living can result when the benefits of associating with conspecifics outweigh the costs. Some of the benefits of sociality include protection from predators, access to resources, and thermoregulation (Stander 1992; Sonerud et al. 2001; Hass and Valenzuela 2002; Scantlebury et al. 2006). Costs of sociality include increased conspicuousness to predators, competition for resources, and exposure to parasites and infections (Brown and Brown 1986; Cresswell 1994; Rieucau and Giraldeau 2009). Aggregations can occur passively, when individuals group together regardless of the identity of other individuals, due to a shared interest in a common resource. For example, male emperor penguins, *Aptenodytes forsteri*, aggregate to save energy (Gilbert et al. 2008). Alternatively, groups can form actively when individuals preferentially associate with specific individuals (Burns and Broders 2015). Additionally, groups can vary in their stability. Meerkats, *Suricata suricatta*, and baboons, *Papio ursinus*, form long-term stable groups (Drewe et al. 2009; Silk et al. 2010), whereas many other vertebrates show fission-fusion dynamics, in which groups of variable size and composition frequently break up and reform (Croft et al. 2003; Krützen et al. 2004; Conradt and Roper 2005; Amici et al. 2008; Smith et al. 2008; Aplin et al. 2012). If individuals maintain preferred social associations despite fission-fusion dynamics, this suggests social structure is not driven by mere aggregation to resources. Here, we follow Kappeler and van Schaik 2002 definition of social structure; a pattern of social interactions and the resulting relationships among members of a society.

One factor that can influence social structure is kinship (Whitehead 2008). The link between relatedness and social relationships is expected to vary according to dispersal patterns. Across mammals, males tend to disperse more frequently and/or further than females (Greenwood 1980), and since females tend to be philopatric, there are increased opportunities for females to associate with kin. Several studies have demonstrated nonrandom associations among female kin (Archie et al. 2006; Carter et al. 2013; Godde et al. 2015). Associations between related females are likely to evolve as living in close proximity to kin can provide inclusive fitness benefits (Hamilton 1964), as long as the social benefits are not outweighed by increased competition between kin (West et al. 2002). Furthermore, associating with kin can enhance direct fitness such as increased offspring survival and reproductive success (Pope 2000; Silk et al. 2009; Viblanc et al. 2010).

Fission-fusion dynamics are prevalent in many bat species (Willis and Brigham 2004; Rhodes 2007; Popa-Lisseanu et al. 2008; Kerth et al. 2011; Patriquin and Ratcliffe 2016) and analyzing association metrics can reveal underlying social relationships and interactions. Recently, Wilkinson et al. (2018) conducted a multi-species comparison and demonstrated that all nine species included in the study had nonrandom associations and emergent social structure. Furthermore, studies have also determined that in some of these species groups composition can be very stable, with group relationships remaining unchanged for years (Chaverri 2010; Kerth et al. 2011). Whether kinship predicts the associations among the species studied by Wilkinson et al. (2018) has also been investigated. For example, female vampire bats, *Desmodus rotundus*, and Bechstein's bat, *Myotis bechsteinii*, and male Jamaican fruit-eating bats, *Artibeus jamaicensis* preferentially associate with related individuals that are of their same sex (Wilkinson et al. 2018). Additionally, both females and males in Spix's disk-winged bats, *Thyroptera tricolor*, associate with same sex related individuals. However, some males with high association rates are not related, while females with high association rates are rarely unrelated (Wilkinson et al. 2018). These findings reveal interesting differences between sexes that could result from differences in dispersal; male *D. rotundus* and *M. bechsteinii* are the dispersing sex (Wilkinson

1985; Kerth et al. 2002), whereas female *A. jamaicensis* are more likely to disperse (Ortega et al. 2003), and neither sex in *T. tricolor* disperses (Chaverri and Kunz 2011).

Although there are over 1,300 described bat species the patterns of associations and the factors influencing these have been investigated in a few bat species. These studies do not reflect the species diversity among Chiroptera. Furthermore, even though several bat species have been found to associate by kinship (Zeus et al. 2018; Wilkinson et al. 2018), kinship does not always predict association. Despite female philopatry and fission-fusion dynamics (Willis and Brigham 2004), roosting associations among female big brown bats, *Eptesicus fuscus*, are not strongly correlated with relatedness (Metheny et al. 2008). Evidently, further studies are needed to provide insight into the relationship between social structure and dispersal in bats.

Fringe-lipped bats (*Trachops cirrhosus*) are part of the ecologically diverse family of leaf-nosed bats (Phyllostomidae). Recently, Halczok et al. (2018) revealed that unlike many Neotropical bats (McCracken and Bradbury 1981; Dechmann et al. 2007; Nagy et al. 2007; Günther et al. 2017), gene flow in fringe-lipped bats is male-mediated. Hence, investigating the association patterns among *T. cirrhosus* can shed light into dispersal patterns among bats. In addition, this species has been studied intensely for its foraging behavior, predator-prey dynamics, and social learning (recently reviewd in Page and Jones 2016) yet its social structure is unknown. As the associations between individuals likely influence its social learning and foraging behavior, describing the social structure is of interest.

The aim of this study was to elucidate group composition and social structure of fringe-lipped bats. First, we described group characteristics, such as group size and roost use. Second, we tested whether *T. cirrhosus* have preferred roosting partners and if so, whether these were influenced by sex or relatedness. We expected that females would have preferred roosting partners similar to other bats with fission-fusion dynamics (Patriquin and Ratcliffe 2016; Wilkinson et al. 2018). If social structure is driven by female associations then adult females will have differentiated social associations (i.e. associations consistent with preferred roosting partners) with other adult females, but adult males will not. The third goal of this study was to determine whether relatedness predicts association, and if so, what is the effect of sex? Since genetic data suggest that male *T. cirrhosus* are the dispersing sex (Halczok et al. 2018), we predicted that associations among females would be higher.

METHODS

Study Species

Fringe-lipped bats roost in mixed-sex groups of up to 50 individuals in hollow trees, culverts, buildings, and caves (Kalko et al. 1999; Cramer et al. 2001). Although both sexes roost together, reproductive males do not court females at the day roost (Flores and Page 2017). As is the case in most leaf-nosed bats (Phyllostomidae) the mating system of *T. cirrhosus* is not known (McCracken and Wilkinson 2000). Females give birth to one offspring at a time coinciding with the start of the rainy season (Flores and Page 2017), however the gestation length is unknown. During the putative mating season reproductive males have enlarged testes and create an odorous substance that is smeared on their forearm, called a forearm crust (Flores and Page 2017). Individuals have large home ranges (average of 60 ha) and throughout the radio-tracking study bats were found to fly an average of 218 m from their day roost to foraging areas (Jones et al. 2017). Fringe-lipped bats are known to conduct short flights hunting for insects, lizards, and frogs (Cramer et al. 2001; Jones et al. 2017).

Captures and Study Area

Fieldwork was conducted from July 2012 to September 2017. We captured bats opportunistically using mist nets (Avinet, Dryden, NY) set at the exits of roosts along Pipeline Road (PLR), and Gamboa Road (GAMRD), in Soberanía National Park, Panamá (9.0743° N, 79.6598° W) and in a roost in the town of Gamboa (GAM), Colón, Panamá (Fig. 4.1). The Chagres River divides GAMRD from the town of GAM and PLR. Additionally, the GAMRD population is a distinct genetic populations from the GAM and PLR population (Halczok et al. 2018).

We identified juveniles by the presence of epiphyseal gaps in the phalanges (Brunet-Rossinni and Wilkinson 2009). We marked each bat with a passive integrated transponder (Biomark, Boise, ID) to allow for identification of recaptured bats. All bats were released at the site of capture. Although capture and marking may impact animal movement, Jones et al. (2017) observed that *T. cirrhosus* frequently switch roosts regardless of capture. All sampling protocols followed guidelines approved by the American Society of Mammalogists for capture, handling, and care of mammals (Sikes et al. 2016) and were approved by the Smithsonian Tropical Research Institute (STRI) Institutional Animal Care and Use Committee (#20100816-1012-16, #2014-0101-2017, #2017-0102-2020). All research was licensed and approved by the government of Panama (SE/A-94-11, SE/A-58-12, SE/A-19-13, SE/A-86-14, SE/A 69-15, SE/AH-2-6).

Roost use

When describing group composition we only included captures along PLR and GAMRD as these roosts structures were all similar and differed in scale from GAM. Since some roosts were very close to each other (0.084 km) while others are farther apart (0.44 km) we calculated whether males and females differed in the distance traveled between roosts. Additionally, to estimate the relative number of roosts used we calculated the proportion of roosts used as the number of roosts visited by each bat divided by the number of total roost observations visited per bat. Results report mean and standard error. We tested for differences between groups using a permuted t-test.

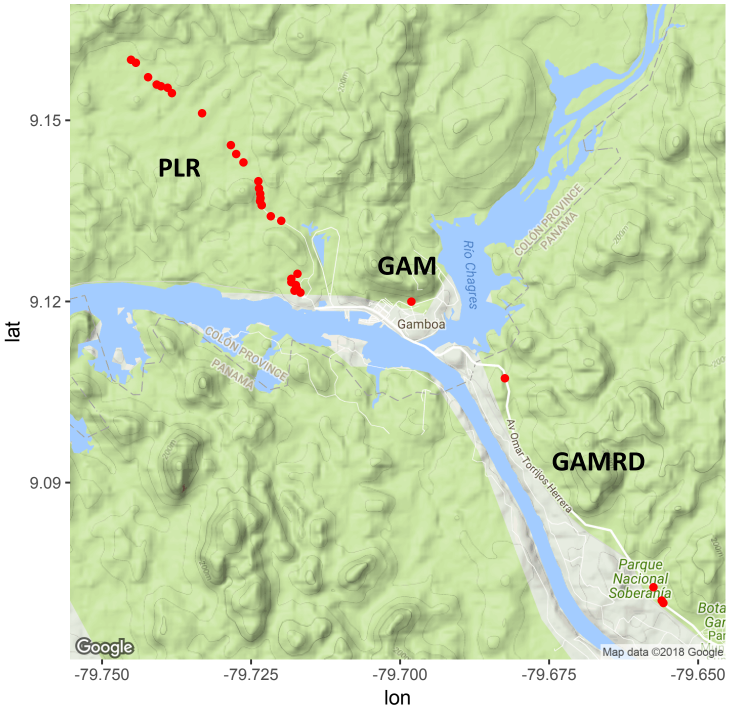


Figure 4.1 Map of study area. Red circles denote roost locations in three populations: along Pipeline Road (PLR), in the town of Gamboa (GAM), and along Gamboa Road (GAMRD).

Genetic relatedness

We obtained wing tissue samples by using a sterilized 4mm biopsy punch. Tissue samples were stored in >95% ethanol until DNA extraction using an ammonium acetate precipitation method (Nicholls et al. 2000). Individuals were genotyped using 16 microsatellite markers as described in Halczok et al. (2018). Relatedness between all pairs of individuals was determined using TrioML (Wang, 2007), as implemented in the COANCESTRY 1.0.1.5 software package (Wang, 2011).

Analyses of Associations

We defined groups as individuals that maintain spatiotemporal proximity (Whitehead 2008). Individuals were considered to be associated (i.e. in the same group) if they occupied the same roost at the same time (Whitehead 2008; Franks et al. 2010). Although presence in the same space does not always mean individuals are interacting (Whitehead 2008) many of the bats captured were in small culverts where individuals were frequently roosting in small clusters in close proximity. Many of the bats captured were in small culverts where we observed that individuals frequently roosted in clusters. We assessed pairwise association using the simple ratio index, the probability of observing both individuals together given that one has been seen (Hoppitt and Farine 2017). We calculated social differentiation as the coefficient of variation (standard deviation divided by the mean) of the simple ratio index (Whitehead 2008). Social differentiation is an important attribute of the social structure of a community and is defined as the variation in the probability of a dyad being associated (Whitehead 2008).

Testing for nonrandom association (preferred roosting partners)

To control for the effect of year and population structure on association, we compared the observed social differentiation with those expected from a null model in which we swapped the roost locations of two individual observations of two bats within the same population and within the same year. Although we have adequate samples for testing overall correlations between relatedness and association, we have low resolution on the true association between any given pair of bats in the social network due to a lack of repeated observation of the same individuals; most bats were captured only once and only one bat was captured as many as 10 times (Fig. 4.A.1). To estimate social differentiation, we therefore reported the results for the subset of bats seen at least four times (n= 33 bats). To test significance, we used the asnipe R package (Farine 2013) to conduct 5000 permutations of the data (Farine 2017).

Testing effects of relatedness on association

To test the effect of relatedness on association, we used the same permutations of the raw data as above, but for comparison, we also used node permutations, which do not control for the effects of time, population, or roost location. A correlation between relatedness and association could be driven by a continuous relationship with closer kin being more associated (correlated nonzero values of relatedness and association), or by a categorical relationship distribution where non-kin are more often not associated (many zero-zero values). To help disentangle these possibilities, we tested the correlation first as a continuous variable, then again with either association, relatedness, or both as a binary variable. To make binary variables, we binned association as zero or nonzero, and relatedness close kin or not (relatedness above or below 0.3).

If the correlation for the binary variables was stronger than the continuous variables, this would indicate that the correlation was largely driven by zeros-nonzero values.

Testing for effects of sex on relatedness, association, and the relatedness-association correlation

To test for effects of sex on association and relatedness, we first tested for an effect of pair type (female-female, male-male, or female-male) on relatedness using node permutations (Mantel test in vegan R package (Oksanen et al. 2017)) and on association (pre-network permutation using QAP in the asnipe R package). These tests indicate whether relatedness or association differs by the type of pair. To detect differences by age, we also conducted separate social differentiation and relatedness-association tests for either adult pairs: adult female-female pairs, or adult male-male pairs.

Testing for female dispersal

To compare whether there was evidence for sex biased philopatry we conducted a permuted F test to test whether males and females differed in the time between the first juvenile capture date and the last capture date as an adult. We conducted 10,000 permutations and report results of a two-sided p-value.

RESULTS

We captured 172 individual bats (80 females, 92 males). Females ($\bar{x}$ = 2.90 ± 0.26 captures, range = 1 – 9) were recaptured more than males ($\bar{x}$ = 2.15 ± 0.19 captures, range = 1 –

10; mean difference = -0.75, df = 1, $p < 0.02$). Using 108 group captures, we sampled 430 individual observations across 30 different roosts.

For roosts on PLR and GAMRD group size estimates ranged from 1 to 13 individuals ($\bar{x} = 3.95 \pm 0.23$, range = 1 – 13). However, this was often only part of the larger group in a roost as individuals can escape and 4 bats may therefore be an underestimate of true mean group size in roosts. In all seven cases when only one individual was captured in the roost, it was a reproductive male with an odorous forearm crust.

We captured 39 groups with reproductive males with forearm crusts and adult females present. Thirty of these groups (77%) had only one reproductive male with a forearm crust present. Groups with two males with a forearm crust had similar number of females present in the group (mean difference = -0.13, n = 39, $p = 0.7$). We captured 14 groups with only reproductive males with a crust present ($\bar{x} = 1.86 \pm 0.39$ individuals, range of males in a group = 1 – 6). The number of males in a group did not vary according to month. Both adult males and females that were captured at least four times, were found in several different roosts (males: range = 2 – 7 roosts; females: range = 2 – 5 roosts), but adult males used more roosts (mean difference = - 0.18, n = 31, $p < 0.006$). Adult males and females traveled similar distances between roosts (mean difference = -.394 km, n = 31, $p = 0.08$).

Nonrandom association

We only tested social differentiation within the PLR population because this is the only population where bats were captured more than three times. When controlling for roost and year, social differentiation was evident among adults of both sexes (CV = 2.50, n = 33 bats, $p < 0.002$), and for female-female pairs (CV = 2.23, n = 16 bats, $p < 0.0002$), but not for male-male pairs (CV= 3.08, n = 17 bats, $p = 0.48$).

Effects of sex on association and relatedness

Among all captures, patterns of association and relatedness differed for adult pairs that were either female-female or included males. We found a correlation between association index and relatedness in females ($r_\tau = .36$, $p < 0.0001$) but not in males ($r_\tau = -0.02$, $p = 0.73$, Fig. 4.2). This was true whether we included all individuals or just individuals captured four or more times. Adult female-female dyads were more often associated (QAP coefficient = 0.01, n = 4624 dyads, $p < 0.004$) than other pairs, whereas male-male pairs were not (QAP coefficient = -0.005, n = 6241 dyads, $p = 0.21$). We also found evidence that adult female-female pairs were more related (Pearson's r = 0.04, n = 4624 dyads, $p < 0.01$). In contrast, male-male dyads were not more related than dyads with females (Pearson's r = -0.009, n = 6241 dyads, $p = 0.71$). Based on these results, we did separate analyses for female-female and male-male pairs.

Association and relatedness

Relatedness predicted association between females (Mantel coefficient = 0.41, n = 16, $p < 0.002$), but not between males (Mantel coefficient = -0.04, n = 17, $p = 0.62$). The correlations between association and relatedness were similar or higher when the continuous variables were coded as binary (Table 4.A.1). We also observed the same results when we included the entire dataset with individuals captured one or more times (Table 4.A.2). This suggests that the effect of relatedness on association was largely driven by unrelated females being unassociated more than expected by chance, a pattern consistent with female philopatry.

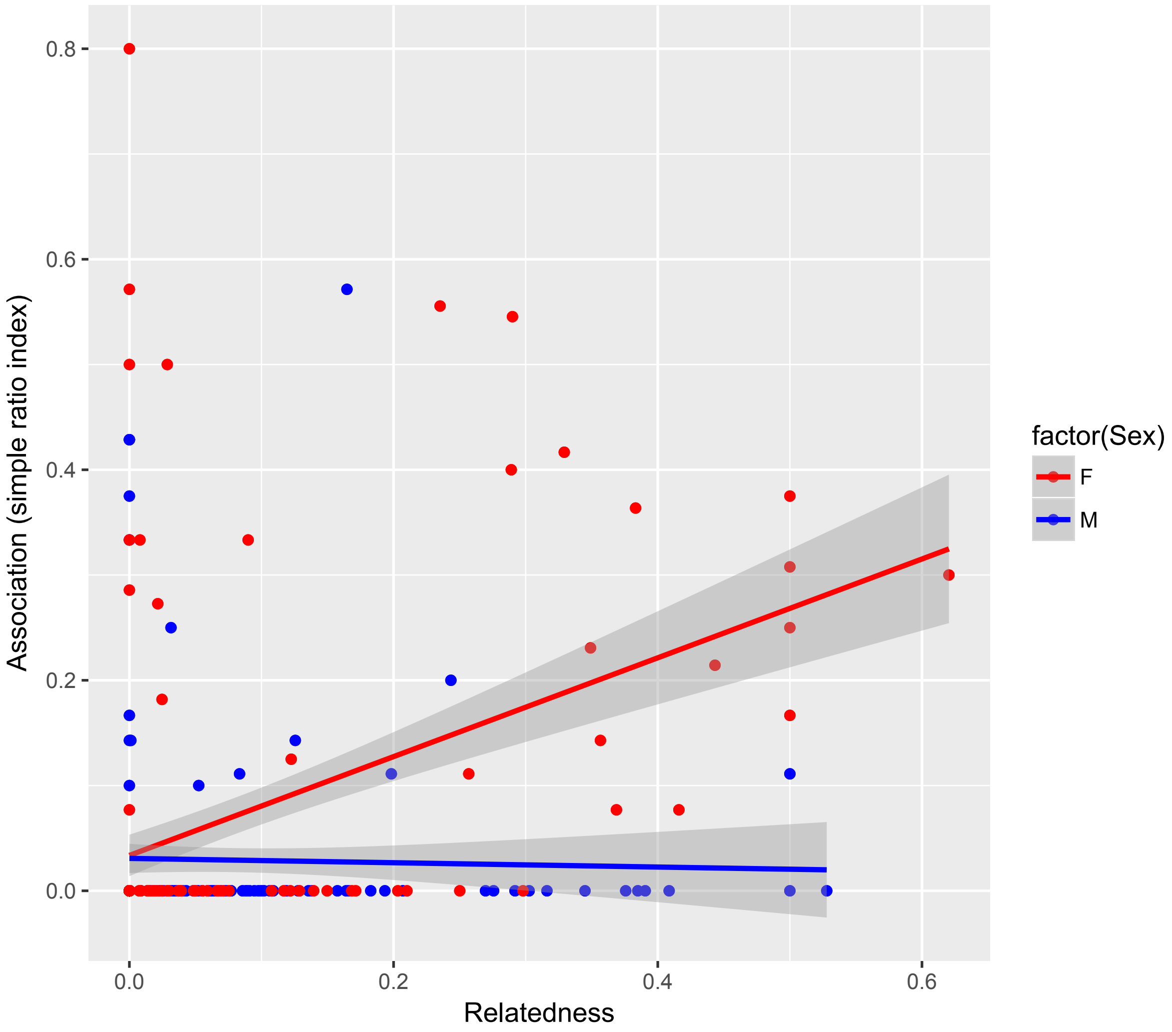


Figure 4.2. Relationship between association (from simple ratio index) and relatedness for male (blue dots and line) and female (red dots and line) fringe-lipped bats (*Trachops cirrhosus*). Lines represent regression slopes with 95% confidence limits.

Female philopatry

Fifty-two bats were captured as juveniles, and 25 of these were re-captured as adults (males = 13, females = 12). We found evidence for female philopatry as the range of time between first juvenile capture and last adult capture was greater for females than males (permutation test: F = 6.59, n = 25, $p < .0005$, Fig 4.3). Juvenile males were re-captured as adults up to 619 days later (range = 195 – 691, x̅ = 451.69) while juvenile females were re-captured up to 1514 days later (range = 165 – 1514, x̅ = 629.54).

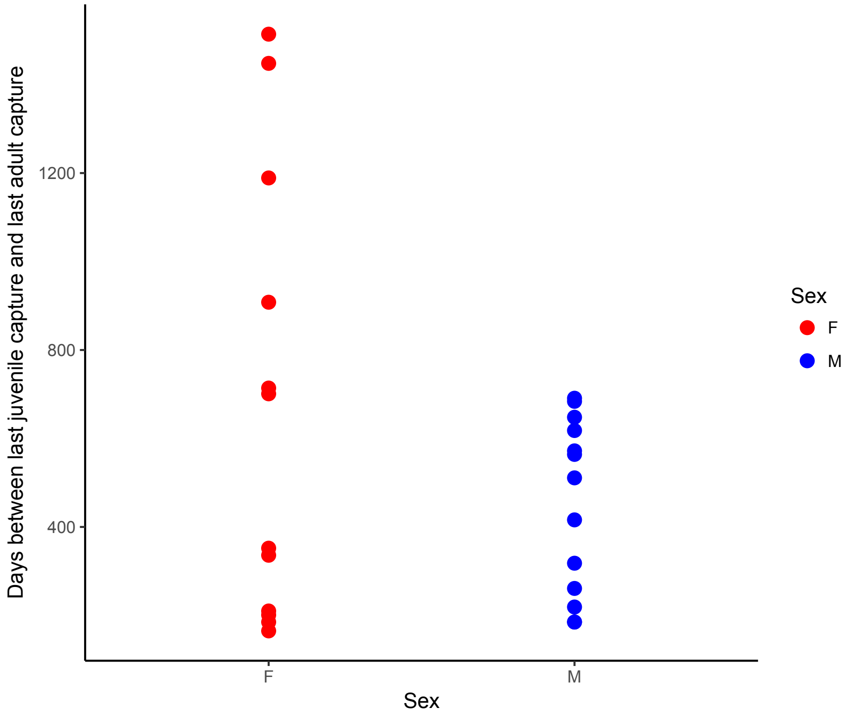


Figure 4.3 Days between the first capture as a juvenile and the last captured as an adult for female and male fringe-lipped bats (*Trachops cirrhosus*).

DISCUSSION

In this study, we captured bats in their roosts over a 5-year period to characterize the group composition and social structure of *T. cirrhosus*. Our results suggest that the majority of fringe-lipped bats roost in either all male bachelor groups or in single-male/multi-female groups. Groups in this species do not appear to be stable as individuals switch roosts frequently, males more frequently than females. We analyzed association metrics in combination with genetic data and found that relatedness predicted associations among female-female dyads, but not male-male dyads. In addition, we found evidence for female philopatry from our long-term capture data.

Most groups in which adult females were present had one reproductive male with forearm crust (Flores and Page 2017). However, in a small number of groups (22%) there were two reproductive males present. Perhaps the difference in the number of males per roost is due to the age of males: roosts with two reproductive males might include an older dominant male and a younger male. Age is one of the factors that can affect reproductive success in mammals (Hollister-Smith et al. 2007; Spong et al. 2008), including greater sac-winged bats, *Saccopteryx bilineata.* Although both peripheral and territorial male *S. bilineata* mate with females in the group, territorial male *S. bilineata* are older and sire more offspring (Heckel and von Helversen 2002). Likewise, although harem males in *P. hastatus* are not morphologically distinct from subordinates, harem males are older and attain more copulations, which suggests that age is an important factor in attaining dominance in the group (McCracken and Bradbury 1977; McCracken and Bradbury 1981). Whether both *T. cirrhosus* males in the roost mate with females is worth investigating.

Male mammals usually mate with multiple females and polygynous and promiscuous mating systems are correlated with male-biased dispersal (Dobson 1982). Recently, Halczok et al. (2018) determined that gene flow in fringe-lipped bats is male mediated suggesting male-biased dispersal. In our study, we found evidence for female philopatry. Mating systems can strongly influence dispersal patterns and, thus, population structure. The mating system of *T. cirrhosus* has not yet been described. The fact that we found that males switch roosts more often than females suggests that males are not defending a particular roost as in resource defence polygyny. This is in contrast to a closely related species to *T. cirrhosus,* white-throated round-eared bats, *Lophostoma silvicolum*, in which males excavate active termite nests that are then colonized by females. In this case, both sexes disperse because male tenure exceeds the age of first breeding in females (Dechmann et al. 2007). Although some groups in our study included two reproductive males with a forearm crust, we suggest that *T. cirrhosus* have a single-male/multi-female mating system. The mating system of *T. cirrhosus* differs to other Neotropical bat species. Unlike, greater spear nosed bats, *Phyllostomus hastatus*, fringe-lipped bats groups are not highly stable (McCracken and Bradbury 1981). Furthermore, we did not detect a seasonal difference to the group composition. Bachelor male groups in *T. cirrhosus* were present year-round and hence did not vary with the female reproductive season. This contrasts to the Honduran white bat, *Ectophylla alba*, in which after parturition groups divide into either all male colonies or into maternity colonies with a single adult male, several females and their young (Brooke 1990). Furthermore, Günther et al. (2016) demonstrated that mating strategies can be flexible and can shift between day and night roosts. Previous work in *T. cirrhosus* did not find evidence of males displaying or courting females at the roost suggesting that copulations might occur outside the day roost (Flores and Page 2017). The behavior and social dynamics of *T. cirrhosus* while out of the roost are worthy of further study.

Our results that relatedness is correlated with associations among female-female dyads demonstrate that *T. cirrhosus* have nonrandom associations. These findings support the conclusions of a recent multispecies comparison in which all nine species investigated had nonrandom associations (Wilkinson et al. 2018). Similar to our results, Wilkinson et al. (2018) found that relatedness predicted association patterns among four of the species studied. However, among these species the patterns were different for males and females, and the authors concluded that these differences are likely due to differences in dispersal patterns between the sexes. For example, relatedness was correlated with associations among female-female dyads only in *M. bechsteinii* and in *D. rotundus*, two species in which males disperse (Wilkinson 1985; Kerth et al. 2002). Our results corroborate the findings of Wilkinson et al. (2018). Male *T. cirrhosus* disperse (Halczok et al. 2018), we found evidence for female biased philopatry, and relatedness predicted association only among female-female dyads. In addition, Wilkinson et al. (2018) found that the strength of the relationship between association and relatedness was best explained by the degree of roost switching. This pattern was strongest for *M. bechsteinii* and *D. rotundus* where males use fewer roosts (Kerth and Morf 2004; Wilkinson et al. 2018). These results contrast to our findings that male *T. cirrhosus* switch roosts more often than females.

The fact that *T. cirrhosus* switched roosts often yet females remain associated with the same individuals suggests that associations are the result of social preferences. Furthermore, roosts did not appear to be limited. Culvert roosts were close (see methods) yet the group of bats would only occupy one culvert at a time, instead of individuals roosting alone throughout different culverts. What factors determine roost selection in *T. cirrhosus* are unknown. Experiments on other bat species have demonstrated that roost selection can vary according to temperature (Kerth et al. 2001) and ectoparasite abundance (Reckardt and Kerth 2007). Furthermore, roost selection can also be aided by conspecific calls which have been shown to help bats in finding tree holes (Ruczynski et al. 2007) and attract individuals to a roost (Chaverri et al. 2010). An interesting future direction would be to determine how female *T. cirrhosus* choose roosts and whether this involves a group decision process (Strandburg-Peshkin et al. 2015). For example, despite living in similar environments, two bat species coordinate group movements differently; brown long-eared bats, *Plecotus auritus*, stay together when switching roosts while Bechstein's bats, *M. bechsteinii*, readily break into sub-groups based on their individual preferences and past experiences (Fleischmann and Kerth 2014).

Preferred relationships in the context of fission-fusion dynamics are consistent with individual recognition among female *T. cirrhosus*. In bats, odors (Safi and Kerth 2003) and vocalizations (Prat et al. 2016) can contain information about an individual's identity. Additionally, several species have been demonstrated to have colony specific odors. For example, female greater bulldog bats, *Noctilio leporinus*, roosting in the same colony mark each other with bodily secretions (Brooke and Decker 1996; Brooke 1997). Colony specific odors can result in recognition of roostmates (De Fanis and Jones 1995b; Englert and Greene 2009). Recognition abilities can also vary between sexes. Female Angola free-tailed bats, *Mops condylurus*, prefer the scent of roostmates, while males did not show a preference (Bouchard 2001). In addition, the complex characteristics of vocalizations make them ideal candidates for individual recognition (Chaverri et al. 2018). Vampire bats preferentially chose the calls of frequent food donors suggesting that *D. rotundus* can recognize individuals from contact calls (Carter and Wilkinson 2016).

Kin-biased association is consistent with, but does not demonstrate, kin discrimination. In all bats studied to date, mothers appear to recognize pups individually by scent (Gustin and McCracken 1987) and by distinct isolation calls (Balcombe 1990). Perhaps similar recognition develops in *T. cirrhosus*, as pups are altricial and dependent on maternal care for the first month (VF pers. obs.). Furthermore, although there is currently no evidence that bats can discriminate unfamiliar kin, studies from other social vertebrates suggest that there are mechanisms, such as prior association and phenotype matching, through which individuals could recognize unfamiliar kin (Mateo 2002; Leclaire et al. 2013; Mateo 2015).

Our results of the roosting dynamics in *T. cirrhosus* can also inform patterns outside the roost, such as foraging patterns and social learning. *T. cirrhosus* can learn to associate novel cues of prey (Page and Ryan 2005) and information about novel cues can spread via social learning (Page and Ryan 2006; Jones et al. 2013). Associations can influence foraging behavior, as socially connected individuals can have a higher rate of food discovery (Aplin et al. 2012) and roosts might act as information centers (Marzluff et al. 1996; Ratcliffe and ter Hofstede 2005). Data from proximity sensors indicate that individual *T. cirrhosus* from the same social group (roostmates) also associate while foraging (Ripperger et al. 2016). It remains unclear if co-roosting associations impact foraging success or social learning.

Many bats are long lived (Wilkinson and South 2002; Podlutsky et al. 2005), and long-term relationships may favor the evolution of cooperative behaviors such as social grooming (Carter and Leffer 2015), food sharing (Wilkinson 1984; Carter and Wilkinson 2013), and alloparental care (Wilkinson 1992; Bohn et al. 2009). Our results that relatedness predicted associations rates among females in conjunction with the long life span of *T. cirrhosus* (survival of over 9 years, VF pers. obs.) are exciting as they inform future studies of cooperation, social learning, and foraging behavior.

APPENDIX 4A: RESULTS FROM MANTEL TESTS OF CORRELATIONS BETWEEN RELATEDNESS AND ASSOCIATION AS CONTINUOUS AND BINARY VARIABLES

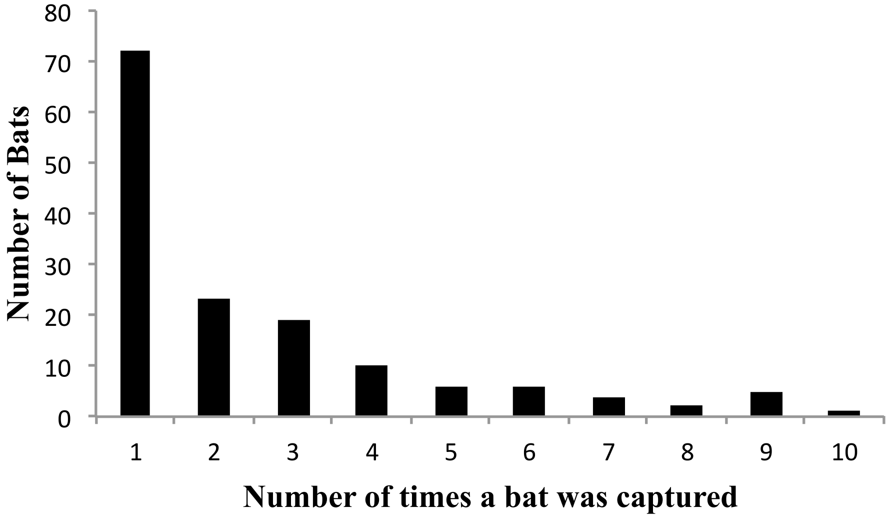
Table 4.A.1. Results of association and relatedness Mantel tests for fringe-lipped (*Trachops cirrhosus*) for bats captured four or more times

Dyad	n = bats	Mantel test correlation with relatedness and association as continuous (p-value)	Mantel test correlation with relatedness as continuous and association as binary (p-value)	Mantel test correlation with both relatedness and association as binary (p-value)
Adult-Adult	33	0.27 (0.001)	0.26 (0.001)	0.26 (0.001)
Female-Female	16	0.41 (0.002)	0.61 (0.001)	0.60 (0.001)
Male-Male	17	-0.04 (0.615)	0.01 (0.416)	-0.03 (0.756)

Table 4.A.2. Results of association and relatedness Mantel tests for fringe-lipped (*Trachops cirrhosus*) bats captured one or more times

Dyad	n = bats	Mantel test correlation with relatedness and association as continuous (p-value)	Mantel test correlation with relatedness as continuous and association as binary (p-value)	Mantel test correlation with both relatedness and association as binary (p-value)
Adult-Adult	130	0.15 (0.001)	0.16 (0.001)	0.16 (0.001)
Female-Female	60	0.23 (0.001)	0.29 (0.001)	0.27 (0.001)
Male-Male	70	0.02 (0.139)	0.03 (0.127)	0.01 (0.384)

Figure 4.A.1. Number of times each fringe-lipped bat (*Trachops cirrhosus*) was captured.



CHAPTER 5: CONCLUSION AND FUTURE DIRECTIONS

In this chapter, I briefly summarize my findings and then highlight potential future directions from both proximate and ultimate perspectives. This dissertation explored the roles of male chemical signaling, conspecific scent preference, and social structure in fringe-lipped bats. In Chapter 2, I characterized a novel odorous forearm crust on male fringe-lipped bats that coincides with the expression of an enlarged chest gland and enlarged testes. In addition, I found that the prevalence of males with a crust increased during the putative female reproductive season. Together, these findings suggest that the forearm crust plays a role in female mate choice or male-male competition. In Chapter 3, I tested both adult females and males with a forearm crust and found that only 33% of females preferred the odor of a male with a forearm crust. Contrary to my prediction, I found strong evidence that males with forearm crusts avoid other males with forearm crust. Unlike the few studies that have investigated the role of odorous traits in other bat species, my results suggest that the chemical compounds in the forearm crust could mediate male-male interactions rather than those between males and females. Finally, to elucidate the role that social structure can play on male behavior, Chapter 4 characterized the group composition and associations among fringe-lipped bats. I found that females have preferred roosting partners despite often switching roosts. Furthermore, relatedness predicted association rates among females but not for males.

One interesting aspect of the forearm crust described in Chapter 2 is that, in comparison with visual secondary sexual traits, such as bright feathers which are molted each season, the forearm crust in male *T. cirrhosus* varies temporally, even within a season. The crust might therefore be dynamically indicative of a male's current condition. From 2014 to 2017, 25 bats were captured multiple times with a forearm crust present. Of these, seven (28%) were subsequently captured without a forearm crust present, and in six of these males, this change was within the same season (range: 12 – 69 days). This is a surprising finding because it indicates that, contrary to other secondary sexual traits used in signaling, the forearm crust can be a facultative trait that changes in presence/absence within a season. One possible advantage of odor communication in contrast to visual signals is that scents such as urine can provide current information about an individual's immediate condition. Evidence from small mammals suggests that males can modulate their scent displays depending on their condition (Zala et al. 2004). In return, this provides an honest indicator of a male's current condition. The factors that control whether a male has a forearm present or absent are unknown and worthy of future investigation. I observed males with forearm crust stereotypically licking their forearms multiple times a day; perhaps this is an energetically costly behavior that some males cannot perform after unsuccessful foraging attempts (Vehrencamp et al. 1989). In addition, this behavior could be mediated by costly hormones, such as testosterone (Wingfield et al. 2001).

In Chapter 2, I also described the chest gland (Fig. 2.2) in fringe-lipped bats. My results can lead to research into the evolutionary history of this gland. I found that glands in both sexes of juveniles are somewhat enlarged and produce a white odorless exudate. In adults, the condition of the gland varies between sexes: in females it is rudimentary while in males it is enlarged and produces a white odorless secretion. In Chapter 3, I analyzed the chemical composition of this exudate and, given the results of Chapter 2 that the development of this gland is correlated with the presence of a forearm crust in males, I predicted that secretions from the chest gland contributed to the forearm crust. Contrary to my expectation, there was no link between the compounds in the forearm crust and the secretion from the chest gland. I suggest that future studies could investigate the evolutionary history of this gland to determine its function. A number of species in the family Molossidae have sexually dimorphic sternal glands (Horst 1966; Dapson et al. 1977). Evidence from black mastiff bats, *Molossus rufus*, suggests that sternal glands are mediated by hormones as the gland regresses after castration (Horst 1966). In Pallas's mastiff bat, *Molossus molossus*, the sternal gland has a cyclical pattern of hypertrophy and active secretion which is synchronous with the breeding season, indicating a possible role in mate choice or territoriality (Krutzsch et al. 2002). Although the sternal gland has been documented and studied in Molossidae, this family is distantly related to leaf-nosed bats (Teeling et al. 2005). While conducting fieldwork in Panama, I also captured a Davies's big-eared bat, *Glyphonycteris daviesi*, with an enlarged gland, indicating this trait has broader distribution within Phyllostomidae. These results warrant future study on the distribution and evolutionary history of this gland, including its ancestral state.

Other than *T. cirrhosus*, sexually dimorphic chest glands have been documented in two species of Phyllostomidae (Goodwin and Greenhall 1961; Valdivieso and Tamsitt 1965). For example, in pale spear-nosed bats, *Phyllostomus discolor,* male glands exude a white, thick, strong-smelling secretion (Valdivieso and Tamsitt 1965). These results contrast with my observations that the exudate in *T. cirrhosus* is odorless. Future studies should conduct further chemical analyses to determine whether the secretions from different species vary in chemical composition. Additionally, the types of chemicals involved could inform the function of the gland.

In Chapter 3, I tested the response of males with forearm crusts to the odors of a foreign male with or without a forearm crust. I found that 8 out of 9 bats preferred the odor of a male without forearm crust. I inferred that males with a forearm crust avoid the odor of another male with forearm crust because the odor of a male with a forearm crust signals a well-conditioned potential rival, whereas those lacking a crust are apt to be in poorer condition. An alternative inference is that males with a forearm crust find the odor of non-reproductive males more interesting and are not avoiding other males with forearm crusts. Although this is a possibility, one of the crusty males in the study demonstrated a distinctive change in behavior towards the crust odor, advancing and retreating vigorously enough to strike the fan used to disperse odors. I interpret this behavioral sequence as signaling aggression towards the scent of a male rival. A potential flaw of this experiment is that the preference of reproductive males without a forearm crust was not tested. However, from 2014-2017, 96 adult male bats were captured between October to February (the timeline of the study in Chapter 3); fifty-eight (58%) males with a forearm crust, 29 (32%) males without a crust, and 9 (10%) males without a crust but with enlarged testes. An appropriate study would only test the latter category, as males without a forearm crust and without enlarged testes are likely younger males or sub-adults. The results from this experiment would not impact our conclusions as these non-reproductive younger males do not represent competitors and are tolerated in social groupings. Although an appropriate test would be to test the latter category of males, this test would not be biologically relevant as only a small number of males in this reproductive state are present in the population during the timeframe of my experiment.

The preference test also demonstrated that the majority of females (6 out of 9, 67%) did not prefer the scent of a male with a forearm crust. However, indicators from other bats (Voigt and Schwarzenberger 2008) suggest that female *T. cirrhosus* might only be sexually receptive for a few days per year. Given the small sample size of my study, future work could conduct further experiments and test a greater number of females to confirm this female preference and conduct studies with females whose estrous state is known. However, an alternative explanation to the lack of preference in females is that perhaps the forearm crust odor is only part of a complex display. Although the roost observations conducted in Panama noted in Chapter 2 did not demonstrate this, behavioral displays could occur at night outside of the roost. To conclusively demonstrate that the forearm crust does not play a role in female mate choice, the reproductive success of males with and without forearm crust should be investigated.

One crucial topic for future research is whether males with a forearm crust have increased reproductive success. The challenge of collecting these data is determining whether a male had a forearm crust present at the time of copulation, especially in view of findings that the forearm crust varies over the course of a season. My results from Chapter 2 will inform future studies as I have characterized the putative female mating period. Investigating the reproductive success of male *T. cirrhosus* and whether this is correlated to forearm crust presence would be interesting, as reproductive success can vary significantly between males of other bat species investigated (Storz et al. 2007; Ward et al. 2014; Fasel et al. 2016; Günther et al. 2017). For example, in Seba's short-tailed bats, *Carollia perspicillata*, harem males sire 60% of all offspring, while bachelors sire 28% (Fasel et al. 2016). In *C. perspicillata,* bachelor males gather together in all-male groups during the daytime (Fasel et al. 2016). This is similar to my results from Chapter 4 in which I observed 14 groups comprised exclusively of adult reproductive males with forearm crusts. Potentially, *T. cirrhosus* with a forearm crust roosting with females have a higher reproductive success than males with forearm crust roosting in bachelor groups or males without forearm crust. The different reproductive success of these reproductive tactics in fringe-lipped bats need to be investigated.

Overall the results from my work can inform the mating system of fringe-lipped bats. The results from Chapter 4, that females have nonrandom associations, could imply that males defend groups of females, and thus constitute evidence of female defense polygyny. Although this mating system has been observed in other bat species (McCracken and Bradbury 1981; McWilliam 1988), females in these species exhibited a high rate of site fidelity. As reported in Chapter 4, both male and female *T. cirrhosus* switch roosts often, suggesting that males do not defend females. Many bat species in the tropics have been reported to have a resource defense polygyny system (Morrison 1979; Williams 1986; Kunz and McCracken 1996; Muñoz-Romo et al. 2008). For these species, protected roost sites constitute a limiting factor and males maintain high roost fidelity to control this resource. Two findings from my work reject the possibility that *T. cirrhosus* have a resource defense polygyny system. First, roosts did not appear to be limited. Culverts were favored roosting sites, and at any given time, many culverts would be empty. Second, males did not show high roost fidelity. I found that males switched roosts significantly more often than females. However, I cannot exclude the possibility that males defend other resources at night, such as foraging areas (Bradbury and Vehrencamp 1976; O'Shea 1980). Furthermore, *T. cirrhosus* could differ in social structure between day and night. Recently, Günther et al. 2016 demonstrated that proboscis bats, *Rhynchonycteris naso*, exhibit alternative strategies depending on the time of day. At night, territorial males actively defend an area. These territories can be occupied by females or can occasionally be visited by females. During the day, groups can be classified as multi-male/multi-female groups where territorial males defend females from other males. These alternating strategies are thought to result from the roosting ecology of this species as *R. naso* roosts on exposed tree trunks where roosting in groups provides a clustered social dispersion allowing individuals to camouflage themselves on the tree trunk. This variation highlights a current issue with the classification of mating systems in bats. Most bats are classified as single male/multi-female, however genetic data can demonstrate variations to these patterns. For example, although most groups of Thomas' fruit-eating bat, *Dermanura watsoni*, had only one male present, suggesting polygyny, genetic data indicate that females mate with multiple males, consistent with polygynandry (Chaverri et al. 2008). Likewise, recent work by Garg et al. 2018 disputed the notion that greater short-nosed fruit bats, *Cynopterus sphinx*, have a harem mating system, combining long-term behavior observations and genetic analyses to show that they aggregate and mate in flexible multi-male/multi-female groups.

The results from Chapter 4 two males with forearm crusts sometimes shared the same roost with females contradict my results from Chapter 3 that males with forearm crusts avoid each other. I think that this discrepancy is likely explained by distance and familiarity. The preference tests conducted in Chapter 3 included odor samples from males originating in different populations. Given that I also found groups of bachelor males, and that bachelor males are often younger (Kunz et al. 1983), it is possible that males roosting with females are older but had previously roosted in all-male bachelor groups. If so, males are already familiar with each other and males used in the preference test were responding to the odors of strangers. This is plausible as bats are able to distinguish familiar individuals based on prior experiences (Carter and Wilkinson 2016). In support of this prediction, several studies demonstrate that familiarity can reduce aggression between individuals (López and Martín 2002; Siracusa et al. 2017). An interesting future direction would be to test the response of males towards odors of a male captured nearby (i.e. local) versus a male captured from a different population (i.e. foreign).

The results from Chapter 2 and 4 indicate that female *T. cirrhosus* could possibly participate in cooperative behaviors such as alloparenting. First, I demonstrated that there is an increase in captures of pregnant female *T. cirrhosus* in March and April, and several females were captured with pups attached to their nipples in May. This suggests that female *T. cirrhosus* reproduce synchronously and have one birth peak per year. Then I showed that female *T. cirrhosus* have preferred roosting partners. If reproduction is synchronous, I would expect that reproductive condition will also influence association rates. Similar results were found in female Northern long-eared bats, *Myotis septentrionalis*, which have nonrandom associations (Garroway and Broders 2007) that vary with reproductive condition (Patriquin et al. 2010). Comparable patterns have been identified in other mammals such as Grevy's zebra, *Equus grevyi*, in which females associate with other females in similar reproductive state (Sundaresan et al. 2007). Together, these observations in which females in a similar reproductive condition roost together, create a context where alloparenting is possible. Although alloparenting behaviors have been demonstrated in bats, however, whether these behaviors are more likely in species in which females associate with other females is not known. Evening bats, *Nycticeius humeralis*, are known to nurse female non-descendent pups (Wilkinson 1992) and female greater spear-nosed bats, *Phyllostomus hastatus*, delay their foraging to 'babysit' pups at the roost (Wilkinson et al. 2016). These cooperative behaviors have also been observed in other social vertebrates, such as meerkats, *Suricata suricatta* (Clutton-Brock et al. 2000). A potential future study could investigate whether female *T. cirrhosus* delay exiting the roost in the presence of pups, whether female presence in the roost is correlated with pup presence, and whether pups are ever left alone in the roost.

In Chapter 4, I demonstrated that *T. cirrhosus* have nonrandom associations and that relatedness predicted degrees of association among females. My work could be expanded by looking at the social interactions between females. Since bats are long lived, an interesting future direction is to determine whether there are dominance-rank relationships among female fringe-lipped bats (Archie et al. 2006). In Bechstein's bats, another species with fission-fusion dynamics and high association rates among females, older females mediate social relationships by linking subunits and maintaining the cohesion of groups (Kerth et al. 2011).

In this dissertation, I explored male chemical signaling using museum collections, long-term capture data, behavioral observations and experiments, and chemical analyses. I also investigated the preference of conspecifics to these male odors and finally I determined the social structure and association patterns in fringe-lipped bats. Although my work has focused on one species, my results can broadly inform other research areas. For example, chemical communication is the most widespread form of communication, present in all multicellular organisms, yet its diversity is poorly understood (Steiger et al. 2011). My characterization of a novel odorous trait suggests that chemical communication and sexually dimorphic chemical traits could be more prevalent in bats than previously thought. Additionally, the results of my preference test demonstrate that odors can also mediate male interactions. The field of sexual selection is biased towards visual and auditory displays yet there is a need for future studies to explore the use of odors in female choice and male-male interactions. Finally, the evolution of sociality is a central theme in evolutionary biology. As the vast majority of bats are social, they are ideal candidates to study this topic. Characterizing the social structure and group dynamics of fringe-lipped bats can inform broader patterns and the causes and consequences of the evolution of sociality.

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