ABSTRACT

Title of Dissertation:	CONSEQUENCES OF SEXUAL SELECTION WITHIN AND BETWEEN SPECIES OF PHYLLOSTOMID BATS
	Danielle Margret Adams, Doctor of Philosophy, 2019
Dissertation directed by:	Professor Gerald Wilkinson, Department of Biology

To understand the myriad effects of sexual selection in the evolution and diversification of life, we must investigate variation within and among diverse animal taxa. Here, I study sexual selection in phyllostomid bats (Family Phyllostomidae), a diverse radiation comprised of 216 species that vary widely in their social behavior and roosting ecology, but whose mating behavior is largely unknown. First, I investigate whether socio-ecological traits predict variation in the intensity of sexual selection among phyllostomid species. In the absence of behavioral data, I use measures of sexual dimorphism as an indicator of precopulatory sexual selection and testes size as a proxy for postcopulatory competition. Taking a phylogenetic approach, I find that roosting aggregation size, but not roost structure permanence, explains family-wide variation in both pre- and postcopulatory sexual selection. Next, I examine the distribution of extra-group paternity in a single species, *Phyllostomus hastatus*, whose social mating system of female-defense polygyny has been well

described. Through molecular parentage assignment of 241 offspring from three wild colonies in Trinidad, West Indies, I find that most harem-holding males are unable to monopolize mating in their social groups, resulting in paternity by extra-group males. Furthermore, variation in the rate of extra-group paternity is associated with harem male body condition as well as the composition of the female group. Finally, I investigate the variation in a male-specific chemical signal found in *P. hastatus*, which has been implicated in male-male competition and female choice of this species. Results show that in addition to individual variation, harem males have significantly different chemical profiles from males found roosting in all-male groups (bachelors). Through the examination of both family-wide and species-specific patterns, we can broaden our understanding of how sexual selection has contributed to the diversity within the Phyllostomidae.

CONSEQUENCES OF SEXUAL SELECTION WITHIN AND BETWEEN SPECIES OF PHYLLOSTOMID BATS

by

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Preface

This dissertation includes previously published work. Chapter three, Male scent gland signals mating status in greater spear-nosed bats, *Phyllostomus hastatus*, was previously published as "Adams, D. M., Y. Li, and G. S. Wilkinson. 2018. Male Scent Gland Signals Mating Status in Greater Spear-Nosed Bats, *Phyllostomus hastatus*. Journal of Chemical Ecology, 44:975-986." DMA conceived the study, collected samples, conducted analyses, and drafted the manuscript. YL advised sample collection procedures and performed the gas chromatography-mass spectrometry. GSW contributed to the design, analysis and editing.

Chapter one, Patterns of sexual dimorphism and mating systems in New World leaf-nosed bats (Phyllostomidae), is submitted for publication as part of a book, the current citation is as follows: Adams, D.M., Nicolay, C., and Wilkinson, G.S. (in press) Patterns of sexual dimorphism and mating systems. *Phyllostomid Bats, A Unique Mammalian Radiation.* (T.H. Fleming, L. Davalos, and M. Mello, eds.) Chicago University Press, Chicago. DMA contributed to idea development, data collection and analysis, and writing of the manuscript. GWS developed the initial idea, collected data, assisted with analyses, and edited the manuscript. CN contributed data and edited the manuscript.

Dedication

In loving memory of my grandmother,

Hedwig Tucker,

whose fruit bat story will always be one of my favorites.

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Introduction

Sexual selection is a powerful evolutionary force resulting from differential reproductive success among individuals within a population. It is responsible for the evolution of extreme traits, such as the elaborate train of the peacock and the formidable mandibles of stag beetles (Darwin 1871), and promotes rapid diversification and speciation (Seddon et al. 2013; Seehausen et al. 2008). By examining patterns of variation in traits that influence competitive success before or after mating between species, we can determine the extent to which species differences arise through natural versus sexual selection. Studies within a single species can then reveal the process of selection in action.

Mating system classifications (e.g. monogamy, female-defense polygyny, scramble competition, etc.) describe the variance in the number of male and female mating partners and, to a lesser degree, the nature of those relationships. The evolution of these different mating system types has been attributed to a variety of factors, including variation in the spatial and temporal distribution of resources (Emlen and Oring 1977), the need for biparental care (Trivers 1972), and the encounter rate between the sexes (Arnold and Duvall 1994; Sutherland 1985).

In my first chapter, I examine the effect of roosting ecology on sexual selection among the phyllostomid bats (family Phyllostomidae). Also known as New World leaf-nosed bats, the phyllostomids are a remarkably diverse radiation of 216 species. In parts of their Neotropical range, phyllostomid bats are the most common

and diverse mammals. For example, phyllostomids account for half of all bat species in Southeast Mexico and Central America (Reid 1997). This diversity and abundance allows them to fill a variety of ecological niches and provide important ecosystem services such as pollination and seed dispersal. Within the family, variation in nearly every life history trait can be found. Diets range from nectarivory in the glosophagine bats, to sanquinivory in the vampire bats, and carnivory in some of the large phyllostomine bats, such as *Vampyrum spectrum*, which feeds on birds and small mammals (Nowak 1994). Roosting habits range from large cave-roosting colonies to small groups in tents constructed from leaves (Kerth 2008; Kunz et al. 2003).

This diverse ecology has likely contributed to their diverse mating behaviors, which range from monogamy to lek polygyny and almost everything in between (McCracken and Wilkinson 2000). The challenge of studying mating systems is that it often requires detailed behavioral observations, which can be especially difficult for nocturnal animals that often roost in difficult to reach places. As such, our understanding of phyllostomid mating systems is based on only a small number of species. Furthermore, even fewer studies have examined the genetic mating systems.

In the absence of detailed behavioral and genetic studies, we can rely on morphological traits to infer the intensity of pre- and post-copulatory sexual selection. Increasing precopulatory competition for mates results in increasing sexual size dimorphism (Clutton-Brock et al. 1977; Dunn et al. 2001; Weckerly 1998) and evolution of weaponry (Rico-Guevara and Hurme 2019). Although bats do not have specialized weapons like the horns and antlers of ungulates, they do have canine teeth

which may serve as weapons and be subject to sexual selection (Gittleman and Van Valkenburgh 1997; Plavcan and van Schaik 1992). Species that form multi-male multi-female groups also have opportunities for multiple mating which can lead to males competing indirectly via sperm competition and result in larger testes (Hosken 1997; Parker and Ball 2005; Wilkinson and McCracken 2003).

In Chapter 1, I used data from museum collections and previously published records to quantify sexual size dimorphism, canine length dimorphism, and testes size for over 87 phyllostomid species. Using phylogenetic comparative methods, I found that roost aggregation size has a significant impact on the strength of sexual selection, such that species roosting in larger aggregations have evolved greater sexual dimorphism. However, the effect of aggregation size on testis size is non-linear, with medium-sized aggregations experiencing the greatest sperm competition. In this chapter, I also provide a more in-depth review of the species for which we have detailed behavioral knowledge and I examine how they fit within the family-wide patterns.

Categorical mating systems based on social associations are convenient and may relate broadly to differences in sexual selection pressures, but we now know that they can be misleading. Molecular parentage studies reveal that mating often occurs outside the social breeding unit, resulting in extra-group paternity (Clutton-Brock and Isvaran 2006; Griffith et al. 2002). Extra-pair paternity is one well-known type of extra-group paternity, in which the breeding group is a single male-female pair. In socially polygamous species, the group includes three or more individuals. The

frequency of this extra-group behavior varies among species, with some showing remarkably high rates, as in the superb fairy wren (*Malurus cyaneus*) in which over 70% of chicks are sired by extra-pair males (Dunn and Cockburn 1998), while others, such as the crimson rosella parrots (*Platycercus elegans*) remain faithful to their social mates (Eastwood et al. 2018). Extra-group paternity thus has the ability to profoundly affect the strength of sexual selection in a population because it alters the distribution of reproductive success within each sex. If extra-group sires are also successful within their social group, the opportunity for sexual selection increases (Webster et al. 2007). Alternatively, the opportunity for sexual selection may decrease if males face a trade-off between within- and extra-group reproduction or extra-group sires are males that are unable to obtain social mates, and would otherwise not reproduce (Jones et al. 2001).

In my second chapter, I examine the frequency and distribution of extra-group paternity in a sexually dimorphic phyllostomid bat whose social mating system has been previously described as female-defense polygyny. The greater spear-nosed bat, *Phyllostomus hastatus*, is a large, omnivorous species with wide distribution throughout the lowland Neotropics (Santos et al. 2003). They often roost in large highly structured colonies, in which females form stable groups of 15-20 unrelated individuals (McCracken and Bradbury 1981) and participate in cooperative foraging (Wilkinson and Boughman 1998) and offspring defense (Bohn et al. 2009) with their groupmates. The clustering of females makes them highly defensible and each group is defended by a single adult male (harem male). Males compete for access to the

limited number of harems, leaving unsuccessful males to roost in all-male bachelor groups (McCracken and Bradbury 1981). This system of female-defense polygyny creates the potential for high within-season variance in male reproductive success. However, harem males are replaced every two to four years, potentially reducing the lifetime variance in reproductive success among males.

The size and stability of *P. hastatus* social groups afford a unique opportunity to examine the genetic mating system in a highly polygynous species. I use mitochondrial haplotypes and microsatellite genotypes to assign parentage to 241 pups from 18 different harems. With these data, I found that harem males typically are unable to fully control paternity within the harem and the ability to exclude extra-group sires is related to male body condition and the age structure of the females within the harem.

In my final chapter, I examine a potential communication signal shaped by sexual selection. Many secondary sexual traits are communication signals because communication is essential to reproduction in nearly all animals. Communication signals facilitate species recognition (Ryan and Rand 1993), sex recognition (Ratterman et al. 2009) and advertisement of receptivity (Nunn 1999). Signals of mate quality and competitive ability are arguably the most interesting signals and countless intraspecific studies have examined the effects of signal variation on reproductive success (Bradbury and Vehrencamp 1998). Visual and acoustic signals have garnered the most attention in vertebrate studies, but most mammals are drab in color and rarely produce elaborate songs (but see Smotherman 2016); they do,

however, possess a diversity of glands. There is a growing body of evidence that chemical signals are targets of sexual selection as they can reveal a variety of attributes, including social status (Buesching et al. 2002a; Setchell et al. 2010), body condition (Buesching et al. 2002a; Buesching et al. 2002b; Ferkin et al. 1997), parasite load (Kavaliers and Colwell 1995; Munoz-Romo and Kunz 2009; Penn and Potts 1998), immunocompetence (Rantala et al. 2002; Zala et al. 2004), and genetic diversity (e.g. Lanyon et al. 2007; Radwan et al. 2008; Setchell et al. 2011).

For my final chapter, I examine the sexually dimorphic scent gland of *P. hastatus*. Using gas chromatography-mass spectrometry, I examined the chemical composition of the glandular secretion and found that variation in the secretion has the ability to signal male mating status (bachelor versus harem male), as well as individual identity. Using our current knowledge of the social mating system, I discuss the potential utility of this chemical signal.

Chapter 1: Patterns of sexual dimorphism and mating systems in New World leaf-nosed bats (Phyllostomidae)

Abstract

Of the 216 species of phyllostomid bats, fewer than 10% have had their mating systems studied in any great detail; however, some species exhibit mating systems ranging from apparent monogamy to extreme polygyny. Paternity studies reveal that the social mating system is generally indicative of the genetic mating system although in some cases, subordinate males father some offspring. These findings suggest that mate selection can involve both male competition and female choice. To estimate the strength of precopulatory and postcopulatory sexual selection, we use measures of sexual dimorphism in relative body mass and canine length as indicators of direct male competition, and relative testes mass as a proxy for sperm competition. We then evaluate the influence of aggregation size and permanence of the roosting structure on the intensity of sexual selection using phylogenetically-informed analyses. Even though females are often larger than males, male-biased sexual dimorphism for relative mass and canine length is widespread and associated with large roosting aggregations. In contrast, sperm competition is greatest in species with intermediate sized aggregations. These patterns of sexual dimorphism are largely consistent with what is known about phyllostomid mating systems, but exceptions provide potential opportunities for future study.

Introduction

Bats exhibit a diverse range of mating systems from monogamy to extreme polygyny (McCracken and Wilkinson 2000). Describing a species' mating system typically requires a long-term study to determine the spatial distributions and behavioral interactions among males and females, in addition to assigning parentage. Relatively few phyllostomid species have been studied in such detail, but the available evidence suggests that species in the family exhibit much of the mating system variation present in the order. In the absence of additional studies, patterns of mating behavior can be inferred by examining variation in traits likely to influence male mating success before and after mating.

Socio-ecological factors can offer insight into expected mating systems because they influence the spatial and temporal distribution of resources and the females that depend on them. When resources or females are spatially clumped and limiting, they become defensible, thus promoting resource or female defense polygyny (Emlen and Oring 1977). Bats form aggregations ranging from a few to thousands of individuals (Kerth 2008) and occupy a range of roost structures that vary in size and longevity (Kunz et al. 2003). Both roost abundance and permanence are known to affect social associations (Chaverri and Kunz 2010), such that species that roost in abundant but ephemeral roosts, such as foliage or leaf tents, tend to have more fluid social structures with short term associations (Chaverri et al. 2007; Sagot and Stevens 2012). By contrast, species in less abundant but more permanent structures, such as caves or hollow trees, exhibit more stable social associations

(Brooke 1997; McCracken and Bradbury 1981; Wilkinson 1985a), often amidst a much larger assemblage of individuals.

When resources or females are defensible, males are expected to compete to control them (Emlen and Oring 1977). Because male mammals are largely liberated from the demands of parental care, they are free to invest in competition to maximize mating opportunities (Trivers 1972), and thus most mammals exhibit some form of polygyny (Clutton-Brock 1989). In both female and resource defense polygyny, selection typically favors large, aggressive males that can compete effectively to control access to females (Andersson 1994; Clutton-Brock et al. 1977; Plavcan and van Schaik 1997). Thus, precopulatory sexual selection has been inferred to be the primary cause of male-biased sexual size dimorphism (SSD) in mammals (Lindenfors et al. 2007; Lindenfors et al. 2002; Plavcan and van Schaik 1997; Weckerly 1998). Alternative explanations based on ecological differences between the sexes have also been proposed (Isaac 2005; Ralls 1977), but have typically received less empirical support. Among bats, however, females are often larger than males (Ralls 1976). One explanation for why female bats are larger is to carry additional weight during and after pregnancy given that bat litters can approach 50% of maternal body mass at birth (Kunz and Kurta 1987). This idea is commonly referred to as the Big Mother hypothesis (Ralls 1976; Stevens et al. 2013). Therefore, even modest male-biased sexual dimorphism may be indicative of strong sexual selection in bats.

In addition to body size, precopulatory sexual selection often promotes the development of weapons (Andersson 1994; Darwin 1871). Unlike large terrestrial

mammals that wield obvious weapons, such as horns or antlers, bats are constrained by aerodynamics given their need to fly, which leaves their canine teeth, and possibly their thumbs, as potential weapons. Primates and carnivores also use their canines as weapons and the degree of sexual dimorphism in canine length is associated with differences in their mating behavior (Gittleman and Van Valkenburgh 1997; Kappeler 1996; Plavcan 2012; Plavcan and van Schaik 1992). Among carnivores, canine sexual dimorphism is greatest in polygynous species with single-male, multi-female groups (Gittleman and Van Valkenburgh 1997). A similar pattern is seen among many primates in which increasing canine dimorphism is correlated with increasing levels of intrasexual aggression (Plavcan and van Schaik 1992), although lemurs and lorises are an exception (Kappeler 1996). Therefore, sexual dimorphism in canine length can serve as an additional indicator of the strength of precopulatory sexual selection.

In situations where males cannot control female mating, precopulatory sexual selection can result from females choosing traits that reflect attributes of male quality other than fighting ability, such as the amount of carotenoid pigment (Blount 2003) or the length of feathers (Andersson 1982). There is evidence that female choice occurs in some bat species and has resulted in sexually dimorphic traits used for signaling, such as the enlarged rostrum of male hammer-headed bats, *Hypsignathus monstrosus* (Bradbury 1977), the wing-sacs of some male emballonurid bats (Bradbury and Vehrencamp 1977; Voigt and von Helversen 1999), and the complex songs produced by some male molossid bats (Smotherman 2016). The role of female choice is largely

unexplored among phyllostomid species; the presence of sexually dimorphic features that can act as signals may reveal candidates worthy of further study.

Reproductive success is not guaranteed by acquiring copulations, because multiple mating by females creates opportunities for postcopulatory sexual selection via sperm competition (Ginsberg and Huck 1989). In many taxa, including bats (Wilkinson and McCracken 2003), there is a strong positive correlation between the opportunity for female promiscuity and size of the testes (Harcourt et al. 1995; Moller and Briskie 1995; Stockley et al. 1997), as males with larger testes are able to produce more sperm (Moller 1988) and are thus more likely to successfully sire offspring. Given the challenges of observing copulations of bats in the wild, measures of relative testis size can provide insight into the degree of female promiscuity and the resulting sperm competition among species of phyllostomids.

Information on roosting habits, particularly aggregation sizes and the structures used for roosting, is more readily available than detailed observations of mating behavior. Therefore, the aim of this chapter is to examine how roosting habits may shape mating systems by influencing opportunities for precopulatory and postcopulatory sexual selection. We infer the strength of sexual selection from measures of sexual dimorphism and testis size using both museum collections and live, wild bats. Finding strong associations will improve our ability to predict mating behavior from simple observations of roosting behavior. We hypothesize that increasing aggregation size increases opportunities for male-male competition and thus promotes precopulatory sexual selection for larger, heavier males with longer

canines. Additionally, we expect larger aggregations to facilitate opportunities for multiple mating by females, thus increasing postcopulatory selection for larger testes. Whether or not such postcopulatory selection occurs depends on whether males can control females within aggregations. When roosts are ephemeral, social groups are likely to be more labile, which may decrease direct competition among males but could increase sperm competition as females will have the opportunity to mate sequentially with multiple males as the group composition changes. Therefore, as roost permanence decreases, we expect sexual dimorphism in body mass and canine length to become less prominent and testes mass to increase. Because the Phyllostomidae include several groups of species that have undergone recent radiations (Rojas et al. 2016), we incorporate phylogenetic similarity (Pagel 1999) into our analyses to determine if relationships among traits or factors are due to recent selection or are the result of gradual evolutionary change that occurred in proportion to the time since a common ancestor. We further examine how the patterns uncovered in our analyses align with what is known about the subset of phyllostomid bats whose mating behavior has been studied.

Methods

Data Collection

To evaluate the role of roost permanence and aggregation size on precopulatory and postcopulatory sexual selection we utilized data from several sources. We downloaded 212,823 phyllostomid specimen records from VertNet

(http://www.vertnet.org) and then added 29,721 records from the United States National Museum of Natural History (USNM, http://collections.nmnh.si.edu/search/mammals/). We used species names provided by Simmons and Cirranello (In press). From these records we extracted sex, lifestage, forearm length, mass, testis size and capture location whenever it was available. We supplemented these data with direct measurements of canine length or testes that we made on specimens at the USNM, the American Museum of Natural History, the University of Kansas Museum of Natural History, and the Carnegie Natural History Museum. For each species, we selected at least 10 adult skulls of each sex that showed little or no evidence of tooth wear to measure the length of the left canine to at least 0.05 mm using calipers. When available, we measured specimens from the same collecting excursion to a single country. We measured length and width of one testis either from fluid specimens or from live animals that either DMA or GSW captured in Trinidad, West Indies, or Costa Rica. After eliminating records without useable data or irreconcilable species names, our dataset contained 60,338 specimen records on 154 species including 149 phyllostomids, two species of *Noctilio* and three mormoopids (Table S1.1). We then examined the range of measurements for each trait and removed obvious outliers, i.e. greater than ± 3 SD from the mean, to ensure that data entry errors did not distort mean values. In Table 1.1 we summarize the number of species and number of specimens for each character in the dataset.

To measure sexual dimorphism in canine length and body size, we first perform a phylogenetic size correction (Revell 2009) because canine length and body

mass are not independent of body size. Using phylogenetic generalized least squares (PGLS), as implemented in CAPER for R (Orme et al. 2013), we regressed species mean canine length on mean forearm length. Because PGLS operates on species means rather than sex-specific means, we used the resulting coefficients and the sex-specific trait means to calculate the sex-specific residuals. We then measured sexual dimorphism as the residual male trait – residual female trait divided by the average value of the trait multiplied by 100, so that each dimorphism measure would represent the percent difference in the trait between the sexes independent of body size. We calculated percent difference in mass similarly, after excluding pregnant females, except that we estimated residuals from the PGLS of log mass on log forearm to account for the nonlinear relationship between mass and forearm. All phylogenetic analyses are based on the phylogeny of Rojas, Warsi, and Dávalos (2016).

As a measure of the intensity of postcopulatory sexual selection, we used natural log (combined testes mass), estimated as double the volume of a prolate spheroid. To compensate for the fact that testes regress during the nonbreeding season and expand during the breeding season, we used the 90% quantile of combined testes mass to represent an average breeding male for each species. This correction likely still underestimates maximum testes mass. For example, average combined testes mass for 174 *Phyllostomus discolor* was 0.597 g while the 90% quantile was 1.053 g and the maximum was 1.868 g. Because testes mass increases with body size, we also include male body mass (natural log-transformed) as a covariate in all models.

Finally, we only used measures of dimorphism or testes in subsequent analyses if there were three or more measurements per sex per species.

We used information from the literature or from museum records to score each species with regard to the degree of permanence of a roosting site and the relative number of individuals typically found in a roosting site (Table S1.2). For each species, we scored roost permanence on an ordinal scale with (1) foliage or roots, (2) tents, (3) hollow trees, logs or excavated termite nests, and (4) caves, culverts, mines or buildings according to reports (Arita 1993; Eisenberg 1989; Kunz et al. 2003; Reid 1997; Tuttle 1976). We calculated the average roost score for species that have been observed in multiple types of roosts. We also scored aggregation size on an ordinal scale with (1) small or family groups less than 10, (2) groups containing 11-25 individuals, (3) small colonies of 25-100, (4) large colonies greater than 100 based on comments in Reid (1997), Eisenberg (1989), Goodwin and Greenhall (1961), or in a Mammalian Species Account (mspecies.oxfordjournals.org; see Table S1.2 for references). In cases where sources differed, we again used the average of the ordinal scores.

Following McCracken and Wilkinson (2000), we also used information from the literature to characterize the mating system as either single male/single female (SM-SF), single male/multi-female (SM-MF), or multi-male/multi-female (MM-MF). In addition, in cases where paternity studies have been conducted, we required harem male paternity to exceed 60% before characterizing a species as SM-MF. As a consequence, some species that were previously described as harem-forming or SM-

MF are now scored MM-MF here (Table S1.2). We made this change because reduced paternity means that sperm competition is likely to be greater and precopulatory selection on body mass or canine length is likely to be lower in such species.

Statistical analyses

To determine the extent to which sexual dimorphism for a trait in any extant species is due to phylogenetic history, i.e. closely related species are more likely to exhibit similar degrees of dimorphism, we estimated Pagel's lambda (λ) using CAPER (Orme et al. 2013). This parameter ranges from 0 to 1, such that λ=0 represents no phylogenetic signal and λ=1 represents strong phylogenetic signal consistent with gradual evolution via a Brownian motion model (Harvey and Pagel 1991; Pagel 1999).

We used phylogenetic generalized least squares (PGLS), implemented in CAPER (Orme et al. 2013) to examine the effects of aggregation size and roost permanence on measures of dimorphism and testes mass. As before, we used the recent noctilionoid tree by Rojas, Warsi, and Dávalos (2016). In the context of PGLS, λ represents the degree to which the phylogeny influences the regression, which may differ from the phylogenetic signal of a particular trait (Symonds and Blomberg 2014). We used AICc for model selection to evaluate the candidate models (Burnham and Anderson 2002), such that the model with the lowest AICc is preferred and models with $\Delta AICc < 2$ are considered equivalent. Because the number of species for which we have data differs depending on which traits are considered, we use only

those species for which we have complete data during model selection, but then apply the selected model to all possible species.

Results

We find that different traits vary in the degree to which phylogenetic similarity has an effect (Table 1.1). Average forearm length has a high lambda value, indicating it is highly influenced by phylogenetic relationships. By contrast, forearm sexual dimorphism has a low lambda value, indicating that SSD varies independently of phylogenetic relationships, i.e. has evolved rapidly among phyllostomid bats. Sexual dimorphism in both mass and canine length exhibits moderate phylogenetic signal, while relative testes mass is also influenced by phylogeny, an observation consistent with the large family-level differences in relative testes mass reported by Wilkinson and McCracken (2003). In addition to these morphological traits, both roost permanence and aggregation size are influenced by phylogeny, with aggregation size having a lambda value not significantly different from 1. Similarities between related species could be due to genetic constraints or to similar patterns of selection; regardless, this finding highlights the need to control for phylogeny rather than assume species values represent independent observations in comparative analyses.

Patterns of dimorphism

Phyllostomid bats vary greatly in body size as measured both by length and sexual dimorphism of forearms. One of the smallest bats in the group, *Ametrida centurio*,

exhibits the greatest female-biased sexual size dimorphism (SSD, male forearm (mean ± SD): 25.56 ± 0.48 mm, female forearm: 31.95 ± 0.76 mm, % difference: -22.22%). By contrast, the largest bat, *Vampyrum spectrum*, exhibits only weak SSD (male forearm: 105.56 ± 2.98 mm, female forearm: 104.33 ± 3.02 mm, % difference: 1.17%). Rensch's rule predicts that among species with male-biased SSD, larger species will show greater degrees of SSD, while among female-biased species, larger species will show less dimorphism (Rensch 1959). We did not find support for this predicted pattern among either female-biased species (PGLS: $F_{1,39} = 0.01$, $p = 0.93$, $\lambda=0.00$) or male-biased species (PGLS: $F_{1,87} = 3.39$, $p = 0.07$, $\lambda=0.31$; Fig. 1.1).

Sexual dimorphism in mass ranges from extreme female bias in *Macrophyllum macrophyllum* (-20.76%), to minimal sex bias in *Diphylla ecaudata* (-0.01%), to extreme male bias in *Monophyllus redmani* (20.67%). Similarly, canine sexual dimorphism ranges from moderately female-biased (*Centurio senex*: -7.51%) to strongly male-biased (*Phyllonycteris poeyi:* 23.24%), with males possessing relatively longer canines than females in most species. Additionally, canine sexual dimorphism is positively associated with mass sexual dimorphism (PGLS: $F_{1,80} = 4.33$, $p = 0.04$, $\lambda = 0.66$, $R^2 = 0.05$; Fig. 1.2).

Although Phyllostomidae tend to have smaller testes than other bat families (Wilkinson and McCracken 2003), they still span a broad range from 0.07% of body mass (*Leptonycteris yerbabuenae*) to 3.67% of body mass (*Diaemus youngi*) indicating that postcopulatory sexual selection is likely important for many species. As expected, log testes mass increases with log body mass (PGLS: $F_{3,72} = 9.01$, $\lambda =$

0.65, $p < 0.01$, $R^2 = 0.27$; coefficient ± SE: 0.61 ± 0.15, $p < 0.01$), but does not covary with measures of canine dimorphism (coefficient ± SE: -0.02 ± 0.02, $p = 0.22$) or mass dimorphism (coefficient ± SE: 0.02 ± 0.01, $p = 0.23$).

Effect of roosting ecology on sexual dimorphism and testis size

As expected, variation in both canine sexual dimorphism and mass sexual dimorphism is best explained by relative aggregation size (Table S1.3), but measures of roost permanence do not improve model fits, as per AICc. Species that form large aggregations are more likely to exhibit male-biased mass dimorphism (PGLS: $F_{1,69} = 19.90$, $p < 0.001$, $R^2=0.22$, $\lambda=0.00$, Table 1.2, Fig. 1.3). Similarly, canine dimorphism increases with aggregation size (PGLS: $F_{1,59} = 10.70$, $p = 0.002$, $R^2=0.15$, $\lambda=0.63$, Table 1.2, Fig. 1.3).

The best-fit model (Table S1.3) that explains variation in relative testes mass includes a positive effect of body mass and a negative quadratic relationship with aggregation size (PGLS: $F_{3,61} = 10.77$, $p < 0.001$, $R^2=0.35$, $\lambda=0.55$, Table 1.2). The model including body mass plus quadratic effects of both aggregation size and roost permanence has a ΔAICc of 0.92 and is considered equivalent (PGLS: $F_{5,59} = 7.60$, $p < 0.001$, $R^2=0.39$, $\lambda=0.51$, Table S1.3). Upon examination of the effect sizes (Table 1.2), it is clear that aggregation size has a stronger effect on relative testes mass than roost permanence. Species with moderate aggregation sizes tend to have larger testes for their body size than species that form very small or very large aggregations (Fig.

1.4). However, there is considerable variation among species that roost in small groups, with combined testes mass ranging from 0.07% to 2.90% of body mass.

Discussion

Dimorphism as a signature of precopulatory selection

We hypothesized that both larger aggregations and more permanent roosting structures would promote competition among males for access to reproductive females and thereby select for larger body mass and longer canines in males relative to females. We found that as roost aggregations increase in size, males become heavier and have longer canines for their size, thus supporting our hypothesis of greater competition in larger groups. We did not, however, find such support for the effect of the roost structure permanence.

For 18 of the 149 species included in our analyses we have more detailed information on mating behavior and can thus examine where these species lie in the family-wide patterns we have found for sexual dimorphism. Two of the least dimorphic species are *Vampyrum spectrum* and *Chrotopterus auritus,* both of which roost in small groups in hollow trees or caves. *V. spectrum* is socially monogamous and the roosting group typically consists of a single male and female along with recent offspring that have not yet dispersed (Vehrencamp et al. 1977). *C. auritus* also appears to be socially monogamous as accounts indicate roosting groups consist of family groups similar to those of *V. spectrum* (Reid 1997). How pairs form in either

species is still unknown, but the lack of sexual dimorphism suggests very limited direct competition between males.

Several species show no sex bias or female bias in dimorphism, including *Uroderma bilobatum*, *Ectophylla alba*, and three of the four *Artibeus* species for which some information on mating system is available (*A. watsoni, A. literatus, A. phaeotis*). These species all roost in small groups and construct leaf tents, except *A. literatus,* which often roost in foliage, or occasionally in hollow trees or caves. Leaf tents cannot accommodate the large aggregations found in more permanent roosting structures, such as hollow trees, caves, and buildings. Additionally, the limited life span of tents requires movement between roosts, which may limit the stability of social groups (Sagot and Stevens 2012). Both of these attributes would limit opportunities for direct competition among males. However, precopulatory sexual selection may act on males if females are choosing a mate based on his tent. Kunz and McCracken (Kunz and McCracken 1996) suggest that tent roosts are a defendable resource and thus likely to be constructed by males to attract females. Observations of *A. watsoni* support this claim. Male *A. watsoni* construct and defend leaf tents and roosting groups generally consist of a single male with multiple females, which suggests a mating system based on resource-defense (Chaverri and Kunz 2006). Although males invest in tent construction, they do not restrict themselves to a single tent and both males and females frequently switch among roost sites (Chaverri and Kunz 2006). By contrast, *E. alba* roost in mixed-sex groups (Brooke 1990) and both males and females engage in tent construction, with multiple individuals making

modifications to a single tent (Rodríguez-Herrera et al. 2011). How group composition and tent construction influence individual mating success is still unknown, but the lack of male-biased dimorphism in mass or canine length among these species implies that small aggregations limit opportunities for direct male-male competition.

The other tent-roosting bats, *A. phaeotis, U. bilobatum, Vampyriscus nymphaea* and occasionally *Artibeus jamaicensis,* appear to form small harem groups consisting of a single male with multiple females. These species exhibit female-biased mass dimorphism, but *V. nymphaea* and *A. jamaicensis* have male-biased canine dimorphism. Little is known about the details of *V. nymphaea*'s mating behavior, but *A. jamaicensis* has been well-studied (Kunz et al. 1983; Morrison 1979; Ortega and Arita 1999, 2000, 2002; Ortega and Maldonado 2006; Ortega et al. 2003). *A. jamaicensis* roosts in a variety of structures ranging from leaf tents to caves and, as a result, aggregation sizes also vary (Kunz et al. 1983). Dominant males aggressively defend groups of females from other males (Ortega and Arita 2000), but the composition of the female groups is labile (Ortega and Arita 1999). In large harems, the dominant male tolerates the presence of a subordinate male, whose presence allows the dominant male to maintain control of the large female group in exchange for a fraction of the paternity (Ortega and Arita 2002; Ortega et al. 2003). The males' enlarged canines are presumably valuable for female defense. Our measures of dimorphism are based on species averages, but given the widespread geographic range of this species along with the diversity of roosting structures and aggregation

sizes, a within-species examination of variation in mass and canine length could reveal interesting patterns. Population differences in relative testes mass has already been reported for this species (Wilkinson and McCracken 2003) and geographic variation in sexual dimorphism is known in two other phyllostomids (Willig and Hollander 1995).

Male *Lophostoma silvicolum* also build roosts to attract females, but rather than modify leaves, they excavate the underside of arboreal termite nests. The size of these roosts constrains aggregation size, but they are more permanent than most leaf tents. Roost construction appears to be under sexual selection as only males perform the excavation and reproductive success is greater for males with roosts (Dechmann and Kerth 2008). Unlike tent-making bats, males are larger than females and have larger canines. Moreover, males with roosts are heavier than those without roosts and females prefer to associate with larger roost holders (Dechmann and Kerth 2008). While these observations have been interpreted as a consequence of female choice, the patterns of dimorphism are consistent with a history of male-male competition.

Another species in which female choice may be important is *Erophylla sezekorni,* which forms labile mixed-sex groups with lek-like mating behavior. Males perform multimodal displays that involve visual wing flapping and display flights, vocalizations, acoustic wing buzzes, and olfactory signals (Murray and Fleming 2008). Similar to classic lekking species, *E. sezekorni* males often perform these displays in small aggregations within the cave. According to our analyses, *E. sezekorni* males and females have a similar body mass, but males have much larger

canines (Fig. 1.2). However, Murray and Fleming (2008) found that males are heavier and in better condition than females. This difference may be due to population differences or seasonal variation. Regardless, their large aggregation size and male-biased canine dimorphism suggest the presence of precopulatory sexual selection, but how mass and canine size play a role in mating success remains to be determined.

In addition to *L. silvicolum*, the species with the greatest degree of mass dimorphism for which mating behavior has been reported are *Phyllostomus hastatus*, and the two outgroup species, *Noctilio leporinus* and *N. albiventris.* All three of these species form harem groups often within larger colony aggregations (Brooke 1997; McCracken and Bradbury 1981; Schad et al. 2012). In both *P. hastatus* and *N. leporinus* female aggregations are remarkably stable over several years and remain intact despite turnover of the harem male. When in residence, the harem male fiercely defends the group by aggressively driving away any approaching males (Brooke 1997; McCracken and Bradbury 1981). This guarding behavior enables the harem male to secure most of the paternity within the group, thus making harem defense critical to reproductive success (McCracken and Bradbury 1977). In turn, competition among males to obtain harem status is expected to be fierce and observations of *P. hastatus* males with injuries during the breeding season support this expectation (personal observation). Large body mass and long canines are thus a likely advantage in these competitive interactions. Less is known about the mating behavior of *N. albiventris*, but its similarity to both to *P. hastatus* and *N. leporinus* in dimorphism, testes mass, and roosting ecology suggests it is similar with respect to mating system.

Overall as aggregation size increases, sexual dimorphism becomes increasingly male-biased. However, within a narrow range of aggregation sizes we still find variation in the degree of dimorphism, particularly among species with small aggregation sizes. Thus, even basic knowledge of roosting aggregations can provide insight into the degree of precopulatory selection a species may have experienced, and simple measures of sexual dimorphism can improve inferences. However, comprehensive understanding of the mating system and precopulatory selective pressures still requires long-term careful observation.

Testes mass as a signature of postcopulatory selection

We expected species that use more ephemeral roosts to have larger testes due to increased opportunities for multiple mating. Additionally, roosting in large aggregations might provide females access to more potential mates and make it more difficult for males to defend their mates, resulting in increased post-copulatory sexual selection. We found that aggregation size has a greater effect on testes mass than does roost permanence. In our analysis, moderate aggregations have higher levels of postcopulatory competition as indicated by the negative quadratic relationship between relative testes mass and increasing aggregation size. However, an additional pattern emerges when we examine the variation within each aggregation level. In doing so, we see that species known to have MM-MF groups have consistently larger testes than SM-MF groups, which in turn have larger testes than SM-SF species (Fig.

1.4). This observation is consistent with previously reported patterns for the entire order (Wilkinson and McCracken 2003).

Of the species for which we have mating system information, some of the largest testes relative to body size are found in *E. alba*, *A. watsoni,* and *L. silvicolum*. As described above, these three species occur in small groups in roosts constructed from leaves or termite mounds. The relatively large testes of *E. alba* are consistent with multiple mating by females and strong sperm competition, which may be expected in their mixed-sex roosting groups. Interestingly, after parturition the composition of roosting groups changes; groups of nursing females associate only with a single adult male, while males form separate aggregations (Brooke 1990). To date, no studies have examined paternity in *E. alba*, so it is unknown what proportion of the pups in a group are sired by the resident male. Both *A. watsoni* and *L. silvicolum* form groups that appear to be harems (Chaverri et al. 2008; Dechmann et al. 2005); however, females do not remain loyal to a single male and frequent roost switching creates opportunities for multiple mating. As a consequence, less than 30% of the pups in an *A. watsoni* tent are sired by the resident male (Chaverri et al. 2008). Similarly, less than half of the pups in a *L. silvicolum* termite roost have been sired by the resident male or by a male the female was known to roost with previously (Dechmann et al. 2005). Harems with labile female membership are also observed in *P. discolor* (GSW, personal observation) and *C. perspicillata* (Fleming 1988; Porter 1979), and males of both species also have relatively large testes. Additionally, *C. perspicillata,* bachelor males are able to successfully sneak copulations further

increasing the degree of sperm competition (Fasel et al. 2016). Flexible harems create opportunities for multiple mating and thus sperm competition; as a consequence, selection is expected to favor males with larger testes (Fig. 1.4).

In contrast, *P. hastatus, N. leporinus,* and *N. albiventris*, all form stable harem groups within permanent roosts (hollow trees and caves) and harem males aggressively defend their female groups. The relatively small testes of these males suggest their defensive behavior limits multiple mating while still monopolizing paternity. For example, *P. hastatus* harem males are able to successfully exclude most intruders and secure 70-100% paternity of pups born in their harems (McCracken and Bradbury 1977).

Both *E. sezekorn*i and *D. rotundus* form large multi-sex aggregations, but appear to have small testes relative to the other multi-male/multi-female species. Small testes may be expected in lekking species because females can exercise mate choice freely and the remating rate is expected to be low; however, studies of lekking birds reveal that females may mate multiply even when able to choose freely (Hess et al. 2012; Lank et al. 2002; Petrie et al. 1992). The testes of *E. sezekorni* are relatively small which supports the hypothesis that remating rates are low when females are able to choose. However, paternity analyses reveal that reproductive skew is relatively weak and thus this species does not conform to a classic lek (Murray and Fleming 2008). These observations do not preclude low female remating rates, but further research is needed. *D. rotundus* also has moderately sized testes for a species with MM-MF groups. Unlike many bats, *D. rotundus* reproduction is asynchronous

and year-round (Wilkinson 1985b). Increasing asynchrony may allow males to sequentially defend preferred females to reduce remating and limit sperm competition (Emlen and Oring 1977). This hypothesis is consistent with their substantial canine length sexual dimorphism, although behavioral observations indicate that females sometimes remate or reject males (Wilkinson 1985b).

As expected, the two monogamous species, *V. spectrum* and *C. auritus*, have relatively small testes. Pair bonding and low population densities drastically reduce opportunities for sperm competition, thus selection should not favor large testes. Other species with small testes include several nectar feeders (*Anoura cultrata, Leptonycteris yerbabuenae,* and *Monophyllus redmani*) and *Centurio senex*. The first three are nectar-feeders that all roost within caves, but with varying aggregation sizes. While little is known about their mating behavior, sexual dimorphism in canine length is substantially male-biased in all three species (14.35% - *A. cultrata*, 15.22% - *L. yerbabuenae*, 12.27% - *M. redmani*) suggesting that males likely fight to control access to groups of females. *Centurio senex* roosts in small aggregations among the foliage. In addition to males having small testes, females are heavier and have relatively larger canines than males, suggesting weak sexual selection on males, but again little is known about their mating behavior.

In addition to variation in testis size, postcopulatory sexual selection could be influenced by the rate of sperm production and sperm morphology (Anderson et al. 2005; Tourmente et al. 2011). Bats are known to exhibit the full range of reproductive delays, from delayed fertilization (sperm storage) to delayed development after

implantation (Orr and Zuk 2013; Racey and Entwistle 2000). Consequently, some of the variation in testis size could be related to species differences in sperm storage or fertilization. For example, *Noctilio albiventris* can delay fertilization (Badwaik and Rasweiler 2000; Rasweiler 1979), which should increase the opportunity for sperm competition. Similarly, *Glossophaga soricina* can delay implantation (Badwaik and Rasweiler 2000; Rasweiler 1979), which should allow females to bet-hedge by mating with multiple males before committing to a pregnancy. Finally, at least three phyllostomids (*Artibeus jamaicensis* (Fleming 1971); *Carollia perspicillata* (Rasweiler and Badwaik 1997; Roellig et al. 2011); *Macrotus californicus* (Bleier 1975)) can delay development, which could allow females to compare developing embryos and only invest in the most successful one. This could favor multiple mating if each embryo was fathered by a different male. Given that females of these species typically give birth to a single pup, this scenario would also require selective embryo resorbtion, which has been reported in some bats (van der Merwe and Rautenbach 1987). The extent to which females use some form of reproductive delay to manipulate postcopulatory sexual selection is an interesting topic worthy of further investigation.

Other consequences of sexual selection: signaling and mate choice

Sexual selection can lead to some of the most elaborate ornaments and armaments (Darwin 1871) and while most bats are drab in color and their morphology is constrained by the demands of flight, several species possess sexually dimorphic

traits and behaviors that may function as signals in agonistic interactions or for mate attraction. Vocalizations are a common sexual signal in many taxa and several studies have documented vocalizations for courtship and territory defense in bats (Behr and von Helversen 2004; Bradbury 1977; Davidson and Wilkinson 2004). Among phyllostomids, two species known to use vocalizations are *E. sezekorni* and *C. perspicillata*, both of which perform multimodal displays. As mentioned previously, *E. sezekorni* males perform a visual display consisting of wing movements and short display flights, which are accompanied by acoustic signals produced both vocally and percussively by vibrating the wings. These signals are directed toward females, suggesting a role in mate attraction; however, the importance of these signals for acquiring mates is unclear because as noted above, reproductive skew is low (Murray and Fleming 2008). *Carollia perspicillata* males also perform wing displays, which include poking with the wings and hovering flights, along with a courtship-specific trill vocalization. These trills exhibit acoustic differences among individuals (Knörnschild et al. 2014), but again, how these calls influence male reproductive success is still not fully understood. An additional set of vocalizations are used in aggressive contexts (Knörnschild et al. 2014) and playback experiments demonstrate that males are able to discriminate individuals by their aggressive calls, which may mitigate conflict between territorial neighbors (Fernandez et al. 2014; Porter 1979).

Olfactory signals are common among bats (Bloss 1999), but their role in mate defense or courtship is poorly understood. The best-studied example is the wing-sac odor of *Saccopteryx bilineata*, an emballonurid bat (Caspers et al. 2008; Voigt et al.

2005a; Voigt and von Helversen 1999), but there is evidence that phyllostomid bats also employ olfaction in both competitive and courtship interactions. Some species have sexually dimorphic scent-producing structures, while others have monomorphic structures with dimorphic chemical profiles. Within the subfamily Phyllostominae, adult males of multiple species have a glandular throat sac that is absent or rudimentary in females (*Phyllostomus discolor* (Holler and Schmidt 1993); *Phyllostomus hastatus* (McCracken and Bradbury 1981), *Chrotopterus auritus* (Medellin 1989), *Phylloderma stenops* (Nowak 1994)). In both *P. discolor* and *P. hastatus*, males rub this gland on their roost site and *P. hastatus* males also rub the gland on their harem females. Holler and Schmidt (1993) report that *P. discolor* males are able to discriminate their own scent from that of other males, which implies utility in territorial defense; furthermore, females can discriminate the scent of familiar and unfamiliar harem males suggesting a potential role in mate recognition or choice. Similarly, the chemical composition of *P. hastatus* gland secretions indicates that males possess individually distinct scent profiles that could facilitate discrimination (Adams et al. 2018). The chemical profile also reflects variation in social status as either a bachelor or harem male, but further work is needed to determine how individuals respond to these signals.

Many chemical signals are associated with a visual component, such as swellings or hairs specialized for disseminating the odor (Fig. 1.5c, d). In two nectar-feeding bats, *Leptonycteris curasoae* and *L. yerbabuenae,* males create a visible patch between their shoulder blades by smearing secretions from various glands on their

backs during the mating season (Munoz-Romo and Kunz 2009; Nassar et al. 2008). In *L. curasoae,* the presence of this dorsal patch is correlated with larger testes, lower body condition, and fewer ectoparasites (Munoz-Romo and Kunz 2009; Munoz-Romo et al. 2012). The secretion mixture contains compounds that act as natural insecticides in other taxa, which might contribute to the lower parasite load, but this causal relationship is yet to be confirmed (Munoz-Romo et al. 2012). Although little is known about the mating system of this species, this visual and chemical signal is expected to serve a role in either mate attraction or choice, as females preferentially associate with odors from males with a dorsal patch over odors from males without a patch (Munoz-Romo et al. 2011). In both species, males are relatively heavier and possess longer canines than females, but have small testes, with *L. yerbabuenae* having the smallest testes (0.07% of body mass) of the phyllostomid bats for which we have data. This implies strong precopulatory competition but weak postcopulatory competition among males despite large aggregations.

Males and females of several *Sturnira* species possess epaulettes, tufts of hair on the shoulders that are associated with underlying sebaceous glands (Fig. 1.5). There is no sexual dimorphism in the structure of these epaulettes (Scully et al. 2000), but there is significant sexual dimorphism in the bacterial colonies present in these glandular regions (Gonzalez-Quinonez et al. 2014). Bacteria play an important role in olfactory communication by altering chemical signals through the metabolism of secretory products, and as a result, odor can serve as a signal of condition or infection status (Penn and Potts 1998; Zala et al. 2004).

Some of the most bizarre sexually dimorphic traits in bats are found among the short-faced bats within the family Stenodermatinae. For example, male *Pygoderma bilabiatum* have glandular tissue around their eyes, under their chins, and on their wrists that undergo seasonal swelling (Tavares and Tejedor 2009)(Fig. 1.5c), during which time males emit a subtle musky odor (R. L. M. Novaes, personal communication). Their small testes and weak female-biased size dimorphism suggest that sexual selection is weak, but the function of these structures and the behavior of this species are largely unknown. Close relatives of *P. bilabiatum* also possess a variety of intriguing facial structures. Male *Sphaeronycteris toxophyllum*, appropriately named the visored bat, have a large sexually dimorphic outgrowth on their foreheads (Fig. 1.5a, b). In addition to the forehead protrusion, males also have a fleshy flap under their chin which can be raised to cover their faces (Nowak 1994). Unfortunately, little is known about the behavior of this species, but we can infer there is some element of male-male competition due to the strong male bias in relative mass, despite weak sexual dimorphism in skeletal and canine size.

In addition to choosing males based on morphological features and behavioral displays, females may also select mates based on the quality of the roosts they construct. As described above, males of several species construct roosts from leaves or termite mounds and these roosts may serve as an extended phenotype (Schaedelin and Taborsky 2009). Because roosts are an essential resource for females, they may prefer males that are able to build suitable roosts. Furthermore, roosts may signal male quality. However, this requires that the resident male roosts only in tents that he

has constructed. While this is clearly the case for *L. silvicolum* males that excavate termite nests (Dechmann and Kerth 2008), it is unlikely to be true for many tent-making bats that frequently switch between several roosts at any given time.

Conclusion

Even though females have larger forearms than males in 69% of phyllostomid species, males have relatively longer canines than females in 85% of species and males have relatively greater body mass than females in 57% of species. These patterns of sexual dimorphism are consistent with strong precopulatory sexual selection acting via male competition for access to mates. Moreover, these two measures of sexual dimorphism are correlated with each other and both are better predicted by size of roosting aggregations than by the degree of permanence of the roosting site. These results indicate that proximity within a roost facilitates male competition and likely enables males to defend larger groups of females. Postcopulatory sexual selection appears to operate independently of precopulatory sexual selection as testis size is uncorrelated with sexual dimorphism in either relative canine length or mass. In contrast to our predictions, postcopulatory sexual selection is not positively related with aggregation size. Instead, species that form relatively small to intermediate sized aggregations exhibit some of the largest testes, although considerable variation in testis size is unexplained. Variation in reproductive delay among species might explain some of that variation.

The patterns of sexual dimorphism we observed are consistent with what is known about the mating systems of phyllostomid bats. Species that form single-male, multi-female (or harem) groups exhibit some of the most extreme male-biased sexual dimorphism for canine length and body mass while species that form single-male, single-female or multi-male, multi-female groups typically show much less sexual dimorphism for these traits. In contrast, testis size tends to be largest in species that form multi-male, multi-female groups in various sized aggregations. The sexually dimorphic traits of the stenodermatine species, such as extended brow ridges, swollen eye tissue, or glandular tissue in males, may be used by females for mate selection; unfortunately, too little is known about their mating systems, but this possibility is certainly worthy of further study. Despite the limited behavioral information available, morphological signatures of sexual selection and commonly collected information on roosting ecology reveal widespread patterns among the phyllostomids, but also highlight the need for further research.

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Tables

Table 1.1 Phylogenetic signal and sample sizes for each trait

Character	No. Species (No. Specimens)	Pagel's λ	(CI)
Forearm Length	137 (17,999)	0.98	(0.92, 1.00)
Forearm Dimorphism (% difference)	129 (17,974)	0.00	(0.00, 0.55)
Mass Dimorphism[2]	110 (17,990)	0.60	(0.29, 0.83)
Canine Dimorphism[1]	87 (2,111)	0.86	(0.56, 0.99)
Testes Mass[3]	107 (6,339)	0.89	(0.72, 0.97)
Roost permanence	105	0.60	(0.33, 0.82)
Aggregation size	83	0.96	(0.85, 1.00)

[1]percent difference of sex-specific residuals from regression on forearm length
[2]percent difference of sex-specific residuals from regression on ln(forearm length)
[3]ln(combined testes mass)

Table 1.2 Effect sizes of roost permanence, aggregation size, and ln(body mass) on measures of dimorphism and testes mass from four phylogenetic generalized least squares models

Model	Response	Predictor	Estimate ± SE	t	p
1	Mass dimorphism	Aggregation	3.67 ± 0.82	4.46	<0.001
2	Canine dimorphism	Aggregation	3.03 ± 0.93	3.27	0.002
3	Testes mass	Aggregation	1.10 ± 0.52	2.10	0.040
		$Aggregation^2$	-0.28 ± 0.11	-2.57	0.012
		ln(Body Mass)	0.64 ± 0.14	4.60	<0.001
4	Testes mass	Roost	1.48 ± 0.78	1.91	0.062
		$Roost^2$	-0.28 ± 0.15	-1.86	0.068
		Aggregation	1.24 ± 0.55	2.26	0.028
		$Aggregation^2$	-0.29 ± 0.11	-2.63	0.011
		ln(Body Mass)	0.65 ± 0.14	4.74	<0.001

Figures

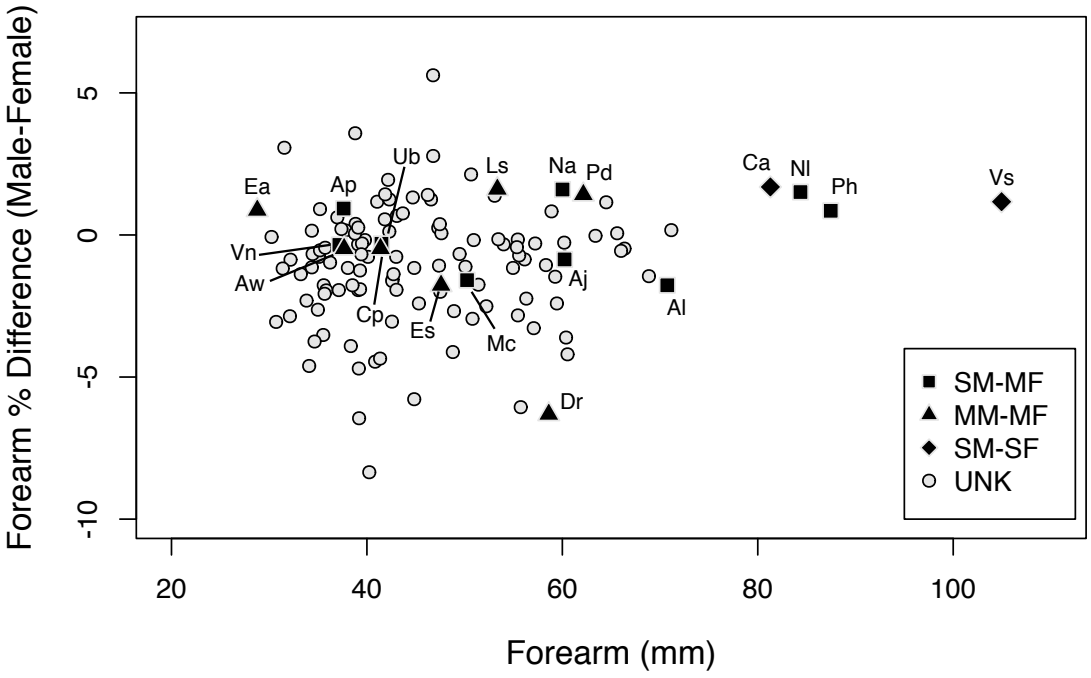

Figure 1.1 Relationship between sexual size dimorphism (SSD) and body size for 129 species. SSD is measured as the difference in forearm length between males and females expressed as a percentage of the species average. Symbols represent mating system types: single male - multifemale (SM-MF), multimale - mulitfemale (MM-MF), single male - single female (SM-SF) and unknown (UNK). Species labels indicate genus and species as follows: Aj – *Artibeus jamaicensis*, Ap – *A. phaeotis*, Aw – *A. watsoni*, Cp – *Carollia perspicillata*, Ca – *Chrotopterus auritus*, Dr – *Desmodus rotundus*, Ea – *Ectophylla alba*, Es – *Erophylla sezkorni*, Ls – *Lophostoma silvicolum*, Mc – *Macrotus californicus*, Nl – *Noctilio leporinus*, Na – *N. albiventris*, Pd – *Phyllostomus discolor*, Ph – *P. hastatus*, Ub – *Uroderma bilobatum*, Vn – *Vampyriscus nymphaea*, Vs – *Vampyrum spectrum*.

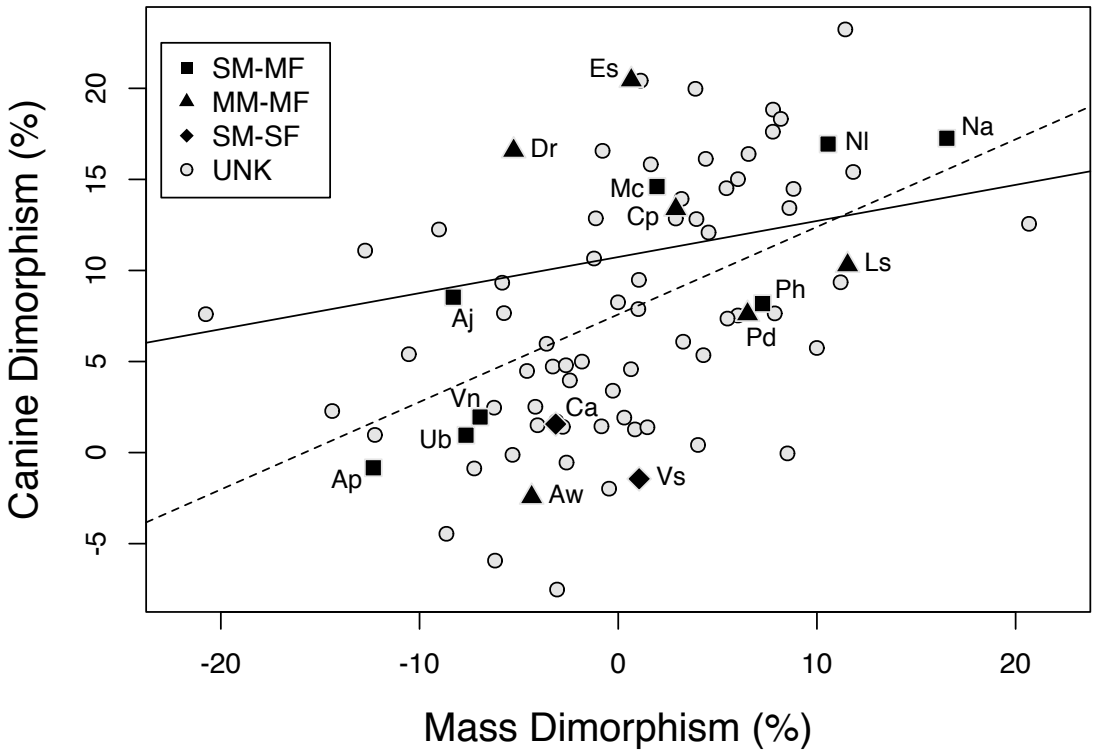

Figure 1.2 Relationship between measures of sexual dimorphism in canine length and mass among 82 species. Lines are fit by ordinary least squares (dashed) or phylogenetic generalized least squares (solid). Symbols represent mating system types and species labels indicate genus and species as in Fig. 1.1

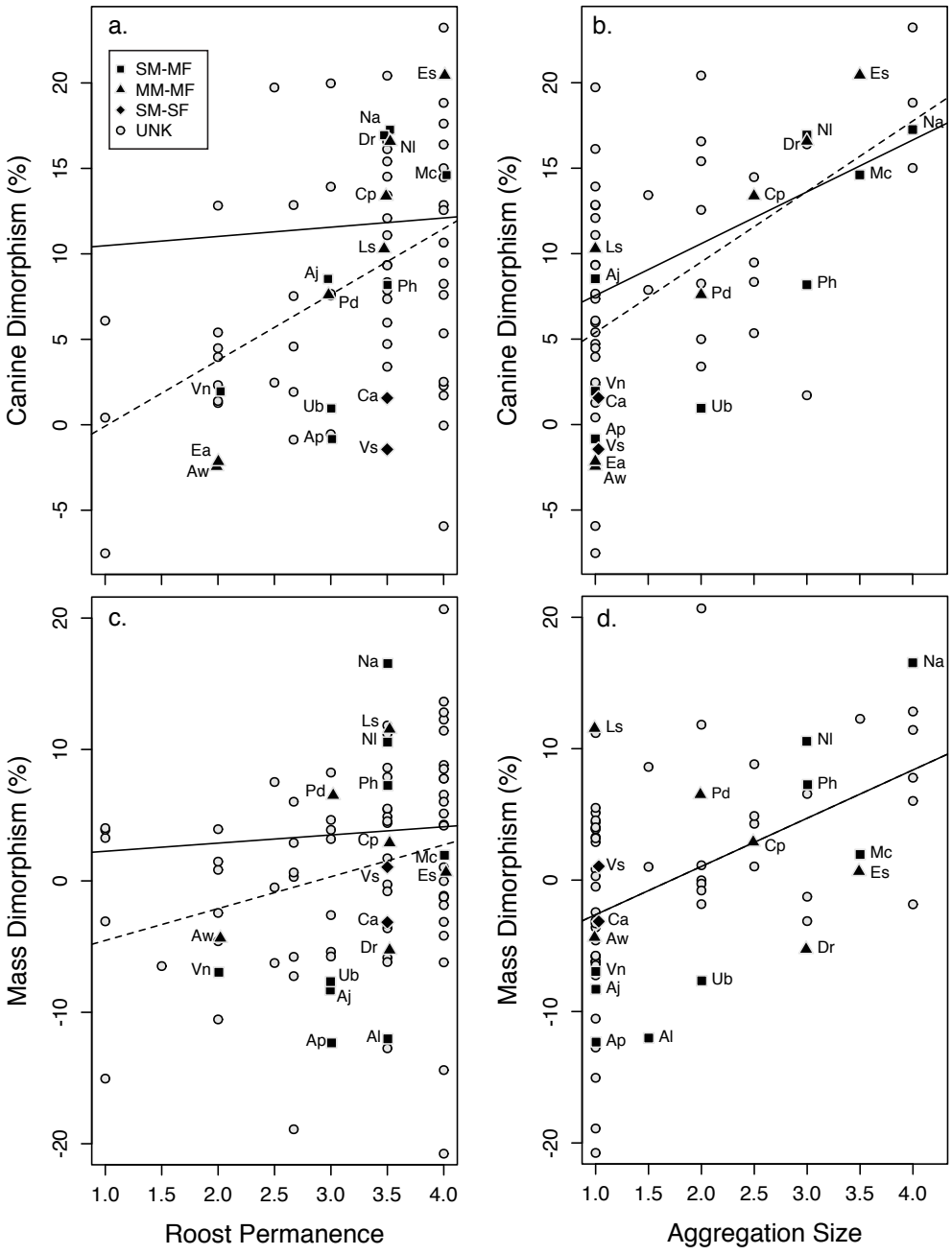

Figure 1.3 Sexual dimorphism for weaponry and weight increase with opportunity for male-male competition due to roost permanence and aggregation size. Canine dimorphism plotted against (A) degree of roost

permanence for 73 species and (B) aggregation size category for 61 species. Mass dimorphism plotted against (C) degree of roost permanence for 90 species and (D) aggregation size category for 71 species. Lines are fit by ordinary least squares (dashed) or phylogenetic generalized least squares (solid) and are identical when lambda is zero. Model details available in Table S1.4. Symbols represent mating system types and species labels indicate genus and species as in Fig. 1.1

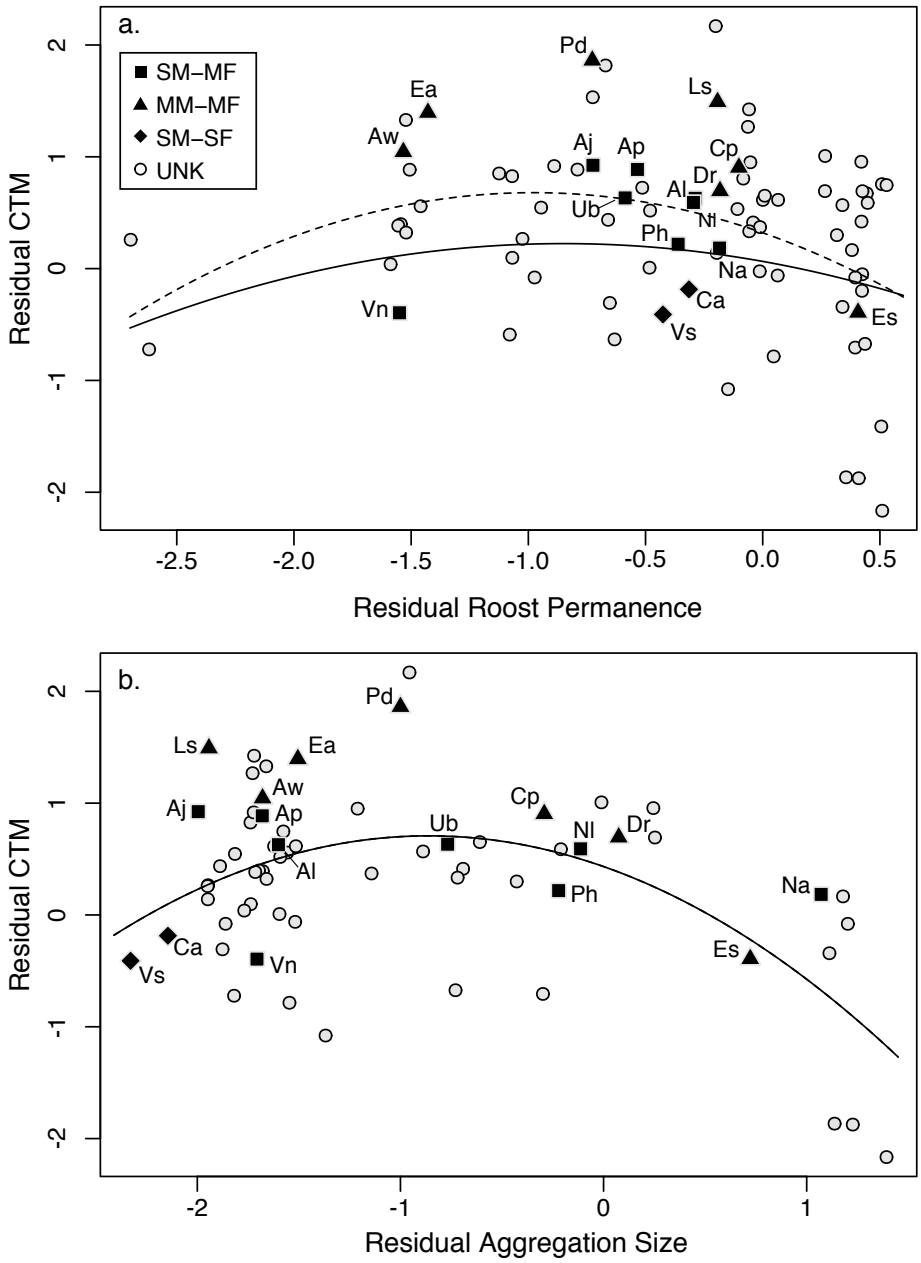

Figure 1.4 Effect of roost permanence (A) and aggregation size (B) on combined testes mass (CTM). To account for the effect of male body mass, ln(CTM), roost permanence, and aggregation size are each regressed on ln(body mass) via PGLS and the residuals are used for plotting. (A) Residual testes mass plotted against residual

roost permanence for 83 species. (B) Residual testes mass plotted against aggregation size for 65 species. Lines fit by ordinary least squares (dashed) and phylogenetic generalized least squares (solid) are identical when lambda is zero. Symbols represent mating system types and species labels indicate genus and species as in Fig. 1.1. Additional model details available in Table S1.5.

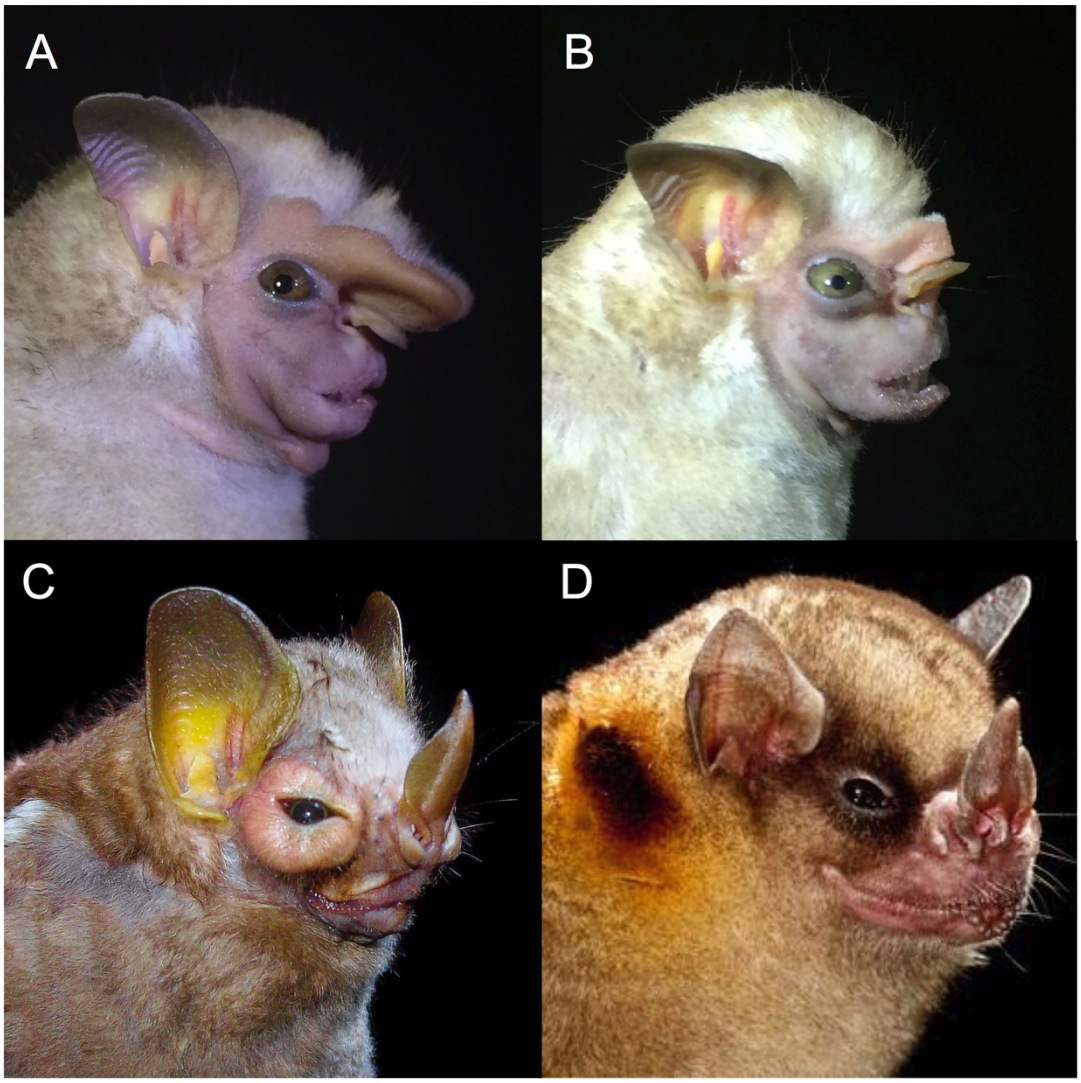

Figure 1.5 Examples of sexually dimorphic traits in phyllostomids. A) Male *Sphaeronycteris toxophyllum* with enlarged browridge. B) Female *Sphaeronycteris toxophyllum* without pronounced browridge. C) Male *Pygoderma bilaboatum* with swollen tissue surrounding the eyes. Males also have swollen tissue around their wrists. D) Male *Sturnira tildae* with shoulder epaulettes caused by glandular secretions. Photos courtesy of Rodrigo Medellin (A, B), Roberto L. M. Novaes (C), and Merlin Tuttle (D).

Chapter 2: Male condition and group heterogeneity predict extra-group paternity in a Neotropical bat

Abstract

Extra-group paternity, in which offspring are sired by a male outside the breeding group, may alter the distribution of reproductive success in a population, thus affecting the opportunity for sexual selection. Both inter- and intraspecific studies have focused largely on mating systems in which females choose their social mates and less is known about extra-group paternity in polygynous systems in which social mates are dictated by male-male competition. In this study, we examine the frequency and distribution of extra-group paternity in a harem-forming bat, *Phyllostomus hastatus*. We find that despite aggressive harem defense, males are unable to fully monopolize reproduction within their harem and over 70% of harems contain extra-group offspring. Harem males in better body condition suffered less paternity loss, but we found no effect of male age or body size. While the age and size of individual females did not predict offspring paternity, we found a significant effect of age heterogeneity within the group. Harems composed of differently aged females were more likely to contain extra-group offspring. Our results provide evidence for the role of male defense in preventing extra-group paternity, but also raise interesting questions about the effect of group composition.

Introduction

In species that form social groups for mating purposes, mating by males from outside the group can result in extra-group paternity, or if the social group consists of only a single male and single female, extra-pair paternity. Extra-group mating behavior has been documented in a wide variety of taxa [e.g. *birds* (Griffith et al. 2002), *mammals* (Clutton-Brock and Isvaran 2007), *fish* (Bose et al. 2019), and *reptiles* (Uller and Olsson 2008)] and causes the genetic mating system to deviate from the social mating system. Because such extra-group mating alters the variance in male reproductive success, it affects the opportunity for sexual selection (Wade and Arnold 1980), and thus can have evolutionary consequences.

Much research has focused on the adaptive value of extra-pair mating from the female perspective (Griffith et al. 2002; Jennions and Petrie 2000; Petrie and Kempenaers 1998), because multiple mating is typically not expected to increase female reproductive success (Trivers 1972). Potential benefits to females include fertility assurance (Hasson and Stone 2009), good genes (Brouwer et al. 2010; Reid and Sardell 2012; Richardson et al. 2005), and compatible genes (Cohas et al. 2007; Jennions and Petrie 2000; Mays and Hill 2004). This emphasis on female choice in the literature is largely driven by a taxonomic bias toward socially monogamous birds, in which female choice has been inferred or demonstrated experimentally (Ferree and Dickinson 2011; Hasselquist and Sherman 2001).

However, not all extra-group copulations are the result of female choice and coercive extra-group copulations are known to occur (Arnqvist and Kirkpatrick 2005;

McKinney et al. 1983; Westneat and Stewart 2003). Socially polygynous species are predicted to exhibit greater variance in male reproductive success, as fewer males have access to reproductive females (Shuster and Wade 2003; Wade and Shuster 2004). As the likelihood of obtaining access to social mates decreases, less competitive males may seek to exploit coercive mating tactics (Clutton-Brock and Parker 1995; Parker 1990). Therefore, the pressures shaping extra-group mating will likely differ among different social mating system types.

Coercive extra-group mating can be costly for females, the coercive extra-group males, and the social mates. Cost to females include risk of injury from aggressive males (Leboeuf and Mesnick 1991), production of genetically inferior offspring (Townsend et al. 2010), and risk of retaliation by the social mate (Valera et al. 2003). Avoiding or resisting coercive mating attempts is an obvious response, but if the energetic or physical costs of resisting exceed the benefits, females are likely to comply with coercive males, thereby reducing the strength of sexual selection (Thornhill and Alcock 1983). To prevent losing reproductive success, social mates are predicted to develop tactics to defend their mates. The effects of coercion and mate defense on extra-group paternity are complicated (Kokko and Morrell 2005) because males are faced with a variety of trade-offs. Socially paired males seeking extra-group copulations must balance investment in pursuing extra-group mating with protecting within-pair mating (Hasselquist and Bensch 1991). If the coercive males do not have social mates, they have no within-group reproductive opportunities to guard. However, when the coercive strategy is plastic and a male has the ability to

obtain social mating status in the future, he is faced with a trade-off between current investment in coercive matings and future competition for access to social mates (Candolin and Vlieger 2013). Under some circumstances, sneaking and coercive mating are as successful as defending social mates, which can lead to stable alternative reproductive strategies (Alonzo 2008).

Bats are a diverse mammalian order that exhibit the full range of social mating systems, from monogamy to lek polygyny (McCracken and Wilkinson 2000). Both female choice (Bradbury 1977; Murray and Fleming 2008; Rossiter et al. 2006; Voigt et al. 2005b) and mate defense (e.g. Dechmann et al. 2005; Kunz et al. 1983; McCracken and Bradbury 1981) have been documented within the order. Extra-group paternity has been investigated in a few socially polygynous species and estimates vary widely among species (Dechmann et al. 2005; Heckel et al. 1999; Heckel and Von Helversen 2003; McCracken and Bradbury 1977; Ortega et al. 2003; Storz et al. 2001). For example, male greater sac-winged bats (*Saccopteryx bilineata*) defend small harems, but also court females with elaborate, multi-modal displays. The rate of extra-group paternity can exceed 60% in *S. bilineata* harems and is likely due to female choice for high quality displays and the inability of males to effectively defend their harems (Heckel et al. 1999; Voigt et al. 2005b). Female defense plays a more prominent role in the mating system of Jamaican fruit-eating bats (*Artibeus jamaicensis*), in which the rate of extra-group paternity is positively correlated with harem size. However, the largest harems have reduced rates of extra-group paternity due to the presence of a subordinate male who helps deter intrusions by extra-group

males in exchange for reproductive opportunities (Ortega and Arita 2002; Ortega et al. 2008).

The greater spear-nosed bat (*Phyllostomus hastatus*) is a large sexually dimorphic bat with female-defense polygyny. Females roost in groups (harems) of 15-20 individuals and each group is defended by a single adult male. Group formation is not initiated or controlled by the harem male, as both natural turnover and experimental removal of males does not disrupt group composition. Instead, groups appear to form for cooperative benefits associated with foraging (McCracken and Bradbury 1981; Wilkinson and Boughman 1998) and pup defense (Bohn et al. 2009). Competition to become a harem male is intense and unsuccessful competitors reside in bachelor groups. On average, harem males are heavier than bachelor males, despite having the same skeletal body size (Adams et al. 2018). Harem males defend their females year-round and may retain tenure for multiple years. Although they aggressively drive away approaching males, they do not appear to restrict or police the movement of females. This affords females the opportunity to visit other males in pursuit of extra-group copulations.

Initial estimates indicate extra-group paternity is rare (McCracken and Bradbury 1977; McCracken and Bradbury 1981), suggesting that either extra-group copulations are rare or extra-group copulations rarely result in successful fertilizations. Extra-group copulations may be rare if females do not pursue them or if harem males successfully prevent them. Alternatively, harem males may rely on repeated copulations to outcompete extra-group males via sperm competition

(Birkhead and Møller 1991; Parker 1990). In the latter case, we would expect males to have large testes to successfully fertilize the large number of females despite sperm competition (Parker and Ball 2005). However, compared to other species in the same family (Phyllostomidae), male *P. hastatus* have small testes for their body size (see Chapter 1), suggesting that sperm competition is not a major selective force. Moreover, male-biased sexual dimorphism in body size and canine length, along with their aggressive behaviors are consistent with intense pre-copulatory competition. These traits are likely beneficial for harem acquisition as well as long-term harem defense.

In this study, we consider the potential causes of extra-group paternity from three perspectives. First, we examine the effect of harem male attributes, specifically male age, body size, and body condition, on extra-group paternity. If male defense is important for excluding extra-group males, we expect older, larger, heavier males to suffer less paternity loss. Next, we examine the effect of female age, body size, and relative timing of parturition. An increase in extra-group paternities among smaller, younger females may, for example, suggest that extra-group matings are coercive and those females are less able to resist. Finally, because variation and interactions within a female group may influence their defensibility or willingness of individuals to engage in extra-group mating, we investigate the effect of collective harem attributes. Specifically, we consider if the composition of each group, with respect to age, body size, and birth synchrony, affects the rate of extra-group paternity.

Methods

Study Population and Sample Collection

For this study, we observed and sampled *P. hastatus* individuals over a 25-year period (1990-2015, cf. Wilkinson et al. 2016) from three wild, cave-roosting colonies in Trinidad, West Indies: Tamana cave (10.4711°N, 61.1958°W), Caura cave (10.7019°N, 61.3614°W), and Guanapo cave (10.6942°N, 61.2654°W). Tamana cave is the largest and contains approximately 20-30 harem groups, which are unevenly distributed among three connected chambers. Guanapo cave is the smallest, but contains the second largest colony with 15-20 harems. Caura cave is intermediate in physical size, but houses the smallest colony with only 4 harem groups. In Trinidad, *P. hastatus* exhibit a single breeding season from November to January, with most pups born in April (McCracken and Bradbury 1981; Porter and Wilkinson 2001).

Each harem typically occupies a separate solution depression in the cave ceiling (Figure 2.1), which allows us to capture the entire group at once using a bucket trap extended to the cave ceiling on a pole. Immediately after capture, bats were held individually in cloth bags while each bat was processed. Previously banded bats were identified by band number and unbanded bats were fitted with a numbered metal band (Monel, National Band and Tag, Newport, KY, USA) on their forearm, with males banded on the right wing and females banded on the left. We recorded the mass (Pesola spring scale), forearm length (digital caliper; Chicago Brand, Medford, OR, USA), and degree of tooth wear (using a 5-category scale, cf. McCracken and Bradbury 1981) for each individual. Unless individuals were banded as newborn

pups, tooth wear is the only way at present to estimate the age of living adult bats (Brunet-Rossinni and Wilkinson 2009). We also collected one or two wing tissue samples from each individual, including pups, using a 3mm diameter biopsy punch. Samples were stored in saturated DMSO solution or 95% ethanol and kept at -4 to -80°C. In total, 18 harem groups consisting of 595 individuals were captured in April, shortly after the pups were born. Twelve harems were from Tamana cave (2001 and 2013), three harems from Caura cave (2013), and three harems from Guanapo cave (1995 and 2001).

Parturition is highly synchronized within colonies and further synchronized within harem groups, such that female group-mates typically give birth within 19 days of each other (Porter and Wilkinson 2001). We used forearm length (FA) to estimate pup age, in days, using the formula (age = 0.77*FA - 24.605) developed by Stern and Kunz (1998), then determined each pup's likely birthdate by counting back from the day they were captured and measured. For each group, we determined the birth peak for a given year as the median birth date. We then calculated the number of days before or after the peak each pup was born. To assess each group's overall degree of synchrony, we calculated the mean absolute deviation from the group median.

Body mass is correlated with skeletal body size and also decreases throughout the day, as the time since last feeding increases. To estimate male body condition, we calculated relative body mass via the residuals from a linear regression of mass on forearm length, time of capture, and their interaction (Table 2.1). We used all

measurements of harem males captured between 1990 and 2015 to fit the regression (N = 168), then predicted the mass of the harem males in this study using their forearm length and time of capture. Harem males were captured multiple times so we calculated a single average forearm length, as this is not expected to change once males reach adult size. Because repeat capture events were at different times of day and male mass can fluctuate between days, we used the time of capture and the male's average forearm length to predict body mass and calculate the residual mass for each independent capture event. To generate a single condition score for each male, the residuals were averaged across all captures within one year of the relevant parturition month (e.g. April 2012 - April 2014).

Maternity Assignment

Pups are non-volant until 6-7 weeks of age (Stern et al. 1997) and spend much of their time nursing. As a result, we could determine the mother's identity when the pup was still nursing during capture. However, as pups get older, they spend less time nursing and are more likely to separate from their mother during capture. Because they were still non-volant during the capture period, we infer that the mother is a member of the harem group, but rely on genetic analyses to identify the specific female (see below).

We extracted DNA wing punches using a PureGene tissue kit (Qiagen). To identify potential mothers, we amplified a region of the mitochondrial control region via polymerase chain reaction (PCR) using primers P* (Wilkinson et al. 1997) and E

(Wilkinson and Chapman 1991). All reactions had a final volume of 25 μL with 1.5mM $MgCl_2$, 0.2 mM dNTP mix, 1.25 U Taq polymerase (2X Taq Master Mix, Apex Bioresearch) and 0.24mM of each primer. We used an annealing temperature of 55 °C and the thermal cycling program described by Meyer and colleagues (2009). PCR product were subsequently purified and sequenced from the P* end on an ABI 3730xl by Eton Bioscience Inc. We screened and aligned sequences using Sequencher v. 5.4 (Gene Codes Corp).

We used eight microsatellite loci for genotyping, of which three were previously used in this species, one was previously developed for *Desmodus rotundus*, and four were newly developed based on 454 junior pyrosequencing data (Table 2.2, Table S2.1). All PCR were performed using either a fluorescently-labeled forward primer or M-13 labeled forward primer with an M-13 labeled fluorophore (Schuelke 2000). All reactions had a total volume of 10 μL with 1.5mM $MgCl_2$, 0.2 mM dNTP mix and 1.25 U Taq polymerase (2X Taq Master Mix, Apex Bioresearch). When the forward primer was fluorescently labeled, the final concentration of each primer was 0.5 mM. When using the M-13 method, the forward primer had a final concentration of 0.13 mM while the reverse primer and the M-13 labeled fluorophore each had final concentrations of 0.5 mM. All PCR reactions were run on a touchdown thermal cycling program with the annealing temperature descending from 64 to 50 °C. (3 min at 95, (15 cycles: 30s at 95, 45s at annealing, 1 min extension at 72), (26 cycles: 30s at 95, 45s at 50°C, 1 min extension at 72), 5 min final extension at 72.) The fluorescently labeled PCR products were separated on an ABI 3730xl DNA

Analyzer (Applied Biosystems) and we used GeneMapper 4.0 (Applied Biosystems) to size and score alleles.

We used GenAlEx v6.5 (Peakall 2012) to calculate Jost's pairwise D_{est} to evaluate genetic differentiation between the three cave populations (Jost 2008). Because populations show significant genetic structuring (D_{est}=0.04, $p<0.01$), the subsequent genetic analyses were performed separately for each cave. To estimate allele frequencies and test for deviations from Hardy-Weinberg equilibrium (HWE) we used only adult genotypes to avoid introducing bias from mother-offspring and half-sibling relatedness. Tests for HWE were performed in GENEPOP for R (Rousset 2008) and rate of allelic drop-out was estimated with CERVUS 3.0.7 (Kalinowski et al. 2007; Marshall et al. 1998).

For each pup, the initial pool of candidate mothers included all females in the harem group that did not have a known pup. Although we were often able to capture all females present at the harem, it is possible that some members of the group were not present at the time of capture; therefore, we also included females that were present in the harem during the January census when available. We narrowed the pool of candidate mothers to only those with the same haplotype as the given pup. If pups had two or more candidate mothers, we used CERVUS to identify the most probable mother at a minimum of 95% confidence allowing for a 1% genotyping error rate.

Paternity Assignment

To evaluate paternity, we used the likelihood-based exclusion method described by Lemons et al. (2015). Typically, CERVUS is used to assign parentage to specific individuals, however, with a slight modification, the same computational framework can be used to estimate the likelihood that a pup is not directly related to the focal male. Pups were classified as extra-group offspring if we could exclude the resident harem male with 95% confidence. Because this approach requires the user to estimate the number of candidate males and the proportion of candidates sampled, we repeated the simulation under a range of parameter estimates to assess the robustness of the results. With the exception of two pups, assignments were consistent across parameter variations. The two pups with inconsistent results were classified as extra-group offspring under most parameter estimates and each mismatched their respective harem males at one locus. As a result, we classified them as extra-group offspring.

Based on the social mating system and previous evidence (McCracken and Bradbury 1977), the harem male is expected to sire most offspring. If the harem male captured with the group is not the likely sire of at least 30% of the offspring (i.e. extra-group paternity rate exceeds 70%), then we did not consider that male to be the resident harem male. It is more likely that he is either a temporary visitor or became the resident male after the end of the mating period. These groups were thus re-analyzed assuming the harem male is unknown.

Without a paternal genotype, we could not directly assess paternity. Instead, we inferred the paternal genotype that could best account for the greatest number of

offspring in the group. This method is likely to underestimate the rate of extra-group paternity, as we are more likely to infer a heterozygous genotype when the group of pups has more than 2 alleles at a given locus. We used this method for two harems that were captured with a harem male and three groups for which the initial extra-group paternity estimates exceeded 70%. When a group contained three or more extra-group pups, regardless of whether the harem male was known, we used a similar approach to infer the minimum number of extra-group sires necessary to account for all extra-group pups in a harem.

Because we captured multiple harems from Tamana in 2013, we could then ask if other harem males are potential sires of the extra-group offspring. To address this question, we used the same exclusion methods as above, but the set of candidate sires included all harem males sampled from Tamana in 2013.

Statistical analyses

Using generalized linear models (GLM), we evaluated the effect of male attributes on the rate of extra-group paternity (number of extra-group offspring / total number of offspring); independent variables include male tooth wear, forearm length, body condition, group size and all first-order interactions. Continuous predictors were scaled prior to analysis and, because the response is the proportion of extra-group paternity per group, we fit the model with a binomial error distribution, logit link function, and weighted it by total number of pups in the group. All possible models were ranked via the corrected Akaike's information criterion (AICc) and models

within four AICc of the top model (ΔAICc < 4) were averaged (Barton 2017). We report full averages, such that coefficients are averaged across all models, not just the subset in which the variable appears.

At the individual level, we examined the effect of maternal attributes on the likelihood of producing an extra-group offspring using a generalized linear mixed model (GLMM) with a binomial error distribution, logit link function, and harem identity included as a random effect. Predictors included maternal forearm length, maternal tooth wear, the difference between the mother's tooth wear and the group median, and the absolute difference between the pup's estimated birthdate and the harem's median birthdate (measured in days). As before, AICc scores were used to rank and select models for averaging.

To evaluate the effect of harem attributes, we fit another set of GLMs and made use of a larger dataset, which includes the five harems for which the harem male was not captured. Independent predictors include group size, birth synchrony (mean absolute deviation from group median), mean female tooth wear, and mean forearm length. Standard deviations of tooth wear and forearm length were also included as measures of group heterogeneity. All statistical analyses were performed in R version 3.5.1 (R Core Development Team, 2018).

Results

Maternity Assignment

A portion of the mtDNA control region was sequenced for 82 pups and 127 adult females captured in Tamana Cave in 2013. A total of 31 variable sites were identified in the 325 bp alignment, producing 27 unique haplotypes. On average, each harem group contained 9.44 ± 0.60 (mean ± SE) haplotypes. Within a harem, each detected haplotype was shared by 1.47 ± 0.08 adult females (Figure 2. 2). Sixteen behaviorally determined mother-pup pairs had matching haplotypes, supporting our expectation that females captured with a nursing pup were indeed mother-pup pairs. For pups captured unattached to a female, the number of candidate mothers within the harem ranged from zero to five females (1.76 ± 0.15 females/pups) based on haplotype data. None of the bats caught prior to 2013 were sequenced, but only six of the 84 pups were captured unattached to a female.

After defining the pool of candidate mothers based on haplotypes, 46 pups were assigned to females based on microsatellite genotypes and 36 pups could not be assigned to any females with at least 95% confidence presumably because their mother evaded capture. Of the 159 mother-pup co-captures, only six showed genotypic inconsistencies that could not be explained by allelic dropout. Allonursing is not known in this species, but pups can grab onto another female during the disruption of capture. For subsequent paternity analyses, these six pups were treated as though the mother was unassigned. In total, 199 of the 241 pups were assigned a mother prior to paternity analyses.

Paternity Assignment

Among the 241 genotyped pups, we identified 34 pups (14.1%) for which the harem male could be excluded as the sire. Of the 18 groups examined, 13 (72.2%) had at least one extra-group offspring, with an average of 1.89 ± 0.48 (14.77% ± 3.48%) extra-group pups per group. The four harems with the greatest number of extra-group offspring (3 - 5 offspring) had at least two different extra-group sires. Tamana and Guanapo caves showed similar rates of extra-group paternity, but no extra-group offspring were detected in Caura (Table 2.3). Within Tamana, the average per-harem rate of extra-group paternity varied among the three chambers (4.1% - 27.9%), but not significantly ($F_{2,7}$=2.34, p=0.17).

Among the ten harems (122 pups) sampled in Tamana in 2013, we detected 19 extra-group offspring. When all harem males from that cave and year are included as potential candidate sires, all males were excluded at 95% confidence for 14 of the 19 extra-group pups. The remaining five had at least one harem male from a different group that could not be excluded as the potential sire. Therefore, most of the extra-group offspring were sired by males that were not included in our analyses; these could be bachelor males or harem males from 10-15 unsampled harems.

Predictors of extra-group paternity

When evaluating the effect of male morphology on the rate of extra-group paternity, seven models were within four AICc of the top model. Male condition was the most important variable, appearing in all seven models, while forearm length, tooth wear and group size each appeared in a subset of the models (Table 2.4). After model averaging, male condition was the only predictor with a coefficient different from zero, such that males with greater body condition experience lower rates of cuckoldry (Figure 2. 3, Table 2.5).

When examining harem attributes, the top model set (ΔAICc < 4) contained 13 models (Table 2.6). Among these models, the standard deviation of female tooth wear and birth synchrony were the most important predictors, but tooth wear variation was the only predictor with a non-zero coefficient in the averaged model (Table 2.7). Although all other terms appeared within the averaged model, their effects were smaller and importance scores were lower (Table 2.7). When considering male and female attributes simultaneously, male condition is the only significant predictor of extra-group paternity rate. Variation in female tooth wear is no longer significant, but this difference is likely due to the reduced sample size (N = 13) and increased number of models contributing to the average model (Table S2.2 & S2.3).

Modeling paternity at the level of the individual produced 14 models with ΔAICc <4, of which, the null model had the lowest AICc (Table 2.8). Measures of female tooth wear and forearm length, both absolute and relative to group medians, do not predict offspring paternity and neither does timing of parturition relative to the

group. Morphologically, mothers of within-group pups are not different from mothers of extra-group pups with regard to forearm length ($t = 0.20$, $df = 35.04$, $p = 0.84$) and tooth wear ($t = 0.79$, $df = 33.58$, $p = 0.44$).

Discussion

We examined the occurrence of extra-group paternity in three wild colonies of greater spear-nosed bats, *Phyllostomus hastatus*. This species' social mating system is one of strong female-defense polygyny, but genetically, we find that males are often unable to defend female groups completely. Across all groups, at least 14% of the offspring are sired by a male other than the resident harem male, although the rate of extra-group paternity varies between caves and among harems. We did not detect any extra-group offspring within one colony, while the others averaged 16.5% extra-group paternity. Most harems had at least one extra-group offspring, with the average harem having approximately two extra-group offspring. Variation in the rate of extra-group paternity within a harem is inversely related to the body condition of the harem male and positively correlated with variation in female age (as measured by toothwear) within the harem. We found no effect of male age or group size. Additionally, female age, body size, and birth synchrony did not predict paternity of individual pups.

Compared to other mammals, the observed rate of extra-group paternity in P. hastatus is relatively low. Across the 26 species reviewed by Isvaran and Clutton-Brock (2007), the mean extra-group paternity rate is 18%; however, that mean rises to

29% when considering only those species that have at least some extra-group paternity. Species with larger female breeding groups tend to have higher rates of extra-group paternity. Many of the species included in recent interspecific analyses have small harems of 4-5 females (Isvaran and Clutton-Brock 2007; Isvaran and Sankaran 2017; Lukas and Clutton-Brock 2014) with one notable exception, the Southern elephant seal (*Mirounga leonina*). With harems often exceeding 40 individuals, extra-group paternity rates range from 25-40% among seal populations (Fabiani et al. 2004; Hoelzel et al. 1999). Blue monkeys (*Cercopithecus mitis*) also defend large harems (5 - 25 females) and approximately 40% of offspring in single-male groups are sired by an extra-group male (Roberts et al. 2014). Given the large harems of *P. hastatus*, averaging 19 females per harem, one might have predicted a greater rate of extra-group paternity than we observed. One caveat is that our estimates of extra-group paternity are minimum estimates, as some extra-group paternity may have gone undetected given our methods.

Among harems, the rate of extra-group paternity is influenced by harem male body condition, but not skeletal body size. Harem males that are heavier for their size have lower rates of extra-group paternity within their harem than lighter males. This finding is consistent with the expectation that stronger, more competitive males will be heavier and better at defending females (Andersson 1994). Compared to bachelors, harem males are in better condition (Adams et al. 2018). Although correlational, this evidence suggests that good condition is necessary for harem acquisition. Given the energetic demands of defense (Kunz et al. 1998), it seems less likely that condition

increases as a result of harem acquisition. The effect of variation in condition that we find in this study indicates that condition is important for the continued defense of the harem.

From behavioral observations, we know that the resident male will approach and repel males that attempt to enter his harem site (McCracken and Bradbury 1981). Extra-group matings have not been directly observed in this species, but it seems unlikely that extra-group mating would successfully occur while the harem male is present, regardless of his condition. These interactions, however, may allow males to communicate their strength and the risk of retaliation to opportunistic extra-group males.

At night, females typically forage in one or two long bouts, but harem males forage in short bouts, making frequent trips back to the roost. This strategy is energetically costly, but allows males to maintain vigilance at the roost site (Kunz et al. 1998). Furthermore, we have observed males temporarily occupying an empty harem site that is not their own at night, perhaps waiting for unattended females to return. Daytime interactions between males may communicate the harem male's strength and dissuade extra-group males from seeking copulations with unattended females. Greater body condition may also allow males to reduce foraging trips and allocate more time to vigilance. A more careful examination of which locations are visited by extra-group males at night, as well as when and where extra-group copulations occur will allow us to better understand how males in good condition maintain better control over within-group paternity. While male-male competition

and female defense are clearly important in this mating system, we also cannot exclude the possibility that heavier males are more attractive to females and thus females are less likely to seek extra-group matings when the harem male is in good condition.

We found no effect of male age on the rate of extra-group paternity in his harem. The harem males included in our study, however, have limited variation in tooth wear (2 - 3.5 on a 5-point scale). Our extensive mark-recapture records (see Wilkinson et al. 2016) show that the average male's harem tenure is two years and their lifespan is short compared to females. In that dataset, we have 464 males for which we have minimum age estimates and less than 2% of males reach five years of age and only a single male reached nine years of age. By contrast, the females in this study show much greater variation in tooth wear (1 - 5) and the mark-recapture records indicate that females live considerably longer than males. Of the 1835 females for which we have minimum age estimates, 18% lived at least 5 years and 5% lived at least 10 years. These differences in longevity and reproductive life histories are consistent with intense male-male competition (Clutton-Brock and Isvaran 2007; Lukas and Clutton-Brock 2014; Promislow 1992) and given the limited time during which males retain harem status, a lack of an age effect is perhaps unsurprising. How age influences a male's ability to gain extra-group fertilizations is not yet known, as we could not identify extra-group sires. In other species, older males are more successful at securing extra-group paternities, particularly when older

males are better at competing for the resources necessary to attract females (Cleasby and Nakagawa 2012; Ward et al. 2014).

If extra-group matings are coerced, we expect that larger or older females would be better able to resist coercive males, and thus be less likely to have extra-group offspring. Alternatively, we might expect the reverse pattern if a female's ability to express choice for extra-group sires is restricted by a physically controlling social mate. Among our study population, we found no relationship between maternal age or size and the paternity of her offspring. Thus, if extra-group copulations are coerced, larger females do not have any resistance advantage. However, males are larger than females and possess larger canine teeth (see Chapter 1), so it is also possible that females, regardless of size or age, do not attempt to resist due to the risk of injury (Smuts and Smuts 1993; Wong and Candolin 2005). Male *P. hastatus* do not restrict female movement (McCracken and Bradbury 1981) and thus females do not need to be larger or older to overcome or escape their social mate.

Although we found no effect of the average age and size of females within a harem, the variation in female age within the group influences the rate of extra-group paternity. Harems with a heterogeneous age structure have higher rates of extra-group paternity than more uniformly-aged groups. However, neither the oldest nor youngest members of the harem are more likely to produce extra-group offspring. The overall effect of age heterogeneity suggests extra-group paternity rate is a 'meta-trait' of the group, driven by the social dynamics within the harem, rather than individual mating strategies (Maldonado-Chaparro et al. 2018). One potential explanation for this

pattern is that group heterogeneity reflects its history. When females disperse from their natal group, they may form new groups with members of the same cohort, or join existing groups (McCracken and Bradbury 1981). As a result, new harems will have less age variation than existing harems with recent additions. Why new harems would have less extra-group paternity is unclear, but further examination of the ecological and social factors that influence harem formation and stability may explain the observed relationship between age heterogeneity and paternity.

As mentioned previously, interspecific (Isvaran and Clutton-Brock 2007) and intraspecific (Ortega and Arita 2002) patterns reveal that monopolizing paternity is more difficult when harems are large. However, we did not find an effect of group size; large harems were no more susceptible to cuckoldry than small harems. Among blue monkeys, the number of females simultaneously in estrus affected the siring success of the resident male (Roberts et al. 2014); therefore, female synchrony may be the more important measure. We found no significant effect of birth synchrony, but this may not be a reliable proxy of estrus synchrony, as bats are often able to manipulate the timing of fertilization and gestation (Racey and Entwistle 2000). Additionally, increasing the number of females in the group may not actually increase defense requirements because they are spatially discrete and females roost in tightly packed groups.

The spatial distribution of breeding pairs has been proposed to explain variation in extra-pair paternity among birds (Birkhead 1978); however, support has been mixed (Schlicht et al. 2015; Westneat and Mays 2005; Westneat and Sherman

1997). Qualitatively, the difference in extra-group paternity rates between the caves suggests that the density and spatial distribution of harems within a colony may influence defensibility. The colony in Caura cave, in which we detected no extra-group paternity, is the smallest of the colonies with only 3-5 harems present each year we visited. The solution depressions in which they roost are in close proximity to one another, but are very deep. In contrast, Guanapo cave is smaller, but houses a much larger colony. Many of the roost sites are in very close proximity and the depressions are relatively shallow, making it easier to move between sites. Tamana cave is much larger and also contains a large colony with the harems distributed among three connected chambers. Although the extra-group paternity rate did not differ significantly between chambers, the variation qualitatively reflects the spatial distribution of the harems. The chamber with the fewest harems and most space between harems had the lowest rate of extra-group paternity (4.2%), while the chamber with the most tightly packed groups had the highest rate of extra-group paternity (27.8%).

Furthermore, different roost sites may vary in quality. When social mate pairing is based on resource defense, location within the colony may reflect male quality. For example, male great cormorants (*Phalacarocorax carbo sinensis*) that nest at the periphery of the colony are inferior quality and also suffer greater rates of extra-pair paternity, regardless of overall nesting density (Minias et al. 2016). Within the caves used by *P. hastatus* different roost sites may be more favorable than others due to risk of predation. However, in this female-defense system, males are not

defending roost sites to attract females. Instead the females occupy the roost and are subsequently defended by the male (McCracken and Bradbury 1981). The location and quality of the roost site may influence recruitment and stability of the female group. The potential effect of spatial distribution may be worthy of further study, especially if additional colonies of varying density and distribution can be located.

In summary, we found that despite their large size and highly mobile females, *P. hastatus* harems contain few extra-group offspring. Variation between harems suggests that prevention of extra-group paternity is driven by a male's ability to defend his harem, as males in better body condition suffer fewer paternity losses. While this finding is also consistent with a female preference for high condition males, teasing apart these effects will require further investigation. Furthermore, the effect of female age heterogeneity on the rate of extra-group paternity raises several interesting questions about how within-group dynamics affect the genetic mating system.

Tables

Table 2.1 Model results relating harem male body mass to forearm length and time of day ($F_{3,164} = 13.8$, $p < 0.001$, $R^2=0.20$). This model was fit using measurements from 168 harem males captured between 1990 and 2015, and then used to calculate the condition (residual mass) for the harem males included in this study (see text for details).

	Estimate ± SE	t	p
Intercept	368.81 ± 128.89	2.86	<0.01
Forearm length	-3.36 ± 1.54	-2.18	0.03
Time of day	-26.54 ± 8.67	-3.06	<0.01
Forearm * Time	0.32 ± 0.10	3.10	<0.01

Table 2.2 Summary of microsatellite loci

Primer	Total alleles	Population	N	Alleles	H_o	H_e	f(Null)
AjA74	7	Tamana	78	5	0.65	0.75	0.0721
		Guanapo	7	5	0.86	0.77	
		Caura	-	-	-	-	-
Ts3Ca2	9	Tamana	207	9	0.64	0.64	0.0001
		Guanapo	118	7	0.75	0.68	-0.0576
		Caura	55	7	0.64	0.65	0.0084
Ts2Ca1	8	Tamana	200	7	0.77	0.76	-0.0048
		Guanapo	61	7	0.74	0.69	-0.0433
		Caura	53	6	0.76	0.73	-0.0145
Dr19	7	Tamana	200	6	0.68	0.64	-0.0352
		Guanapo	95	6	0.70	0.66	-0.0248
		Caura	55	4	0.53	0.62	0.0912
Phast01	5	Tamana	207	4	0.71	0.69	-0.0156
		Guanapo	120	5	0.75	0.73	-0.0141
		Caura	54	4	0.72	0.72	0
Phast04	2	Tamana	164	2	0.40	0.44	0.0417
		Guanapo	14	2	0.36	0.45	0.0997
		Caura	-	-	-	-	-
Phast07	5	Tamana	205	5	0.42	0.40	-0.0364
		Guanapo	114	5	0.44	0.50	0.0614
		Caura	49	4	0.61	0.59	-0.0271
Phast09	6	Tamana	205	6	0.64	0.65	0.0042
		Guanapo	116	6	0.56	0.60	0.0313
		Caura	55	5	0.75	0.73	-0.0127

N = number of adult individuals sampled. Pups were excluded from these measures due to non-independence resulting from parent-offspring sampling. Observed and expected heterozygosity (H_o and H_e, respectively) and the frequency of null alleles were all calculated via CERVUS v. 3.0.7

Table 2.3 Distribution of extra-group paternity

	Tamana	Guanapo	Caura
N groups	11	4	3
N pups	137	69	35
Total extra-group offspring	22	12	0
Overall extra-group paternity rate	16.1%	17.4%	0.0%
Range of extra-group paternity rates per-group	0.0 - 53.8%	5.8 - 27.8 %	0.0%
Mean extra-group paternity rate per-group	16.5%	16.6%	0%

Table 2.4 GLMs predicting the effect of three male attributes and group size on the proportion of extra-group offspring present in the harem. Presented are the top-ranked models used for model averaging (ΔAICc < 4). C = body condition, T = tooth wear, F = forearm length, G = group size

Model	df	logLik	AICc	delta	weight
C	2	-21.46	48.11	0	0.32
C + F	3	-19.97	48.61	0.50	0.25
C + T	3	-20.50	49.66	1.55	0.15
C + F + T	4	-18.51	50.02	1.91	0.12
C * F	4	-19.12	51.24	3.12	0.07
C + G	3	-21.44	51.54	3.43	0.06
C * F + T	5	-16.64	51.85	3.73	0.05

Table 2.5 Model-averaged coefficients ± SE for models of the rate of extra-group paternity within groups (Table 3). Coefficient estimates are full averages calculated over the set of selected models.

Variable	Estimate ± SE	95% CI	Importance
Intercept	-2.18 ± 0.37	(-2.91, -1.45)	
Male condition	**-0.33 ± 0.14**	**(-0.61, -0.05)**	**1.00**
Male forearm	-0.09 ± 0.24	(-0.55, 0.38)	0.48
Male tooth wear	-0.26 ± 0.52	(-1.27, 0.76)	0.32
Male forearm * condition	0.02 ± 0.09	(-0.16, 0.21)	0.11
Group size	-0.00 ± 0.02	(-0.03, 0.03)	0.06

Table 2.6 GLMs predicting the effect of harem attributes on the proportion of extra-group offspring present in the harem. Presented are the top-ranked models used for model averaging (ΔAICc < 4). B = birth synchrony, T = average female tooth wear, F = average female forearm length, sT = standard deviation of tooth wear, sF = standard deviation of forearm length, G = group size

Model	df	logLik	AICc	delta	weight
sT + B	3	-30.39	68.50	0	0.23
sT	2	-32.48	69.75	1.25	0.13
sT + B + G	4	-29.70	70.48	1.98	0.09
sT + B + T	4	-29.72	70.51	2.01	0.09
sT + G	3	-31.43	70.58	2.08	0.08
sT + sF	3	-31.59	70.89	2.39	0.07
sT + T	3	-31.66	71.03	2.53	0.07
sT + B + F	4	-30.30	71.67	3.17	0.05
sT + B + sF	4	-30.39	71.85	3.35	0.04
sT + B + F * T	6	-26.11	71.86	3.36	0.04
sT + F	3	-32.19	72.09	3.59	0.04
sT + T * F	5	-28.58	72.16	3.66	0.04
sT + F + T	4	-30.63	72.33	3.83	0.03

Table 2.7 Coefficients of average model of harem attributes on the proportion of extra-group offspring present in the harem. Coefficient estimates are full averages calculated over the set of selected models.

Variable	Estimate ± SE	95% CI	Importance
Intercept	-1.98 ± 0.22	(-2.45, -1.52)	
Toothwear SD	**2.59 ± 1.00**	**(0.45, 4.73)**	**1.00**
Birth sync	0.22 ± 0.26	(-0.30, 0.74)	0.54
Mean tooth wear	-0.16 ± 0.40	(-0.99, 0.66)	0.27
Group size	0.01 ± 0.03	(-0.05, 0.07)	0.20
Mean forearm	0.09 ± 0.30	(-0.54, 0.72)	0.17
Forearm SD	-0.06 ± 0.32	(-0.72, 0.60)	0.11
Mean forearm * mean toothwear	-0.23 ± 0.92	(-2.08, 1.61)	0.08

Table 2.8 GLMMs predicting paternity (extra-group or within-group) in response to maternal attributes and birth timing. Presented are the top-ranked models used for model averaging (ΔAICc < 4). TD = difference between maternal tooth wear and group median, T = maternal tooth wear, BD = difference between birth date and group median birth date, and F = maternal forearm.

Model	df	logLik	AICc	delta	weight
Null	2	-75.01	154.1	0	0.21
TD	3	-74.48	155.1	1.02	0.12
T	3	-74.65	155.42	1.35	0.11
BD	3	-74.74	155.60	1.52	0.10
F	3	-75.00	156.13	2.06	0.07
T*F	5	-72.95	156.20	2.13	0.07
TD + BD	4	-74.36	156.92	2.85	0.05
T + TD	4	-74.41	157.02	2.95	0.05
F + TD	4	-74.48	157.16	3.09	0.04
T + BD	4	-74.50	157.20	3.13	0.04
T + F	4	-74.7	157.50	3.43	0.04
F + BD	4	-74.74	157.68	3.60	0.03
T*F + TD	6	-72.64	157.72	3.65	0.03
T*F + BD	6	-72.77	157.96	3.89	0.03

Figures

Figure 2.1 Photograph of several harems in Tamana cave (second chamber). Each harem occupies a discrete solution depression. In this part of the cave, harems are tightly clustered, whereas other areas (not shown) have more space between solution depressions. (Photo credit: G. Wilkinson)

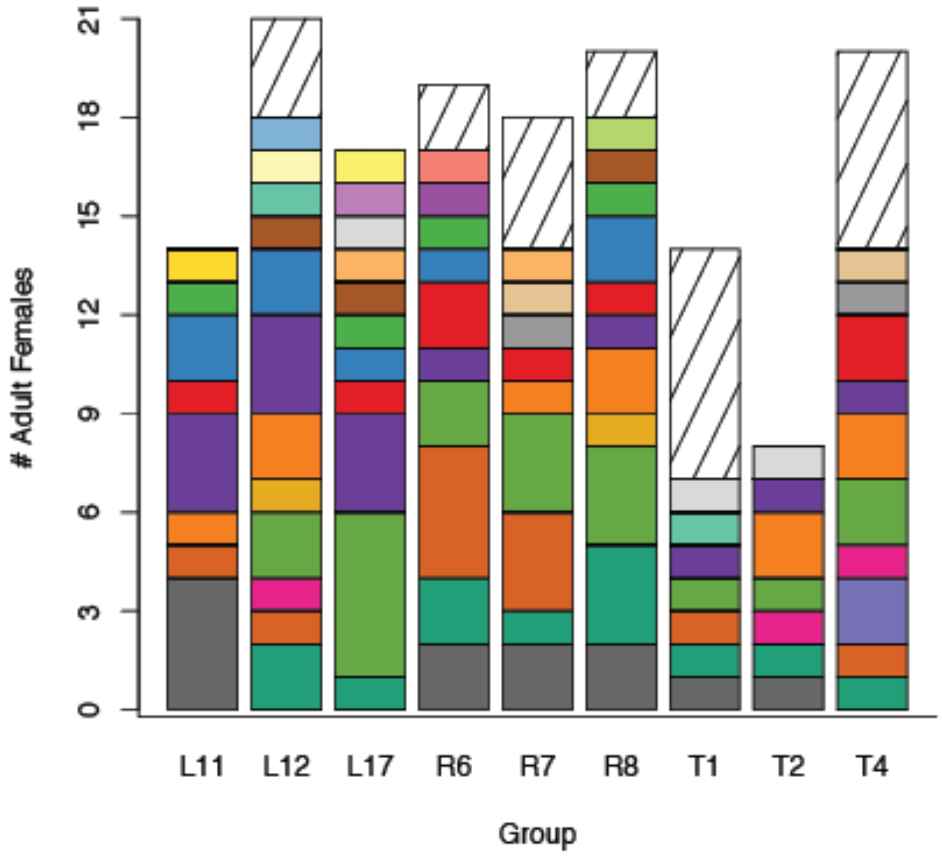

Figure 2.2 Distribution of unique haplotypes among adult females of nine harems in Tamana Cave. Each color represents a unique haplotype, except white with black hatching represents unknown haplotypes. Total height of each bar represents the number of females in the harem.

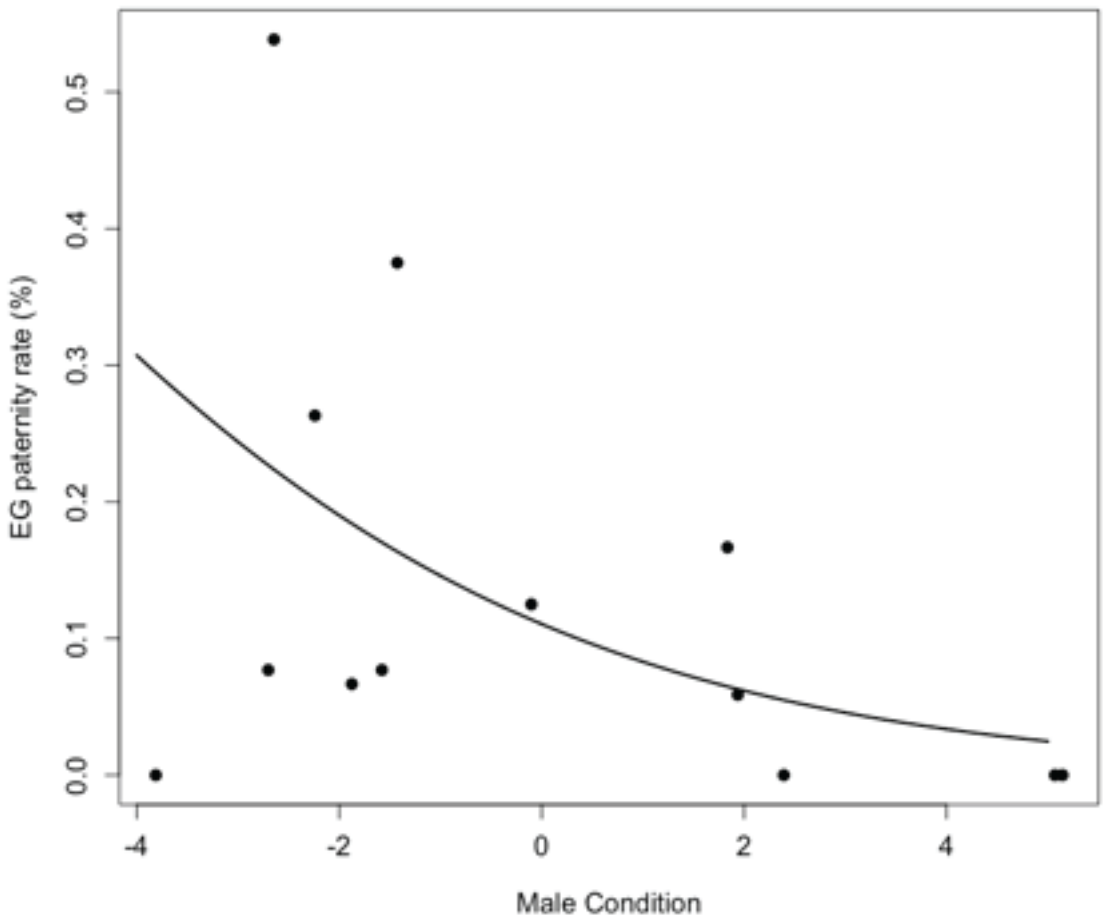

Figure 2.3 The rate of extra-group paternity detected within a harem as a function of harem male body condition. Each point represented a single harem and the line represents the extra-group paternity rate predicted by the average model, while holding the other terms at zero (i.e. the mean after centering the data).

Chapter 3: Male scent gland signals mating status in greater spear-nosed bats, *Phyllostomus hastatus*

Abstract

Chemical signals are ubiquitous, but often overlooked as potentially important for conveying information relevant for sexual selection. The male greater spear-nosed bat, *Phyllostomus hastatus*, possesses a sexually dimorphic gland on the chest that produces an odoriferous secretion. Here, we investigate the potential for this glandular secretion to act as a sexually selected olfactory signal by examining gland activity in and out of the mating season and determining if variation in its chemical composition reflects variation in male mating status or attributes of the individual. Based on gas chromatography-mass spectrometry (GC-MS) measurements of samples collected from wild bats roosting in caves in Trinidad, West Indies, we find that males that defend and roost with groups of females (harem holders) have significantly different chemical profiles from males found roosting in all male groups (bachelors). Additionally, profiles differed significantly among individuals. Taken together, these results suggest that this chemical signal has the potential to communicate both mating status and individual identity and thus could be used to mediate interactions among individuals within this harem-based social mating system.

Introduction

Sexual selection has produced elaborate signals for advertising competitiveness to rivals or quality to potential mates (Darwin 1871). Communication between rivals is important for mediating intrasexual competition by reducing direct conflict, particularly in species where strong male-male competition may cause injuries (Andersson 1994). In the presence of female choice, signals may reveal desirable features to potential mates, such as genetic quality or provisioning ability. While acoustic and visual signals are commonly studied modalities, increasing evidence indicates that olfactory signals can also play a role in sexual selection and provide information about competitiveness and mate quality (Johansson and Jones 2007; Martin et al. 2018; Penn and Potts 1998; Rich and Hurst 1998).

Olfactory signals can communicate information in multiple ways, ranging from the rate and placement of signals to their chemical content. In territorial species, scent marking the perimeter of a territory is a common behavior. Because investment in odorant production and time spent depositing scent marks is costly (Gosling and Roberts 2001; Gosling et al. 2000; Harris et al. 2018; Radwan et al. 2006), the abundance of marks and the ability to countermark act as a signal of competitive ability to both rivals and potential mates (Fisher et al. 2003; Rich and Hurst 1998, 1999). Similarly, the height of a scent mark left on a vertical substrate by a terrestrial mammal can reveal the signaler's body size, which is often correlated with competitive ability (Sharpe 2015). Many mammalian olfactory signals are complex

chemical blends, and variation in the presence/absence or relative abundance of constituents can reveal attributes of the signaler relevant to intra- and inter-sexual selection, including social status (Buesching et al. 2002a; Setchell et al. 2010), body condition (Buesching et al. 2002a; Buesching et al. 2002b; Ferkin et al. 1997), age (Caspers et al. 2011; Leclaire et al. 2014), parasite load (Kavaliers and Colwell 1995; Munoz-Romo and Kunz 2009; Penn and Potts 1998), immunocompetence (Rantala et al. 2002; Zala et al. 2004), and hormone levels (Burgener et al. 2009). The connection between these traits and the chemical profile is mediated by shared physiological and genetic mechanisms. For example, several studies report an association between chemical profiles and genetic diversity at major histocompatibility complex (MHC) loci (e.g. Lanyon et al. 2007; Radwan et al. 2008; Setchell et al. 2011). The MHC influences immune function in vertebrates, thus affecting the parasite load (Kurtz et al. 2004; Westerdahl et al. 2005) and microbial community (Bolnick et al. 2014; Kubinak et al. 2015), which can subsequently affect olfactory profiles (Archie and Theis 2011; Lanyon et al. 2007; Penn and Potts 1998). Through various, often unknown, physiological connections, odor can also indicate genetic diversity or heterozygosity at non-MHC loci (Leclaire et al. 2012; Overath et al. 2014; van Bergen et al. 2013), as well as genetic relatedness and compatibility (Charpentier et al. 2008; Charpentier et al. 2010; Penn 2002; Thomas and Simmons 2011).

Bats, the second most speciose order of mammals, possess a diversity of scent-producing glands on the face, around the genitals, and in the subaxillary region (Bloss 1999; Brooke and Decker 1996; Rehorek et al. 2010; Scully et al. 2000) and

rely on chemical communication in multiple contexts (Bloss 1999; Dechmann and Safi 2005). Prior studies have demonstrated the utility of scent in the discrimination and recognition of colony members (Bloss et al. 2002; Bouchard 2001; Safi and Kerth 2003), individuals (Safi and Kerth 2003), and offspring (Gustin and McCracken 1987). However, with few exceptions (Caspers et al. 2008; Santos et al. 2016; e.g. Voigt and von Helversen 1999), the role of chemical communication in courtship and mating interactions is largely unknown among bats. The sexual dimorphism of the glands and scent-dispersing structures on many species (Bouchard 2001; Hickey and Fenton 1987; Scully et al. 2000; Tavares and Tejedor 2009), suggests that odor plays a role in intra- and intersexual interactions of many bats, which have diverse mating system types (McCracken and Wilkinson 2000).

In this study, we focus on the greater spear-nosed bat, *Phyllostomus hastatus*, in which adult males possess a large sebaceous gland on their chest that produces a thick white secretion with a pungent odor (Figure 3.1). This gland is sexually dimorphic, as it is rudimentary and lacks secretory elements in females (James 1977). In addition to the glandular dimorphism, *P. hastatus* exhibits sexual size dimorphism (SSD), with males larger than females (McCracken and Bradbury 1981). Although this male-biased SSD is common among mammals (Weckerly 1998), it is atypical for bats, which often show reversed SSD due to the demands of flight during pregnancy and lactation (Ralls 1976). These dimorphic features are consistent with strong sexual selection in *P. hastatus*, which is further supported by their social behavior.

Like many other phyllostomid bats, *P. hastatus* exhibits female-defense harem polygyny, but the large size and long-term stability of the harem groups is relatively unique (McCracken and Wilkinson 2000) and creates ample opportunity for sexual selection (Shuster and Wade 2003; Wade and Shuster 2004). Each harem group consists of 10-25 unrelated females (McCracken and Bradbury 1977; McCracken and Bradbury 1981) and is defended by a single male, who can retain tenure at a harem for up to four years (Wilkinson et al. 2016). Harem males smear the secretion from the chest gland onto the fur of the females within their harems, thus giving both males and females a pungent odor (McCracken and Bradbury 1981). Harem males attempt to monopolize matings, but the close proximity of neighboring harems and presence of large bachelor male groups may limit the degree to which harem males control paternity (McCracken and Bradbury 1977). Males in bachelor groups may roost together for many years and some may never attain harem status (Wilkinson et al. 2016). Given the priority access harem males have to mating opportunities, competition for access to females is intense. Evidence of fights is obvious in the many wounds and scars found on males' faces, bodies, and wings (personal observation).

Our aim is to determine if male chest gland secretions can communicate mating status, body condition, or individual identity. Males occasionally make forays into the harems of other males and the resident male drives away the intruders (McCracken and Bradbury 1981). Therefore, advertising status and physical attributes, such as body size and condition, may allow males to assess their opponents

without escalation. By scent-marking the females, harem males may still be able communicate the risk of retaliation to intruders while absent from the harem. Currently, little is known about how males acquire harems or who else sires offspring within harems, but the ability to advertise indicators of quality could potentially facilitate both of these events. Additionally, individuals in neighboring harems are likely to have repeated interactions due to the stability of roosting locations (McCracken and Bradbury 1981). Signals of individual identity would facilitate recognition of neighbors, which could mitigate potential conflict (Temeles 1994; Tibbetts and Dale 2007).

In this study we examine the composition of the glandular secretions via gas chromatography-mass spectrometry (GC-MS) and assess the potential information content of the signal by examining how variation in chemical profiles relates to variation in mating status (harem vs. bachelor), body size, body condition, and age of males. Using repeated samples taken from the same individuals over several days, we also evaluate the potential for the odor to reveal individual identity.

Materials and Methods

Study Population

Bats were captured in three caves, Caura (10.7019°N, 61.3614°W), Guanapo (10.6942°N, 61.2654°W), and Tamana (10.4711°N, 61.1958°W) on the island of Trinidad, West Indies from December-January, 2012-2015. Each cave contained a

colony of *P. hastatus* with up to 30 harem groups, some of which were previously banded (Wilkinson et al. 2016). Typically, each harem occupies a separate solution depression in the cave ceiling, but multiple groups may share large depressions and still remain spatially segregated. In Trinidad, *P. hastatus* exhibit a single breeding season from November to January, with most pups born in April (McCracken and Bradbury 1981; Porter and Wilkinson 2001).

We determined male mating status as either harem or bachelor by their roosting associations at the time of capture. We captured entire groups in the cave during the day (11:00 - 19:00) using a bucket extended on poles to the cave ceiling. A single adult male caught with a group of adult females was defined as a harem male. Males from groups containing multiple adult males, and occasionally non-reproductive females, were classified as bachelor males (McCracken and Bradbury 1981).

After capture, bats were held individually in cloth bags while each bat was processed. Previously banded bats were identified by their band number, and unbanded bats were fitted with a numbered metal band (Monel, National Band and Tag, Newport, KY, USA) on their forearm, with males banded on the right wing and females banded on the left. We recorded the mass (Pesola spring scale), forearm length (digital caliper; Chicago Brand, Medford, OR, USA), and degree of tooth wear (using a 5 category scale, cf. McCracken and Bradbury 1981) for each individual. Unless individuals were banded as newborn pups, tooth wear is currently the only way to estimate the age of living adult bats (Brunet-Rossinni and Wilkinson 2009).

Male testes length and width were measured when possible, and testes volume was estimated assuming a prolate spheroid ($V=4/3\pi r_w^2 r_l$, where r_w is half the width and r_l is half the length). The mating season for *P. hastatus* in Trinidad is from November to January (James 1977; McCracken and Bradbury 1981), and so testes were at or near their maximal size during the period of capture. To estimate body condition, we used the residuals from a linear regression of male body mass on forearm length from 154 adult males captured in the three caves. All measurements and samples used in these analyses were collected during the breeding season; however, additional observations of chest gland activity were made during the non-breeding season (April-June 2013, April 2018).

Sample Collection

The male chest gland forms a deep pocket, which can be everted when palpated (Figure 3.1). By gently squeezing the area around the gland, we extruded the white secretion and scooped it directly into a pre-cleaned glass vial with PTFE-lined septum. We wore a fresh pair of powder-free nitrile gloves for each sample to prevent contamination from our skin. After collection, the vials were immediately stored on ice until we returned to the field station where they were stored at -4°C. To identify any potential contaminants, we collected one or more blanks on each sampling day in which we handled the vial exactly as during sample collection, but no sample was added to the vial. Samples and blanks were kept frozen during shipment to the US and then stored at to -80°C until analysis.

In January 2015, we collected 50 samples from 31 individuals, including 20 bachelor and 11 harem males from Tamana Cave. Replicate samples were collected from males recaptured in this cave on subsequent days. Additionally, some males were brought back to the field station (William Beebe Tropical Research Station, Trinidad, West Indies, 10.69253°N, 61.28956°W) and held in individual cages for up to 6 days for behavioral testing, during which time additional samples were collected every two days. We were able to collect two or more replicate samples from 3 bachelor and 11 harem males.

GC-MS Analysis

We isolated the non-polar and weakly polar compounds via an ether-water extraction, using 99.9% extra-pure methyl *tert*-butyl ether (MTBE, Acros Organics) and chromatography-grade water (Fisher Scientific). All glassware was doubly rinsed with MTBE prior to use. For the extraction, we added 500 μL of MTBE and 500 μL of water to each sample. Samples were then vortexed for 45 s and centrifuged for 5 min at 3000 rpm. The MTBE supernatant was transferred to a new solvent-rinsed tube and stored on ice. An additional 500 μL of MTBE was added to the aqueous phase, mixed, centrifuged, and subsequently the MTBE phase was pooled with the previous extraction on ice. This process was repeated for a third round, resulting in approximately 1.5 mL of MTBE extract. Each sample was concentrated to dryness on ice under a stream of ultra-high purity nitrogen, and the dried product was redissolved in 100 μL of MTBE before GC-MS measurements.

Samples were loaded into vials with solvent-rinsed glass chromatography inserts and stored at -80°C until analysis. Just prior to analysis, an internal standard of 2.5 µL of hexachlorobenzene solution (2 mg HCB/mL MTBE) was added to each sample. To prevent extended delays between extraction and GC-MS analysis, the samples were processed in 3 batches, and all samples were analyzed within 36 h of extraction.

The gas chromatography measurements were performed on an Agilent 6890N system coupled with a JEOL high-resolution magnetic sector mass spectrometer (JMS-700 MStation) with the EI ion source (70 eV). The mass spectrometer was operated in the mode of high scan speed and low resolution (1000) with the mass range from 50 to 600 daltons. A silica capillary column (Agilent HP-5MS, 30 m length, 250 µm I.D.) was used with helium (at 1 ml/min) as the carrier gas. Analysis was performed as follows: injection volume was 1 µl, the inlet temperature was 280 °C in splitless mode, the column temperature was programmed from 50 °C at 1.0 min, then increased to 310 °C at the rate of 16 °C/min and then held at 310 °C for another 2.75 minutes.

All chromatographic data pre-processing was done using MALDIquant for R (Gibb and Strimmer 2012). Although this package is designed for MALDI-TOF data, many of the pre-processing functions are also appropriate for chromatographic data. First, we corrected for baseline shift using the SNIP algorithm. Because of slight variations in elution times between samples, chromatograms were aligned first by the internal standard and then further refined using a peak-based method, which employs

a LOWESS warping function, utilizing a preliminary peak list (5-point half-window and a signal-to-noise ratio of 2). After alignment, peaks were automatically detected in all samples using a half-window of 2 and SNR of 0.5. We then used MStation software (JEOL, USA) to obtain the mass spectra, which we used to ensure the aligned peaks represent the same compounds. Peaks that show inconsistent spectra across samples were removed from subsequent statistical analyses. As a result, we retained only the subset of peaks that could be reliably matched and quantified across samples. By ignoring rare or low intensity compounds, differences among individuals or groups will be more conservative. To account for variation in total sample intensity, all analyses are based on relative abundance values, where the total abundance for each sample is defined as the sum of intensities for all retained peaks. Relative abundance proportions were transformed using the arcsine square-root prior to analysis. The sum of the raw intensities (total intensity) was recorded to account for variation in signal strength between samples.

Statistical Analysis

All statistical analyses were performed in R (version 3.3.2, R Core Team 2016) via RStudio (RStudio Team 2015). We fit a generalized linear mixed model (GLMM) with a binomial error distribution and logit link function to test for effects of body size and condition on male mating status, including cave site as a random effect. Some males were captured and measured multiple times and so we calculated their average body size and condition. The significance of each predictor is evaluated via a

likelihood ratio test (LRT) comparing a model with a term of interest to a model without that term. Because tooth wear is scored on a 5-point scale, we used a Fisher's exact test to assess differences between bachelor and harem males. We also compare the degree of testes development using a generalized linear model (GLM) with mating status and season as predictors of testes state (scrotal vs. abdominal) with a binomial error distribution. The significance of each variable is evaluated via LRT as above. We use a t-test to evaluate the difference in testes size between bachelor and harem males that have scrotal testes.

To evaluate the effect of male traits on the chemical profile, we used a permutational multivariate analysis of variance (PERMANOVA) with a Bray-Curtis distance matrix and 9999 permutations. To avoid negative eigenvalues, a constant was added to all non-diagonal dissimilarities (Legendre and Anderson 1999; Oksanen et al. 2016). We chose a Bray-Curtis distance because similarity is based only on compounds that are present in at least one sample, such that the absence of a compound in a pair of samples does not contribute to their similarity. This is especially important when zeros may be the result of detection ability, rather than true absence. Because PERMANOVA models lack AIC values, we used backward model selection, sequentially removing terms with the least explanatory power until all remaining terms were below an α_{crit} of 0.30. We chose a conservative α_{crit} to limit biases resulting from stepwise regression. When multiple terms had similar *p*-values ($p \pm 0.1$), we dropped each one alternately to examine their effects on the remaining terms before progressing. PERMANOVA is sensitive to differences in multivariate

dispersion (Warton et al. 2012), so we also performed an analysis of multivariate homogeneity of group dispersions (betadisper function, vegan pacakage, Oksanen et al. 2016). Although we have replicate gland samples from some males, we included only the first sample collected from each individual in these analyses to remove potential effects of sample order or sample location (captive vs. wild). In the full model, we included male status, body size (forearm length), body condition, presence of scrotal testes, and age (tooth wear) as potential explanatory variables, as well as batch number, to account for potential variation between the three separate GC-MS runs, and the total signal intensity (sum of all raw peak intensities). To evaluate the effect of testes volume, we repeated the analyses using only the samples for which we had testes measurements.

To determine how well male mating status can be discriminated based on chemical profiles, we used a canonical analysis of principal coordinates (CAP: Anderson and Robinson 2003; Anderson and Willis 2003). This constrained ordination method, as implemented by the CAPdiscrim function in the BiodiversityR package (Kindt and Coe 2005), first performs a principal coordinate analysis (PCoA) followed by a linear discriminant analysis (LDA). We selected the number of PCoA axes (m) used in the LDA that provided the highest reclassification rate and significance was evaluated based on 999 permutations. Because this method can use only a single constraint, the effect of signal intensity is not accounted in this method. To further identify which compounds are associated with the status of each male, we

performed an indicator species analysis (Dufrene and Legendre 1997) using the INDICSPECIES package (Caceres and Legendre 2009).

To evaluate the secretion's potential to signal individual identity, we used the same PERMANOVA and CAP methods as above; however, we included males with replicate samples. Factors included in the full model are individual identity nested within mating status, replicate number, batch number, and total intensity. To remove the effect of male mating status, the permutations are constrained by mating status.

Results

Mating status, Morphology, and Age

Bachelor and harem males do not differ in body size, as measured by forearm length (GLMM, $X^2 = 0.00$, $df = 1$, $p = 0.99$); however, they do differ in body condition (GLMM, $X^2 = 4.73$, $df = 1$, $p = 0.03$), such that harem males are heavier than bachelors given their skeletal body size. Additionally, harem males exhibit greater tooth wear than bachelor males (Median score: harem = 3, bachelor = 2; Fisher's exact, $N = 127$, $p < 0.001$), which suggests they tend to be older. The presence of scrotal testes is influenced by both mating status and season, such that harem males are more likely to have scrotal testes (GLM, $X^2 = 39.22$, $df = 1$, $p < 0.001$), and scrotal testes are more common in the breeding season (GLM, $X^2 = 35.03$, $df = 1$, $p < 0.001$). There is no significant interaction between season and mating status (GLM, $X^2 = 1.54$, $df = 1$, $p = 0.21$). When considering only the males with scrotal, and thus

measurable testes, we find no significant difference in testes volume with respect to mating status ($t = 1.18$, $df = 90$, $p = 0.24$).

We observed that harem males maintain a continuously active chest gland, regardless of season ($N = 64$). Most bachelor males also possess an active chest year-round, but 13 of 122 bachelor observations showed an inactive chest gland during the breeding season. These bachelor males also had abdominal testes and tooth wear indicative of young age (score: 2), with one exception who had slightly greater tooth wear (score: 3).

Mating status and Chemical Composition

Automated peak detection detected 62-102 peaks per sample. However, after alignment and manual inspection, several peaks could not be aligned reliably across samples (i.e. the mass spectra were inconsistent) due to low intensity or the tendency for isomers to co-elute. Two peaks (retention times: 16.75 s and 18.09 s) were detected in the blanks run between samples and thus excluded. As a result, only 43 peaks from the GC-MS were retained for statistical analysis (Figure 3.2, Table 3.1). Of these 43 peaks, 33 were detected in all samples, and the remainder were detected in at least 80% of the samples.

We found significant effects of male mating status and batch number on the chemical profile, but no significant effects of body size, condition, presence of scrotal testes, or tooth wear (PERMANOVA, Table 3.2). Similarly, the CAP discriminant analysis on the chemical profiles correctly classified 18 of 20 bachelors and eight of

the 11 harem males, for an overall success rate of 84% ($m = 7$, $p = 0.02$). We found no significant difference in multivariate dispersion between each group (betadisp analysis, $F_{1,29} = 0.01$, $p = 0.97$), indicating there is as much variation in chemical profile among harem males as there is among bachelor males (Figure 3.3). Other male attributes (body size, condition, presence of scrotal testes, and tooth wear) do not significantly affect the GC-MS chemical profile (Table 3.2). Using the subset of males with testes size measurements, we find that testes size is also not a significant predictor of the GC-MS profile ($F_{1,13} = 1.48$, $p = 0.20$). Indicator species analysis identified 10 compounds that show significantly different associations between the two male classes; six are more abundant in harem males and four are more abundant in bachelor males (Table 3.1, Figure 3.4). Interestingly, all six of the harem male indicator compounds are apparently more volatile than the four bachelor male indicator compounds (Table 3.1). Such a sequence is highly nonrandom (Runs test, $Z = 2.67$, $p = 0.01$).

Individual Identity and Chemical Composition

The GC-MS profiles among individual males for which we have replicate samples ($N_{bach} = 3$, $N_{harem} = 9$) differed significantly independent of the effect of status (Table 3.3). We also found a significant effect of the batch, but not replicate. The CAP discriminant analysis, which is unable to account for the batch effect, was able to successfully classify 18 of the 29 samples (62.1%) to the correct individual ($m = 5$, $p > 0.05$).

Discussion

Adult male greater spear-nosed bats maintain an active chest gland throughout the year, regardless of their mating status. The chemical composition of the gland's secretion, however, differs significantly between bachelor and harem males. Based on our analysis of 43 compounds, we found that samples could be assigned to the correct mating status for 84% of individuals tested. This chemical signal thus has the potential to communicate male mating status to both rivals and potential mates. Ten compounds show significant differences between mating statuses; of these, harem male secretions contain greater relative abundance of the earlier eluting (smaller, lighter) compounds, while those of bachelor males exhibit greater abundance of the later eluting (larger, heavier) compounds. This non-random pattern may reflect differences in volatility; however, further analysis of the chemical structures will be needed.

Although we did not find any significant association between the chemical profile and other male attributes, such as body size and condition, we did detect enough inter-individual variation to facilitate individual discrimination or recognition, even after accounting for variation due to mating status. The discriminant analysis for individual identity only had a 62% success rate; however, this method is limited in its ability to account for multiple effects simultaneously. Additionally, the compounds included in our analyses represent only a fraction of the total variation present in the

chemical composition of the glandular secretion. By using MTBE during the extraction, we specifically targeted non-polar and weakly polar compounds, which include volatile compounds. However, less volatile, polar compounds can also play a key role in communication by modifying the rate at which volatiles are released (Greene et al. 2016; Hurst et al. 1998), influencing the microbial breakdown of signal precursors (Ezenwa and Williams 2014), and facilitating the transport of volatiles to olfactory receptors (Briand et al. 2004; Lazar et al. 2004). For example, house mice secrete highly variable, nonvolatile, major urinary proteins (MUPs) that alter the release of volatile compounds from the urine to create individual odor signatures (Hurst et al. 2001; Roberts et al. 2018).

Testosterone can influence glandular signals (Ebling 1977; Lewis 2009), and testis size is often correlated with circulating testosterone levels in mammals (e.g. Lewis 2009; Morrow et al. 2016; Preston et al. 2012), including bats (Martin and Bernard 2000). However, we did not detect an effect of either the presence of scrotal testes or testes size on the chemical profile of the chest gland. All of our samples were taken during the breeding season, when testes reach their maximal size. In *P. hastatus*, testes regress into the abdominal cavity during the non-breeding season (McCracken and Bradbury 1981), but we have observed that the chest gland remains active. Tooth wear of the few males with inactive glands during the breeding season, indicates they are young males whose gland may not yet be fully developed. These males were likely born the preceding spring and recently dispersed to join a bachelor group. We did not observe any inactive chest glands during the non-breeding season;

at this time pups are clearly distinguished from adults, and all adults are at least one year of age. Sampling the gland and hormone levels during different seasons could reveal any seasonal variation in gland composition due to differences in circulating testosterone or reproductive state.

In Trinidad, greater spear-nosed bats mate only during a three month period, but harem males actively defend their females throughout the year (McCracken and Bradbury 1981). Because the chest gland remains active year-round and has the ability to signal status, we believe this sexually dimorphic gland is likely to play a role in mate defense. The use of scent in territoriality is well-documented in several mammals (reviewed by Gosling and Roberts 2001), including another harem-forming bat, the greater sac-winged bat (*Saccopteryx bilineata*). *S. bilineata* harem males rub facial gland secretions on the periphery of their harem site in the evening. Because females have already departed by that time, this behavior has been interpreted to be a signal to potential intruders rather than potential mates (Caspers and Voigt 2009). *P. hastatus* harem males mark their roost sites, as evidenced by stains on the cave ceiling at roost sites, as well as the females within their harem (McCracken and Bradbury 1981). When the harem male is present at the harem site, scent marks may be redundant to other visual or vocal cues; however, nightly foraging trips leave females unattended for periods of time. Because olfactory cues will persist in the male's absence, fresh scent marks on females or the roost site could signal his residency to potential intruders. In addition to the presence or freshness of the scent deposit, territorial scent marks often reveal attributes of the territory holder. However,

of the male traits we measured, only mating status was significantly associated with variation in the chemical profile.

In many species with stable territories, it is common for individuals to be less aggressive to their neighbors, a phenomenon often referred to as the "dear enemy phenomenon" (reviewed by Temeles 1994). One explanation is that neighbors pose less of a threat than vagrants or floaters because established neighbors already have a territory and are less motivated to encroach upon their neighbors' resources (Jaeger 1981; Temeles 1990). Alternatively, reduced aggression may result from recognition of neighbors and remembrance of previous interactions (Getty 1989; Ydenberg et al. 1988). Although the behavioral outcome may be the same, these different causes rely on different signals. The former requires a signal to assess threat level or categorically discriminate territory holders from floaters. For example, the anal gland secretion of territorial Eurasian beavers (*Castor fiber*) signals a male's mating status, and males spend more time investigating the scent of an unfamiliar subordinate than a more dominant, but equally unfamiliar, male (Tinnesand et al. 2013). In *P. hastatus*, harem males may be less of a threat because they already have females with which to mate, whereas bachelors may attempt to steal copulations or usurp the harem male, and thus may present a greater threat. Therefore, signaling mating status may help harem males avoid confrontation when moving throughout the colony. If this is the case, then we would expect the scent of a bachelor male to elicit a stronger defensive response from a harem male than that of another harem male.

If the 'dear enemy effect' arises from recognition of previous rivals, there must be a mechanism to discriminate familiar and unfamiliar individuals, such as an individually-distinct scent (Carazo et al. 2008; Lopez and Martin 2002; Palphramand and White 2007; Rosell and Bjorkoyli 2002). Within the roost, harem males are likely to experience repeated interactions with their neighbors due to the long-term use of specific roosting sites. We have found that *P. hastatus* glandular secretions have the potential to encode identity, but behavioral testing is needed to determine if this variation is relevant to the receivers. Male pale spear-nosed bats, *Phyllostomus discolor*, which possess the same sexually dimorphic chest glands as *P. hastatus*, are able to discriminate familiar and unfamiliar males from scent marks applied at the roost site (Holler and Schmidt 1993).

In addition to functioning for intra-sexual communication, the chest gland may also produce an inter-sexual signal. In harem-based polygyny, it is often assumed that male-male competition selects for the most competitive mates and any role of female choice is often overlooked. However, growing research on sexual conflict reveals that males preferred by females are not always the most competitive in male-male interactions (Hunt et al. 2009; Okada et al. 2014; Swedell et al. 2014; Wong and Candolin 2005). In *P. hastatus*, opportunities for female choice may arise via copulations with males other than the harem resident given that an estimated 10-40% of pups are fathered by a male other than the harem male (McCracken and Bradbury 1981). Although we found the male chest gland secretion does not reflect male age, size, or condition, it may still provide signals for mate assessment if

individual variation in male scent reveals attributes of male quality that are not correlated with competitive ability, such as genetic compatibility.

Additionally, male chemical signals may directly influence females' reproductive state. While there is no evidence of induced ovulation, sperm storage, or extended reproductive delay in *P. hastatus* (James 1977), parturition is highly synchronized (Porter and Wilkinson 2001). The mechanism underlying this synchrony is unknown, but chemical signals are a likely candidate, given their role in reproductive synchrony in other mammals (deCatanzaro 2015; deCatanzaro et al. 2014; Dodge et al. 2002).

The sexually dimorphic development and activity of the chest gland suggests it has been shaped by sexual selection, but once established, the signal may be co-opted for other social functions. Because all females in a harem are marked by the same male, a group signature scent is inadvertently created. This scent may serve as a redundant signal of group identity, as *P. hastatus* females vocally signal group identity via screech calls while flying to and from feeding sites (Boughman 1997; Boughman and Wilkinson 1998). While the message of the two signals may be redundant, the different modalities have advantages in different contexts. Scent marks are produced only by the males and are long-lasting, and short-range signals can be used in a crowded cave. Screech calls, which are typically given outside the cave to coordinate foraging behavior (Wilkinson and Boughman 1998), are independent of the male, of short duration, and signal over a much longer range. A group scent might facilitate cooperation among members of a long-term group via non-vocal recognition

within the roost. Similarly, the greater bulldog bat, *Noctilio leporinus*, forms stable female groups, and females create a group-specific scent by rubbing their heads on the subaxial glands of their group-mates (Brooke 1997). *Noctilio leporinus* females also appear to coordinate movements to and from foraging areas (Brooke 1997). Additionally, adult *P. hastatus* females can discriminate pups from their harem from those of other harems via the pups' isolation calls (Bohn et al. 2007). This group signature is especially important for facilitating cooperative defense of pups (Bohn et al. 2009). Given that pup defense is a costly behavior, especially since group-mates are non-kin, accurate discrimination of group versus non-group is paramount, and signal redundancy may be favored.

Here we have provided chemical evidence to show that male greater spear-nosed bats can advertise both mating status and individual identity by the application of secretions from a scent gland. The observed sexual dimorphism in the gland and scent marking behaviors suggest that this signal serves a role in mate defense, and possibly the acquisition of a harem or the attraction of additional mates. However, further examination of the chemical composition of the secretion and behavioral studies are needed to confirm these possibilities. Exploration of the proximate causes of scent variation, such as hormones, genotype, and microbial community, may also be fruitful.

Compliance with ethical standards

All samples were collected and exported under permits from the Wildlife Section of Forestry Division of Trinidad and Tobago. All methods of capture, handling, and sample collection follow the guidelines set forth by the American Society of Mammalogists and were approved by the University of Maryland Institutional Animal Care and Use Committee (FR-13-77).

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Tables

Table 3.1 Compounds detected via GC-MS and retained for statistical analyses

			Relative Abundance[b] (%)		
Peak No.	Retention Time	HOS[a]	Harem	Bachelor	Indicator[c]
1	8.96		0.64	0.52	
2	9.38	240	0.15	0.16	
3	9.49	210	1.18	1.18	
4	10.73	268	0.47	0.44	
5	10.98	268	0.18	0.24	
6	11.69	258	2.03	1.70	
7	11.94	272	1.78	1.76	
8	12.37	308	4.70	5.13	
9	12.59	308	1.36	1.78	
10	12.69	386	0.37	0.41	
11	12.81	285	2.50	2.04	H*
12	13.46	336	1.08	0.88	
13	13.64	336	1.16	1.46	
14	13.74	324	3.20	2.73	H*
15	13.96	386	2.34	2.25	
16	14.09	386	2.36	2.26	
17	14.29	362	3.20	2.53	H**
18	14.42	327	2.30	1.79	H**
19	14.61	327	1.09	1.01	
20	14.71	341	3.52	2.67	H***
21	14.92	341	4.42	4.45	
22	15.11	358	3.15	3.57	
23	15.24	341	2.28	1.65	H**

			Relative Abundance[b] (%)		
Peak No.	Retention Time	HOS[a]	Harem	Bachelor	Indicator[c]
24	15.50	355	3.39	3.16	
25	15.62	325	2.62	2.33	
26	15.79	386	4.20	4.18	
27	15.97	367	4.92	5.32	
28	16.05	367	1.74	1.21	
29	16.35	383	2.27	2.39	
30	16.45	430	2.37	2.49	
31	16.59	414	2.84	3.35	B*
32	16.90	451	2.73	2.94	
33	17.37	508	4.82	4.96	
34	17.55	508	4.30	5.09	B***
35	17.69	465	1.50	2.39	B**
36	17.82	522	3.57	3.17	
37	17.92	479	2.47	2.37	
38	17.97	479	1.52	1.57	
39	18.20	493	2.23	1.95	
40	18.42	548	3.92	4.29	
41	18.60	548	1.82	2.54	B*
42	19.25	562	0.40	0.41	
43	19.68	576	0.94	1.26	

[a] Highest observed signal in the mass spectrum of the compound
[b] Abundance relative to the total abundance of the 43 peaks retained for statistical analysis.
[c] H indicates positive association with harem males, B indicates positive association with bachelor males in indicator species analysis. Symbols indicate level of significance (* $p<0.05$, ** $p<0.01$, *** $p<0.001$)

Table 3.2 Full and reduced models of male trait effects on chemical profiles

Effect	df	SS	pseudo-F	p
Full Model	10, 20	0.063	1.327	0.046
Status	1	0.007	1.511	0.104
Size	1	0.005	1.113	0.305
Condition	1	0.003	0.759	0.668
Age	3	0.012	0.897	0.653
Testes	1	0.006	1.393	0.148
Batch	2	0.016	1.709	0.034
Intensity	1	0.006	1.349	0.179
Reduced Model	6, 24	0.048	1.745	0.001
Status	1	0.009	2.034	0.033
Size	1	0.005	1.161	0.261
Condition	1	0.005	1.192	0.265
Batch	2	0.016	1.693	0.030
Intensity	1	0.007	1.417	0.127

Table 3.3 Effect of individual identity on chemical profiles

Effect	df	SS	pseudo-F	p
	13, 14	0.059	2.949	<0.001
Individual (Status)	10	0.041	2.824	<0.001
Replicate	1	0.001	0.864	0.516
Batch	2	0.007	2.297	0.025

Figures

Figure 3.1 Adult male *Phyllostomus hastatus* in flight (ventral surface). Arrow indicates the location of the chest gland, which is visible as a small bare patch. Inset shows close up of secretion being expressed from a chest gland by gloved fingers

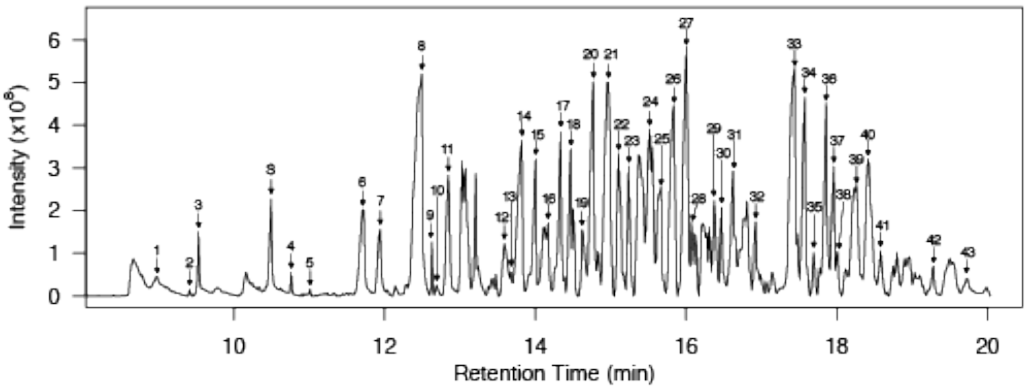

Figure 3.2 Representative GC-MS chromatogram of male chest gland secretion. Numbered arrows indicate peaks used in statistical analyses. Peak labeled "S" denotes the internal standard.

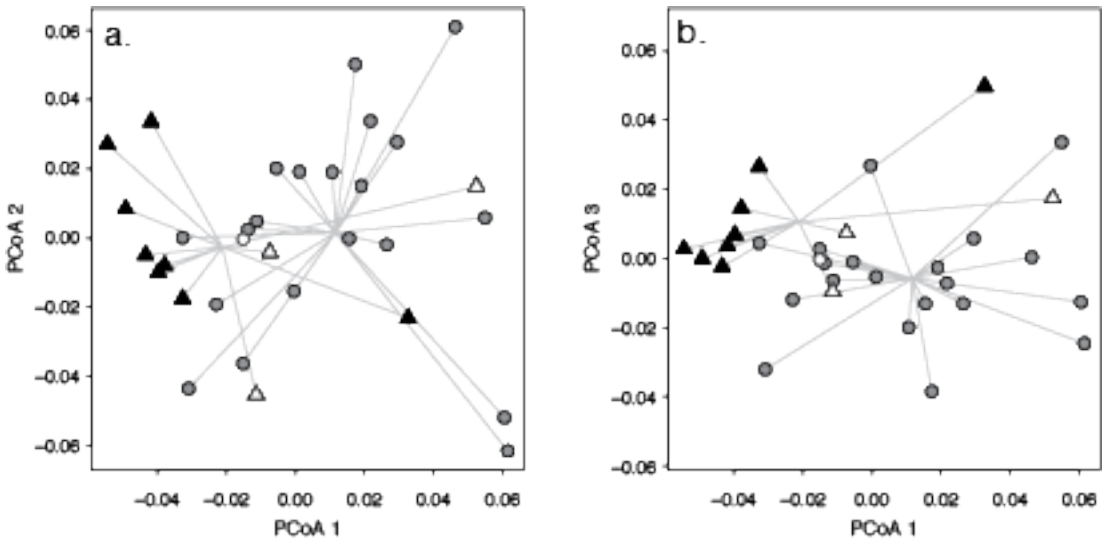

Figure 3.3 Principal coordinate ordination plots showing variation between bachelor (circles) and harem (triangles) males. Open symbols indicate individuals that were incorrectly classified via CAP discrimination. Grey lines connect each point to the group centroid. (a) The first axis (PCoA1) explains 25.1% of the total variance and second axis (PCoA2) explains 16.7% of the total variance. (b) The third axis (PCoA3) explains 9.7% of the variation

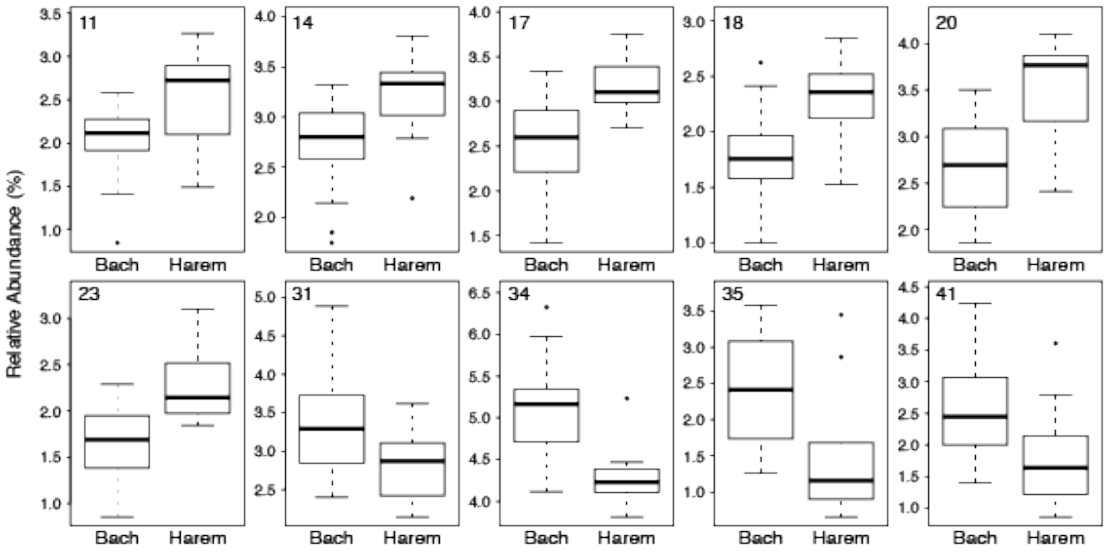

Figure 3.4 Relative abundance of the ten compounds significantly associated with a mating status. Numbers correspond to the peak numbers listed in Table 3.1

Conclusion

Sexual selection has captured the interest of behavioral ecologists and evolutionary biologists due to its ability to shape the morphology and behavior of animals. To understand how sexual selection contributes to the vast biological diversity we observe, we need to examine both the factors that influence the opportunity for sexual selection as well as the behavioral and morphological traits shaped by it. Comparative studies provide an excellent framework for investigating how ecological factors affect variation in the intensity of sexual selection across taxa, while species-specific studies afford that ability to examine the connection between trait variation and reproductive success. In this dissertation, I examine both the causes and consequences of sexual selection among the diverse species of phyllostomid bats. Using a phylogenetic comparative approach, I have examined the ecological drivers of variation among nearly 150 species within the family Phyllostomidae. I then narrowed my focus to a single species, P*hyllostomus hastatus*, to examine variation in male reproductive success, specifically measuring the ability of harem males to monopolize paternity within their harems, as well as the potential for sexually dimorphic gland to act as a communication signal.

Using data accumulated from various sources, I demonstrate a positive correlation between social aggregation size and the intensity of pre-copulatory competition, as inferred by sexual dimorphism of body mass and canine length (Chapter 1). While this pattern is clear across the family, there is still substantial

variation among the species that roost in small aggregations (Figure 1.3b, d). For example, *Anoura caudifer* and *Musonycteris harrisoni* exhibit pronounced male-biased canine sexual dimorphism (greater than 15%), whereas *Centurio senex* and *Hylonycteris underwoodi* exhibit strong female bias (less than -5%), but all roost in small aggregations of ten or fewer individuals (Figure 1.3b, d; Tables S1.1, S1.2). Similarly, relative testes mass ranged from 0.07% to 2.90% of body mass among species with small aggregations (Tables S1.1, S1.2). Clearly, we must examine additional socio-ecological factors, such as the abundance and distribution of food resources, to explain variation among species with similar aggregation sizes. For example, the distribution of food resources affects the distribution of roost sites (Kunz et al. 2003), movements between roosts and foraging sites (Tschapka 2004), as well as the potential benefits of information sharing information within a roost (Dechmann et al. 2010; Safi and Kerth 2007). These interactions between foraging behavior and roosting ecology will undoubtedly affect the opportunities for pre- and post-copulatory selection.

Testes mass is a commonly used proxy of sperm competition (Hosken 1998; Hosken and Ward 2001; Wilkinson and McCracken 2003); however, variation in sperm traits (Anderson et al. 2005), seminal products (Perry et al. 2013), and female reproductive tract morphology (Hellriegel and Ward 1998) are also expected reflect variation in post-copulatory competition. For example, the sperm midpiece contains the mitochondria and is highly variable among species (Anderson et al. 2005; Tourmente et al. 2011). Mammal species with multiply mating females have greater

mid-piece volumes relative to single-mating species, thus suggesting an enlarged midpiece is an adaption for sperm competition (Anderson et al. 2005). Although mid-piece volume and other sperm attributes are correlated with relative testes size across mammals (Anderson et al. 2005; Tourmente et al. 2011)., bats are underrepresented in these broad studies (one and zero bat species included, respectively). Forman and Genoways (1979) describe sperm morphology for 37 phyllostomid species and note variation in the acrosome length. They also report midpiece length, which varies from 5.57 microns to 12.51 microns, but only qualitatively report width and shape, making it difficult to assess volume without further measure.

Orr and Zuk (2013, 2014) have recently reviewed the potential for reproductive delays to increase opportunity for post-copulatory selection via sperm competition and cryptic female choice. For example, delayed fertilization increases the time for sperm competition, thus promoting post-copulatory sexual selection (Orr and Zuk 2014). Bats are known to employ a variety of reproductive delays, from delayed fertilization to delayed development (Racey and Entwistle 2000). Most bats, including phyllostomids, produce only a single offspring per cycle, but if females ovulate multiple eggs, then delayed implantation and development could allow for cryptic female choice via selective implantation or differential resource allocation during early developmental stages. Cryptic female choice is an exciting topic within sexual selection (Firman et al. 2017), and the diversity within phyllostomid bats makes them interesting candidates for such tactics.

Among the phyllostomids, *Phyllostomus hastatus* is one of the best studied

species with regard to social and mating behavior, and yet many questions remain. Based on the social mating system, we would predict the harem male controls all mating opportunities within the harem. However, through molecular parentage analysis, I have shown that extra-group paternity occurs in most harems, but at relatively low levels (Chapter 2). The observation that harem males in better condition suffer less paternity loss is consistent with our expectations based on the aggressive defense behavior of harem males, although we cannot yet exclude the possibility that low extra-group paternity is due to a female preference for high quality males. How this extra-group paternity affects the overall intensity of sexual selection is still an open question. To address this, we must better quantify the variance in both male and female lifetime reproductive success. Identifying the extra-harem sires and the rate of harem male turnover are important next steps.

Beyond the observation that harem males aggressively drive away intruders, the behavioral interactions between males are not well known (McCracken and Bradbury 1981). Examination of the interactions between neighboring harem males may inform our understanding of harem defense, and extra-group paternity. These interactions may be mediated by olfactory communication, as secretions from the male chest gland have the potential to signal male mating status (bachelor vs. harem male) as well as individual identity (Chapter 3). Behavioral tests are still needed to determine how males and females respond to this olfactory signal. Studies on captive populations of the congeneric species *P. discolor* reveal that both males and females can discriminate familiar and unfamiliar individuals via scent marks from the chest

gland (Holler and Schmidt 1993). Although their social and mating systems differ, it is plausible that *P. hastatus* use their chest gland in a similar fashion. Behavioral tests on wild *P. hastatus* are challenging, but well-designed experiments are likely to yield interesting and informative results.

Much of the remarkable diversity within the family Phyllostomidae represents an adaptation to an array of ecological niches. However, sexual selection has clearly shaped the family's repertoire of mating behaviors, secondary sexual traits, and sexual dimorphism. Using a diverse set of analytical tools, I have examined several of these traits to illustrate patterns within and among species. Although this work has broadened our understanding of how sexual selection has contributed to the diversity within the Phyllostomidae, there is still much to learn.

Appendices

Appendix 1: Supplementary Tables for Chapter 1

Table S1.1 Morphometric data for 149 phyllostomid species and five outgroup species. Female (F) and male (M) trait means and measures of dimorphism. Forearm dimorphism is presented as the percent different from the mean of the sexes. Mass and canine dimorphism (D) presented as percent difference from the mean using sex-specific residuals from a PGLS of trait means on forearm length (see text for details). Testes mass is presented as combined testes mass (CTM) and testes mass as a percentage of body mass (% BM).

	Forearm Length (mm)			Mass (g)			Canine Length (mm)			Testes Mass	
Species	**F**	**M**	**% Diff**	**F**	**M**	**D**	**F**	**M**	**D**	**CTM**	**% BM**
Ametrida centurio	31.95	25.56	-22.22	10.62	7.55	-9.02	2.30	2.16	12.25	0.013	0.17
Anoura caudifer	36.85	37.08	0.62	10.40	10.99	4.40	1.96	2.32	16.13	0.095	0.86
Anoura cultrata	41.71	41.94	0.55	16.36	18.02	8.82	2.46	2.86	14.48	0.045	0.25
Anoura geoffroyi	42.95	42.28	-1.57	14.54	15.08	6.56	2.28	2.64	16.40	0.209	1.39
Anoura latidens	43.16	42.83	-0.77	14.98	15.01	1.64	2.05	2.38	15.83	0.380	2.53
Ardops nichollsi	46.27	46.85	1.25	19.93	17.54	-15.04					
Ariteus flavescens	41.92	38.56	-8.35	15.46	12.73	-5.32	2.31	2.09	-0.12	0.144	1.13
Artibeus aequatorialis	64.12	63.37	-1.18	44.80	42.41						
Artibeus amplus	69.36	68.36	-1.45	52.00	54.55		4.20	4.58	10.13	0.462	0.84
Artibeus anderseni		38.30		12.25	11.88					0.101	0.84

	Forearm Length (mm)			Mass (g)			Canine Length (mm)			Testes Mass	
Species	**F**	**M**	**% Diff**	**F**	**M**	**D**	**F**	**M**	**D**	**CTM**	**% BM**
Artibeus aztecus	46.13	43.54	-5.78	20.75	20.50	7.52				0.234	1.14
Artibeus cinereus	38.76	38.91	0.39	11.00	10.25		2.57	2.64	2.32	0.231	2.25
Artibeus concolor	47.98	47.03	-2.00	19.17	17.93	-2.79	2.94	2.92	1.41	0.105	0.58
Artibeus fimbriatus	66.52	66.20	-0.48	57.26	54.74	-3.77					
Artibeus fraterculus	56.38	55.90	-0.86	33.91	32.59	-2.55					
Artibeus glaucus	39.53	39.04	-1.25	12.96	12.79	0.84	2.49	2.49	1.27	0.105	0.82
Artibeus gnomus	37.77	37.83	0.16	11.29	10.19	-10.54	2.45	2.59	5.40	0.084	0.82
Artibeus hirsutus	55.78	55.38	-0.72		31.30						
Artibeus inopinatus	51.55	50.05	-2.95	26.38	26.47	5.13					
Artibeus jamaicensis	60.49	59.97	-0.86	44.45	40.36	-8.30	3.76	4.06	8.53	0.407	1.01
Artibeus lituratus	71.36	70.11	-1.77	71.87	62.07	-12.02				0.411	0.66
Artibeus obscurus	60.15	58.72	-2.41	38.84	35.79	-4.06	4.18	4.15	1.50	0.411	1.15
Artibeus phaeotis	37.44	37.79	0.93	12.36	11.10	-12.33	2.71	2.71	-0.84	0.157	1.42
Artibeus planirostris	63.40	63.38	-0.03	55.72	53.95	-3.18				0.486	0.90
Artibeus ravus	39.25	38.50	-1.93	11.42	10.20						
Artibeus rosenbergi				10.50							
Artibeus schwartzi	66.19	65.82	-0.56	53.86	49.09	-8.36					
Artibeus toltecus	39.49	38.73	-1.94	14.58	14.08	-0.50				0.175	1.25
Artibeus watsoni	37.76	37.58	-0.48	11.63	11.04	-4.36	2.84	2.76	-2.44	0.183	1.67
Brachyphylla cavernarum	64.12	64.86	1.15	42.57	42.93	-1.26	3.19	3.12		0.462	1.07
Brachyphylla nana	58.63	58.01	-1.06	29.93	30.58	4.29	3.12	3.25	5.35	0.179	0.59
Carollia brevicauda	39.83	39.76	-0.18	14.68	15.46	5.44	1.82	2.10	14.52	0.183	1.19
Carollia castanea	37.35	37.43	0.21	11.51	12.59	8.61	2.11	2.42	13.43	0.183	1.46
Carollia perspicillata	41.49	41.29	-0.48	17.17	17.55	2.90	2.05	2.33	13.38	0.221	1.26
Carollia sowelli	40.81	41.29	1.17	16.61	17.74	4.88					

	Forearm Length (mm)			Mass (g)			Canine Length (mm)			Testes Mass	
Species	**F**	**M**	**% Diff**	**F**	**M**	**D**	**F**	**M**	**D**	**CTM**	**% BM**
Carollia subrufa	39.67	38.92	-1.91	17.31	18.29	7.90				0.157	0.86
Centurio senex	42.03	42.56	1.25	19.87	19.60	-3.08	2.53	2.38	-7.52	0.047	0.24
Chiroderma doriae	53.70	52.00			32.90						
Chiroderma salvini	49.56	48.25	-2.68	26.19	25.03	-0.46	3.88	3.72	-1.98	0.242	0.97
Chiroderma trinitatum	39.16	39.03	-0.33	14.98	12.90	-14.40	2.53	2.58	2.28	0.141	1.09
Chiroderma villosum	45.86	44.77	-2.41	22.92	21.57	-2.60	3.60	3.51	-0.55	0.055	0.25
Choeroniscus godmani	34.21	33.43	-2.31	8.91	8.40	-1.83	1.40	1.42	5.00	0.013	
Choeroniscus minor	35.23	34.96	-0.77	7.44	7.85		1.60	1.60		0.051	0.65
Choeronycteris mexicana	45.10	44.58	-1.16	16.21	16.03	1.13	2.06	2.49	20.42	0.047	
Chrotopterus auritus	80.60	81.97	1.69	74.35	74.39	-3.15	5.12	5.29	1.57	0.207	0.28
Desmodus rotundus	60.46	56.76	-6.31	35.93	30.30	-5.26	2.61	2.82	16.58	0.264	0.87
Diaemus youngi	51.86	50.96	-1.75	31.20	34.30	11.83	2.00	2.27	15.41	1.259	3.67
Diphylla ecaudata	54.08	53.90	-0.33	26.23	26.06	-0.01	2.00	2.16	8.25	0.209	0.80
Ectophylla alba	28.65	28.90	0.87	5.75	5.42		1.69	1.67	-2.15	0.157	2.89
Enchisthenes hartii	39.56	39.44	-0.30	16.83	16.62	-0.84	2.60	2.63	1.44	0.221	1.34
Erophylla bombifrons	47.24	47.36	0.25	15.17	17.25	12.27					
Erophylla sezekorni	48.01	47.17	-1.77	17.09	16.56	0.66	2.11	2.53	20.44	0.058	0.35
Gardnerycteris crenulatum	49.64	49.31	-0.67	13.80	13.09	-3.30	2.72	2.83	4.72	0.302	2.32
Gardnerycteris koepckeae	47.40	48.05									
Glossophaga commissarisi	34.55	34.32	-0.67	9.18	8.75	-3.60	2.03	2.14	5.97	0.101	1.15
Glossophaga leachii	36.40	36.05	-0.97	9.62	9.61	1.72				0.057	0.59
Glossophaga longirostris	38.27	37.83	-1.16	11.90	11.63	-0.27	2.28	2.33	3.40	0.101	0.86
Glossophaga morenoi				8.50	7.00						
Glossophaga soricina	35.89	35.26	-1.77	9.72	9.51	1.01	1.98	2.10	7.88	0.084	0.88
Glyphonycteris daviesi	56.23	54.66	-2.83	30.00	24.75		2.80	2.83		0.084	0.34

	Forearm Length (mm)			Mass (g)			Canine Length (mm)			Testes Mass	
Species	**F**	**M**	**% Diff**	**F**	**M**	**D**	**F**	**M**	**D**	**CTM**	**% BM**
Glyphonycteris sylvestris	41.75	39.93	-4.46	9.65	10.50		1.53	1.54	8.35	0.047	
Hsunycteris cadenai		33.00		6.95	7.70						
Hsunycteris thomasi	32.32	32.04	-0.87	6.45	6.73	6.02	2.03	2.17	7.53	0.110	1.63
Hylonycteris underwoodi	33.46	33.00	-1.38	8.05	7.37	-6.20	1.76	1.63	-5.93	0.033	
Lampronycteris brachyotis	40.26	39.95	-0.77	11.50	13.00					0.101	0.77
Leptonycteris curasoae	53.49	53.41	-0.15	24.13	26.01	7.79	2.24	2.70	18.83	0.084	0.32
Leptonycteris nivalis	54.81	55.99	2.13	24.40	26.58		2.39	2.64		0.157	0.59
Leptonycteris yerbabuenae	52.70	53.43	1.38	21.44	23.45	6.02	2.15	2.55	15.01	0.017	0.07
Lichonycteris obscura	32.58	31.66	-2.86	6.62	6.45	3.27	1.46	1.49	6.09	0.013	
Lionycteris spurrelli	35.44	34.52	-2.63	9.10	8.30	-4.17	1.58	1.56	2.52	0.112	1.35
Lonchophylla concava	33.29	33.15	-0.42	7.93	7.94		1.87	2.01		0.183	2.32
Lonchophylla robusta	42.91	43.20	0.67	14.99	15.01	-1.13	2.25	2.58	12.86	0.122	0.81
Lonchorhina aurita	50.97	50.88	-0.18	15.14	14.60	-3.12	2.08	2.11	1.71	0.157	1.07
Lonchorhina orinocensis	42.93	42.24	-1.62	9.01	8.46	-1.22	1.84	2.00	10.66	0.013	0.15
Lophostoma brasiliense	36.19	35.49	-1.95	9.65	9.68	3.88	2.62	3.15	19.97	0.121	1.25
Lophostoma carrikeri	46.13	47.43	2.78	21.20	24.03	8.24				0.302	
Lophostoma occidentalis	53.60	54.25	1.21	23.30	25.47						
Lophostoma silvicolum	52.92	53.78	1.61	28.24	32.53	11.54	3.70	4.16	10.29	0.616	1.90
Macrophyllum macrophyllum	36.15	34.90	-3.52	9.62	7.26	-20.76	1.57	1.61	7.60	0.101	1.39
Macrotus californicus	50.65	49.85	-1.59	12.17	11.74	1.95	1.75	1.97	14.61		
Macrotus waterhousii	50.36	49.80	-1.12	12.88	12.55	1.04	2.19	2.37	9.48	0.127	1.01
Mesophylla macconnelli	31.18	30.24	-3.06	6.80	6.70	3.93	1.72	1.89	12.82	0.079	1.17
Micronycteris buriri	41.00	39.00		8.27	7.77						
Micronycteris hirsuta	43.21	41.91	-3.05	12.86	13.50	11.19	2.53	2.69	9.35	0.264	1.95
Micronycteris megalotis	34.87	33.30	-4.61	6.09	5.64	4.54	1.69	1.80	12.09	0.074	1.31

	Forearm Length (mm)			Mass (g)			Canine Length (mm)			Testes Mass	
Species	**F**	**M**	**% Diff**	**F**	**M**	**D**	**F**	**M**	**D**	**CTM**	**% BM**
Micronycteris microtis	34.55	34.16	-1.14	6.69	5.72	-12.74	1.59	1.75	11.09	0.038	0.66
Micronycteris minuta	35.29	35.10	-0.54	6.90	6.42	-5.84	1.66	1.81	9.33	0.020	0.31
Micronycteris schmidtorum	35.04	35.36	0.91	7.00	6.73	-6.17				0.013	
Mimon bennettii	57.43	54.05	-6.06	23.91	22.83					0.017	
Mimon cozumelae	57.30	57.13	-0.30	24.50	24.00					0.038	0.16
Monophyllus plethodon	41.76	42.58	1.94	13.09	14.69	7.78	2.08	2.54	17.62	0.075	0.51
Monophyllus redmani	38.77	38.79	0.05	11.00	13.55	20.67	1.85	2.10	12.56	0.038	0.28
Mormoops blainvillei	44.00	47.00		7.35	8.23					0.006	0.07
Mormoops megalophylla	55.48	55.39	-0.16	16.64	16.25	-1.84				0.013	0.08
Musonycteris harrisoni	42.26	42.31	0.12				1.77	2.16	19.73		
Noctilio albiventris	59.54	60.50	1.60	25.07	30.73	16.54	3.51	4.24	17.26	0.160	0.52
Noctilio leporinus	83.73	85.00	1.51	56.63	65.41	10.56	4.81	5.79	16.94	0.411	0.63
Phylloderma stenops	71.09	71.21	0.17	48.49	53.77	9.99	2.57	2.73	5.74	1.533	2.86
Phyllonycteris aphylla	45.90	46.55	1.41	16.94	19.93	13.64				0.026	
Phyllonycteris poeyi	47.60	47.63	0.06	17.62	19.78	11.43	2.35	2.97	23.24	0.115	0.58
Phyllops falcatus	42.25	40.45	-4.35	18.16	16.63	-2.44	2.19	2.16	3.96	0.101	
Phyllostomus discolor	61.71	62.59	1.42	37.52	41.09	6.51	3.18	3.49	7.60	1.053	2.56
Phyllostomus elongatus	65.59	65.63	0.06	38.70	40.59	4.64				0.751	1.84
Phyllostomus hastatus	87.10	87.84	-1.48	93.25	101.77	7.26	4.93	5.40	8.18	0.387	0.38
Phyllostomus latifolius	59.70	58.83	-1.47	24.20							
Platalina genovensium	48.00	48.84		14.50	15.50		2.42				
Platyrrhinus albericoi	61.47	59.29	-3.61	55.00						0.101	
Platyrrhinus angustirostris	37.70	36.96	-1.98	13.86	12.25		2.96	2.95		0.084	0.68
Platyrrhinus aurarius	52.87	51.56	-2.51	34.12	33.07	0.30	3.90	3.89	1.92	0.183	0.55
Platyrrhinus brachycephalus	38.10	39.49	3.58	15.10	13.20	-18.90				0.183	1.39

	Forearm Length (mm)			Mass (g)			Canine Length (mm)			Testes Mass	
Species	**F**	**M**	**% Diff**	**F**	**M**	**D**	**F**	**M**	**D**	**CTM**	**% BM**
Platyrrhinus chocoensis	51.92	50.26	-3.25	28.00			4.58	4.64		0.290	1.03
Platyrrhinus dorsalis	50.12	51.20	2.13	29.22	28.46	-5.78					
Platyrrhinus fusciventris	38.83	38.26	-1.48	13.58	12.31		3.07	3.02		0.084	0.68
Platyrrhinus helleri	38.86	38.18	-1.77	15.29	14.00	-6.25	2.99	3.02	2.47	0.084	0.60
Platyrrhinus incarum	37.48	36.76	-1.94	14.79	12.73	-12.25	2.75	2.73	0.97	0.038	0.30
Platyrrhinus infuscus	56.94	55.68	-2.24	42.64	43.20	4.20				0.339	0.79
Platyrrhinus ismaeli		59.60									
Platyrrhinus lineatus	47.63	47.12	-1.08	25.42	23.27	-7.25	3.13	3.07	-0.87	0.101	0.43
Platyrrhinus matapalensis	39.06	39.16	0.26	15.37	14.15	-8.65	3.12	2.99	-4.46	0.148	1.05
Platyrrhinus nigellus	43.43	42.60	-1.93	19.90	18.68						
Platyrrhinus umbratus	44.95	45.16	0.47	25.86	23.69		3.85	3.73		0.101	0.42
Platyrrhinus vittatus	61.80	59.26	-4.20	50.33	47.84	0.64	4.66	4.71	4.58	0.101	0.21
Pteronotus parnellii	60.48	60.00	-0.80	16.28	17.95	12.83	2.91	5.43		0.084	0.47
Pygoderma bilabiatum	40.46	37.93	-6.45	21.62	19.02	-5.75	2.89	2.59		0.046	0.24
Rhinophylla alethina	36.04	35.30	-2.07	12.15	12.57	6.33	2.06	2.24		0.226	
Rhinophylla fischerae	30.25	30.23	-0.07	8.25	8.06					0.112	1.39
Rhinophylla pumilio	35.29	33.99	-3.75	9.66	9.19	1.47	1.76	1.70	1.39	0.137	1.49
Scleronycteris ega	34.70	35.20					1.77	1.75			
Sphaeronycteris toxophyllum	40.09	38.25	-4.70	14.58	14.79	8.52	2.37	2.25	-0.04	0.065	0.44
Stenoderma rufum	49.79	47.78	-4.12	21.07	20.28	3.88				0.047	
Sturnira erythromos	39.56	39.29	-0.68	14.40	15.42	7.88	1.99	2.13	7.64	0.215	1.39
Sturnira lilium	41.57	42.17	1.43	18.34	19.26	2.89	2.61	3.01	12.85	0.165	0.86
Sturnira ludovici	44.39	44.98	1.32	21.97	24.27	8.18	2.37	2.89	18.32	0.157	0.64
Sturnira luisi	43.50	43.83	0.76	18.72	19.73	4.11					
Sturnira magna	58.00	56.13	-3.28	43.50	52.50					0.396	0.76

	Forearm Length (mm)			Mass (g)			Canine Length (mm)			Testes Mass	
Species	**F**	**M**	**% Diff**	**F**	**M**	**D**	**F**	**M**	**D**	**CTM**	**% BM**
Sturnira mordax	45.45	48.08	5.62	21.00	27.00						
Sturnira nana	34.33	34.38	0.15								
Sturnira oporaphilum	45.00			20.00	21.00						
Sturnira tildae	47.37	47.55	0.38	25.06	26.01	3.18	2.66	3.07	13.93	0.183	0.70
Tonatia bidens	55.27	54.63	-1.16	24.00	29.00		3.48	3.71	7.56	0.513	
Tonatia saurophila	58.64	59.13	0.83	29.03	28.01	-5.39				0.767	2.75
Trachops cirrhosus	60.27	60.11	-0.27	31.65	33.26	5.50	3.68	3.95	7.36	0.162	0.49
Trinycteris nicefori	39.10	37.60	-3.91	9.19	8.30	-0.79	1.31	1.44	16.57	0.101	1.21
Uroderma bilobatum	41.55	41.42	-0.31	17.24	15.89	-7.66	2.97	2.99	0.95	0.157	0.99
Uroderma magnirostrum	43.01	42.42	-1.38	17.10	15.96	-4.59	2.82	2.91	4.48	0.087	0.54
Vampyressa elisabethae											
Vampyressa melissa	37.96	36.50									
Vampyressa pusilla	31.06	32.03	3.07	8.14	8.01	-6.48	1.97				
Vampyressa thyone	31.56	31.19	-1.18	8.33	7.72	-5.75	1.94	2.07	7.66	0.084	1.08
Vampyriscus bidens	35.81	35.65	-0.45	11.64	11.26	-2.63	2.45	2.56	4.79	0.051	0.45
Vampyriscus brocki	33.43				8.00						
Vampyriscus nymphaea	37.24	37.11	-0.35	13.31	12.35	-6.95	2.97	3.02	1.95	0.047	0.38
Vampyrodes caraccioli	55.47	55.23	-0.43	32.35	33.44	4.01	3.71	3.71	0.42	0.183	0.55
Vampyrum spectrum	104.33	105.56	1.17	153.09	157.89	1.05	7.61	7.58	-1.44	0.282	0.18

Table S1.2 Mating systems and roost data for 149 phyllostomid species and five outgroup species. Average roost permanence scores reflect use of four roost types: foliage (F), tents (T), hollow trees (HT), and caves (C). Average aggregation size scores reflect average of reported aggreagation size categories. Values in parentheses indicate score given to each roost type or aggregation size.

Species	Mating System	Roost Perm.	F (1)	T (2)	HT (3)	C (4)	Aggr. Size	1-10 (1)	11-25 (2)	26-100 (3)	>100 (4)	References
Ametrida centurio												
Anoura caudifer		3.50			x	x	1.00	x				1, 2
Anoura cultrata		4.00				x	2.50		x	x		3, 4
Anoura geoffroyi		4.00				x	3.00		x	x	x	1, 4, 5, 6, 7
Anoura latidens												
Ardops nichollsi		1.00	x				1.00	x				1, 8
Ariteus flavescens												
Artibeus aequatorialis												
Artibeus amplus												
Artibeus anderseni		2.00		x			1.00	x				9, 10
Artibeus aztecus		2.50	x			x						1, 4
Artibeus cinereus		2.00		x			1.00	x				1, 3, 5, 10
Artibeus concolor												
Artibeus fimbriatus												
Artibeus fraterculus												
Artibeus glaucus		2.00		x			1.00	x				9, 10
Artibeus gnomus		2.00		x			1.00	x				9, 10
Artibeus hirsutus		4.00				x						1
Artibeus inopinatus		4.00				x	1.00	x				1, 4

Artibeus jamaicensis	SM-MF	3.00		x	x	x	1.50	x	x			1, 4, 5, 10
Artibeus lituratus	SM-MF	2.67	x		x	x	1.50	x	x			1, 3, 4, 5
Artibeus obscurus												
Artibeus phaeotis	SM-MF	3.00		x		x	1.00	x				1, 3, 4, 9, 10
Artibeus planirostris												
Artibeus ravus												
Artibeus rosenbergi												
Artibeus schwartzi												
Artibeus toltecus		2.50	x	x	x	x	1.00	x				1, 4, 9, 10
Artibeus watsoni	MM-MF	2.00		x			1.00	x				4, 10, 11
Brachyphylla cavernarum		4.00				x	3.00		x	x	x	1, 12
Brachyphylla nana		4.00				x	2.50		x	x		1, 13
Carollia brevicauda		3.50			x	x						1, 4
Carollia castanea		3.50			x	x	1.50	x	x			1, 4, 14
Carollia perspicillata	MM-MF	3.50			x	x	2.50		x	x		1, 4, 5
Carollia sowelli		3.50			x	x	1.50	x	x			4
Carollia subrufa		3.50			x	x						1, 4
Centurio senex		1.00	x				1.00	x				1, 4, 5
Chiroderma doriae												
Chiroderma salvini												
Chiroderma trinitatum		4.00				x						1, 15
Chiroderma villosum		3.00			x							15
Choeroniscus godmani							2.00		x			3
Choeroniscus minor		3.00			x		1.00	x				16
Choeronycteris mexicana		3.50			x	x	2.00		x			1, 4, 6
Chrotopterus auritus	SM-SF	3.50			x	x	1.00	x				4, 6, 17
Desmodus rotundus	MM-MF	3.50			x	x	3.00		x	x	x	1, 4, 5

Diaemus youngi		3.50			x	x	2.00		x			1, 4, 5, 18
Diphylla ecaudata		4.00				x	2.00	x	x	x		1, 4
Ectophylla alba	MM-MF	2.00		x			1.00	x				3, 4, 10
Enchisthenes hartii												
Erophylla bombifrons		4.00				x	3.50			x	x	1
Erophylla sezekorni	MM-MF	4.00				x	3.50			x	x	1, 19
Gardnerycteris crenulatum		3.50			x	x	1.00	x				1, 3, 4
Gardnerycteris koepckeae												
Glossophaga commissarisi		3.50			x	x	1.50	x	x			1, 3, 4, 6
Glossophaga leachii		3.50			x	x						6
Glossophaga longirostris		3.50			x	x	2.00		x			1, 20
Glossophaga morenoi		3.50			x	x						4, 6
Glossophaga soricina		3.50			x	x	1.50	x	x			1, 3, 4, 5, 6
Glyphonycteris daviesi		3.00			x		1.00	x				1, 4
Glyphonycteris sylvestris		3.50			x	x	2.50		x	x		1, 3, 4
Hsunycteris cadenai												
Hsunycteris thomasi		2.67	x		x	x						1, 4
Hylonycteris underwoodi		4.00				x	1.00	x				1, 4, 6, 21
Lampronycteris brachyotis		3.50			x	x	2.00	x	x	x		1, 3, 4, 5, 6
Leptonycteris curasoae		4.00				x	4.00				x	3, 6, 22
Leptonycteris nivalis		4.00				x	4.00				x	1, 4, 6
Leptonycteris yerbabuenae		4.00				x	4.00				x	4, 23
Lichonycteris obscura		1.00	x				1.00	x				24
Lionycteris spurrelli		4.00				x						3
Lonchophylla concava		4.00				x						1, 4
Lonchophylla robusta		4.00				x						1, 3, 4
Lonchorhina aurita		4.00				x	3.00		x	x	x	1, 3, 4, 5, 6

Lonchorhina orinocensis		4.00				x						1, 3
Lophostoma brasiliense		3.00			x							3, 4
Lophostoma carrikeri		3.00			x							1
Lophostoma occidentalis												
Lophostoma silvicolum	MM-MF	3.50			x	x	1.00	x				1, 3, 4
Macrophyllum macrophyllum		4.00				x	1.00	x				1, 3, 4, 6
Macrotus californicus	SM-MF	4.00				x	3.50			x	x	1, 6, 25
Macrotus waterhousii		4.00				x	2.50	x	x	x	x	1, 4, 6, 26
Mesophylla macconnelli		2.00	x	x	x		1.00	x				1, 4, 10
Micronycteris buriri												
Micronycteris hirsuta		3.50			x	x	1.00	x				3, 4, 14
Micronycteris megalotis		3.50			x	x	1.00	x				1, 5, 6
Micronycteris microtis		3.50			x	x	1.00	x				4
Micronycteris minuta		3.50			x	x	1.00	x				1, 4
Micronycteris schmidtorum		3.50			x	x	1.00	x				1, 4
Mimon bennettii		3.50			x	x	1.00	x				1, 6, 27
Mimon cozumelae		3.50			x	x	1.50	x	x			1, 4
Monophyllus plethodon		4.00				x						1
Monophyllus redmani		4.00				x	2.00		x			1, 28
Mormoops blainvillei		4.00				x	4.00				x	29
Mormoops megalophylla		4.00				x	4.00				x	6
Musonycteris harrisoni		2.50	x			x	1.00	x				1, 6
Noctilio albiventris	SM-MF	3.50			x	x	4.00				x	30
Noctilio leporinus	SM-MF	3.50			x	x	3.00		x	x	x	4, 5
Phylloderma stenops												
Phyllonycteris aphylla		4.00				x						1
Phyllonycteris poeyi		4.00				x	4.00				x	1, 31

Phyllops falcatus		2.00	x		x		1.00	x				1, 32
Phyllostomus discolor	MM-MF	3.00			x		2.00	x	x	x		1, 3, 4, 5
Phyllostomus elongatus		3.00			x							1, 3
Phyllostomus hastatus	SM-MF	3.50			x	x	3.00		x	x	x	1, 4, 5
Phyllostomus latifolius												
Platalina genovensium		4.00				x	2.00	x	x	x		1
Platyrrhinus albericoi												
Platyrrhinus angustirostris												
Platyrrhinus aurarius		2.67	x		x	x	1.00	x				3
Platyrrhinus brachycephalus		2.67	x		x	x	1.00	x				3
Platyrrhinus chocoensis												
Platyrrhinus dorsalis		2.67	x		x	x	1.00	x				1, 3, 10
Platyrrhinus fusciventris		2.67	x		x	x	1.00	x				15
Platyrrhinus helleri		2.50	x	x	x	x	1.00	x				1, 4
Platyrrhinus incarum												
Platyrrhinus infuscus		4.00				x						1
Platyrrhinus ismaeli												
Platyrrhinus lineatus		2.67	x		x	x	1.00	x				1, 3
Platyrrhinus matapalensis												
Platyrrhinus nigellus												
Platyrrhinus umbratus		2.67	x		x	x	1.00	x				3
Platyrrhinus vittatus		2.67	x		x	x						1, 4
Pteronotus parnellii		4.00				x	4.00				x	6
Pygoderma bilabiatum												
Rhinophylla alethina												
Rhinophylla fischerae												
Rhinophylla pumilio		2.00		x								10

Scleronycteris ega										
Sphaeronycteris toxophyllum		4.00				x				1
Stenoderma rufum		1.00	x				1.00	x		32
Sturnira erythromos										
Sturnira lilium		2.67	x		x	x	1.00	x		1, 3, 4
Sturnira ludovici										
Sturnira luisi										
Sturnira magna										
Sturnira mordax										
Sturnira nana										
Sturnira oporaphilum										
Sturnira tildae		3.00			x		1.00	x		15
Tonatia bidens		3.00			x					1, 3
Tonatia saurophila		3.00			x					4
Trachops cirrhosus		3.50			x	x	1.00	x		1, 3, 4, 5, 6
Trinycteris nicefori		3.50			x	x	2.00		x	3, 4, 5
Uroderma bilobatum	SM-MF	3.00		x		x	2.00		x	1, 4, 5, 10
Uroderma magnirostrum		2.00		x			1.00	x		4, 10
Vampyressa elisabethae										
Vampyressa melissa										
Vampyressa pusilla		1.50	x	x			1.00	x		1, 10
Vampyressa thyone		3.00		x		x	1.00	x		4
Vampyriscus bidens										
Vampyriscus brocki										
Vampyriscus nymphaea	SM-MF	2.00		x			1.00	x		4, 10
Vampyrodes caraccioli		1.00	x				1.00	x		1, 3, 4, 5
Vampyrum spectrum	SM-SF	3.50			x	x	1.00	x		1, 4, 5

References contained in table

1. Tuttle, M. D. 1976. Collecting Techniques. Pp. 71-88 *in:* Biology Bats of the New World Family Phyllostomatidae (R. J. Baker, J. K. Jones, Jr., and D. C. Carter, eds.). Texas Tech Press, Lubbock, Texas.
2. Oprea, M., L. Aguliar, and D. E. Wilson. 2009. *Anoura caudifer*(Chiroptera: Phyllostomidae). Mammalian Species, 844:1-8.
3. Eisenberg, J. F. 1989. Mammals of the Neotropics: the northern Neotropics. The University of Chicago Press, London.
4. Reid, F. 1997. A field guide to the mammals of Central America and Southeast Mexico. Oxford University Press.
5. Goodwin, G. G., and A. M. Greenhall. 1961. A review of the bats of Trinidad and Tobago. Bulletin of the American Museum Natural History, 122:195-301.
6. Arita, H. T. 1993. Conservation biology of the cave bats of Mexico. Journal of Mammalogy, 74:693-702.
7. Ortega, J., and I. Alarcón-D. 2008. *Anoura geoffroyi*(Chiroptera: Phyllostomidae). Mammalian Species, 818.
8. Jones, J. K., Jr., and H. H. Genoways. 1973. Ardops nichollsi. Mammalian Species, 24:1-2.
9. Timm, R. M. 1987. Tent construction by bats of the genera Artibeus and Uroderma.
10. Kunz, T. H., L. F. Lumsden, T. Kunz, and M. Fenton. 2003. Ecology of cavity and foliage roosting bats. Pp. 3-89 *in:* Bat ecology (T. H. Kunz and M. B. Fenton, eds.). The University of Chicago Press, Chicago.
11. Chaverri, G., and T. H. Kunz. 2006. Roosting ecology of the test-roosting bat*Artibeus watsoni*(Chiroptera: Phyllostomidae) in Southwestern Costa Rica. Biotropica, 38:77-84.
12. Swanepoel, P., and H. H. Genoways. 1983. *Brachyphylla cavernarum*. Mammalian Species, 205:1-6.
13. Swanepoel, P., and H. H. Genoways. 1983. *Brachyphylla nana*. Mammalian Species, 206:103.
14. Kelm, D. H., K. R. Wiesner, and O. von Helversen. 2008. Effects of artificial roosts for frugivorous bats on seed dispersal in a Neotropical forest pasture mosaic. Conservation Biology, 22:733-741.
15. Gomes, G. A., F. A. Reid, and M. D. Tuttle. 2015. Bats of Trinidad and Tobago: A Field Guide and Natural History. Trinibat, Trinidad.
16. Solmsen, E.-H., and H. Schliemann. 2008. *Choeroniscus minor*(Chiroptera: Phyllostomidae). Mammalian Species, 822:1-6.
17. Medellin, R. A. 1989. *Chrotopterus auritus*. Mammalian Species, 343:1-5.
18. Greenhall, A. M., and W. A. Schutt. 1996. *Diaemus youngi*. Mammalian Species, 533:1-7.
19. Murray, K. L., and T. H. Fleming. 2008. Social structure and mating system of the buffy flower bat,*Erophylla sezekorni*(Chiroptera, Phyllostomidae). Journal of Mammalogy, 89:1391-1400.
20. Webster, W. D., C. O. H. Jr., and P. J. Soriano. 1998. *Glossophaga longirostris*. Mammalian Species, 576:1-5.
21. Jones, J. K., Jr., and J. A. Homan. 1974. *Hylonycteris underwoodi*. Mammalian Species, 32:1-2.
22. Cole, F. R., and D. E. Wilson. 2006. *Leptonycteris yerbabuenae*. Mammalian Species, 797:1-7.
23. Cole, F. R., and D. E. Wilson. 2006. *Leptonycteris curasoae*. Mammalian Species, 796:1-3.
24. Villalobos-Chaves, D., B. Rodriguez-Herrera, and M. Tschapka. 2013. First Records of Day Roosts of the Nectar-Feeding Bat*Lichonycteris obscura*(Phyllostomidae: Glossophaginae). Caribbean Journal of Science, 47:335-338.
25. Bell, G. P. 1985. The sensory basis of prey location by the California leaf-nosed bat Macrotus californicus (Chiroptera: Phyllostomidae). Behav. Ecol.

Sociobiol., 16:343-347.
26. Anderson, S. 1969. *Macrotus waterhousii*. Mammalian Species, 1:1-4.
27. Ortega, J., and H. T. Arita. 1997. *Mimon bennettii*. Mammalian Species, 549:1-4.
28. Homan, J. A., and J. K. Jones, Jr. 1975. *Monophyllus redmani*. Mammalian Species, 57:1-3.
29. (Online Database). Available from National Museum of Natural History Division of Mammals Collection. Retrieved 1 Dec 2016 http://collections.nmnh.si.edu/search/mammals/
30. Aguirre, L. F., L. Lens, and E. Matthysen. 2003. Patterns of roost use by bats in a neotropical savanna: implications for conservation. Biological Conservation, 111:435-443.
31. Mancina, C. A. 2010. *Phyllonycteris poeyi*(Chiroptera: Phyllostomidae). Mammalian Species, 42:41-48.
32. Tavares, V. d. C., and C. A. Mancina. 2008. *Phyllops falcatus*(Chiroptera: Phyllostomidae). Mammalian Species, 811:1-7.

Table S1.3 Candidate models testing the effect of aggregation size and roost permanence on measures of sexual dimorphism and testes mass. Models of testes mass also include natural log-transformed body mass, ln(BM), as an independent variable.

Dependent	Independent	AICc	ΔAICc
Canine dimorph	**Aggregation**	380.71	
	Aggregation + Roost	382.84	2.13
	Aggregation + Roost + Aggregation*Roost	384.11	3.40
	Roost	385.14	4.43
Mass dimorph	**Aggregation**	476.03	
	Aggregation + Roost	478.04	2.01
	Aggregation + Roost + Aggregation*Roost	479.70	3.67
	Roost	484.63	8.60
Testes mass	**Aggregation^2 + Aggregation + ln(BM)**	144.81	
	Aggregation^2 + Aggregation + Roost^2 + Roost + ln(BM)	145.72	0.92
	Aggregation^2 + Aggregation + Roost + ln(BM)	146.92	2.11
	Roost^2 + Roost + ln(BM)	147.90	3.09
	Aggregation + ln(BM)	148.27	3.46

Dependent	Independent	AICc	ΔAICc
	ln(BM)	148.54	3.74
	Aggregation + $Roost^2$ + Roost + ln(BM)	149.21	4.40
	Aggregation + Roost + ln(BM)	149.32	4.51
	Roost + ln(BM)	150.53	5.72

Table S1.4 Phylogenetic least squares (PGLS) and ordinary least squares (OLS) models testing the effect of roost permanence and aggregation sizes on measures of canine dimorphism and mass dimorphism

Dependent	Independent	Model	Estimate ± SE	t	df	F	p	R^2	λ (CI)
Canine Dimorph.	Roost	PGLS	0.54 ± 0.79	0.68	1, 71	0.46	0.50	0.01	1.00 (0.84, 1.00)
Canine Dimorph.	Roost	OLS	3.85 ± 0.93	4.15	1, 71	17.25	< 0.001	0.20	-
Canine Dimorph.	Aggregation	PGLS	3.03 ± 0.93	3.27	1, 59	10.70	0.002	0.15	0.63 (0.00, 1.00)
Canine Dimorph.	Aggregation	OLS	4.13 ± 0.77	5.32	1, 59	28.32	< 0.001	0.32	-
Mass Dimorph.	Roost	PGLS	0.62 ± 1.07	0.58	1, 88	0.34	0.56	0.004	0.43 (0.06, 0.76)
Mass Dimorph.	Roost	OLS	2.43 ± 0.95	2.55	1, 88	6.51	0.01	0.07	-
Mass Dimorph.	Aggregation	PGLS	3.67 ± 0.82	4.46	1, 69	19.90	< 0.001	0.22	0.00 (0.00, 0.47)
Mass Dimorph.	Aggregation	OLS	3.67 ± 0.82	4.46	1, 69	19.90	< 0.001	0.22	-

Table S1.5 Phylogenetic least squares (PGLS) and ordinary least squares (OLS) models testing the effect of roost permanence and aggregation sizes on testes mass (natural log-transformed). Natural log transformed body mass (ln(BM) is included as covariate in all models.

Dependent	Independent	Model	df	F	Estimate ± SE	t	p	R^2	λ (CI)
Testes Mass	$Roost^2$ + Roost + ln (BM)	PGLS	3, 79	8.24			< 0.01	0.24	0.72 (0.30, 0.93)
	$Roost^2$				-0.20 ± 0.12	-1.70	0.09		
	Roost				1.13 ± 0.68	1.67	0.09		
	ln(BM)				0.63 ± 0.13	4.74	< 0.01		
Testes Mass	$Roost^2$ + Roost + ln (BM)	OLS	3, 79	15.69			< 0.01	0.37	-
	$Roost^2$				-0.38 ± 0.13	-2.93	< 0.01		
	Roost				1.97 ± 0.75	2.63	0.01		
	ln(BM)				0.74 ± 0.12	6.00	< 0.01		
Testes Mass	$Aggr^2$ + Aggr + ln (BM)	PGLS	3, 61	10.77			< 0.01	0.35	0.55 (0.00, 0.89)
	$Aggr^2$				-0.28 ± 0.11	-2.57	0.01		
	Aggr				1.10 ± 0.52	2.10	0.04		
	ln(BM)				0.64 ± 0.14	4.60	<0.01		

Dependent	Independent	Model	df	F	Estimate ± SE	t	p	R^2	λ (CI)
Testes Mass	$Aggr^2$ + Aggr + ln (BM)	OLS	3, 61	17.49			< 0.01	0.46	-
	$Aggr^2$				-0.35 ± 0.11	-3.27	< 0.01		
	Aggr				1.34 ± 0.50	2.26	< 0.01		
	ln(BM)				0.73 ± 0.13	5.57	<0.01		

Appendix 2: Supplementary Tables for Chapter 2

Table S2.1 Microsatellite primer sequences designed for *Phyllostomus hastatus*

Label	Forward/ Reverse	Primer sequence (5' - 3')	T_m	Motif	Product size
Phast01	F	AGATGAGCTCAGATGGGTGG	59.6	$(AC)_{11}$	133 - 144
	R	AAATCAGGCCAGCACATGTG	59.9		
Phast04	F	GATCCACTCGTCCTCCACTC	60.0	$(AC)_{13}$	162 - 165
	R	AAAGCTCTCTACTGCCGGTC	60.2		
Phast07	F	GCCAGAGTCACGTCTTTAAGC	60.0	$(AC)_{14}$	131 - 141
	R	CAGGCTGGGTAGTGTTTGTC	59.2		
Phast09	F	GAATCCAGGCAAACAAATGGG	58.8	$(AC)_{17}$	96 - 109
	R	CCTAGAGTCCGGAAGAAGCC	60.0		

Table S2.2 GLMs predicting the effects of both male and female attributes on the proportion of extra-group offspring present in the harem. Presented are the top-ranked models used for model averaging (ΔAICc < 4). C = male body condition, B = birth synchrony, G = group size, T = tooth wear, F = forearm length, sT = standard deviation of tooth wear, sF = standard deviation of forearm length. Subscripts indicate male (m) and average female (f) attributes.

Model	df	logLik	AICc	delta	weight
C	2	-21.46	48.1	0	0.13
$C + sF_f$	3	-19.79	48.3	0.13	0.12
$C + F_f + T_m$	4	-17.68	48.36	0.25	0.11
$C + F_f$	3	-19.92	48.51	0.39	0.11
$C + sF_f + sT_f$	4	-18.09	49.18	1.07	0.08
$C + sF_f + T_f$	4	-18.29	49.58	1.47	0.06
$C + T_m$	3	-20.50	49.66	1.55	0.06
C + B	3	-20.60	49.87	1.76	0.05
$C + sT_f$	3	-20.63	49.92	1.81	0.05
$C + T_f$	3	-21.01	50.69	2.58	0.04
$C + sF_f + F_f$	4	-18.90	50.81	2.70	0.03
$C * F_f + T_m$	5	-16.24	51.05	2.94	0.03
$C + sT_f + F_f$	4	-19.13	51.26	3.15	0.03
$C + sT_f + B$	4	-19.26	51.52	3.4	0.02
C + G	3	-21.44	51.54	3.43	0.02
$C * T_f + sF_f$	5	-16.55	51.67	3.56	0.02
$C + F_f + T_m$	4	-19.37	51.73	3.62	0.02
$C + sF_f + sT_f + T_f$	5	-16.72	52.01	3.9	0.02

Table S2.3 Coefficients for averaged model of Table S2.2

Variable	Estimate ± SE	95% CI	Importance
Intercept	-2.21 ± 0.49	(-3.18, -1.24)	
Male condition	**-0.36 ± 0.15**	**(-0.66, -0.06)**	**1**
Female forearm SD	-0.58 ± 1.02	(-2.59, 1.42)	0.35
Mean female forearm	0.36 ± 0.71	(-1.04, 1.76)	0.31
Female tooth wear SD	0.41 ± 1.10	(-1.74, 2.56)	0.20
Male tooth wear	-0.21 ± 0.50	(-1.20, 0.78)	0.22
Mean female tooth wear	0.19 ± 0.64	(-1.07, 1.44)	0.1
Birth sync	0.02 ± 0.09	(-0.16, 0.20)	0.08
Mean fem. forearm * male condition	-0.02 ± 0.14	(-0.30, 0.26)	0.03
Group size	-0.00 ± 0.01	(-0.02, 0.02)	0.02
Mean fem. tooth wear * male condition	-0.01 ± 0.13	(-0.26, 0.23)	0.02

Bibliography

Adams, D. M., Y. Li, and G. S. Wilkinson. 2018. Male Scent Gland Signals Mating Status in Greater Spear-Nosed Bats, *Phyllostomus hastatus*. Journal of Chemical Ecology, 44:975-986.

Alonzo, S. H. 2008. Conflict between the sexes and alternative reproductive tactics within a sex. Pp. 435-450 *in:* Alternative Reproductive Tactics: An Integrative Approach (R. F. Oliveira, M. Taborsky, and H. J. Brockmann, eds.). Cambridge University Press, Cambridge.

Anderson, M. J., J. Nyholt, and A. F. Dixson. 2005. Sperm competition and the evolution of sperm midpiece volume in mammals. Journal of Zoology, 267:135-142.

Anderson, M. J., and J. Robinson. 2003. Generalized discriminant analysis based on distances. Australian and New Zealand Journal of Statistics, 45:301-318.

Anderson, M. J., and T. J. Willis. 2003. Canonical analysis of principal coordinates: a useful method of constrained ordination for ecology. Ecology, 84:511-525.

Andersson, M. 1982. Female choice selects for extreme tail length in a widowbird. Nature, 299:818-820.

Andersson, M. 1994. Sexual Selection. Princeton University Press, Princeton.

Archie, E. A., and K. R. Theis. 2011. Animal behaviour meets microbial ecology. Animal Behaviour, 82:425-436.

Arita, H. T. 1993. Conservation biology of the cave bats of Mexico. Journal of Mammalogy, 74:693-702.

Arnold, S. J., and D. Duvall. 1994. Animal mating systems: a synthesis based on selection theory. The American Naturalist, 143:317-348.

Arnqvist, G., and M. Kirkpatrick. 2005. The evolution of infidelity in socially monogamous passerines: The strength of direct and indirect selection on extrapair copulation behavior in females. The American Naturalist, 165:S26-S37.

Badwaik, N. K., and J. J. I. V. Rasweiler. 2000. Pregnancy. Pp. 221-293 *in:* Reproductive Biology of Bats. (E. G. Crichton and P. H. Krutzsch, eds.). Academic Press, San Diego.

Barton, K. 2017. MuMIn: Multi-Model Inference.

Behr, O., and O. von Helversen. 2004. Bat serenades - complex courtship songs of the sac-winged bat (*Saccopteryx bilineata*). Behavioral Ecology and Sociobiology, 56:106-115.

Birkhead, T. R. 1978. Behavioural adaptations to high density nesting in the common guillemot *Uria aalge*. Animal Behaviour, 26:321-331.

Birkhead, T. R., and A. P. Møller. 1991. Frequent copulations and mate guarding as alternative paternity guards in birds: a comparative study. Behaviour, 118:170-186.

Bleier, W. J. 1975. Fine structure of implantation and the corpus luteum in the California Leaf-nosed bat, *Macrotus californicus*. PhD Thesis. Texas Tech University.

Bloss, J. 1999. Olfaction and the use of chemical signals in bats. Acta Chiropterologica, 1:31-45.

Bloss, J., T. E. Acree, J. M. Bloss, W. R. Hood, and T. H. Kunz. 2002. Potential use of chemical cues for colony-mate recognition in the big brown bat, *Eptesicus fuscus*. Journal of Chemical Ecology, 28:819-834.

Blount, J. D., Metcalfe, N. B., Birkhead, T. R., Surai, P. F. 2003. Carotenoid modulation of immune function and sexual attractiveness in zebra finches. Science, 5616:125-127.

Bohn, K. M., C. F. Moss, and G. S. Wilkinson. 2009. Pup guarding by greater spear-nosed bats. Behavioral Ecology and Sociobiology, 63:1693-1703.

Bohn, K. M., G. S. Wilkinson, and C. F. Moss. 2007. Discrimination of infant isolation calls by female greater spear-nosed bats, *Phyllostomus hastatus*. Animal Behaviour, 73:423-432.

Bolnick, D. I., L. K. Snowberg, J. G. Caporaso, C. Lauber, R. Knight, and W. E. Stutz. 2014. Major Histocompatibility Complex class IIb polymorphism influences gut microbiota composition and diversity. Molecular Ecology, 23:4831-4845.

Bose, A. P. H., J. M. Henshaw, H. Zimmermann, K. Fritzsche, and K. M. Sefc. 2019. Inclusive fitness benefits mitigate costs of cuckoldry to socially paired males. BMC Biology, 17.

Bouchard, S. 2001. Sex discrimination and roostmate recognition by olfactory cues in the African bats, *Mops condylurus* and *Chaerephon pumilus* (Chiroptera : Molossidae). Journal of Zoology, 254:109-117.

Boughman, J. W. 1997. Greater spear-nosed bats give group-distinctive calls. Behavioral Ecology and Sociobiology, 40:61-70.

Boughman, J. W., and G. S. Wilkinson. 1998. Greater spear-nosed bats discriminate group mates by vocalizations. Animal Behaviour, 55:1717-1732.

Bradbury, J., and S. L. Vehrencamp. 1998. Principles of Animal Communication. First edition. Sinauer Associates, Sunderland, Massachusetts.

Bradbury, J. W. 1977. Lek mating behavior in the hammer-headed bat. Zeitschrift Fur Tierpsychologie-Journal of Comparative Ethology, 45:225-255.

Bradbury, J. W., and S. L. Vehrencamp. 1977. Social organization and foraging in emballonurid bats: 3. Mating systems. Behavioral Ecology and Sociobiology, 2:1-17.

Briand, L., J.-C. Huet, V. Perez, G. Lenoir, C. Nespoulous, Y. Boucher, D. Trotier, and J.-C. Pernollet. 2004. Odorant and pheromone binding by aphrodisin, a hamster aphrodisiac protein. FEBS Letters, 476:179-185.

Brooke, A. P. 1990. Tent selection, roosting ecology, and social organization of the tent-making bat, *Ectophylla alba*, in Costa Rica. Journal of Zoology, 221:11-19.

Brooke, A. P. 1997. Organization and foraging behaviour of the fishing bat, *Noctilio leporinus* (Chiroptera:Noctilionidae). Ethology, 103:421-436.

Brooke, A. P., and D. M. Decker. 1996. Lipid compounds in secretions of fishing bat, *Noctilio leporinus* (Chiroptera: Noctilionidae). Journal of Chemical Ecology, 22:1411-1428.

Brouwer, L., I. Barr, M. van de Pol, T. Burke, J. Komdeur, and D. S. Richardson. 2010. MHC-dependent survival in a wild population: evidence for hidden genetic benefits gained through extra-pair fertilizations. Molecular Ecology, 19:3444-3455.

Brunet-Rossinni, A. K., and G. S. Wilkinson. 2009. Methods for age estimation and the study of senescence in bats. Pp. 315-325 *in:* Ecological and Behavioral Methods for the Study of Bats (T. H. Kunz and S. Parsons, eds.). Johns Hopkins University Press, Baltimore.

Buesching, C. D., C. Newman, and D. W. Macdonald. 2002a. Variations in colour and volume of the subcaudal gland secretion of badgers (*Meles meles*) in relation to sex, season and individual-specific parameters. Mammalian Biology, 67:147-156.

Buesching, C. D., J. S. Waterhouse, and D. W. MacDonald. 2002b. Gas-chromatographic analyses of the subcaudal gland secretion of the European badger (*Meles meles*) part II: Time-related variation in the individual-specific composition. Journal of Chemical Ecology, 28:57-69.

Burgener, N., M. Dehnhard, H. Hofer, and M. L. East. 2009. Does anal gland scent signal identity in the spotted hyaena? Animal Behaviour, 77:707-715.

Burnham, K. P., and D. R. Anderson. 2002. Model selection and multimodel inference: a practical information–theoretic approach. Springer, New York.

Caceres, M. D., and P. Legendre. 2009. Associations between species and groups of sites: indices and statistical inference. Ecology, 90:3566-3574.

Candolin, U., and L. Vlieger. 2013. Should attractive males sneak: the Ttade-off between current and future offspring. PLoS One, 8:e57992.

Carazo, P., E. Font, and E. Desfilis. 2008. Beyond 'nasty neighbours' and 'dear enemies'? Individual recognition by scent marks in a lizard (*Podarcis hispanica*). Animal Behaviour, 76:1953-1963.

Caspers, B., S. Franke, and C. C. Voigt. 2008. The wing-sac odour of male greater sac-winged bats *Saccopteryx bilineata* (Emballonuridae) as a composite trait: seasonal and individual differences. Pp. 151-160 *in:* Chemical Signals in Vertebrates 11 (J. L. Hurst, R. J. Beynon, S. C. Roberts, and T. D. Wyatt, eds.). Springer New York.

Caspers, B. A., F. C. Schroeder, S. Franke, and C. C. Voigt. 2011. Scents of adolescence: the maturation of the olfactory phenotype in a free-ranging mammal. PLoS One, 6.

Caspers, B. A., and C. C. Voigt. 2009. Temporal and spatial distribution of male scent marks in the polygynous greater sac-winged bat. Ethology, 115:713-720.

Charpentier, M. J. E., M. Boulet, and C. M. Drea. 2008. Smelling right: the scent of male lemurs advertises genetic quality and relatedness. Molecular Ecology, 17:3225-3233.

Charpentier, M. J. E., J. C. Crawford, M. Boulet, and C. M. Drea. 2010. Message 'scent': lemurs detect the genetic relatedness and quality of conspecifics via olfactory cues. Animal Behaviour, 80:101-108.

Chaverri, G., and T. H. Kunz. 2006. Roosting ecology of the test-roosting bat *Artibeus watsoni* (Chiroptera: Phyllostomidae) in Southwestern Costa Rica. Biotropica, 38:77-84.

Chaverri, G., and T. H. Kunz. 2010. Ecological determinants of social systems: perspectives on the functional role of roosting ecology in the social behavior of tent-roosting bats. Pp. 275-318 *in:* Advances in the Study of Behavior: Behavioral Ecology of Tropical Animals (R. Macedo, ed.). Academic Press, Burlington.

Chaverri, G., O. E. Quiros, M. Gamba-Rios, and T. H. Kunz. 2007. Ecological correlates of roost fidelity in the tent-making bat *Artibeus watsoni*. Ethology, 113:598-605.

Chaverri, G., C. J. Schneider, and T. H. Kunz. 2008. Mating system of the tent-making bat, *Artibeus watsoni* (Chiroptera: Phyllostomidae). Journal of Mammalogy, 89:1361-1371.

Cleasby, I., and S. Nakagawa. 2012. The influence of male age on within-pair and extra-pair paternity in passerines. Ibis, 154:318-324.

Clutton-Brock, T. H. 1989. Review lecture: mammalian mating systems. Proceedings of the Royal Society of London B: Biological Sciences, 236:339-372.

Clutton-Brock, T. H., P. H. Harvey, and B. Rudder. 1977. Sexual dimorphism, socionomic sex ratio and body weight in primates. Nature, 269:797-800.

Clutton-Brock, T. H., and K. Isvaran. 2006. Paternity loss in contrasting mammalian societies. Biology Letters, 2:513-516.

Clutton-Brock, T. H., and K. Isvaran. 2007. Sex differences in ageing in natural populations of vertebrates. Proceedings of the Royal Society B-Biological Sciences, 274:3097-3104.

Clutton-Brock, T. H., and G. A. Parker. 1995. Sexual coercion in animal societies. Animal Behaviour, 49:1345-1365.

Cohas, A., N. G. Yoccoz, and D. Allaine. 2007. Extra-pair paternity in alpine marmots, Marmota marmota: genetic quality and genetic diversity effects. Behavioral Ecology and Sociobiology, 61:1081-1092.

Darwin, C. 1871. The Descent of Man, and Selection in Relation to Sex. John Murray, London.

Davidson, S. M., and G. S. Wilkinson. 2004. Function of male song in the greater white-lined bat, *Saccopteryx bilineata*. Animal Behaviour, 67:883-891.

deCatanzaro, D. 2015. Sex steroids as pheromones in mammals: the exceptional role of estradiol. Hormones and Behavior, 68:103-116.

deCatanzaro, D., T. Pollock, L. J. Greville, and P. A. Faure. 2014. Estradiol transfer from male big brown bats (*Eptesicus fuscus*) to the reproductive and brain tissues of cohabiting females, and its action as a pheromone. General and Comparative Endocrinology, 208:126-133.

Dechmann, D. K., B. Kranstauber, D. Gibbs, and M. Wikelski. 2010. Group Hunting--A reason for sociality in molossid bats? PLoS One, 5:e9012.

Dechmann, D. K., and K. Safi. 2005. Studying communication in bats. Cognition, Brain, and Behavior, 9:479-496.

Dechmann, D. K. N., E. K. V. Kalko, B. König, and G. Kerth. 2005. Mating system of a neotropical roost-making bat: The white-throated, round-eared bat, *Lophostoma silvicolum* (Chiroptera: Phyllostomidae). Behavioral Ecology and Sociobiology, 58:316-325.

Dechmann, D. K. N., and G. Kerth. 2008. My home is your castle: Roost making is sexually selected in the bat *Lophostoma silvicolum*. Journal of Mammalogy, 89:1379-1390.

Dodge, J. C., M. B. Kristal, and L. L. Badura. 2002. Male-induced estrus synchronization in the female Siberian hamster (*Phodopus sungorus sungorus*). Physiology & Behavior, 77:227-231.

Dufrene, M., and P. Legendre. 1997. Species assemblages and indicator species: the need for a flexible asymmetrical approach. Ecological Monographs, 63:345-366.

Dunn, P. O., and A. Cockburn. 1998. Costs and benefits of extra-group paternity in superb fairy-wrens. Ornithological Monographs:147-161.

Dunn, P. O., L. A. Whittingham, and T. E. Pitcher. 2001. Mating systems, sperm competition, and the evolution of sexual dimorphism in birds. Evolution, 55:161-175.

Eastwood, J. R., M. L. Berg, R. F. H. Ribot, H. S. Stokes, J. M. Martens, K. L. Buchanan, K. Walder, and A. T. D. Bennett. 2018. Pair fidelity in long-lived

parrots: genetic and behavioural evidence from the Crimson Rosella (*Platycercus elegans*). Emu - Austral Ornithology, 118:369-374.

Ebling, F. J. 1977. Hormonal control of mammalian skin glands. Pp. 17-33 *in:* Chemical Signals in Vertebrates (D. Muller-Schwarze and M. M. Mozell, eds.). Springer, Boston, MA.

Eisenberg, J. F. 1989. Mammals of the Neotropics: the northern Neotropics. The University of Chicago Press, London.

Emlen, S. T., and L. W. Oring. 1977. Ecology, sexual selection, and the evolution of mating systems. Science, 197:215-223.

Ezenwa, V. O., and A. E. Williams. 2014. Microbes and animal olfactory communication: Where do we go from here? Bioessays, 36:847-854.

Fabiani, A., F. Galimberti, S. Sanvito, and A. R. Hoelzel. 2004. Extreme polygyny among southern elephant seals on Sea Lion Island, Falkland Islands. Behavioral Ecology, 15:961-969.

Fasel, N., V. Saladin, and H. Richner. 2016. Alternative reproductive tactics and reproductive success in male *Carollia perspicillata* (Seba's short-tailed bat). Journal of Evolutionary Biology, 29:2242-2255.

Ferkin, M. H., E. S. Sorokin, R. E. Johnston, and C. J. Lee. 1997. Attractiveness of scents varies with protein content of the diet in meadow voles. Animal Behaviour, 53:133-141.

Fernandez, A. A., N. Fasel, M. Knörnschild, and H. Richner. 2014. When bats are boxing: aggressive behaviour and communication in male Seba's short-tailed fruit bat. Animal Behaviour, 98:149-156.

Ferree, E. D., and J. L. Dickinson. 2011. Natural extrapair paternity matches receptivity patterns in unguarded females: evidence for importance of female choice. Animal Behaviour, 82:1167-1173.

Firman, R. C., C. Gasparini, M. K. Manier, and T. Pizzari. 2017. Postmating female control: 20 years of cryptic female choice. Trends in Ecology & Evolution, 32:368-382.

Fisher, H. S., R. R. Swaisgood, and H. Fitch-Snyder. 2003. Countermarking by male pygmy lorises (*Nycticebus pygmaeus*): do females use odor cues to select mates with high competitive ability? Behavioral Ecology and Sociobiology, 53:123-130.

Fleming, T. H. 1971. *Artibeus jamaincensis* - Delayed development in a neotropical bat. Science, 171:402-&.

Fleming, T. H. 1988. The Short-tailed Fruit Bat: A Study in Plant-Animal Interactions. University of Chicago Press, Chicago.

Forman, G. L., and H. H. Genoways. 1979. Sperm morphology. Pp. 177-204 *in:* Biology of Bats of the New World family Phyllostomatidae, Part III (R. J. Baker, J. K. J. Jr., and D. C. Carter, eds.). Texas Tech University Press, Lubbock, Texas.

Getty, T. 1989. Are dear enemies in a war of attrition. Animal Behaviour, 37:337-339.

Ginsberg, J. R., and U. W. Huck. 1989. Sperm competition in mammals. Trends in Ecology & Evolution, 4:74-79.

Gittleman, J. L., and B. Van Valkenburgh. 1997. Sexual dimorphism in the canines and skulls of carnivores: Effects of size, phylogeny, and behavioural ecology. Journal of Zoology, 242:97-117.

Gonzalez-Quinonez, N., G. Fermin, and M. Munoz-Romo. 2014. Diversity of bacteria in the sexually selected epaulettes of the little yellow-shouldered bat *Sturnira lilium* (Chiroptera: Phyllostomidae). Interciencia, 39:882-889.

Goodwin, G. G., and A. M. Greenhall. 1961. A review of the bats of Trinidad and Tobago. Bulletin of the American Museum Natural History, 122:195-301.

Gosling, L. M., and S. C. Roberts. 2001. Scent-marking by male mammals: Cheat-proof signals to competitors and mates. Advances in the Study of Behavior, 30:169-217.

Gosling, L. M., S. C. Roberts, E. A. Thornton, and M. J. Andrew. 2000. Life history costs of olfactory status signalling in mice. Behavioral Ecology and Sociobiology, 48:328-332.

Greene, L. K., K. E. Grogan, K. N. Smyth, C. A. Adams, S. A. Klager, and C. M. Drea. 2016. Mix it and fix it: functions of composite olfactory signals in ring-tailed lemurs. R Soc Open Sci, 3:160076.

Griffith, S. C., I. P. F. Owens, and K. A. Thuman. 2002. Extra pair paternity in birds: A review of interspecific variation and adaptive function. Molecular Ecology, 11:2195-2212.

Gustin, M. K., and G. F. McCracken. 1987. Scent recognition between females and pups in the bat *Tadarida brasiliensis mexicana*. Animal Behaviour, 35:13-19.

Harcourt, A. H., A. Purvis, and L. Liles. 1995. Sperm competition: mating system, not breeding season, affects testes size of primates. Functional Ecology, 9:468-476.

Harris, R. L., M. Boulet, K. E. Grogan, and C. M. Drea. 2018. Costs of injury for scent signalling in a strepsirrhine primate. Sci Rep, 8:9882.

Harvey, P. H., and M. D. Pagel. 1991. The Comparative Method in Evolutionary Biology. Oxford University Press, Oxford.

Hasselquist, D., and S. Bensch. 1991. Trade-off between mate guarding and mate attraction the polygynous great reed warbler. Behavioral Ecology and Sociobiology, 28:187-193.

Hasselquist, D., and P. W. Sherman. 2001. Social mating systems and extrapair fertilizations in passerine birds. Behavioral Ecology, 12:457-466.

Hasson, O., and L. Stone. 2009. Male infertility, female fertility and extrapair copulations. Biological Reviews of the Cambridge Philosophical Society, 84:225-244.

Heckel, G., C. C. Voigt, F. Mayer, and O. Von Helversen. 1999. Extra-harem paternity in the white-lined bat *Saccopteryx bilineata* (Emballonuridae). Behaviour, 136:1173-1185.

Heckel, G., and O. Von Helversen. 2003. Genetic mating system and the significance of harem associations in the bat *Saccopteryx bilineata*. Molecular Ecology, 12:219-227.

Hellriegel, B., and P. I. Ward. 1998. Complex female reproductive tract morphology: its possible use in postcopulatory female choice. Journal of Theoretical Biology, 190:179-186.

Hess, B. D., P. O. Dunn, and L. A. Whittingham. 2012. Females choose multiple mates in the lekking greater prairie chicken (*Tympanuchus cupido*). Auk, 129:133-139.

Hickey, M. B. C., and M. B. Fenton. 1987. Scent-dispersing hairs (osmetrichia) in some Pteropodidae and Molossidae (Chiroptera). Journal of Mammalogy, 68:381-384.

Hoelzel, A. R., B. J. Le Boeuf, J. Reiter, and C. Campagna. 1999. Alpha-male paternity in elephant seals. Behavioral Ecology and Sociobiology, 46:298-306.

Holler, P., and U. Schmidt. 1993. Olfactory communication in the lesser spear-nosed bat *Phyllostomus discolor* (Chiroptera, Phyllostomidae). Zeitschrift Fur Saugetierkunde, 58:257-265.

Hosken, D. J. 1997. Sperm competition in bats. Proceedings of the Royal Society B-Biological Sciences, 264:385-392.

Hosken, D. J. 1998. Testes mass in megachiropteran bats varies in accordance with sperm competition theory. Behavioral Ecology and Sociobiology, 44:169-177.

Hosken, D. J., and P. I. Ward. 2001. Experimental evidence for testis size evolution via sperm competition. Ecology Letters, 4:10-13.

Hunt, J., C. J. Breuker, J. A. Sadowski, and A. J. Moore. 2009. Male-male competition, female mate choice and their interaction: determining total sexual selection. Journal of Evolutionary Biology, 22:13-26.

Hurst, J. L., C. E. Payne, C. M. Nevison, A. D. Marie, R. E. Humphries, D. H. L. Robertson, A. Cavaggioni, and R. J. Beynon. 2001. Individual recognition in mice mediated by major urinary proteins. Nature, 414:631-634.

Hurst, J. L., D. H. L. Robertson, U. Tolladay, and R. J. Beynon. 1998. Proteins in urine scent marks of male house mice extend the longevity of olfactory signals. Animal Behaviour, 55:1289-1297.

Isaac, J. L. 2005. Potential causes and life-history consequences of sexual size dimorphism in mammals. Mammal Review, 35:101-115.

Isvaran, K., and T. Clutton-Brock. 2007. Ecological correlates of extra-group paternity in mammals. Proceedings of the Royal Society B: Biological Sciences, 274:219-224.

Isvaran, K., and S. Sankaran. 2017. Do extra-group fertilizations increase the potential for sexual selection in male mammals? Biology Letters, 13.

Jaeger, R. G. 1981. Dear enemy recognition and the costs of aggression between salamanders. American Naturalist, 117:962-974.

James, C. J. W. 1977. Studies of Reproduction in the female neotropical bat *Phyllostomus hastatus hastatus* (Pallas) with observations of general biology and ecoogy in Trinidad, W.I. University of the West Indies, St. Augustine, Trinidad.

Jennions, M. D., and M. Petrie. 2000. Why do females mate multiply? A review of the genetic benefits. Biological Reviews, 75:21-64.

Johansson, B. G., and T. M. Jones. 2007. The role of chemical communication in mate choice. Biological Reviews, 82:265-289.

Jones, A. G., D. Walker, C. Kvarnemo, K. Lindström, and J. C. Avise. 2001. How cuckoldry can decrease the opportunity for sexual selection: Data and theory from a genetic parentage analysis of the sand goby, *Pomatoschistus minutus*. Proceedings of the National Academy of Sciences, 98:9151-9156.

Jost, L. 2008. GST and its relatives do not measure differentiation. Molecular Ecology, 17:4015-4026.

Kalinowski, S. T., M. L. Taper, and T. C. Marshall. 2007. Revising how the computer program CERVUS accommodates genotyping error increases success in paternity assignment. Molecular Ecology, 16:1099-1106.

Kappeler, P. M. 1996. Intrasexual selection and phylogenetic constraints in the evolution of sexual canine dimorphism in strepsirhine primates. Journal of Evolutionary Biology, 9:43-65.

Kavaliers, M., and D. D. Colwell. 1995. Discrimination by female mice between the odors of parasitized and non-parasitized males. Proceedings of the Royal Society of London Series B-Biological Sciences, 261:31-35.

Kerth, G. 2008. Causes and consequences of sociality in bats. Bioscience, 58:737-746.

Kindt, R., and R. Coe. 2005. Tree diversity analysis: A manual and software for common statistical methods for ecological and biodiversity studies. World Agroforestry Centre (ICRAF), Nairobi.

Knörnschild, M., M. Feifel, and E. K. V. Kalko. 2014. Male courtship displays and vocal communication in the polygynous bat *Carollia perspicillata*. Behaviour, 151:781-798.

Kokko, H., and L. J. Morrell. 2005. Mate guarding, male attractiveness, and paternity under social monogamy. Behavioral Ecology, 16:724-731.

Kubinak, J. L., W. Z. Stephens, R. Soto, C. Petersen, T. Chiaro, L. Gogokhia, R. Bell, N. J. Ajami, J. F. Petrosino, L. Morrison, W. K. Potts, P. E. Jensen, R. M. O'Connell, and J. L. Round. 2015. MHC variation sculpts individualized microbial communities that control susceptibility to enteric infection. Nature Communications, 6:8642.

Kunz, T. H., P. V. August, and C. D. Burnett. 1983. Harem social organization in cave roosting *Artibeus jamaicensis* (Chiroptera: Phyllostomidae). Biotropica, 15:133-138.

Kunz, T. H., and A. Kurta. 1987. Size of bats at birth and maternal investment during pregnancy. Pp. 79-106 *in* Symposia of the Zoological Society of London.

Kunz, T. H., L. F. Lumsden, T. Kunz, and M. Fenton. 2003. Ecology of cavity and foliage roosting bats. Pp. 3-89 *in:* Bat Ecology (T. H. Kunz and M. B. Fenton, eds.). The University of Chicago Press, Chicago.

Kunz, T. H., and G. F. McCracken. 1996. Tents and harems: Apparent defence of foliage roosts by tent-making bats. Journal of Tropical Ecology, 12:121-137.

Kunz, T. H., S. K. Robson, and K. A. Nagy. 1998. Economy of harem maintenance in the greater spear-nosed bat, *Phyllostomus hastatus*. Journal of Mammalogy, 79:631-642.

Kurtz, J., M. Kalbe, P. B. Aeschlimann, M. A. Haberli, K. M. Wegner, T. B. Reusch, and M. Milinski. 2004. Major histocompatibility complex diversity influences parasite resistance and innate immunity in sticklebacks. Proceedings of the Royal Society of London, Series B: Biological Sciences, 271:197-204.

Lank, D. B., C. M. Smith, O. Hanotte, A. Ohtonen, S. Bailey, and T. Burke. 2002. High frequency of polyandry in a lek mating system. Behavioral Ecology, 13:209-215.

Lanyon, C. V., S. P. Rushton, G. O'Donnell A, M. Goodfellow, A. C. Ward, M. Petrie, S. P. Jensen, L. Morris Gosling, and D. J. Penn. 2007. Murine scent mark microbial communities are genetically determined. FEMS Microbiology Ecology, 59:576-583.

Lazar, J., L. E. Rasmussen, D. R. Greenwood, I. S. Bang, and G. D. Prestwich. 2004. Elephant albumin: a multipurpose pheromone shuttle. Chemistry & Biology, 11:1093-1100.

Leboeuf, B. J., and S. Mesnick. 1991. Sexual behavior of male northern elephant seals: 1. Lethal injuries to adult females. Behaviour, 116:143-162.

Leclaire, S., T. Merkling, C. Raynaud, H. Mulard, J. M. Bessiere, E. Lhuillier, S. A. Hatch, and E. Danchin. 2012. Semiochemical compounds of preen secretion reflect genetic make-up in a seabird species. Proceedings of the Royal Society B-Biological Sciences, 279:1185-1193.

Leclaire, S., J. F. Nielsen, and C. M. Drea. 2014. Bacterial communities in meerkat anal scent secretions vary with host sex, age, and group membership. Behavioral Ecology, 25:996-1004.

Legendre, P., and M. J. Anderson. 1999. Distance-based redundancy analysis: teting multispecies responses in multifactorial ecological experiments. Ecological Monographs, 69:1-24.

Lemons, P. R., T. C. Marshall, S. E. McCloskey, S. A. Sethi, J. A. Schmutz, and J. S. Sedinger. 2015. A likelihood-based approach for assessment of extra-pair paternity and conspecific brood parasitism in natural populations. Molecular Ecology Resources, 15:107-116.

Lewis, R. J. 2009. Chest staining variation as a signal of testosterone levels in male Verreaux's Sifaka. Physiology & Behavior, 96:586-592.

Lindenfors, P., J. L. Gittleman, and K. E. Jones. 2007. Sexual size dimorphism in mammals. Pp. 16-26 *in:* Sex, Size and Gender Roles: Evolutionary Studies of Sexual Size Dimorphism (D. J. Fairbairn, W. U. Blanckenhorn, and T. Székely, eds.). Oxford University Press, Oxford.

Lindenfors, P., B. S. Tullberg, and M. Biuw. 2002. Phylogenetic analyses of sexual selection and sexual size dimorphism in pinnipeds. Behavioral Ecology and Sociobiology, 52:188-193.

Lopez, P., and J. Martin. 2002. Chemical rival recognition decreases aggression levels in male Iberian wall lizards, Podarcis hispanica. Behavioral Ecology and Sociobiology, 51:461-465.

Lukas, D., and T. Clutton-Brock. 2014. Costs of mating competition limit male lifetime breeding success in polygynous mammals. Proceedings of the Royal Society of London, Series B: Biological Sciences, 281.

Maldonado-Chaparro, A. A., P. O. Montiglio, W. Forstmeier, B. Kempenaers, and D. R. Farine. 2018. Linking the fine-scale social environment to mating decisions: a future direction for the study of extra-pair paternity. Biological Reviews of the Cambridge Philosophical Society, 93:1558-1577.

Marshall, T. C., J. Slate, L. E. B. Kruuk, and J. M. Pemberton. 1998. Statistical confidence for likelihood-based paternity inference in natural populations. Molecular Ecology, 7:639-655.

Martin, J., R. Garcia-Roa, and R. Van Damme. 2018. Sexual selection and the chemical signal design of lacertid lizards. Zoological Journal of the Linnean Society, 183:445-457.

Martin, L., and R. T. F. Bernard. 2000. Endocrine regulation of reproduction in bats: The role of circulating gonadal hormones. Pp. 27-58 *in:* Reproductive Biology of Bats (E. G. Crichton and P. H. Krutzsch, eds.). Academic Press, San Diego.

Mays, H. L., and G. E. Hill. 2004. Choosing mates: good genes versus genes that are a good fit. Trends in Ecology & Evolution, 19:554-559.

McCracken, G. F., and J. W. Bradbury. 1977. Paternity and genetic heterogeneity in the polygynous bat, *Phyllostomus hastatus*. Science, 198:303-306.

McCracken, G. F., and J. W. Bradbury. 1981. Social organization and kinship in the polygynous bat *Phyllostomus hastatus*. Behavioral Ecology and Sociobiology, 8:11-34.

McCracken, G. F., and G. S. Wilkinson. 2000. Bat mating systems. Pp. 321-362 *in:* Reproductive Biology of Bats (E. G. Crichton and P. H. Krutzsch, eds.). Academic Press, London.

McKinney, F., S. r. Derrickson, and P. Mineau. 1983. Forced copulation in waterfowl. Behaviour, 86:250-294.

Medellin, R. A. 1989. *Chrotopterus auritus*. Mammalian Species, 343:1-5.

Meyer, C. F. J., E. K. Kalko, and G. Kerth. 2009. Small-scale fragmentation effects on local genetic diversity in two phyllostomid bats with different dispersal abilities in Panama. Biotropica, 41:95-102.

Minias, P., K. Wojczulanis-Jakubas, R. Rutkowski, K. Kaczmarek, and T. Janiszewski. 2016. Spatial patterns of extra-pair paternity in a waterbird colony: separating the effects of nesting density and nest site location. Behavioral Ecology and Sociobiology, 70:369-376.

Moller, A. P. 1988. Ejaculate quality, testes size, and sperm competition in primates. Journal of Human Evolution, 17:479-488.

Moller, A. P., and J. V. Briskie. 1995. Extra-pair paternity, sperm competition and the evolution of testis size in birds. Behavioral Ecology and Sociobiology, 36:357-365.

Morrison, D. W. 1979. Apparent male defense of tree hollows in the bat, *Artibeus jamaicensis*. Journal of Mammalogy, 60:11-15.

Morrow, G. E., S. M. Jones, and S. C. Nicol. 2016. Interaction of hibernation and male reproductive function in wild Tasmanian echidnas *Tachyglossus aculeatus setosus*. Journal of Mammalogy, 97:852-860.

Munoz-Romo, M., J. F. Burgos, and T. H. Kunz. 2011. Smearing behaviour of male *Leptonycteris curasoae* (Chiroptera) and female responses to the odour of dorsal patches. Behaviour, 148:461-483.

Munoz-Romo, M., and T. H. Kunz. 2009. Dorsal patch and chemical signaling in males of the long-nosed bat, *Leptonycteris curasoae* (Chiroptera: Phyllostomidae). Journal of Mammalogy, 90:1139-1147.

Munoz-Romo, M., L. T. Nielsen, J. M. Nassar, and T. H. Kunz. 2012. Chemical composition of the substances from dorsal patches of males of the curasoaen long-nosed bat, *Leptonycteris curasoae* (Phyllostomidae: Glossophaginae). Acta Chiropterologica, 14:213-224.

Murray, K. L., and T. H. Fleming. 2008. Social structure and mating system of the buffy flower bat, *Erophylla sezekorni* (Chiroptera, Phyllostomidae). Journal of Mammalogy, 89:1391-1400.

Nassar, J. M., M. V. Salazar, A. Quintero, K. E. Stoner, M. Gomez, A. Cabrera, and K. Jaffe. 2008. Seasonal sebaceous patch in the nectar-feeding bats *Leptonycteris curasoae* and *L. yerbabuenae* (Phyllostomidae : Glossophaginae): phenological, histological, and preliminary chemical characterization. Zoology, 111:363-376.

Nowak, R. M. 1994. Walker's Bats of the World. Johns Hopkins University Press, Baltimore, Maryland.

Nunn, C. L. 1999. The evolution of exaggerated sexual swellings in primates and the graded-signal hypothesis. Animal Behaviour, 58:229-246.

Okada, K., M. Katsuki, M. D. Sharma, C. M. House, and D. J. Hosken. 2014. Sexual conflict over mating in Gnatocerus cornutus? Females prefer lovers not fighters. Proceedings of the Royal Society B-Biological Sciences, 281:9.

Oksanen, J., F. G. Blanchet, M. Friendly, R. Kindt, P. Legendre, D. McGlinn, P. R. Minchin, R. B. O'Hara, G. L. Simpson, P. Solymos, M. H. H. Stevens, E. Szoecs, and H. Wagner. 2016. vegan: Community Ecology Package. R.

Orme, D., R. Freckleton, G. Thomas, T. Petzoldt, S. Fritz, N. Isaac, and W. Pearse. 2013. caper: Comparative Analyses of Phylogenetics and Evolution in R.

Orr, T. J., and M. Zuk. 2013. Does delayed fertilization facilitate sperm competition in bats? Behavioral Ecology and Sociobiology, 67:1903-1913.

Orr, T. J., and M. Zuk. 2014. Reproductive delays in mammals: an unexplored avenue for post-copulatory sexual selection. Biological Reviews, 89:889-912.

Ortega, J., and H. T. Arita. 1999. Structure and social dynamics of harem groups in *Artibeus jamaicensis* (Chiroptera : Phyllostomidae). Journal of Mammalogy, 80:1173-1185.

Ortega, J., and H. T. Arita. 2000. Defence of females by dominant males of *Artibeus jamaicensis* (Chiroptera : Phyllostomidae). Ethology, 106:395-407.

Ortega, J., and H. T. Arita. 2002. Subordinate males in harem groups of Jamaican fruit-eating bats (*Artibeus jamaicensis*): Satellites or sneaks? Ethology, 108:1077-1091.

Ortega, J., J. A. Guerrero, and J. E. Maldonado. 2008. Aggression and tolerance by dominant males of *Artibeus jamaicensis*: strategies to maximize fitness in harem groups. Journal of Mammalogy, 89:1372-1378.

Ortega, J., and J. E. Maldonado. 2006. Female interactions in harem groups of the Jamaican fruit-eating bat, *Artibeus jamaicensis* (Chiroptera : Phyllostomidae). Acta Chiropterologica, 8:485-495.

Ortega, J., J. E. Maldonado, G. S. Wilkinson, H. T. Arita, and R. C. Fleischer. 2003. Male dominance, paternity, and relatedness in the Jamaican fruit-eating bat (*Artibeus jamaicensis*). Molecular Ecology, 12:2409-2415.

Overath, P., T. Sturm, and H. G. Rammensee. 2014. Of volatiles and peptides: in search for MHC-dependent olfactory signals in social communication. Cellular and Molecular Life Sciences, 71:2429-2442.

Pagel, M. 1999. Inferring the historical patterns of biological evolution. Nature, 401:877-884.

Palphramand, K. L., and P. C. L. White. 2007. Badgers, *Meles meles*, discriminate between neighbour, alien and self scent. Animal Behaviour, 74:429-436.

Parker, G. A. 1990. Sperm competition games: sneaks and extra-pair copulations. Proceedings: Biological Sciences, 242:127-133.

Parker, G. A., and M. A. Ball. 2005. Sperm competition, mating rate and the evolution of testis and ejaculate sizes: a population model. Biology Letters, 1:235-238.

Peakall, R. a. S. P. E. 2012. GenAlEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research- an update. Bioinformatics, 28:2537-2539.

Penn, D., and W. K. Potts. 1998. Chemical signals and parasite-mediated sexual selection. Trends in Ecology & Evolution, 13:391-396.

Penn, D. J. 2002. The scent of genetic compatibility: Sexual selection and the major histocompatibility complex. Ethology, 108:1-21.

Perry, J. C., L. Sirot, and S. Wigby. 2013. The seminal symphony: how to compose an ejaculate. Trends in Ecology & Evolution, 28:414-422.

Petrie, M., M. Hall, T. Halliday, H. Budgey, and C. Pierpoint. 1992. Multiple mating in a lekking bird: Why do peahens mate with more than one male and with the same male more than once? Behavioral Ecology and Sociobiology, 31:349-358.

Petrie, M., and B. Kempenaers. 1998. Extra-pair paternity in birds: Explaining variation between species and populations. Trends in Ecology and Evolution, 13:52-57.

Plavcan, J. M. 2012. Sexual size dimorphism, canine dimorphism, and male-male competition in primates: where do humans fit in? Human Nature-an Interdisciplinary Biosocial Perspective, 23:45-67.

Plavcan, J. M., and C. P. van Schaik. 1992. Intrasexual competition and canine dimorphism in anthropoid primates. American Journal of Physical Anthropology, 87:461-477.

Plavcan, J. M., and C. P. van Schaik. 1997. Intrasexual competition and body weight dimorphism in anthropoid primates. American Journal of Physical Anthropology, 103:37-67.

Porter, F. L. 1979. Social behavior in the leaf-nosed bat, *Carollia perspicillata* 1. Social Organizaiton. Journal of Comparative Ethology, 49:406-417.

Porter, T. A., and G. S. Wilkinson. 2001. Birth synchrony in greater spear-nosed bats (*Phyllostomus hastatus*). Journal of Zoology, 253:383-390.

Preston, B. T., I. R. Stevenson, G. A. Lincoln, S. L. Monfort, J. G. Pilkington, and K. Wilson. 2012. Testes size, testosterone production and reproductive behaviour in a natural mammalian mating system. Journal of Animal Ecology, 81:296-305.

Promislow, D. E. L. 1992. Costs of sexual selection in natural populations of mammals. Proceedings of the Royal Society of London, Series B: Biological Sciences, 247:203-210.

R Core Team. 2016. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Racey, P. A., and A. C. Entwistle. 2000. Life-history and reproductive strategies of bats. Pp. 636-414 *in:* Reproductive Biology of Bats (E. G. Crichton and P. H. Krutzsch, eds.). Academic Press, London.

Radwan, J., M. Chadzinska, M. Cichon, S. C. Mills, B. Matula, E. T. Sadowska, K. Baliga, A. Stanisz, S. Lopuch, and P. Koteja. 2006. Metabolic costs of sexual advertisement in the bank vole (*Clethrionomys glareolus*). Evolutionary Ecology Research, 8:859-869.

Radwan, J., A. Tkacz, and A. Kloch. 2008. MHC and preferences for male odor in the bank vole. Ethology, 114:827-833.

Ralls, K. 1976. Mammals in which females are larger than males. The Quarterly Review of Biology, 51:245-276.

Ralls, K. 1977. Sexual dimorphism in mammals: avian models and unanswered questions. The American Naturalist, 111:917-938.

Rantala, M. J., I. Jokinen, R. Kortet, A. Vainikka, and J. Suhonen. 2002. Do pheromones reveal male immunocompetence? Proceedings of the Royal Society B-Biological Sciences, 269:1681-1685.

Rasweiler, J. J. 1979. Early embryonic development and implantation in bats. Journal of Reproduction and Fertility, 56:403-416.

Rasweiler, J. J., and N. K. Badwaik. 1997. Delayed development in the short-tailed fruit bat, *Carollia perspicillata*. Journal of Reproduction and Fertility, 109:7-20.

Ratterman, N. L., G. G. Rosenthal, and A. G. Jones. 2009. Sex recognition via chemical cues in the sex-role-reversed Gulf pipefish (*Syngnathus scovelli*). Ethology, 115:339-346.

Rehorek, S. J., T. D. Smith, and K. P. Bhatnagar. 2010. The orbitofacial glands of bats: an investigation of the potential correlation of gland structure with social organization. Anatomical Record-Advances in Integrative Anatomy and Evolutionary Biology, 293:1433-1448.

Reid, F. 1997. A field guide to the mammals of Central America and Southeast Mexico. Oxford University Press.

Reid, J. M., and R. J. Sardell. 2012. Indirect selection on female extra-pair reproduction? Comparing the additive genetic value of maternal half-sib extra-pair and within-pair offspring. Proceedings of the Royal Society B: Biological Sciences, 279:1700-1708.

Rensch, B. 1959. Evolution Above the Species Level. Columbia University Press, New York.

Revell, L. J. 2009. Size-correction and principal components for interspecific comparative studies. Evolution, 63:3258-3268.

Rich, T. J., and J. L. Hurst. 1998. Scent marks as reliable signals of the competitive ability of mates. Animal Behaviour, 56:727-735.

Rich, T. J., and J. L. Hurst. 1999. The competing countermarks hypothesis: reliable assessment of competitive ability by potential mates. Animal Behaviour, 58:1027-1037.

Richardson, D. S., J. Komdeur, T. Burke, and T. von Schantz. 2005. MHC-based patterns of social and extra-pair mate choice in the Seychelles warbler. Proceedings of the Royal Society B-Biological Sciences, 272:759-767.

Rico-Guevara, A., and K. J. Hurme. 2019. Intrasexually selected weapons. Biological Reviews of the Cambridge Philosophical Society.

Roberts, S.-J., E. Nikitopoulos, and M. Cords. 2014. Factors affecting low resident male siring success in one-male groups of blue monkeys. Behavioral Ecology, 25:852-861.

Roberts, S. A., M. C. Prescott, A. J. Davidson, L. McLean, R. J. Beynon, and J. L. Hurst. 2018. Individual odour signatures that mice learn are shaped by involatile major urinary proteins (MUPs). BMC Biology, 16:48.

Rodríguez-Herrera, B., G. Ceballos, and R. A. Medellín. 2011. Ecological aspects of the tent building process by *Ectophylla alba* (Chiroptera: Phyllostomidae). Acta Chiropterologica, 13:365-372.

Roellig, K., B. R. Menzies, T. B. Hildebrandt, and F. Goeritz. 2011. The concept of superfetation: a critical review on a 'myth' in mammalian reproduction. Biological Reviews, 86:77-95.

Rojas, D., O. M. Warsi, and L. M. Dávalos. 2016. Bats (Chiroptera: Noctilionidae) challenge a recent origin of extant neotropical diversity. Systematic Biology, 65:432-448.

Rosell, F., and T. Bjorkoyli. 2002. A test of the dear enemy phenomenon in the Eurasian beaver. Animal Behaviour, 63:1073-1078.

Rossiter, S. J., R. D. Ransome, C. G. Faulkes, D. A. Dawson, and G. Jones. 2006. Long-term paternity skew and the opportunity for selection in a mammal with reversed sexual size dimorphism. Molecular Ecology, 15:3035-3043.

Rousset, F. 2008. genepop'007: a complete re-implementation of the genepop software for Windows and Linux. Molecular Ecology Resources, 8:103-106.

RStudio Team. 2015. RStudio: Integrated Development for R. RStudio, Inc., Boston, MA.

Ryan, M. J., and A. S. Rand. 1993. Species recognition and sexual selection as a unitary problem in animal communication. Evolution, 47:647-657.

Safi, K., and G. Kerth. 2003. Secretions of the interaural gland contain information about individuality and colony membership in the Bechstein's bat. Animal Behaviour, 65:363-369.

Safi, K., and G. Kerth. 2007. Comparative analyses suggest that information transfer promoted sociality in male bats in the temperate zone. The American Naturalist, 170:465-472.

Sagot, M., and R. D. Stevens. 2012. The evolution of group stability and roost lifespan: perspectives from tent-roosting bats. Biotropica, 44:90-97.

Santos, M., L. F. Aguirre, L. B. Vazquez, and J. Ortega. 2003. Phyllostomus hastatus. Mammalian Species:1-6.

Santos, P. S., A. Courtiol, A. J. Heidel, O. P. Honer, I. Heckmann, M. Nagy, F. Mayer, M. Platzer, C. C. Voigt, and S. Sommer. 2016. MHC-dependent mate choice is linked to a trace-amine-associated receptor gene in a mammal. Sci Rep, 6:38490.

Schad, J., D. K. Dechmann, C. C. Voigt, and S. Sommer. 2012. Evidence for the 'good genes' model: association of MHC class II DRB alleles with ectoparasitism and reproductive state in the neotropical lesser bulldog bat, Noctilio albiventris. PLoS One, 7:e37101.

Schaedelin, F. C., and M. Taborsky. 2009. Extended phenotypes as signals. Biological Reviews, 84:293-313.

Schlicht, L., M. Valcu, and B. Kempenaers. 2015. Spatial patterns of extra-pair paternity: beyond paternity gains and losses. Journal of Animal Ecology, 84:518-531.

Schuelke, M. 2000. An economic method for the fluorescent labeling of PCR fragments. Nature Biotechnology, 18:233-234.

Scully, W. M. R., M. B. Fenton, and A. S. M. Saleuddin. 2000. A histological examination of the holding sacs and glandular scent organs of some bat

species (Emballonuridae, Hipposideridae, Phyllostomidae, Vespertilionidae, and Molossidae). Canadian Journal of Zoology, 78:613-623.

Seddon, N., C. A. Botero, J. A. Tobias, P. O. Dunn, H. E. Macgregor, D. R. Rubenstein, J. A. Uy, J. T. Weir, L. A. Whittingham, and R. J. Safran. 2013. Sexual selection accelerates signal evolution during speciation in birds. Proc Biol Sci, 280:20131065.

Seehausen, O., Y. Terai, I. S. Magalhaes, K. L. Carleton, H. D. Mrosso, R. Miyagi, I. van der Sluijs, M. V. Schneider, M. E. Maan, H. Tachida, H. Imai, and N. Okada. 2008. Speciation through sensory drive in cichlid fish. Nature, 455:620-626.

Setchell, J. M., S. Vaglio, K. M. Abbott, J. Moggi-Cecchi, F. Boscaro, G. Pieraccini, and L. A. Knapp. 2011. Odour signals major histocompatibility complex genotype in an Old World monkey. Proceedings of the Royal Society of London, Series B: Biological Sciences, 278:274-280.

Setchell, J. M., S. Vaglio, J. Moggi-Cecchi, F. Boscaro, L. Calamai, and L. A. Knapp. 2010. Chemical Composition of scent-gland secretions in an Old World Monkey (*Mandrillus sphinx*): Influence of sex, male status, and individual identity. Chemical Senses, 35:205-220.

Sharpe, L. L. 2015. Handstand scent marking: height matters to dwarf mongooses. Animal Behaviour, 105:173-179.

Shuster, S. M., and M. J. Wade. 2003. Mating Systems and Strategies. Princeton University Press, Princeton.

Simmons, N. B., and A. L. Cirranello. In press. Species diversity and discovery: a new Golden Age for Phyllostomidae *in:* Phyllostomid Bats, A Unique Mammalian Radiation (T. H. Fleming, L. Dávalos, and M. Mello, eds.). Chicago University Press, Chicago.

Smotherman, M., Knörnschild, M., Smarsh, G., & Bohn, K. . 2016. The origins and diversity of bat songs. Journal of Comparative Physiology A, 202:535-554.

Smuts, B. B., and R. W. Smuts. 1993. Male aggression and sexual coercion of females in nonhuman primates and other mammals: evidence and theoretical implications. Advances in the Study of Behavior, 22:1-63.

Stern, A. A., and T. H. Kunz. 1998. Intraspecific variation in postnatal growth in the greater spear-nosed bat. Journal of Mammalogy, 79:755-763.

Stern, A. A., T. H. Kunz, and S. S. Bhatt. 1997. Seasonal wing loading and the ontogeny of flight in *Phyllostomus hastatus* (Chiroptera: Phyllostomidae). Journal of Mammalogy, 78:1199-1209.

Stevens, R. D., M. E. Johnson, and E. S. McCulloch. 2013. Absolute and relative secondary-sexual dimorphism in wing morphology: a multivariate test of the 'Big Mother' hypothesis. Acta Chiropterologica, 15:163-170.

Stockley, P., M. J. G. Gage, G. A. Parker, and A. P. Moller. 1997. Sperm competition in fishes: The evolution of testis size and ejaculate characteristics. American Naturalist, 149:933-954.

Storz, J. F., H. R. Bhat, and T. H. Kunz. 2001. Genetic consequences of polygyny and social structure in an Indian fruit bat, *Cynopterus sphinx*. II. Variance in male mating success and effective population size. Evolution, 55:1224-1232.

Sutherland, W. J. 1985. Chance can produce a sex difference in variance in mating success and explain Bateman's data. Animal Behaviour, 33:1349-1352.

Swedell, L., L. Leedom, J. Saunders, and M. Pines. 2014. Sexual conflict in a polygynous primate: costs and benefits of a male-imposed mating system. Behavioral Ecology and Sociobiology, 68:263-273.

Symonds, M. R. E., and S. P. Blomberg. 2014. A primer on phylogenetic generalised least squares *in:* Modern Phylogenetic Comparative Methods and Their Application in Evolutionary Biology (L. Z. Garamszegi, ed.). Spring-Verlag, Berlin.

Tavares, V. d. C., and A. Tejedor. 2009. The forelimb swellings of *Pygoderma bilabiatum* (Chiroptera: Phyllostomidae). Chiroptera Neotropical, 15:411-416.

Temeles, E. J. 1990. Northern harriers on feeding territories respond more aggressively to neighbors than to floaters. Behavioral Ecology and Sociobiology, 26:57-63.

Temeles, E. J. 1994. The role of neighbors in territorial systems: when are they 'dear enemies'? Animal Behaviour, 47:339-350.

Thomas, M. L., and L. W. Simmons. 2011. Crickets detect the genetic similarity of mating partners via cuticular hydrocarbons. Journal of Evolutionary Biology, 24:1793-1800.

Thornhill, R., and J. Alcock. 1983. The Evolution of Insect Mating Systems. Harvard University Press, Cambridge, Massechusetts.

Tibbetts, E. A., and J. Dale. 2007. Individual recognition: it is good to be different. Trends in Ecology & Evolution, 22:529-537.

Tinnesand, H. V., S. Jojola, A. Zedrosser, and F. Rosell. 2013. The smell of desperadoes? Beavers distinguish between dominant and subordinate intruders. Behavioral Ecology and Sociobiology, 67:895-904.

Tourmente, M., M. Gomendio, and E. R. S. Roldan. 2011. Sperm competition and the evolution of sperm design in mammals. BMC Evolutionary Biology, 11:12.

Townsend, Andrea K., Anne B. Clark, and Kevin J. McGowan. 2010. Direct benefits and genetic costs of extrapair paternity for female American crows (*Corvus brachyrhynchos*). The American Naturalist, 175:E1-E9.

Trivers, R. L. 1972. Parental investment and sexual selection. Pp. 136-179 *in:* Sexual Selection and the Descent of Man (B. Campbell, ed.). Aldline, Chicago.

Tschapka, M. 2004. Energy density patterns of nectar resources permit coexistence within a guild of Neotropical flower-visiting bats. Journal of Zoology, 263:7-21.

Tuttle, M. D. 1976. Collecting Techniques. Pp. 71-88 *in:* Biology Bats of the New World Family Phyllostomatidae (R. J. Baker, J. K. Jones, Jr., and D. C. Carter, eds.). Texas Tech Press, Lubbock, Texas.

Uller, T., and M. Olsson. 2008. Multiple paternity in reptiles: patterns and processes. Molecular Ecology, 17:2566-2580.

Valera, F., H. Hoi, and A. Krištín. 2003. Male shirkes punish unfaithful females. Behavioral Ecology, 14:403-408.

van Bergen, E., P. M. Brakefield, S. Heuskin, B. J. Zwaan, and C. M. Nieberding. 2013. The scent of inbreeding: a male sex pheromone betrays inbred males. Proceedings of the Royal Society B-Biological Sciences, 280.

van der Merwe, M., and I. L. Rautenbach. 1987. Reproduction in Schlieffen's bat, *Nycticeius schlieffenii*, in the eastern Transvaal lowveld, South Africa. Journal of Reproduction and Fertility, 81:41-50.

Vehrencamp, S. L., F. G. Stiles, and J. W. Bradbury. 1977. Observations on the foraging behavior and avian prey of the neotropical carnivorous bat, *Vampyrum spectrum*. Journal of Mammalogy, 58:469-478.

Voigt, C. C., B. Caspers, and S. Speck. 2005a. Bats, bacteria, and bat smell: Sex-specific diversity of microbes in a sexually selected scent organ. Journal of Mammalogy, 86:745-749.

Voigt, C. C., G. Heckel, and F. Mayer. 2005b. Sexual selection favours small and symmetric males in the polygynous greater sac-winged bat *Saccopteryx bilineata* (Emballonuridae, Chiroptera). Behavioral Ecology and Sociobiology, 57:457-464.

Voigt, C. C., and O. von Helversen. 1999. Storage and display of odour by male *Saccopteryx bilineata* (Chiroptera, Emballonuridae). Behavioral Ecology and Sociobiology, 47:29-40.

Wade, M. J., and S. J. Arnold. 1980. The intensity of sexual selection in relation to male sexual behavior, female choice, and sperm precedence. Animal Behaviour, 28:446-461.

Wade, M. J., and S. M. Shuster. 2004. Sexual selection: harem size and the variance in male reproductive success. The American Naturalist, 164:E83-E89.

Ward, H. L., R. D. Ransome, G. Jones, and S. J. Rossiter. 2014. Determinants and patterns of reproductive success in the greater horseshoe bat during a population recovery. PLoS One, 9.

Warton, D. I., S. T. Wright, and Y. Wang. 2012. Distance-based multivariate analyses confound location and dispersion effects. Methods in Ecology and Evolution, 3:89-101.

Webster, M. S., K. A. Tarvin, E. M. Tuttle, and S. Pruett-Jones. 2007. Promiscuity drives sexual selection in a socially monogamous bird. Evolution, 61:2205-2211.

Weckerly, F. W. 1998. Sexual-size dimorphism: Influence of mass and mating systems in the most dimorphic mammals. Journal of Mammalogy, 79:33-52.

Westerdahl, H., J. Waldenstrom, B. Hansson, D. Hasselquist, T. von Schantz, and S. Bensch. 2005. Associations between malaria and MHC genes in a migratory songbird. Proc Biol Sci, 272:1511-1518.

Westneat, D. F., and H. L. Mays, Jr. 2005. Tests of spatial and temporal factors influencing extra-pair paternity in red-winged blackbirds. Molecular Ecology, 14:2155-2167.

Westneat, D. F., and P. W. Sherman. 1997. Density and extra-pair fertilizations in birds: a comparative analysis. Behavioral Ecology and Sociobiology, 41:205-215.

Westneat, D. F., and I. R. K. Stewart. 2003. Extra-pair paternity in birds: causes, correlates, and conflict. Annual Review of Ecology Evolution and Systematics, 34:365-396.

Wilkinson, G. S. 1985a. The social organization of the common vampire bat. I. Pattern and cause of association. Behavioral Ecology and Sociobiology, 17:111-121.

Wilkinson, G. S. 1985b. The social organization of the common vampire bat. II. Mating system, genetic structure, and relatedness. Behavioral Ecology and Sociobiology, 17:123-134.

Wilkinson, G. S., and J. W. Boughman. 1998. Social calls coordinate foraging in greater spear-nosed bats. Animal Behaviour, 55:337-350.

Wilkinson, G. S., G. G. Carter, K. M. Bohn, and D. M. Adams. 2016. Non-kin cooperation in bats. Philosophical Transactions of the Royal Society of London B: Biological Sciences, 371.

Wilkinson, G. S., and A. M. Chapman. 1991. Length and sequence variation in evening bat D-loop mtDNA. Genetics, 128:607-617.

Wilkinson, G. S., F. Mayer, G. Kerth, and B. Petri. 1997. Evolution of repeated sequence arrays in the D-loop region of bat mitochondrial DNA. Genetics, 146:1035-1048.

Wilkinson, G. S., and G. F. McCracken. 2003. Bats and balls: sexual selection and sperm competition in Chiroptera. Pp. 128-155 *in:* Bat Ecology (T. H. Kunz and M. B. Fenton, eds.). The University of Chicago Press, Chicago.

Willig, M. R., and R. R. Hollander. 1995. Secondary sexual dimorphism and phylogenetic constraints in bats - a multivariate approach. Journal of Mammalogy, 76:981-992.

Wong, B. B. M., and U. Candolin. 2005. How is female mate choice affected by male competition? Biological Reviews of the Cambridge Philosophical Society, 80:559-571.

Ydenberg, R. C., L. A. Giraldeau, and J. B. Falls. 1988. Neighbors, strangers, and the asymmetric war of attrition. Animal Behaviour, 36:343-347.

Zala, S. M., W. K. Potts, and D. J. Penn. 2004. Scent-marking displays provide honest signals of health and infection. Behavioral Ecology, 15:338-344.

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Steve Fetter, Ph.D.
Dean of the Graduate School
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Dear Dean Fetter,

This letter is written to signify that the dissertation committee, committee chair, and the graduate director have all approved the use of previously published co-authored work in the final dissertation of Danielle Adams, Biological Sciences Graduate Program, 111143535. In accordance with the Graduate School's policy the dissertation committee has determined that they made substantial contributions to the included work.

The citation for the co-authored work is:

Adams, D. M., Y. Li, and G. S. Wilkinson. 2018. Male Scent Gland Signals Mating Status in Greater Spear-Nosed Bats, *Phyllostomus hastatus*. Journal of Chemical Ecology, 44:975-986.

Per Graduate School policy the dissertation forward will identify the scope and nature of the student's contributions to the jointly authored work included in the dissertation and a copy of this letter will be submitted with the dissertation.

Sincerely,

Dr. Gerald Wilkinson Dissertation Committee Chair, Professor, Biology

Dr. Zakiya Whatley, Program Manager, Biological Sciences Graduate Program

Danielle Adams, Doctoral Candidate, Biological Sciences