Causes and consequences of sociality in a neotropical bat

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Presented by
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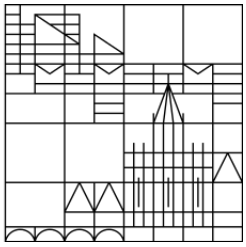

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Summary

In this thesis, I investigated the causes and consequences of sociality in the neotropical bat *Molossus molossus*. Due to the short availability of the insect patches it forages on and the energetic costs of flight, this species is at energetic edge every day. Social foraging of group members, using acoustic information transfer to locate insect patches, was recently proposed as one reason for sociality of this species. The aim of my thesis was to assess the relationship between social structure, foraging efficiency and survival using several social groups studied in the village of Gamboa, Panama. Because species identification in this genus was unreliable, we first used molecular, morphometric and acoustic data to distinguish *M. molossus* from the sympatric species *M. coibensis* and *M. bondae*. The social groups of *M. molossus* typically formed small harems of ten adults and their offspring. Because members of the same social group forage together, small group size may result from a trade-off between benefits of patch detection and costs of conspecific interference. Based on our data collected with automated monitoring systems, one foraging session of ca. one hour after dusk was energetically sufficient for individuals to fast until the following dusk period. We found no correlation between group size and survival which may reflect a similar survival within the natural range of group sizes. The short female lifespan (median of 1.8 years) that we found is likely a result from life at the energetic edge due to a highly specialized diet (patches of ephemeral insects).

Zusammenfassung

Diese Dissertation ist eine Abhandlung meiner Forschungsarbeit in Panama und befasst sich weitestgehend mit der Frage warum neotropische Fledermäuse der Spezies Molossus molossus in sozialen Gruppen leben. M. molossus Individuen leben an einem energetischen Limit. Zum einen ist ihre primäre Futterquelle (abendliche Insektenschwärme) eingeschränkt und nur über einen kurzen Zeitraum verfügbar, und zum anderen ist der aktive Flug eine Fortbewegungsart die viel Energie benötigt. Dieses Dilemma erfordert deshalb hocheffiziente Jagdstrategien. Eine aktuelle Studie mit M. molossus zeigt, dass der akustische Informationsaustausch über vorhandene Insektenschwärme vermutlich einer der Hauptgründe für Sozialverhalten in dieser und anderen Fledermausspezies ist. Das Ziel dieser Dissertation war es, die Beziehung zwischen der Sozialstruktur und Jagdeffizienz mehrerer, individueller Fledermausgruppen zu untersuchen. Mithilfe der gesammelten Daten konnten außerdem Rückschlüsse über die Evolution von Sozialverhalten in dieser Spezies gezogen werden. Da die Unterscheidung zwischen den einzelnen Spezies der Gattung Molossus bis dato relativ schwierig war, wurde am Anfang der Forschungsarbeit ein neuer Ansatz entwickelt. Mithilfe einer Kombination aus morphologischen, akustischen und genetischen Daten wurde M.molossus von zwei sympatrischen Spezies, M. coibensis und M. bondae, unterschieden. Dieser Ansatz machte es möglich gezielte Studien an M. molossus durchzuführen.

Die gesammelten Daten zeigen, dass M. molossus Gruppen kleine Harems sind, die aus 10+ ausgewachsenen Tieren und ihrem Nachwuchs bestehen. Die konsistent kleine Gruppengröße

lässt vermuten [In unseren Studien gingen meist die Mitglieder derselben sozialen Gruppe zusammen auf Nahrungsjagd, was vermuten lässt], das kleine Gruppen selektiv bevorzugt sind und dass die Vorteile dieser Gruppengröße (z.B. gemeinsame, effiziente Jagd) die Nachteile (z.B. Konkurenz um Fortpflanzungsmöglichkeiten) überwiegen. Während der Feldarbeit wurden automatische Erkennungssysteme an den Schlafplätzen individueller Gruppen installiert, die Daten über Identität und individuelles Gewicht sammelten. Anhand dieser Daten konnte gezeigt werden, dass M. molossus Individuen in einer Jagdperiode (eine Stunde nach Sonnenuntergang) genug Energie in Form von Insekten bis zur nächsten Jagdperiode zu sich nehmen . Des Weiteren wurde keine Wechselbeziehung zwischen Gruppengröße und individueller Lebenspanne in dieser Spezies gefunden. Dies könnte eine ähnliche Lebensspanne innerhalb des natürlichen Bereichs der Gruppengröße reflektieren. Die in unseren Ergebnissen geringe Lebenspanne bei weiblichen Fledermäuse(im Durchschnitt 1,8 Jahre) könnte ein Resultat des energetischen Limits aufgrund der hoch-spezialisierten Diät (Patchs von kurzlebigen Insekten) sein, an dem sich die Tiere tagtäglich befinden.

General introduction

Social living characterizes many species on Earth. Why animals are social and how group interactions are balanced has been the focus of many empirical and theoretical studies (Wilson 2000, Alcock 2003, Rubenstein 2009). A common theoretical condition for social living implies that net benefits of group living outweigh the costs in terms of evolutionary fitness (Hamilton 1964, Lehmann & Keller 2006). Costs may include higher parasitism rates and infection risk, risk of inbreeding, increased detectability by predators and competition for resources, such as food and mating opportunities. Benefits of sociality can be either simple density-dependent effects that result from aggregations of individuals (e.g. improved microclimate) or cooperative behavior including alloparenting, allogrooming and joint efforts in the building of shelters (Ward & Zahavi 1973, Milinski 1987, Axelrod & Dion 1988, Crowley 1996, Dugatkin & Mesterton-Gibbons 1996, Beauchamp et al. 1997, Buckley 1997, Hatchwell & Komdeur 2000).

One adaptive mechanism favoring sociality is increased foraging success through active or passive information transfer (Wilkinson & Boughman 1998, Safi & Kerth 2007, Dechmann et al. 2009). In addition to personal information that an individual gains from direct interaction with its physical environment, social animals can benefit from information transfer about food resources (type, location and amount; (Conradt & Roper 2003, Dall et al. 2005) to increase their foraging efficiency. This enhanced food acquisition through information transfer can theoretically provide benefits in the short-term (i.e. improved foraging success over a feeding

bout) and also in the long-term by leading to increased fitness. Therefore, social foraging should be favored over solitary foraging under certain ecological circumstances like diet ephemerality (unpredictable resource in space and time but abundant). A comprehensive social foraging theory has emerged in the last decade, with models predicting the size of foraging groups and predictions regarding the use of food resources for group members and whole groups (Giraldeau & Caraco 2000). However, little empirical evidence is available in mammals, despite the richness of social mammalian species. Moreover, the majority of studies on social foraging are biased towards the use of visual information (Fernández-Juricic et al. 2004) whereas mechanisms for nocturnal species that rely on auditory perception for social foraging is understudied (but see (Cvikel et al. 2015)).

As flying nocturnal mammals, bats constitute an ideal system to the evolution of group living, with more than 1300 species described ("1331 and counting" 2015). Bats exhibit a broad range of social systems, from a solitary lifestyle over seasonal aggregations to stable societies. These variations in sociality are found along ecological, environmental, and morphological gradients over different habitats (Kerth 2008). When social, bats can obtain information about food from other individuals through various sensory modalities (vision, sound and olfaction). The use of information transfer has been proposed as an important driving force in social evolution of bats. Information transfer about food patches appears as a promoter of group living in male groups in the temperate zones (Safi & Kerth 2007), segregated female or male colonies in the subtropics (Levin et al. 2013, Cvikel et al. 2015) as well as mixed groups in the tropics

(Dechmann et al. 2009, 2010). By acquiring social information about food, bats can theoretically reduce foraging time and increase their energy intake. Over the long-term, an improved foraging efficiency could ultimately enhance fitness and promote social foraging. But is information transfer about food really a route for the evolution of sociality in bats? The aim of my thesis was to investigate this question in mixed groups from the tropical *Molossus molossus*. I focused on four key questions in my different chapters:

- **Chapter 1**: What are the knowns and unknowns of social foraging in bats?
- **Chapter 2**: How to distinguish between three species of sympatric bats, including *Molossus molossus*, a species proven to forage with group members?
- **Chapter 3**: What is the relationship between group size, survival and longevity in the socially foraging bat *M. molossus*?
- **Chapter 4**: What are the foraging patterns and efficiency of the socially foraging bat *M. molossus*?

In **Chapter 1**, we reviewed the literature on social foraging in bats and its implications on the evolution of social group living. We examined the different mechanisms of information transfer by describing the location (i.e. in the roost or on the wing) and the diversity of information transfer (i.e. social cues or signals). We also discussed the costs and benefits of actors and recipients facing different situations of social foraging. Finally, we identified three main gaps to the study of social foraging: resource properties, interactions between individuals and fitness consequences.

Social foraging has been recently characterized in the neotropical bat *Molossus molossus*. A telemetry study showed that group members were foraging in close proximity to each other (Dechmann et al. 2010). By eavesdropping on the change in echolocation calls emitted during prey capture (Barclay 1982, Wilkinson & Boughman 1998, List 2004), conspecifics can enhance prey searching (Cvikel et al. 2015). The narrow-shaped wing morphology of *M. molossus* results in high energetic requirements within an open-air foraging niche (Voigt & Holderied 2012). Additionally, the foraging activity of *M. molossus* is limited to a short environmental window around dusk and dawn (Dechmann et al. 2010, Esbérard & Bergallo 2010, Holland et al. 2011) that follow the peaks of emergence of ephemeral insects (Jones & Rydell 2003). Taken together, the energetic costs of flight as well as the patchiness and the short availability of insect patches may put individuals of *M. molossus* at an energetic edge. Therefore, we hypothesize that the use of social information should be highly beneficial for this species to increase foraging efficiency in short-term, and ultimately increase fitness.

As a first step to work on the species *M. molossus*, we needed to be able to correctly identify them. The taxonomy of the genus *Molossus* is challenging because species are morphologically similar and often occur in sympatry. As a prerequisite for further research on this species, we used a multi-method approach to validate the taxonomy of several groups captured in the village of Gamboa, Panama (**Chapter 2**). We compared molecular data from DNA-based markers with morphometric and echolocation call information to assess the reliability of each

type of information to reliably differentiate *M. molossus* from the other sympatric species of the genus.

We then used a set of social groups reliably identified as *M. molossus* (**Chapter 2**) to investigate group size and its relationship with survival and longevity (**Chapter 3**). This central aspect of evolutionary ecology is highly dependent on demographic and ecological circumstances. A previous telemetry study characterized that individuals from the same social group forage together (Dechmann et al. 2010). For this socially foraging species, we expected a (small) stable group size (Sibly 1983), which is directly related to the potential for information transfer, social coordination, and costs of conspecific interference (Cvikel et al. 2015). Unlike most bats, *M. molossus* can be recaptured from the same roost repeatedly and across multiple years, allowing to obtain reliable survival data over a long period of time. In a capture-recapture study, we recaptured the social groups multiple times over several years to obtain variations of group size as well as the survival of individually tagged bats. We used survival analyses on this mark-recapture dataset to evaluate the relationship between group size and survival.

Finally, we assessed the foraging patterns and foraging efficiency of individuals from five social groups of *M. molossus* (**Chapter 4**). We wanted to understand the behavioral and energetic strategies this species used to cope with the energetic costs of flight as well as the patchiness and short availability of insect patches. Foraging efficiency is a currency that is usually difficult to obtain in free-ranging animals. Because these bats crawl on their bellies to enter and exit the

roost, we could use automated scales installed at the exits of some roots to automatically identify and weigh animals via PIT-tag. By recording body mass gain over time, we identified foraging patterns and energetic strategies of this socially foraging animal.

This original research covers a wide range of disciplines, ranging from taxonomy to evolutionary ecology. Relevant to taxonomists and fieldwork biologists, we provided a multi-method approach to identify traits relevant to the differentiation of sympatric species that will be relevant to taxonomist and fieldwork biologists. We also provided novel information on unique behavioral and energetic strategies for a species extremely specialized for insect patches.

Chapter 1 - Information transfer: a reason for sociality in bats?

In preparation for submission

Chapter 1

Information transfer: a reason for sociality in bats?

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Abstract

Information transfer about ephemeral food sources has been proposed as a driver for the evolution of sociality. Given their remarkable range of social systems and ecological niches, bats constitute an ideal group to study sociality and social foraging in a bigger evolutionary context. Here we review current literature about information transfer connected to sociality and foraging behavior in bats, and the potential implications on the evolution of sociality in this taxa. Generally, information transfer about food sources appears crucial especially in male aggregations of insectivorous species in temperate zones and several tropical bat species. In some species, coordinated foraging (assessed with light-emitting diode or radio-tracking) is also closely associated with communal nursing. The gaps in knowledge in socially foraging bats are identified. Studying the interplay between social information transfer and foraging behavior in bats can add to our understanding of information transfer about food sources as a potential promotor of the evolution of sociality.

Sociality and its factors

Group living occurs in many species and the question why animals are social and how group interactions are balanced has been the focus of many empirical and theoretical studies (Wilson 2000, Alcock 2003, Rubenstein 2009). A common theoretical condition for social living implies that overall benefits of group living outweigh the costs in terms of evolutionary fitness (Hamilton 1964, Lehmann & Keller 2006). Costs include for example higher parasitism rates, risk of inbreeding, infection risk, increased detectability by predators, and competition for limited food resources and/or mating opportunities. Benefits of sociality can be density-dependent effects that result from aggregations of individuals (e.g. improved microclimate) or cooperative behavior (e.g. allogrooming, joint effort in the building of shelters (Ward & Zahavi 1973, Milinski 1987, Axelrod & Dion 1988, Crowley 1996, Dugatkin & Mesterton-Gibbons 1996, Beauchamp et al. 1997, Buckley 1997, Hatchwell & Komdeur 2000)). Thereby, group living is considered an advantage in evolutionary fitness if the benefits outweigh the costs.

Bats as model species

With more than 1300 species around the world ("1331 and counting" 2015), bats constitute an appropriate model to study the evolution of sociality. Bats show a broad range of social systems, from a solitary lifestyle over seasonal aggregations to stable closed societies found along with morpho-ecological gradients over different habitats and climates. A recent review stated three potential origins to explain group living in the order Chiroptera: 1) ecological

constraints (i.e. roost limitation), 2) physiological demands (i.e. social thermoregulation) and 3) demographic traits, where long-lived and philopatric animals form multigenerational social groups (Kerth 2008).

Social foraging in bats

Another hypothesis, increased foraging efficiency through active or passive information transfer about ephemeral resources has recently emerged as an alternative driver for the evolution of sociality in bats (Safi & Kerth 2007, Dechmann et al. 2009, 2010). Studies have revealed that by observing the behavior of others, animals are able to acquire social information about the location, quantity, and quality of food (Horn 1968, Krebs 1974). That information can increase feeding efficiency is beneficial for individuals to increase energy intake per unit time, reduce the time exposed to predation risk and competition, and to save metabolic energy which can be allocated to other essential behaviors (e.g. reproduction). Short-term benefits in such cases can be defined as an increase in mean food intake rate (MFIR) and/or a reduction in variance of mean food intake rate (Beauchamp 2005). Long-term effects of information transfer about food sources could improve fitness with increased survival and/or greater numbers of individual offspring.

The location of information transfer

Whether social foraging is beneficial depends on the spatial and temporal distribution and availability of resources. The patchiness of the resource is a crucial parameter that favours grouping in different species of birds and mammals (Johnson et al. 2002). A tight link exists between the habitat and the bat morpho-ecology and the resource and the location of information transfer (Fig. 1-1). Depending on the temporal stability of diet, information transfer can either occur outside or in the roost. If the resource is only briefly available (few hours like ephemeral insects), its exploitation occurs mostly by bats adapted to open-air (fast-flyers with narrow wings). If the resource remains available for long periods (several hours to days, e.g. fruits), knowledgeable bats – with broad wings to manoeuver in cluttered environments - can transfer information in the feeding areas but also in their roosts (Ratcliffe & Ter Hofstede 2005, O'Mara, Dechmann, et al. 2014) following the "information-center hypothesis" (Ward & Zahavi 1973). The distribution and availability of resources not only influence the foraging habitat and the morpho-ecology but also the nature of the information transfer about food sources.

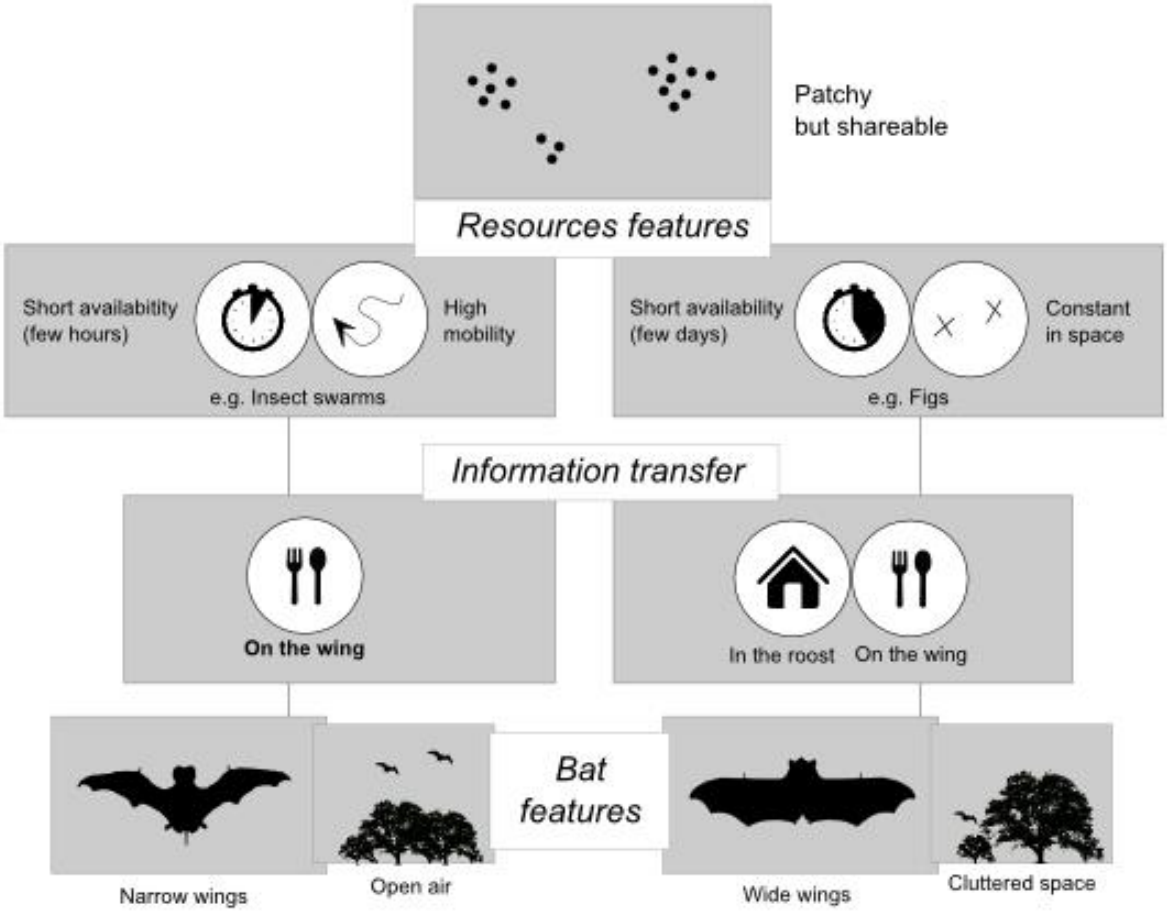

Figure 1-1. A simplified overview of social foraging in bats. The nature of the diet influences the foraging niche of bats, typically the foraging habitat, the morpho-ecology and the location of information transfer.

The nature of information transfer

While bats exploiting their environment, individuals rely on different kinds of social information (summarized in Table 1-1). **Inadvertent social information*** (* = definition in the glossary) via **social cues*** (Galef & Giraldeau 2001) corresponds to passive information transfer, typically

individuals producing inevitable cues that could be utilized when making movement decisions. Social cues can be obtained in the spatially restricted context of the roost. For instance, frugivorous evaluate olfactory cues of conspecifics to learn about novel food sources (Ratcliffe & Ter Hofstede 2005, O'Mara, Dechmann, et al. 2014). In another study, naïve fringe lipped bats (*Trachops cirrhosus*) learned to feed on novel prey (i.e. toads) from experienced individuals based on species specific prey calls (Page & Ryan 2006). Moreover, the smell of fresh urine was perceived and identified as a social cue related to foraging success by evening bats (*Nycticeus humeralis*) (Wilkinson 1992a). Unsuccessful individuals of this species have been observed to follow successful group members leaving for another foraging bout (Wilkinson 1992a). The majority of bat species are echolocating insectivores that emit echolocation pulses that constitute another prime target to eavesdrop upon (Fenton 2003, Gillam 2007, Dechmann et al. 2009). These pulses have the potential to convey information about species specific traits, group affiliation and individual characteristics like sex, body size and age (Voigt-Heucke et al. 2010, Jones & Siemers 2011, Knornschild et al. 2012). In a foraging context, these echolocating bats rely on **terminal phases*** (or feeding buzzes) for the final acquisition of prey (Schnitzler et al. 2003). By eavesdropping, individuals can learn not only about the location but also the density of individuals which may be correlated with food. The echoes of the feeding buzz can only be perceived from a short distance by the echolocating bat (e.g. respectively 4-6 m in *Noctilio albiventris* and 0.5-2 m in *Molossus molossus* (Dechmann et al. 2009, 2010)), but can be transferred inadvertently over a much larger distance to other echolocating bats (e.g. respectively 35-40 m in *Noctilio albiventris* and 54 m in *Molossus molossus* (Dechmann et al.

2009, 2010)). The number of feeding buzzes could be an indication about the quality of the food patch and has the potential to influence the decision of other bats in the vicinity to explore the unknown area. Thus, bats from different species have been shown to react strongly to playbacks of these terminal phases (Gillam 2007, Dechmann et al. 2009). A study combining a GPS combined to an acoustic microphone, mounted on the insectivorous *Rhinopoma microphyllum*, proved through echolocation recordings that these bats actively aggregate (Cvikel et al. 2015).

Contrary to social cues which are inadvertent, social information via **signals*** is **advertent***. Many echolocating bats broadcast social calls to attract or repel individuals (Pfalzer & Kusch 2003). Two types of acoustic signals linked to food are described, the territorial calls and "contact" calls. According to the "food-patch defense hypothesis", territorial calls are used for spacing individuals while they forage and avoid competition between conspecifics as shown for *Corynorhinus rafinesquii, Lasiurus cinereus semotus* and *Pipistrellus pipistrellus* (Belwood & Fullard 1984, Budenz et al. 2009, Loeb & Britzke 2010). The frequency of these agonistic calls increases with the number of bats in the foraging area and also with the diminution of prey availability (Belwood & Fullard 1984, Racey & Swift 1985). Territorial calls are also used by individuals in groups, for example in greater spear nosed bats (*Phyllostomus hastatus)* where individuals apparently defend flower patches they feed upon (Wilkinson & Boughman 1998). The frequency of aggression calls would rise with the augmentation of individuals foraging together in the food patch**.** In parallel, contact calls are crucial to ensure the coordination of

group members or mother/infant pairs. Bats from the same roost can exit simultaneously during clustered departures, using social calls for coordination as observed in common pipistrelles (*Pipistrellus pipistrellus)* (Racey & Swift 1985).

Consequences of information transfer about food

Mechanisms of information transfer about food sources are highly diverse in bats and involve different resources and sensory modes to acquire information. Information transfer – either advertent or inadvertent – has potential fitness consequences, both for the producer and the receiver of this information. In addition, social information is crucial to learn about novel and familiar food sources with strong spatio-temporal availability (Ratcliffe & Ter Hofstede 2005, O'Mara, Dechmann, et al. 2014). Information transfer in the roost (odor transfer on the breath) constitutes a strategy to reduce costs associated with home-range monitoring (Ratcliffe & Ter Hofstede 2005). Food sources already known can be explored preferentially, ensuring increased foraging efficiency. In many cases, information transfer and social foraging can lead to faster food discoveries (Pitcher et al. 1982, Götmark et al. 1986) and also acts as a buffer against variable hunting success (Caraco et al. 1995).

Information transfer and potential consequences

Assessing the fitness consequences of information transfer is challenging in free ranging and flying nocturnal animals like bats. In this paragraph, we discuss benefits and costs of

information transfer about food from the producer and the receiver's perspectives. Producer and receiver can be positively or negatively influenced by information transfer. Four categories of social behaviors can be described, regarding the respective effects of information transfer for the actor and the recipient: mutual benefit (+/+), altruism (-/+), selfishness (+/-) and spite (-/-) (West et al. 2007). Mutual benefits in bats could result from food searching and food patch defense. Fieldwork and comparative studies suggested that male aggregations in temperate bats and mixed-sex groups in the tropics result from benefits of social foraging through enhanced prey searching and potentially more effective tracking of the dynamic resource (i.e. local enhancement) (Safi & Kerth 2007, Dechmann et al. 2009, 2010). Mutual benefits from food-patch defense are also suggested for group members of greater spear-nosed bats (*Phyllostomus hastatus)*. Altruism (-/+), a behavior costly to the actor and beneficial to the recipient, is described in vampire bats through direct food sharing (Carter & Wilkinson 2013) but we did not find examples only for information transfer. Contrary to altruism, selfishness implies only benefits to the producer. This is likely to be the case in the context of territorial calls of single individuals, like in *Pipistrellus pipistrellus* (Racey & Swift 1985). Spite, where both producer and receiver experience negative effects might occurs in the context of aggression from territorial calls, but we did not find direct evidence for this.

Other social behaviors in socially foraging bats

Other social behaviors can be associated to social foraging such as nursing. For example, nursing of nondescendant offspring is observed in group members of *Phyllostomus hastatus*

and *Nycticeus humeralis* despite low levels of relatedness (Wilkinson 1992b, Bohn et al. 2009). Direct benefits from milk dumping – either immediate or delayed – have been suggested by Wilkinson (Wilkinson 1992b). Dumping milk prior to a foraging bout decreases weight to potentially optimize foraging efficiency and favor associated milk production. Delayed direct benefits are increased survival of pups, and subsequent increases in colony size and potential for information transfer.

Table 1-1. Glossary (adapted from Dall et al. 2005, with references included).

Keyword	Description
Altruism	A behavior which is costly to the actor and beneficial to the recipient; in this case and below, cost and benefit are defined on the basis of the lifetime direct fitness consequences of a behavior (West et al. 2007).
Direct fitness	The component of fitness gained through the impact of an individual's behavior on the production of offspring (West et al. 2007).
Inadvertent social information	A class of cues that are produced inadvertently by individuals engaged in some activity, such as foraging, fighting, mating, and so on (Danchin et al. 2004)
Indirect fitness	The component of fitness gained from aiding the reproduction of related individuals
Information-center (hypothesis)	The colony functions as a central place for exchanging information about the location of food patches (Information Center Hypothesis) (Hagan III & Walters 1990).
Local enhancement	How the presence of foragers at a patch makes the patch most obvious to other searchers (Buckley 1997)
Mutual benefit	A behavior which is beneficial to both the actor and the recipient (West et al. 2007).
Signals	Sources of socially acquired information that are elicited to influence the behavior of others. They are generally studied as 'communication'.
Social calls	Social calls are vocalizations produced in addition to echolocation calls, and carry information to conspecifics (Pfalzer & Kusch 2003).
Social cues	A type of inadvertent social information that conveys discrete information about the presence or absence of some feature (e.g. presence or absence of predators or the spatial location of a food patch) (Galef & Giraldeau 2001).

Social foraging	Individuals collect information about food by observing the behavior of other individuals
Social information	Any information that is generated by the behavior of another organism.
Terminal phases (or feeding buzzes):	Calls emitted by echolocating bats immediately before attacking airborne insects, they indicate the potential presence of prey in the nearby area (Gillam 2007).

Table 1-2. A classification of social information about food in bats.

		Behaviour (producer)	Information	Sense (receiver)	Location	Consequences (producer/receiver)	Refs.
Inadvertent	**Social cues**	Eating	Food preferences of group members (e.g. toads)			Competition (-/+) Local enhancement (+/+)	Page et al. 2006
		Breathing waste product	Food preferences of group members (e.g. fruits) Foraging success (urine smell)			Competition (-/+) Local enhancement (+/+)	Ratcliffe et al. 2005 Wilkinson 1992
		Feeding buzzes	Location + quality of food patch (insect swarms)			Competition (-/+) Local enhancement (+/+)	Gillam 2007 Dechmann et al. 2009
Advertent	**Signals**	"Territorial" calls	Location of food patch (e.g. fruits trees)			Intruder repelled (+/-) Competition (-/+) (-/-) Group defence of the food patch (+/+)	Wilkinson et al. 1998
		Contact calls	Location of group members			Competition (-/+) Local enhancement (+/+) Food-patch defence (+/+)	Pfalzer & Kusch 2003

Future avenues of research

Considering the diversity of bat species (“1331 and counting” 2015) and their wide variety of socio-ecology, the current knowledge on mechanisms of social foraging in bats is probably fragmentary. We identify here three main gaps for future research on social foraging in bats: (i) knowledge on resource properties, (ii) interactions between individuals and (iii) fitness

consequences of information transfer.

i. Resource properties

Theoretically, only a resource that is patchy can be shared in the context of social foraging. Different approaches have been used to characterize resource properties. Based on a comparative study, Safi and Kerth characterized diet based on taxonomy to categorize insects as ephemeral food sources or not (Safi & Kerth 2007). Wilkinson estimated insect density based on automated suction traps (Wilkinson 1992a). For a study on the Egyptian fruit bat, Shohami quantified the resource distribution of fruiting trees (Shohami 2015). However, assessing resource properties remains technically challenging and the majority of the studies on social foraging in bats do not provide information about the level of patchiness of the food source. Additional knowledge on the spatial and temporal properties of food sources is required to better understand the conditions for the evolution of social foraging. Several tools are available to understand how the properties of the resource affect individuals and information transfer, including modelling (Torney et al. 2011) and molecular biology to investigate feces and diet properties (Bohmann et al. 2011, Alberdi et al. 2012).

ii. Interactions between individuals

Assessing the use of information by individuals in the wild remain technically challenging. Marking bats with Pit-tags, light tags or rings allows researchers to characterize and describe interactions between individuals, especially around the roost. Infra-red video recordings within

roost communities are likely to reveal different social behaviors like food sharing, communal nursing or mutual allogrooming as shown in different species (Wilkinson 1992b, Bohn et al. 2009, Carter & Wilkinson 2013, Geipel et al. 2013). Telemetry studies are another strategy to assess in-flight dynamics and spatial locations of individuals at specified time points and thereby potential for information transfer (e.g. (Dechmann et al. 2010)). GPS combined with acoustic microphones are emerging methods for investigating social foraging of bat groups on the wing for echolocating bats (Cvikel et al. 2015).

iii. Fitness consequences

Finally, assessing fitness consequences of social foraging constitutes a great challenge for researchers. Food sharing, shown in *Desmodus rotundus* and *Micronycteris microtis* (Carter & Wilkinson 2013, Geipel et al. 2013), suggests direct evidence for short term as well as long term benefits. However, other potential benefits of information transfer are more difficult to characterize. The challenge will be to link the information transfer and its use to how it affects individual fitness. Investigation of fitness-related parameter like foraging efficiency, survival and reproductive output in relation to the social environment like group size are promising avenues of research.

In this review, we summarize a comprehensive amount of data regarding social information transfer related to foraging ecology of bats. Studying the interplay between social information and available food sources in bats can add to our understanding of the importance of

information transfer, its influence on fitness consequences, and the implications for the evolution of animal sociality.

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Chapter 2 - The value of molecular vs. morphometric and acoustic information for species identification using sympatric molossid bats

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Chapter 2

The value of molecular vs. morphometric and acoustic information for species identification using sympatric molossid bats

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Abstract

A fundamental condition for any work with free-ranging animals is correct species identification. However, in case of bats, information on local species assemblies is frequently limited especially in regions with high biodiversity such as the Neotropics. The bat genus *Molossus* is a typical example of this, with morphologically similar species often occurring in sympatry. We used a multi-method approach based on molecular, morphometric and acoustic information collected from 962 individuals of *Molossus bondae*, *M. coibensis*, and *M. molossus* captured in Panama. We distinguished *M.bondae* based on size and pelage coloration. We identified two robust species clusters composed of *M. molossus* and *M. coibensis* based on 18 microsatellite markers but also on a more stringently determined set of four markers. Phylogenetic reconstructions using the mitochondrial gene co1 (DNA barcode) were used to diagnose these microsatellite clusters as *M. molossus* and *M.coibensis*. To differentiate species, morphological information was only reliable when forearm length and body mass were combined in a linear discriminant function (95.9% correctly identified individuals).
When looking in more detail at *M. molossus* and *M. coibensis*, only four out of 13 wing parameters were informative for species differentiation, with *M. coibensis* showing lower values for hand wing area and hand wing length and higher values for wing loading. Acoustic recordings after release required categorization of calls into types, yielding only two informative subsets: approach calls and two-toned search calls. Our data emphasizes the importance of combining morphological traits and independent genetic data to inform the best choice and combination of discriminatory information used in the field. Because parameters

can vary geographically, the multi-method approach may need to be adjusted to local species assemblies and populations to be entirely informative.

Keywords: Chiroptera, DNA barcoding, Molossidae, morphometrics, Neotropics, systematics

Introduction

Molecular biology, with the study of mitochondrial and nuclear genomes, has revolutionized our understanding of the distribution and evolutionary history of worldwide species diversity. In the context of mammalian species diversity, the order Chiroptera (= bats) constitutes an exceptional taxon, with over 1331 species listed in a recent systematic review ("1331 and counting" 2015) representing a fifth of all extant mammals. Molecular studies have also led to the discovery of many cryptic lineages and boosted the number of described bat species. For example, analyses of mitochondrial genes revealed several cryptic species in well-studied areas such as Europe (Kiefer et al. 2002, Ibáñez et al. 2006, Mayer et al. 2007, Puechmaille, Allegrini, et al. 2012, Bogdanowicz et al. 2015). The use of DNA barcoding (Hebert & Gregory 2005) led to a reevaluation of the number of bat species in the tropics (Francis et al. 2010, Clare et al. 2011, Wilson et al. 2014). Based on their sequence similarity, the barcodes can be clustered into Molecular Operational Taxonomic Unit (MOTU) (Floyd et al. 2002). One great advantage of the DNA barcoding is the important database available for comparative purposes (bold: The Barcode of Life Data System, (Ratnasingham & Hebert 2007)). However, DNA barcodes present

pitfalls linked to maternal inheritance (reviewed in (Rubinoff et al. 2006)) and should always be considered in conjunction with other sources of data. For instance, nuclear microsatellite loci were used successfully to identify *Pipistrellus kuhlii* as one biological species with two mitochondrial barcodes (Andriollo et al. 2015). The use of nuclear microsatellites is also powerful to detect potential interspecific hybridization, otherwise undetected via the sole use of mitochondrial barcodes (Berthier et al. 2006). Other taxonomic parameters, such as morphological characters or echolocation calls, should also be combined with molecular data, following for example the framework of Integrated Operational Taxonomic Units (IOTUs) (Galimberti et al. 2012). Integrating traditional taxonomy to molecular taxonomy is seen as the future of taxonomy (Padial et al. 2010).

Despite this recent boost of bat diversity with molecular species identification, the status of many bat taxa is not yet firmly established. The bat genus *Molossus* (family Molossidae; E. Geoffroy, 1805) is a typical example of this. These Neotropical bats occur from Northern Mexico to Southern Argentina. A systematic review from 1913 described a total of 19 species (Miller 1913). Many of these species were later synonymized and seven or eight species, depending on the authors, were recognized in the latest taxonomic reviews (Dolan 1989, Nowak 1994, Simmons 2005, Eger 2008). In addition, one species, *M. alvarezi,* was newly described based on size, pelage coloration and morphological characteristics (González-Ruiz et al. 2011). Despite broad agreement among systematic reviews the taxonomic boundaries and names within the genus are not settled. For example, *M. bondae* (J.A. Allen, 1904) and *M. currentium* (O. Thomas,

1901) can be grouped under the name *M. currentium* (Simmons 2005) or considered as two species based on their distribution in Central or South America (Eger 2008). Similarly, *M. molossus* (Pallas, 1766) has been described as being "desperately in need of revision" (Simmons 2005) and probably represents a species complex; indeed, *M. coibensis* (J. A. Allen, 1904) was treated as a synonym of *M. molossus* (Koopman 1994, Reid 1998) yet is now considered a full species based on recent systematic assessments (Dolan 1989, Simmons 2005, Eger 2008).

To date, few studies have applied molecular information to address questions regarding the taxonomy of the genus *Molossus*. The first molecular investigation of the evolutionary relationships within the genus relied on allozymes (Dolan 1989). A more recent study identified only higher-level relationships between genera of the family Molossidae using one mitochondrial gene and three nuclear genes (Ammerman et al. 2012). More commonly, researchers have distinguished among *Molossus* spp. using morphological characters, especially in the field; however few attempts have been made to verify the reliability of such assignments. Here we compare molecular data from DNA-based markers with more commonly used morphometric and bioacoustic information to assess the reliability of each type of information for the identification of several *Molossus* species in Panama. We distinguished the *Molossus* species at our study site with a set of newly developed microsatellite markers and sequence data from the mitochondrial gene *co1* (DNA barcode) and the mitochondrial region d-loop for *M. molossus* and matched them with common field identification methods, i.e. morphological measurements and echolocation call recordings. While we were able to identify the molossid

species at our site in Panama with our methods, we also find that one or even several field-based methods may not be sufficient for the proper identification of morphologically similar species whose traits may locally vary quite substantially.

Material and methods

Ethics statements

Capture and handling of animals was carried out with permission from the Autoridad Nacional del Ambiente in Panama with approval from the Institutional Animal Care and Use Committee of the Smithsonian Tropical Research Institute (2012-0505-2015). All animals were gently handled during measurements of morphological parameters, photographs of wings, genetic sampling and acoustic recording. All animals were released back in clearings in the same area in which where they were captured. Heart tissue for genetic marker development came from a freshly dead bat found in a private home.

Sampling and data acquisition

During different fieldwork seasons between 2008 and 2013, we captured a total of 962 bats of the genus *Molossus* in Panama. Of these, 935 individuals were captured from various buildings in the village of Gamboa (Panama, 09°07’ N, 79°41’ W), 21 from the roof of the Smithsonian Tropical Research Institute’s (STRI) laboratory building on Barro Colorado Island (09°09’ N, 79°50’ W) as well as a dead tree off the shore of BCI, and seven from the roof of STRI’s dormitory at the Bocas del Toro research station (09°21’ N, 82°15’). We used mist-nets

(Ecotone, Gdynia, Poland) to catch bats during their evening emergence. We determined sex, age, forearm length, mass, reproductive status, and marked each individual with unique subcutaneous passive integrated transponder (Trovan ID-100, Euro ID, Weilerswist, Germany). We also sampled wing membrane tissue using a biopsy punch (2 or 3 mm, © Stiefel, U.S.A.) for genotyping purposes (Worthington Wilmer & Barrett 1996). During some fieldwork seasons, we also collected wing photos and echolocation calls for some individuals. We selected data only for individuals that were genotyped later for microsatellites. We retained size-referenced wing photos for the 116 genotyped bats to obtain measurements for several wing parameters (see below for details on wing morphology evaluation). Finally, we selected echolocation calls for 80 genotyped bats. The recording protocol was as follows: bats were placed individually in a semi-open environment on a cloth wrapped over the end of a 2-meter pole to allow them to orientate and choose their moment of take-off freely. When the bat left the pole, acoustic recordings were made at a sampling rate of 448 kHz with an Acer Aspire One laptop computer (model KAV60, Acer Inc., Taiwan) using the Avisoft-UltraSoundGate 116H and the Avisoft-RECORDER USHG software (Avisoft Bioacoustics, Germany). Recordings were semi-automatic, with manual activation, a pre-trigger of 2 seconds and a post-trigger of 5 seconds to ensure the acquisition of full call sequences. The condenser microphone CM16/CMPA we used (Avisoft Bioacoustics, Germany) had a sensitivity ranging from 10 to 200 kHz. The datasets of wing and echolocation calls overlapped for 35% of the analyzed individuals. The overlap of the datasets in terms of individuals is of minor concern here. We used microsatellite clusters (see methods later) to identify species for the individuals found in the different datasets. Our approach

benefited from larger sample sizes that are more representative of the populations studied.

Molecular analyses and species identification

A subset of the captured individuals (n = 27) was clearly identified as *M. bondae* based on their size and darker pelage coloration (Eger 2008) and use of different roosts. The species status of these 27 individuals was therefore not checked with molecular methods. . For the remaining 935 individuals of *M. molossus* and *M. coibensis*, we used molecular methods; specifically i) for genetic clustering of nuclear microsatellite markers and ii) phylogenetic tree reconstruction with 659 base pairs of the mitochondrial gene cytochrome oxidase subunit 1 (*co1*) and 615 base pairs of the hyper variable fragment of the control region (d-loop). Laboratory work with these markers was initially targeted at different questions, i.e. a study of genetic population structure in *M. molossus* as well as an exploration of fur color variation. This explains the use of different markers as well as protocols and number of individuals in each analysis.

1) Microsatellite development and genotyping

The detailed laboratory protocol for the nuclear microsatellite markers is available in the S1 File. Eighteen primer pairs successfully amplified; we report the sequences, accession numbers for the NCBI Probe database, the fluorescent dyes and the multiplex combinations in S2 Table. We used these 18 microsatellite markers to genotype 935 individuals.

2) Microsatellite evaluation and clustering

To identify the number of species captured, we performed microsatellite-based clustering of 935 genotyped individuals. This aim was achieved in three steps: i) genetic clustering of individuals based on the 18 microsatellite loci, ii) assertion of different assumptions for genetic models (Hardy-Weinberg Equilibrium, low frequency of null alleles and linkage equilibrium) and iii) genetic clustering based on a robust, filtered set of those loci that adhered closely to the respective genetic assumptions. We first determined the number of genetic clusters corresponding to the number of species (at least two). We used the 18 microsatellite loci using a two-step Discriminant Analysis of Principal Components (DAPC (Jombart et al. 2010)), a clustering method that does not require specific genetic assumptions for the loci used (unlike other clustering software that typically make use of patterns in, e.g., Hardy Weinberg and linkage equilibria (Jombart et al. 2010)). The second step consisted of checking three genetic assumptions within each cluster defined by DAPC: Hardy-Weinberg Equilibrium (HWE), low frequency of null alleles and linkage equilibrium. Only loci following these three conditions in each cluster were used for the second, stringent clustering analysis performed using the software STRUCTURE v2.3.4 (Pritchard et al. 2000, Falush et al. 2003).

For the first part of the microsatellite analysis, we selected the number of genetic clusters (corresponding to the different species) based on Bayesian Information Criterion (BIC), a measure of the trade-off between goodness of fit and complexity of the model. We calculated the BIC for 18 clusters (the number of buildings sampled) and 100 PCs with the *adegenet* package (Jombart 2008) in R v.3.1.0 (R Development Core Team 2014). A two-step Discriminant

Analysis of Principal Components DAPC (Jombart et al. 2010) was used to infer the selected number of clusters. We retained the number of principal component axes corresponding to ~80% of the cumulative score in the Principal Component Analysis step and the number of axis corresponding to the optimized *a*-score in the Discriminant Analysis step (Jombart & Collins 2015).

For each cluster defined with the DAPC, we identified the number of alleles at each locus, the heterozygosity (observed and expected), tested for deviations of HWE and estimated the null allele frequency using CERVUS v3.0.3 (Kalinowski et al. 2007). For each cluster, we also tested for linkage disequilibrium between all pairs of loci using the log likelihood ratio statistic and default parameters implemented in GENEPOP ON THE WEB (Raymond & Rousset 1995, Rousset 2008) and we applied a Bonferroni correction to the significance level of 0.05 (0.05 : 9 loci at HWE = 0.00556) to correct for multiple testing. For the following steps, we selected only loci that were in HWE, had an estimated null allele frequency < 0.10, and were in linkage equilibrium for all clusters. It has recently been shown that null allele estimation with CERVUS can be misleading (Dąbrowski et al. 2015). We therefore additionally used the software ML-NULL, which has been shown to perform best among a number of methods (Kalinowski & Taper 2006, Dąbrowski et al. 2015), to obtain additional estimates and confirm frequencies < 0.10. The outcomes of both methods (i.e., CERVUS and ML-NULL) did not differ in our case (results not shown).

The last clustering analyses were based on the selected number of genetic clusters in the data and only those loci following closely the genetic assumptions of HWE, null alleles, and LD. As a complementary method to the two-step DAPC (following the procedure described earlier), we ran an analysis with the software STRUCTURE v2.3.4 (Pritchard et al. 2000, Falush et al. 2003). We used default parameters from the software with an admixture model, a length of burn-in period of 20,000 and a number of MCMC repetitions after burn-in of 80,000. We performed 10 replicate runs for the number of determined genetic clusters and averaged the results in CLUMPP v1.1.2 (Jakobsson & Rosenberg 2007). A few individuals showed lower membership probability to a genetic cluster with STRUCTURE (< 0.9), even though showing a strong assignment with DAPC. We excluded these individuals, potentially attributed to the wrong species, to avoid potential mistakes in subsequent analyses, because it is known that DAPC can be over-confident in making genetic cluster assignments and more than one method should be utilized to check for cluster assignment (Frosch et al. 2014). The pruned dataset was used to identify the number of alleles for each cluster.

3) Sequencing and phylogenetic reconstructions

We sequenced *co1* for 96 individuals and d-loop for 150 individuals. The detailed laboratory protocol for the mitochondrial genes is available in the S1 File. The newly generated sequences are available on GenBank, respectively under the accession numbers KT721362 - KT721412 for

the 51 *co1* sequences and KT721413 - KT721441 and KT721443 – KT721563 for the 150 d-loop sequences.

We obtained 74 *co1* sequences from GenBank, including all sequences for *Molossus* and four outgroups from the molossid family (three species of *Cynomops* and one species of *Promops*). We aligned the 51 *co1* sequences from this study with the 74 GenBank sequences using muscle (Edgar 2004) with default parameters as implemented in Seaview 4.5.4. (Gouy et al. 2010). We aligned the d-loop sequences from this study with MEGA 4.0 (Tamura et al. 2007) and visually checked the alignment for repeated sequence arrays (Wilkinson et al. 1997), a pattern already found in different bat species (Wilkinson et al. 1997).

Three *Cynomops* species (GenBank accession numbers JF447634, JN312044 and EF080319) and *Promops centralis* (JF444936) were used as outgroups to root the tree inferred from *co1* sequences. The last sequence was labelled as *M. rufus* but we verified the species using the Barcode of Life Data System (more than 29000 sequences for the Order Chiroptera, (Ratnasingham & Hebert 2007)). The tree inferred from d-loop sequences was unrooted because we could not find close publicly available sequences of close outgroups that could be satisfactorily aligned with our sequences. In order to find the best-fitting model for each gene, we compared 56 models of nucleotide evolution using jModelTest 2.1.7 and the Bayesian Information Criterion (BIC) (Darriba et al. 2012). The best-fitting model was then used in PAUP* 4b10 (Swofford 2003) to infer the respective phylogenies. Reliability of nodes was measured

using 100 non-parametric bootstraps that were then mapped on the inferred trees using the *plotBS* option in the R package *phangorn* (Schliep 2011). We validated a posteriori the taxonomic identification of the sequences deposited in GenBank (see discussion). The information on genetic clustering from the STRUCTURE analysis was also plotted on the tips of the final trees.

Variation of fur color

We selected a set of eight individuals from the three species with pictures of the fur color for the back. This set of individuals was representative of the whole range of fur color observed in the field. This selection of pictures displayed the intra-species variation but also inter-species overlap in fur color. Our further use of the pictures to quantify colors was limited by the absence of camera calibration (Stevens et al. 2007).

Analyses of body parameters

We investigated morphological species differences based on two parameters: forearm length (mm) and body mass (g). We used these parameters to estimate a linear discriminant function using the "lda" function (library Mass) in R v.3.1.0 (R Core Team 2015) to separate *Molossus* species. We included the *M. bondae* here as well as the genetically identified *M. molossus* and *M. coibensis*. Only adults, but not pregnant females, were used in the analysis. We calculated means and 95% confidence intervals (CI) for each combination of morphological parameter, species and sex. We used the formula provided in the R book to obtain the 95% Confidence

Intervals (Crawley 2007). We also assessed the classification rate of the species by the lda function with the leave-one out cross validation procedure.

Analyses of wing shape

We used the wing photos to extract a series of wing parameters and morphological traits relevant to flight performance and foraging strategy (Norberg & Rayner 1987). We followed an established procedure to define landmarks and obtain the following measurements (Schmieder et al. 2015) from wing photos (Fig 2-1): forearm length (mm), total area (mm^2), total wing length (mm), arm wing area (mm^2), arm wing length (mm), hand wing area (mm^2), hand wing length (mm), wing aspect ratio (wing length^2 / wing area), wing loading (body mass*g / wing area), tip length ratio (hand wing length / arm wing length), tip area ratio (hand wing area / arm wing area), tip shape index (tip area ratio / tip length ratio – tip area ratio), and a circularity index ($4*\pi*$wing area / wing perimeter^2). All measurements were collected by the same person to minimize inter-observer error. For each combination of wing parameter, species and sex, we calculated the mean and the 95% CI (Crawley 2007).

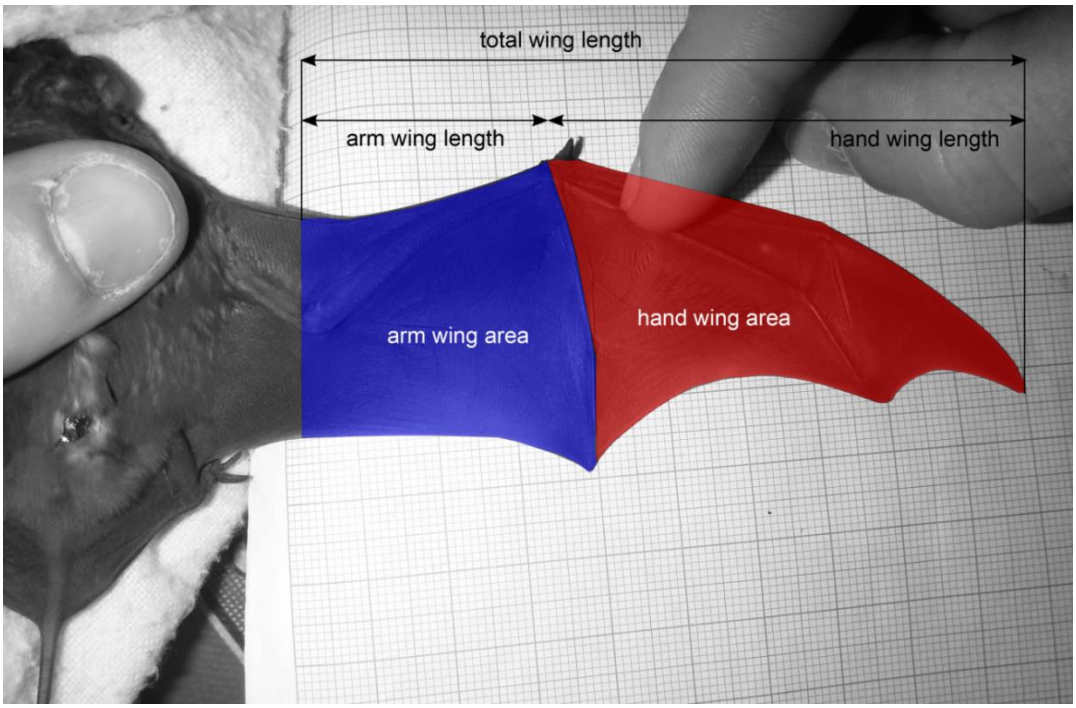

Fig 2-1. Right wing of a *Molossus molossus* showing areas used to analyze wing shape.

Analyses of echolocation calls

We analyzed echolocation calls from a subset of individuals genetically identified as *M. molossus* or *M. coibensis* using the software Batsound 4.1.300 (Pettersson Electronik AB, Uppsala, Sweden). We randomly selected sequences of up to ten calls that contained a sufficient signal to noise ratio for each individual. Sampling frequency was configured at 44.1 kHz, with 16 bits per sample and a 512-point FFT with a Hamming window for analysis. A 112 Hz frequency resolution was obtained for spectrograms and power spectrum. In each call, we measured six echolocation parameters using the software Batsound (Pettersson Elektronik AB, Sweden). From the spectrogram, based on the fundamental call, we measured 1) the Start Frequency (SF; frequency measured at the beginning of the call), 2) the End Frequency (EF;

frequency measured at the end of the call) and 3) the bandwidth (BW; difference between SF and EF) in kHz. From the maximal intensity in the power spectrum, we determined the 4) Peak Frequency (PF). From the oscillogram, we extracted 5) the Duration D and 6) the Pulse Interval (PI; time interval between two consecutive calls) in ms.

First, we analyzed all calls to examine the entire recorded acoustic diversity. We found a great range of variability in the calls, consistent with previous studies on *M. molossus* in Belize and Cuba (Kossl et al. 1999, O'Farrell & Miller 1999). We examined the Pearson's product moment correlation using R v.3.1.0 (R Core Team 2015). Only two of the acoustic parameters (SF and PF) showed a strong correlation of 0.95 (all others ranging from -0.69 to 0.85). We excluded PF and ran a Principal Component Analysis (PCA) of all calls with the five remaining acoustic parameters. Secondly, we categorized our different sequences of calls into call types. A typical sequence of calls started at the release perch with short calls with a downward frequency modulation and a prominent second harmonic, similar to the "approach call" recorded for *M. molossus* in the vicinity of their roosts in Cuba (Mora et al. 2004). We also recorded search flight calls with narrow bandwidths (Mora et al. 2004) when a bat was higher above the ground. Search flight calls were typically two-toned and alternating between a lower frequency pulse (SI) and a higher frequency pulse (SII) (Kossl et al. 1999, Mora et al. 2004). Some search flight calls were also irregularly alternating the SI and SII or were three-toned, a known pattern for this species (Barataud et al. 2013, Jung et al. 2014). For our purpose of species comparisons, we selected only sequences with a clear call structure: the "scanning calls" where all calls had

harmonics and the two-toned search flight calls consistently alternated with a lower and higher frequency pulse (SI and SII, Fig 2-2). For each combination of call type and species, we calculated mean and the 95% CI (Crawley 2007). We disregarded sequences of calls that could not be firmly categorized such as sequences of "scanning calls" that did not always show harmonics, sequences mixing "scanning calls" and search flight calls as well as search flight calls irregularly alternating the tones or showing an uncertain number of tones.

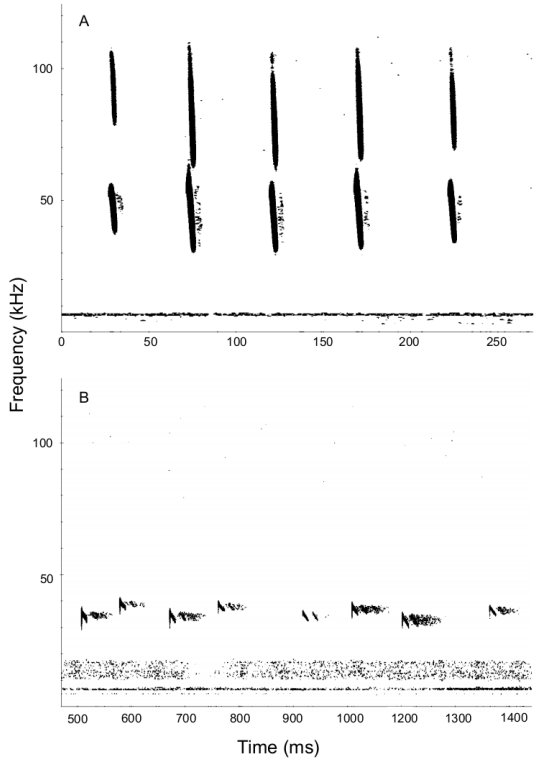

Fig 2-2. Sonograms of the two types of calls informative for identification found in *Molossus molossus* and *coibensis*. (A) Approach calls with harmonics. (B) Search calls alternating a lower and higher frequency pulse.

Results

Microsatellite evaluation and clustering

We genoptyped 935 individuals at 18 microsatellite loci (for dataset, see supplementary file S3 Dataset). Based on the complete dataset with 18 loci, we selected *K* = 2 clusters because of the shape of the BIC curve as a function of the number of clusters (ranging from one to 18), showing a much better likelihood for *K*=2 than for *K* = 1 and only little gain in likelihood for additional clusters (S4 Figure). In the two-step DAPC, we retained 60 axes (~80% of the cumulative variance) in the Principal Component Analysis step and one axis (optimized *α*-score) in the Discriminant Analysis step. From the 935 individuals, 841 were attributed to *Cluster One* and 94 to *Cluster Two*.

For these genetic clusters, we list the number of alleles, the range of allele size, the observed and expected heterozygosities and the estimated null allele frequency in Table 2-2. Two loci from *Cluster One* and three from *Cluster Two* significantly departed from Hardy-Weinberg equilibrium (HWE). Three loci from *Cluster One* and six from *Cluster Two* showed high estimated null allele frequencies (over 10%). Two of the loci from *Cluster Two* departing from the HWE also had high estimated null allele frequency, potentially resulting from null amplification. Of the nine loci at HWE, many pairs showed significant linkage disequilibrium (22 for *Cluster One* and nine for *Cluster Two* out of 36). The only loci in HWE, in linkage equilibrium and with estimated null allele frequency < 0.10 across the two clusters were C56, C77, C115 and C132.

Table 2-2. Cross-amplification and genetic tests for 18 *Molossus molossus* loci grouped in two genetic clusters. The columns respectively represent: A, Number of alleles and AS, range of allele sizes (bp); Ho, observed heterozygosity; He, expected heterozygosity, F(null), estimated null allele frequency. Loci or values highlighted in boldface departed significantly from HWE (following p-values from testing in CERVUS) or had high estimated null allele frequencies (> 0.10).

Genetic cluster	*Cluster One* (n = 841)				*Cluster Two* (n = 94)			
Locus	A	AS (bp)	Ho / He	F(null)	A	AS (bp)	Ho / He	F(null)
Mol_A2	9	191 - 211	0.74 / 0.76	0.02	7	189 - 203	0.65 / 0.68	0.01
Mol_A221	11	286 - 321	0.45 / 0.47	0.02	7	286 - 315	0.14 / 0.18	**0.16**
Mol_B233	4	198 - 209	0.51 / 0.54	0.02	3	198 - 205	0.02 / 0.08	**0.48**
Mol_C3	11	257 - 284	0.81 / 0.80	-0.01	11	261 - 286	0.82 / 0.8	-0.02
Mol_C6	7	101 - 117	0.52 / 0.60	0.06	6	100 - 107	**0.16 / 0.53**	**0.55**
Mol_C20	4	136 - 142	0.71 / 0.72	0.00	5	143 - 151	0.58 / 0.59	0.00
Mol_C27	23	270 - 320	0.83 / 0.87	0.02	7	268 - 304	0.33 / 0.40	0.09
Mol_C56	18	171 - 213	0.88 / 0.84	-0.02	12	186 - 210	0.83 / 0.84	0.00
Mol_C61	19	177 - 214	**0.78 / 0.89**	0.07	6	182 - 198	0.59 / 0.61	0.01
Mol_C77	11	198 - 225	0.71 / 0.73	0.02	5	198 - 231	0.27 / 0.30	0.05
Mol_C109	17	255 - 293	0.60 / 0.87	**0.18**	7	243 - 277	0.31 / 0.79	**0.44**
Mol_C109bis	18	218 - 247	**0.81 / 0.83**	0.01	6	214 - 226	0.61 / 0.63	0.02
Mol_C114	12	268 - 310	0.78 / 0.76	-0.02	10	268 - 313	0.73 / 0.81	0.05
Mol_C115	12	265 - 294	0.75 / 0.78	0.02	7	265 - 282	0.61 / 0.67	0.05
Mol_C117	15	294 - 348	0.28 / 0.85	**0.51**	6	298 - 340	0.57 / 0.55	-0.01
Mol_C118	10	213 - 224	0.55 / 0.81	**0.19**	6	214 - 224	**0.06 / 0.51**	**0.81**
Mol_C132	15	147 - 182	0.77 / 0.81	0.03	4	178 - 186	0.42 / 0.46	0.03
Mol_D109	20	291 - 324	0.86 / 0.90	0.02	6	296 - 317	0.03 / 0.07	**0.42**

We consequently based all following clustering analyses on only four loci and two clusters. We also excluded two individuals with missing data for these specific loci. Some individuals retained

also showed missing data at two loci (n = 6) and one locus (n = 87) of these four. The performance of the DAPC and STRUCTURE analyses on four loci matched that of the analyses with 18 loci resulting in two similar genetic clusters. The majority of individuals were clearly found in *Cluster One* (orange) and *Cluster Two* (blue, (Fig 2-3). Only ten individuals out of 933 (1%) showed discrepancies in clustering, with clear assignment to a cluster in the DAPC but admixture in the STRUCTURE analysis (posterior assignment probability < 0.9). These individuals with uncertain assignment were removed from the subsequent analyses as explained in the methods. The pruned dataset was composed of 923 individuals: 833 in *Cluster One* that occurred in all 18 sampled buildings that we caught bats from and 90 in *Cluster Two* we caught from five of the buildings. These two clusters were used to determine the number of alleles for each of the 18 loci (S5 Table).

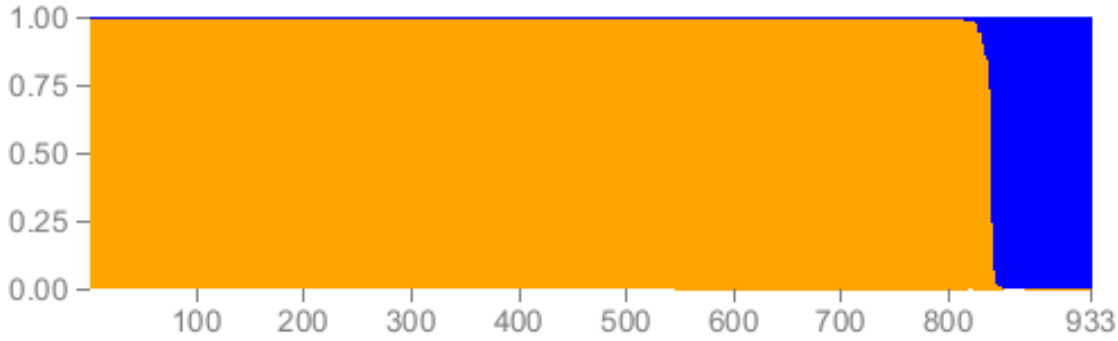

Fig 2-3. Genetic clustering (K = 2) of 933 Molossus bats from the village of Gamboa. Cluster One is represented in orange and Cluster Two in blue. The two clusters were obtained with four microsatellite primers using the software STRUCTURE. The few individuals at the edge of the two clusters are admixed.

Phylogenetic results: the *co1* tree

The final co1 alignment consisted of 659 nucleotides for 125 individuals (S6 Dataset). Phylogenetic reconstruction under a Tamura-Nei TrN+I+Γ substitution model displayed numerous polytomies with most nodes showing low statistical support (<70 %) (S7 Figure).The majority of samples genotyped with co1 (96.1%) were also genotyped using microsatellites, the membership to the microsatellite clusters are represented in color at the nodes of the tree. In the *Molossus* crown, bootstrap values were >70% in only five nodes. The tree consisted mostly of i) deep rooted clades comprising newly sampled *M. molossus* from Panama together with published haplotypes from Ecuador, Suriname and Guyana, ii) a "floating" clade from Panama and iii) a tree crown with two *M. rufus*, four *M. coibensis* and a few more individuals from Panama. The tree crown was composed of a well-supported clade with two *M. rufus* (BS = 100) and a polytomy composed of a *M. sp.* from Venezuela (JF447833), a clade with four *M. coibensis* and a *Molossus sp.* (JF442201) from Ecuador and a clade with 11 of our individuals from Panama. As these 11 bats were also found in *Cluster Two* from the microsatellite clustering analysis, we later considered all these animals to be *M. coibensis*. The deeper branches of the tree consisted of *M. molossus* from Panama, Ecuador, Suriname and Guyana. At the roots of the tree, the 22 individuals from Panama were mixed together with the GenBank sequences of *M. molossus*, with no clear biogeographic pattern. We also found a well-supported clade with 17 bats from Panama (BS = 98), sister to the crown tree. However, the 22 individuals mixed with the GenBank sequences and the 17 individuals from this Panamanian clade were previously grouped in the same *Cluster One* from the microsatellite clustering

analysis. We therefore did not consider the Panamanian clade as a new species, but rather a "floating" clade and defined all individuals from *Cluster One* as *M. molossus*, in this tree and subsequent analyses.

Phylogenetic results: the d-loop tree

The final alignment of d-loop was 615 base pair long and encompassed 150 individuals (S8 Dataset). The 150 new d-loop sequences showed high genetic diversity, with 42 different haplotypes. Most haplotypes (n = 28) were found in one individual, except for a common haplotype that was shared by 38 individuals. Fourteen haplotypes were shared between different roosts, one of them being shared between ten roosts. According to the BIC criterion, the Hasegawa-Kishino-Yano model (HKY) with a proportion of invariable sites (I) was the best fitting model for the tree reconstruction (S9 Figure). The d-loop tree presented a similar topology than the co1 tree, with several clades of *M. molossus* and a clade with *M. coibensis*. One of the individuals from this tree (KT721428) was previously identified as *M. coibensis* in the *co1* tree (KT721364, transponder number EAC87), we therefore assigned its clade in the d-loop tree to the species *M. coibensis* and the rest of the individuals to *M. molossus*. Statistical support for the *M. coibensis*' clade and six subclades of *M. molossus* was high (BS ≥ 90). Most of the d-loop sequences (77.3 %) were also genotyped for microsatellites, the membership to the microsatellite clusters are represented in color at the nodes of the tree.

Variation of fur color

The subset was composed of two *M. bondae* identified morphologically as well as three *M. molossu*s and three *M. coibensis* confirmed genetically. We observed a fur color on the back ranging from light brown to dark brown. The similarity of the fur color emerges clearly from this panel of three species (Fig. 2-4).

Fig. 2-4. Variation of fur color (back) for eight individuals of *M. molossus*, *M. coibensis* and *M. bondae*.

Analyses of body parameters

We obtained morphological data from 617 adults of both sexes: 526 *M. molossus*, 64 *M. coibensis* and 27 *M. bondae* (the first two genetically assigned to species; S10 Dataset). Forearm length (mm) and body mass (g) were normally distributed and overlapped between the three species (Fig 2-5). Forearm length was ranked in increasing order for *M. coibensis*, *M. molossus* and *M. bondae*. Body mass was ranked in increasing order for *M. molossus*, *M. coibensis* and *M. bondae*. Only *M. molossus* showed body mass below 9.5 g while only *M. bondae* had forearm length and body mass above 39.63 mm and 17 g respectively. Twenty-five of the 617 bats (4.1%) were misclassified by the lda function using forearm length and body mass.

Misclassification occurred for animals with extreme values for the species range. Thus, the lightest *M. bondae* (n = 3, range = 10.5 - 12.0 g) and the heaviest *M. molossus* (n = 3, range = 16.0 - 17.0 g) were wrongly identified as well the smallest *M. molossus* (n = 9, range = 31.6 - 36.6 mm) and the largest *M. coibensis* (n = 10, range = 34.5 - 37.02 mm).

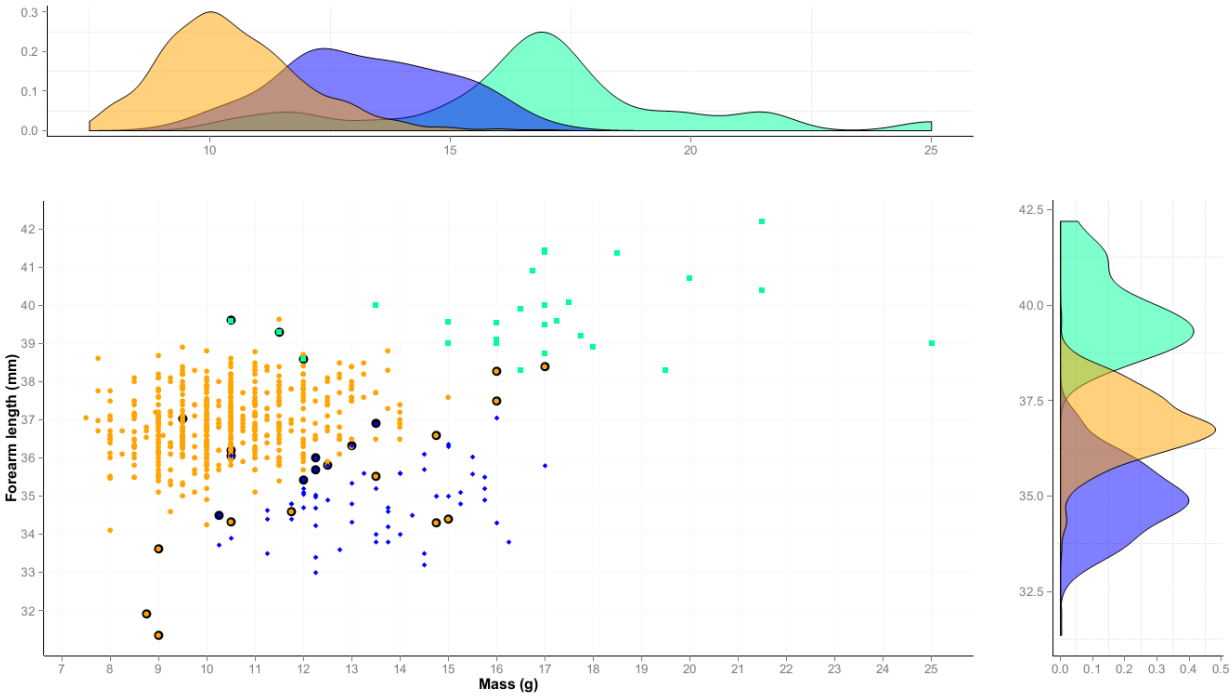

Fig 2-5. Forearm length (mm) plotted against body mass (g) for three Panamanian *Molossus* species. The color code is as follows: *M. molossus* (orange dots), *M. coibensis* (blue diamonds) and *M. bondae* (green squares). Following the same color code, the frequency distribution of body mass is plotted above the graph and the frequency distribution of forearm length on the right side of the graph. Points outlined in black are misclassified individuals based on the linear discriminant function and the leave-one out cross validation procedure (4.1% of the

individuals).

We provide means, 95% CI and range of forearm length and body mass in Table 2-3 for each combination of species and sex. We found a significant male-biased sexual dimorphism in all three species at the intra-specific level, as the 95% CI did not overlap. Inter-specific values differed significantly for both parameters based on the 95% CI.

Table 2-3. Sex-specific means [95% CI] of forearm length and body mass.

Parameter	**Forearm length (mm)**			**Body mass (g)**		
Sex	**Female**		**Male**	**Female**		**Male**
M. coibensis	34.6 [34.3 - 34.8] (n = 41)	<	35.6 [35.3 - 35.9] (n = 23)	12.8 [12.3 - 13.3] (n = 41)	<	14.1 [13.5 -14.8] (n = 23)
M. molossus	36.7 [36.6 - 36.8] (n = 403)	<	37.4 [37.2 - 37.6] (n = 123)	10.2 [10.1 - 10.3] (n = 403)	<	11.4 [11.1 - 11.6] (n = 123)
M. bondae	39.4 [39.0 - 39.8] (n = 18)	<	40.6 [39.8 - 41.3] (n = 9)	16.4 [14.9 - 18.0] (n = 18)	<	17.9 [15.8 - 20.0] (n = 9)

Based on the leave-one-out cross-validation procedure, the overall classification rate of the function was 95.9 % (97.7% for *M. molossus*, 84.4% for *M. coibensis* and 88.9% for *M. bondae*). Only 25 of 617 individuals (4.1%) were misclassified based on the combined two morphological parameters alone (symbols outlined in black in Fig 2-5).

Analyses of wing shape

We analyzed wing photos (S11 Dataset) of 104 *M. molossus* (87 females and 17 males) and 12 *M. coibensis* (8 females and 4 females). The means and 95% CI for each combination of wing parameter, species and sex are summarized in Table 2-4. Four parameters were significantly different between species: *Molossus molossus* had longer forearms (confirming the results outlined in the previous paragraph), larger hand wing area, and a longer hand wing while wing loading was greater in *M. coibensis*.

Table 2-4. Mean [95% CI] of wing parameters for species and sex. The four parameters highlighted in bold differed significantly between species. For each species, the intermediate column compares mean values between sexes.

Species	***Molossus coibensis***			***Molossus molossus***		
Sex	**Female (n = 8)**		**Male (n = 4)**	**Female (n = 87)**		**Male (n = 17)**
Forearm length (mm)	33.8 [32.5 - 35.1]	<	35.3 [35.0 - 35.6]	36.7 [36.6 - 36.9]	<	37.1 [36.7 - 37.5]
Total area (mm²)	2292 [2064 - 2520]	<	2469 [2233 - 2706.0]	2792.1 [2739 - 2845]	>	2660.6 [2531 - 2789]
Total wing length (mm)	94.8 [89.3 - 100.2]	<	99.7 [92.5 - 107.0]	103.8 [102.7 - 104.9]	>	102.7 [100.1 - 105.3]
Arm wing area (mm²)	1086 [939 - 1233]	<	1263 [1103 - 1422]	1354 [1319 - 1388]	>	1321 [1226 - 1416]

Arm wing length (mm)	35.1 [32.0 - 38.2]	<	39.3 [34.4 - 44.2]	38.8 [38.3 - 39.4]	>	38.5 [36.9 - 40.1]
Hand wing area (mm^2)	1179 [1092 - 1266]	<	1211 [1107 - 1315]	1432 [1404 - 1459]	>	1361 [1318 - 1403]
Hand wing length (mm)	59.9 [57.2 - 62.5]	<	60.0 [57.6 - 62.3]	64.9 [64.0 - 65.7]	>	64.1 [62.6 - 65.6]
Circularity	0.49 [0.48 - 0.51]	>	0.48 [0.47 - 0.49]	0.51 [0.50 - 0.51]	>	0.49 [0.48 - 0.50]
Tip area ratio	1.1 [1.0 - 1.2]	>	1.0 [0.8 - 1.1]	1.1 [1.0 - 1.1]	>	1.0 [1.0 - 1.1]
Tip length ratio	1.7 [1.6 - 1.8]	>	1.5 [1.4 - 1.7]	1.7 [1.6 - 1.7]	=	1.7 [1.6 - 1.7]
Tip shape index	1.8 [1.5 - 2.1]	>	1.7 [1.1 - 2.3]	1.9 [1.7 - 2.0]	>	1.7 [1.5 - 1.9]
Aspect ratio	3.9 [3.8 - 4.1]	<	4.0 [3.8 - 4.3]	3.9 [3.8 - 3.9]	<	4.0 [3.9 - 4.1]
Wing loading (Nm^{-2})	53.9 [46.9 - 60.9]	<	56.1 [47.6 - 64.5]	39.3 [37.9 - 40.7]	<	43.2 [40.6 - 45.9]

Analyses of echolocation calls

We recorded echolocation calls of 80 adult bats: 65 *M. molossus* and 15 *M. coibensis*. We measured 8 to 30 calls per individual, resulting in 834 for *M. molossus* and 255 pulses for *M. coibensis* (S12 Dataset). When analyzing unclassified calls, we found no clear species differences based on the Principal Component Analysis (Fig 2-7), with just 51.9% of the variance explained by the first axis and 21.6% by the second axis.

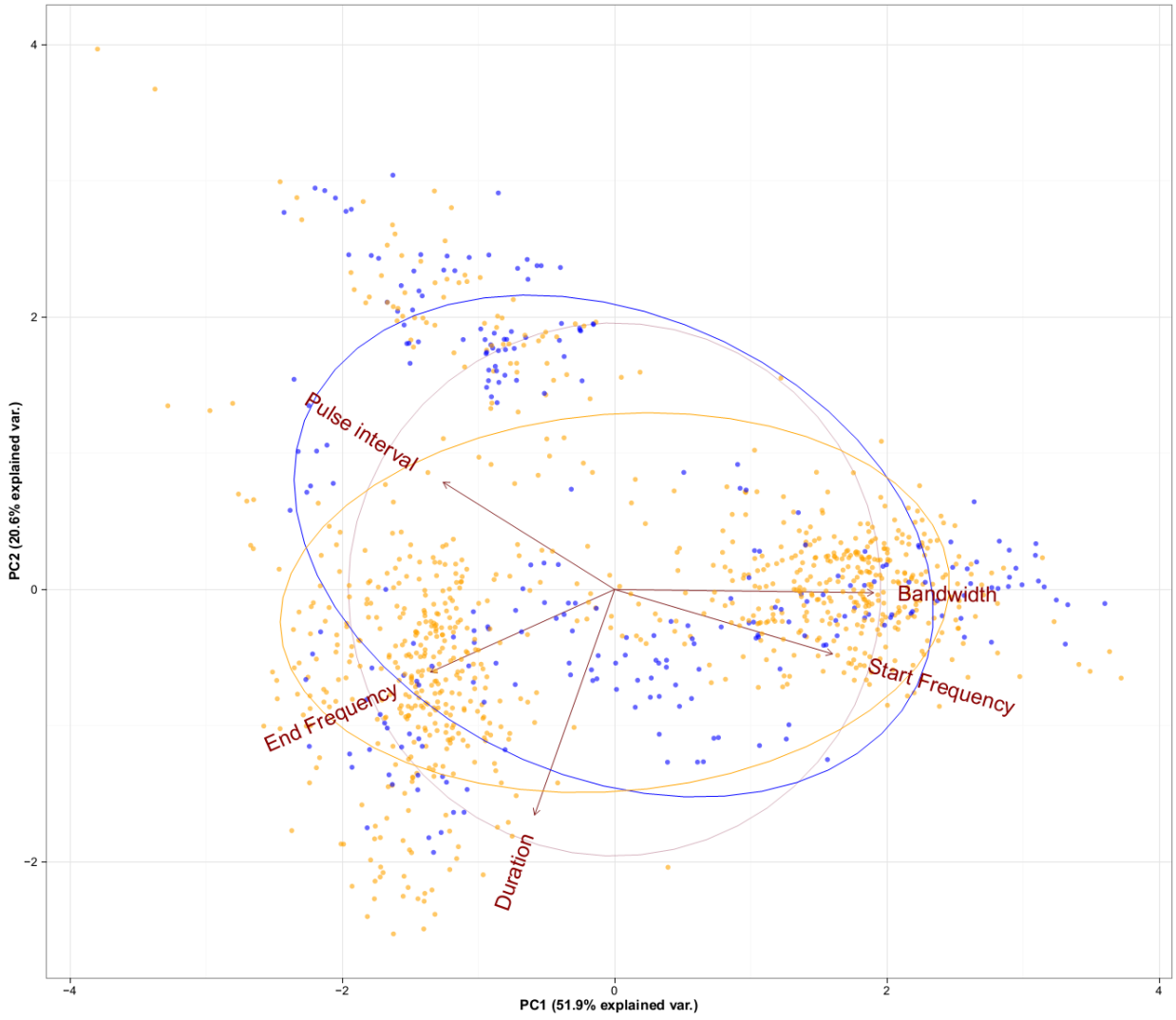

Fig 2-7. Principal Component Analysis of five acoustic parameters for *M. molossus* (orange) and *M. coibensis* (blue).

We found sequences of approach calls with harmonics in 35.4% of the *M. molossus* and 46.7% of the *M. coibensis*. We observed search calls that regularly alternated between the two tones

SI and SII in 18.5% of the *M. molossus* and 40.0% of the *M. coibensis*. Average SF and EF were higher in scanning calls of *M. coibensis* but lower in the two-toned calls. For the two-toned calls call duration was shorter in *M. molossus* and bandwidth was higher in *M. coibensis*. Finally, pulse interval in the SII of the search calls, was higher in *M. coibensis*. Mean values and SD for the five acoustic parameters and the different call types are compiled in Table 2-5 (for results of t-tests see S13 Table).

Table 2-5. Comparison of acoustic parameters between *M. molossus* and *M. coibensis*. Values are means ± 1 standard deviation. **V**alues in boldface represent significant differences between species based on a Student's t-test for the given acoustic parameter. The two figures for sample size indicate the number of individuals and the number of calls.

Call type	Species	Sample size	Start Fr. (kHz)	End Fr. (kHz)	Bandwidth (kHz)	Duration (ms)	Pulse Interval (ms)
"Scanning call"	*M. coibensis*	7 (70)	**56.1 ± 2.9**	**30.6 ± 5.5**	25.5 ± 6.4	0.4 ± 0.05	62.3 ± 54.9
	M. molossus	23 (226)	**52.8 ± 3.7**	**29.0 ± 3.2**	23.8 ± 4.9	0.4 ± 0.08	50.7 ± 37.3
Two-toned SI	*M. coibensis*	6 (29)	**35.4 ± 1.3**	**29.8 ± 1.9**	**5.6 ± 1.3**	**0.4 ± 0.4**	76.2 ± 23.6
	M. molossus	12 (60)	**39.1 ± 3.6**	**34.4 ± 3.8**	**4.7 ± 2.0**	**0.6 ± 0.3**	75.4 ± 24.6
Two-toned SII	*M. coibensis*	6 (29)	**39.7 ± 2.1**	**35.0 ± 1.8**	**4.7 ± 1.2**	**0.3 ± 0.3**	**153.8 ± 58.9**
	M. molossus	12 (60)	**42.8 ± 2.9**	**39.1 ± 3.0**	**3.8 ± 1.4**	**0.6 ± 0.3**	**117.6 ± 44.5**

Discussion

Our combination of morphometric and molecular data confirmed the sympatry of at least three species: *Molossus molossus*, *M. coibensis* and *M. bondae* at our study site in Panama. *Molossus molossus* was more abundant than *M. coibensis* in the sampled buildings while *M. bondae* was rarely captured.

Microsatellite clustering

Microsatellite analyses were useful for detecting species but also to reveal potential interspecific hybrids. Both the DAPC BIC method based on 18 loci and the STRUCTURE method based on four loci, recovered two genetic clusters, with consistent cluster membership with few exceptions. Only 1% of the individuals were clearly assigned to one cluster with the DAPC method but showed admixture based on STRUCTURE. Sequencing a subset of these individuals with the gene *co1* revealed that the two microsatellite clusters corresponded to *M. molossus* and *M. coibensis*. As we successfully genotyped both species with the microsatellites, they offer the potential for cross-species amplification in the genus *Molossus*. Multiple and non-exclusive explanations such as non-random mating could explain why so many of the loci diverged from HWE, and/or showed high prevalence of null alleles (Hartl & Clark 2007). We may have sampled a non-random subset of the males in the gene pool (Storz 1999); in particular, our sampling was biased towards harem social groups occupying buildings (McCracken & Wilkinson 2000) whereas unsampled males were probably solitary and roosting elsewhere. The four loci that stringently followed the genetic assumptions for the STRUCTURE analyses revealed a low

number of admixed individuals (n = 10), representing only 1% of all genotyped individuals. Admixture signatures in STRUCTURE can result from interspecific hybridization or retention of ancestral polymorphism (Muir & Schlötterer 2005, Berthier et al. 2006, Randi 2008, Brown et al. 2010). Admixture can also result from microsatellite size homoplasy, a well-described phenomenon that remains infrequently tested (Adams et al. 2004, Rossiter et al. 2007). Further investigation of admixture in this study, be it technical artifact or biological reality, is not of relevance here because of its low incidence, and therefore out of scope presently. Further analysis will be required to identify the mechanism leading to admixture and the taxonomic status of admixed individuals.

Phylogenetic reconstructions

Sympatry of very similar-looking species is common in bats (Barratt et al. 1997, von Helversen et al. 2001, Ibáñez et al. 2006) and can make species identification in the field extremely difficult. We successfully clustered individuals according to species with the *co1* sequences. In addition, the *co1* tree allowed us to incorporate GenBank sequences from different species and countries. We thus matched our sequences from Panama to *M. molossus* from Guyana, Ecuador and Suriname and to *M. coibensis* from Ecuador. Our phylogenetic reconstructions provided the second piece of molecular evidence that *M. coibensis* and *M. molossus* occur in sympatry in Panama after the allozyme study by Dolan (Dolan 1989). We found that the two species occur in the same buildings where they probably form species-specific social groups. Sympatric individuals of these two species have previously been reported for the province of

Napo in Ecuador (McDonough et al. 2011, Clare et al. 2011). Our phylogenetic tree also revealed a "floating" clade of *M. molossus* that we were not able to match to GenBank sequences. With low statistical support (BS = 23), this phylogenetic uncertainty may represent a soft polytomy that could be resolved with increased character sampling (Lack & Van Den Bussche 2010). The same 17 individuals were assigned to *M. molossus* in the microsatellite clustering analyses. This molecular differentiation may result from the occurrence of two distinct barcodes in the same species, as recently found in the bat *Pipistrellus kuhlii* (Andriollo et al. 2015). The phylogenetic tree also revealed three GenBank sequences that were probably wrongly identified: JF442201 from Ecuador and JF447833 from Venezuela are probably not *M. molossus* but *M. coibensis*, and the inverse is true for JF442240 from Ecuador. Quality control of sequences using phylogenetic analyses (Botero-Castro et al. 2015) could easily avoid taxonomic misidentification of sequences submitted in public databases (Nilsson et al. 2006). Despite its utility for species identification, our phylogenetic reconstructions using *co1* recovered a large polytomy with limited statistical support for the majority of nodes, and further phylogenetic reconstruction based on the fast-evolving mitochondrial region d-loop also recovered clades with low support in most cases. Future studies incorporating nuclear genes (Lack & Van Den Bussche 2010, Ruedi et al. 2013, Velazco & Patterson 2013), combined datasets (i.e. morphology and genetics) (Springer et al. 2001) or even complete genomes (Murphy et al. 2004, Delsuc et al. 2005, Song et al. 2012) will thus be valuable in elucidating further the taxonomy of morphologically similar molossid bats. Whole genome analyses of thousands of species are envisioned since many years (Genome 10K Community of Scientists. 2009) and this

is becoming a reality with the development of next generation sequencing technologies, for example in birds with the B10K initiative (Zhang 2015, Kraus & Wink 2015).

Variation of fur color

The panel of fur color (Fig. 2-4) reveals the overlap between species, namely between *M. molossus* and *M. coibensis*.

Analyses of body parameters

In contrast to the molecular methods, simple morphological parameters such as forearm length and body mass can easily be obtained in the field (Dietz & von Helversen 2004). Used separately, these morphological parameters did not allow good discrimination of the three species due to the overlap in parameter distributions and the flip in ranks. Only when analyzed together in a linear discriminant function, the two parameters led to a high average rate of correct identification of the three species (95.9%). At the species level, the classification rate was ranked in decreasing order for *M. molossus* (97.7%), *M. bondae* (88.9%) and *M. coibensis* (84.4%). Similarly, for example, *Myotis* from Switzerland can be most reliably distinguished using a canonical discriminant function based on the forearm length and the ear length (Arlettaz et al. 1991). *Rhinolophus* from Bulgaria, Greece and Turkey can be correctly assigned using a canonical discriminant function of the forearm length and the first phalanx of the fourth finger (Dietz et al. 2006) but for *Rhinolophus* from Tunisia the second phalanx of the fourth finger has to be added to the discriminant function (Puechmaille, Hizem, et al. 2012). The

discriminant analysis constitutes a powerful approach to differentiate between morphologically similar species, but only if the right combination of parameters from correctly assigned subsets of the individuals can be found. In addition to the species differences in mass and forearm length, we also observed significant sexual size dimorphism with larger and heavier males in all three species. The sample size is low for *M. coibensis* (eight females and four males) but the reference values of the two parameters for each sex (Table 2-3) should allow correct species identification for most individuals of our three focal species in Panama.

Analyses of wing shape

Wing shape is under strong selection for ecological niche use in bats because it underpins flight performance and foraging strategy (Norberg & Rayner 1987). Wing shape can also be useful for species identification (Dietz et al. 2006, Schmieder et al. 2015). However, all molossid bats have narrow wings well-adapted to hunting insects in open spaces (Voigt & Holderied 2012) and, therefore, many of the wing parameters that we measured did not vary between species. However, forearm length (mm), hand wing area (mm^2), hand wing length (mm) and wing loading (Nm^{-2}) differed between *M. molossus* and *M. coibensis*, with the former possessing longer wings on average at our study site. While such variation may potentially be ecologically significant for niche separation between the two species, significant intraspecific geographic variation in wing parameters can be found, for example in rhinolophids (Dietz et al. 2006). Until a dataset from a broader geographic range is available, our values should only be used for comparisons with individuals within Central America or even Panama.

Analyses of echolocation calls

Acoustic recording of free-flying bats is a widespread method for surveys and species differentiation including different species of *Molossus* (O'Farrell & Miller 1999, Jung & Kalko 2011, Jung et al. 2014). Acoustic recordings after release such as ours are also commonly used but not, to date, in molossids. The method is generally criticized as it is not representative of the environment and thus calls in free-flying animals (O'Farrell et al. 1999) but remains invaluable to match acoustic and molecular data of the same individual. Acoustic recordings after release proved useful only for a subset of our recordings after we categorized into different types of calls. The manual categorization of the calls confirmed the previously described call diversity: approach calls with harmonics described in *M. molossus* (Kossl et al. 1999, Mora et al. 2004), two-toned search calls (described in the Molossidae and Vespertilionidae Fenton et al. 1998, Kingston et al. 2003) and three-toned search calls (*M. molossus* (Jung et al. 2014). We selected the only two unmistakeable categories: short calls with a downward frequency modulation and a prominent second harmonic (approach calls) and the alternating two-toned calls (search calls). Despite the apparent utility of these calls to discriminate species, several limitations to this method must be considered. Only a few parameters were found to be significantly different between the two species. For example, mean values and standard deviations for SI and SII strongly overlapped between *M. molossus and M. coibensis* (e.g. 0.3 ± 0.3 ms & 0.4 ± 0.4 ms vs. 0.6 ± 0.3 ms and 0.6 ± 0.3 ms). Start and end frequency were the most reliable for species distinction. Following the recommendation of

Barclay (Barclay 1999), our reference values should only be compared to individuals released under the open sky and away from background clutter.

Comparison with other studies

Previous studies have reported reference values for different morphological parameters. However, several of these studies provided reference values using a different taxonomy from the one we used. For example, Simmons (Simmons 2005) considered *M. bondae* and *M. currentium* grouped under the name *M. currentium* and Reid (Reid 1998) treated *M. coibensis* as a subspecies of *M. molossus*. Studies that followed the same taxonomy as we did provided values consistent with our results (based on molecular validation): for example, a range of 33.2 - 36.0 mm, 33.5 - 34.7 mm or 33.9 – 36.0 mm for *M. coibensis* (Eger 2008, Gregorin et al. 2011, Correa da Costa et al. 2013), 35 - 40 mm for *M. molossus* (Eger 2008) and 38.4 - 41.1 mm or 38 - 43 mm for *M. bondae* (Reid 1998, Eger 2008). Measurements of body mass showed similar values too, with a range of 16 - 21 g for *M. bondae* (Reid 1998). However, larger values of forearm length in *M. coibensis* and *M. molossus* were found in a study from southeastern Brazil (Pimenta et al. 2014). *M. coibensis* showed a range of 35.5 - 38.1 mm for females and 36.5 - 38.1 mm for males while *M. molossus* showed a range of 35.3 - 42.2 mm for females and 38.2 - 42.3 mm for males. The inconsistency between the values reported in our study in Panama and the one in Brazil could be a result of natural geographic variation - a common phenomenon in bats (Storz et al. 2001) - or from incorrect taxonomic attribution leading to wrong values in other studies. Problems in taxonomic attribution can result from unsettled taxonomy, for

example *M. coibensis* from French Guyana referred as a true species (Eger 2008) or as a separate species, *M. barnesi* (Simmons 2005, Gregorin et al. 2011). To tackle these issues, additional comparative studies using molecular validation are required to provide reference values for these species in other regions of their biogeographic ranges.

Conclusions

All methods we used, based on molecular, morphometric or acoustic data, provided useful information for species discrimination. However, all of them, even the genetic methods, had their limitations, too. While we were able to reliably identify *M. bondae* based on size and pelage coloration, the microsatellite analysis led to a reliable genetic clustering of *M. coibensis* and *M. molossus* using two different methods. Individuals were assigned correctly with just a few exceptions when using all 18 microsatellite loci as well as with the more stringently determined subset of markers (n = 4). However, developing microsatellite primers involves a considerable effort. Other molecular methods, PCR-based assays (Boston et al. 2011) or high resolution melting (Wittwer 2009) are promising alternatives that should allow cheaper and quicker results of similar quality. The phylogenetic reconstructions with the *co1* sequences were also useful for species identification but mitochondrial DNA markers alone did not provide strong clade support. Comparison of morphometric parameters, i.e. forearm length and body mass, was a simple and very useful tool for species discrimination. However, they were only discriminatory when combined in a linear discriminant analysis function or when the sex of the individuals was taken into account. Previous work on other species shows that a different trait

combination may need to be found for each local species assembly, which may only allow correct species assignment after fieldwork has been completed, similar to the molecular methods. This may be particularly true for species-rich regions such as our study area where cryptic species are still being described and material for identification is patchy or even lacking. Only four of the 13 wing parameters we included in our analysis differed between species: forearm length, hand wing area, hand wing length and wing loading, again training the dataset on a subset of individuals was necessary to obtain reliable rules of thumb that can be used in the field and then again, potentially only locally. Finally, only a subset of the commonly used call recordings revealed species-specific differences in different acoustic parameters. This may be due to the artificial situation of a release, however as recording of free-flying bats cannot be matched to DNA or morphological measurements, it remains difficult to assess whether analysis of these calls would be more reliable even if species could be reliably identified in such a situation.

Although any single morphological measurement proved to be unreliable for species identification, we found that combining multiple measurements we could resolve reliable identification of the focal *Molossus spp.* in Panama, as verified by the genetic data. Based on these findings we emphasize the importance of combining morphological traits for field identification, as well as using independent genetic data to help decide upon the best combination of these traits in any given location. Proper species identification is the important basis for any work with wild animals and thus distinguishing a focal species within a local

species complex may only be possible using a multi-method approach.

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Authors' contributions

Conceived and designed the experiments: YG ET RHSK AL DKND. Performed the experiments: YG ET DL MM DKND. Analyzed the data: YG FBC MM. Contributed reagents/materials/analysis tools: AL SJR. Wrote the paper: YG ET DL MM FBC SJR RHSK AL DKND.

Chapter 3 - Group size, survival and surprisingly short lifespan in socially foraging bats

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Chapter 3

Group size, survival and surprisingly short lifespan in socially foraging bats

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Abstract

The relationships between group size, survival, and longevity vary greatly among social species. Depending on demographic and ecological circumstances, there are both positive and negative effects of group size variation on individual survival and longevity. For socially foraging species in particular there may be an optimal group size that predicts maximum individual survival that is directly related to the potential for information transfer, social coordination, and costs of conspecific interference. Our aim was to investigate this central aspect of evolutionary ecology by focusing on a socially foraging bat, *Molossus molossus.* This species optimizes foraging success by eavesdropping on the echolocation calls of group members to locate ephemeral food patches. We expected to find the highest survival and longest lifespans in small groups as a consequence of a trade-off between benefits of information transfer on ephemeral resources and costs of conspecific interference. In a mark-recapture study of 14 mixed-sex *M. molossus* social groups in Gamboa, Panama, spanning several years we found the expected relatively small and intermediate, but stable groups, with a mean size of 9.6 ± 6.7 adults and juveniles. We estimated survival proxies using Cox proportional hazard models and multistate-mark recapture models generated with recapture data as well as automated monitoring of roost entrances in a subset of the groups. Median survival of females was very short with 1.8 years and a maximum estimated longevity of 5.6 years. Contrary to our expectations, we found no relationship between variation in group size and survival, a result similar to few other studies. Strong selection towards small group size may result from psychoacoustic and cognitive constraints related to acoustic interference in social foraging and the complexity of coordinated

flight. The short lifespans were unexpected and may result from life at the energetic edge due to a highly specialized diet. The absence of a relationship between group size and survival may reflect a similar but optimized survival within the selected range of group sizes. We expect the pattern of small group sizes will be consistent in future research on species dependent on social information transfer about ephemeral resources.

Keywords: Cox proportional hazard model, fitness, *Molossus molossus*, multistate mark-recapture model, social foraging, sociality.

Introduction

Group living is widespread across the animal kingdom and evolved convergently from an ancestral solitary state in different taxa [e.g. 1]. Many species remain solitary or are only seasonally social (O'Mara, Wikelski, et al. 2014), showing that sociality is only beneficial when benefits outweigh the costs (Silk 2007). For example, in the social cliff swallow (*Hirundo pyrrhonota*), colony size is correlated with at least 13 different types of costs (e.g., parasitic infestation, brood parasitism) and at least 13 different types of benefits (e.g., predator-avoidance, information transfer, Brown & Brown 1996). Thus, group size is an important trait that responds to cost-benefit regimes depending on a species, its ecological niche and life history (Hoogland 1995, Brown & Brown 1996). In fact, the size of animal aggregations can vary from small social groups below ten individuals like the prides of lions (Caraco & Wolf 1975) to huge colonies with millions of seabirds or bats (McCracken et al. 1994, Jovani et al. 2008). Thus,

a crucial step in any study is to distinguish between aggregations of individuals, due to external circumstances such as roost limitation, and "true" social groups with reciprocal relationships (which may be contained in larger aggregations) (Wilson 2000).

Sociality should be adaptive (Silk 2007), we therefore expect fitness benefits of optimal group size resulting in prolonged survival, enhanced reproductive success or both. Life history theory predicts that animals should allocate their energy differently to individual reproduction or survival (Hirshfield & Tinkle 1975, Roff 1992). As a general rule of thumb, small animals are short-lived and produce many offspring (e.g. rodents, *r*-strategists) while large animals are long-lived and have few offspring (e.g. elephants, *K*-strategists) (Pianka 1970). Bats are an exception to this general pattern, being small but long-lived and producing relatively few offspring. However, while life history theory does not incorporate sociality, there are many studies linking group size with survival. Different parameters are used to investigate this, the two most common being maximum lifespan (or maximum longevity) and an averaged estimate for the survival of the group members. Comparative studies on birds and mammals did not find any correlation between maximum lifespan and group size (Wilkinson & South 2002, Blumstein & Møller 2008, Beauchamp 2010, Kamilar et al. 2010). The same is not true for the relationship between group size and survival. Group size is often positively correlated with survival in many taxa, including termites (Miramontes & Desouza 1996), social spiders (Bilde et al. 2007), birds (Brown et al. 2003, Brown & Brown 2004, Serrano et al. 2005) and mammals (Robinette et al. 1995, Clutton-Brock et al. 1999). In all of these examples, social behaviours, such as predator

avoidance, social thermoregulation or social foraging, lead to improved survival. However, there is a limit to the benefits of increasing group size. For instance in certain colonies of Neotropical spiders, survival of the colonies increased with colony size. But above a threshold in colony size (~15 individuals), survival of the colony decreased, presumably because of an increase in intra-colony competition (Bilde et al. 2007). In other species, such as the Seychelles warbler (adults) and a social spider (juveniles), there is even a strictly negative relationship between survival and group size, again probably due to competition for resources (Brouwer et al. 2006, Bilde et al. 2007). Despite this decreased survival, increasing group size brings reproductive benefits in the Seychelles warbler. The reverse situation was observed in Neotropical spider, with survival benefits but reproductive costs with increasing group size leading to a trade-off situation and resulting in maximum fitness at intermediate size (Avilés & Tufiño 1998). Finally, in some species, including wild dogs, juvenile rodents, primates or coatis, group size and survival are independent (Hass & Valenzuela 2002, Borries et al. 2008, Hayes et al. 2009, Gusset & Macdonald 2010), interpreted to be a result of specific ecological conditions such as low competitor density and high food availability.

One important benefit of sociality is information transfer between individuals (Danchin et al. 2004, Dall et al. 2005, McNamara & Dall 2010). In a foraging context, animals can detect conspecifics present at food patches through “local enhancement” (Hoppitt & Laland 2013). The number of animals at a food patch and the modality of the information they use (e.g., sound, vision, olfaction) can have crucial implications for their fitness. Many bird species rely on

local enhancement through vision to detect conspecifics at a food patch (e.g. seabirds, vultures, ospreys and swallows) (Hoffman et al. 1981, Flemming et al. 1992, Haney et al. 1992, Buckley 1996). In an empirical test of recruitment of seabirds to food patches, adjusted estimates for average distance recruitment ranged from 4.9 to 11.3 km (Haney et al. 1992). Therefore, vision, the most commonly used mode of information transfer during foraging leads to the attraction of individuals over long distances and is believed to have led to the evolution of bird colonies (Beauchamp 1999). Echolocating bats, in contrast, cannot use vision during nocturnal foraging. Instead the benefit from information transfer by eavesdropping on changes in each others' echolocation calls that indicate successful localization of a food source (Barclay 1982, Gillam 2007, Safi & Kerth 2007, Dechmann et al. 2009, 2010, Jones & Siemers 2011). Compared to vision, the propagation distance of echolocation calls is very short due to rapid atmospheric attenuation (Griffin 1971). For instance, maximum hearing distance of conspecifics was estimated at 54 m in *M. molossus* and 35-40 m in *Noctilio albiventris* (Dechmann et al. 2009, 2010), however this is ten times the distance from which they can actively localize a prey item. The restriction to different modalities (e.g. vision vs. sound) therefore has direct implications for the foraging strategy. However, the relationship between social foraging, the composition of groups, survival, and group size remains poorly understood in bats despite the wide reliance on social information to locate resources and its effect on the evolution of group living in bats and other animals.

To test if and how social group size affects survival in bats, we studied Pallas's mastiff bat

Molossus molossus (Pallas, 1766), a species that forms stable social groups that roost and forage together (Dechmann et al. 2010). The narrow-shaped wing morphology of *M. molossus* results in high energetic requirements within an open-air foraging niche (Thomas & Suthers 1972). As a result of this specialized morphology, this species depends on ephemeral insect swarms as their only food source that are only available at dawn and dusk, and therefore foraging time is restricted to short activity peaks of less than an hour (Dechmann et al. 2010). These energetic and morphological limitations as well as the narrow foraging niche make increased foraging efficiency through the use of social information from conspecifics highly important. One might thus expect that a large number of foraging partners and correlated increase in social group size would be advantageous. However, theoretical work indicates optimal individual uptake in groups with a small number of signallers in a recruitment scenario (Torney et al. 2011). Due to the short availability window of its resource and the modality of information transfer (acoustical), *M. molossus* must coordinate flight and eavesdrop on echolocating group members on the wing instead of recruiting. This quickly creates a complex system of signallers and receivers and thus a trade-off between benefits of improved indirect prey detection and costs of conspecific acoustic interference (Cvikel et al. 2015). Thus, we hypothesized that there is an ideal group size for *M. molossus,* and that this group size should be small. Individual survival should then be highest in these ideal small groups. To test this we used two approaches: a) We captured 14 social groups multiple times over several years. During a subset of this time period, we b) also monitored four of these groups with automatic transponder readers to get a more precise temporal resolution of changes in group

composition. We modelled the role of group size using these two data sets in two survival analyses based on the Cox proportional hazard and multistate mark-recapture models. We based our survival analysis on all adults present in a group because males and females are known to forage together (Dechmann et al. 2010). However, we only analysed survival and lifespan for adult females because males probably spend time as bachelors before and possibly even after their presence in the groups, which then is probably only indicative of their harem tenure. The unique access to data from free-ranging socially foraging bats thus allowed us to test predictions from theoretical evolutionary models in a system of naturally behaving animals.

Material and methods

Data collection

We collected data in Gamboa, Panama (09°07' N 79°41' W), where *Molossus molossus* roosts in crevices in houses. We defined social groups as the set of individuals roosting in a single building crevice, but sometimes several social groups occupied separate crevices in the same building. We collected data about group size in two ways: repeated captures from roosts and automated monitoring with transponder readers (henceforth called "BaTLis", custom-made by the workshop of the University of Konstanz).

Captures - We captured social groups with mist nets (Ecotone, Gydnia, Poland) at the entrance of roosts during evening emergence. The nets formed a closed space around the roost entrance, thus the entire group was caught in most cases apart from individuals that potentially

remained in the roost. Between 2008 and 2014, we caught 14 mixed-sex social groups, resulting in 81 capture events and 490 individuals (2-9 capture events per group). We determined sex, age and reproductive status, and individually marked all bats with a subcutaneous passive integrated transponder (Trovan ID-100, Euro ID, Weilerswist, Germany) at first capture.

BaTLis - We monitored the roost entrance of four of the 14 groups with the BaTLis between April 2013 and June 2014 and followed the presence of each marked bat. Each BaTLi contained two light beams to determine direction of individuals passing as well as a balance. These two latter datasets allowed us to follow and quantify the number of unmarked bats using the roost entrance, e.g., immigrants into the group as well as freshly fledged juveniles. Over the 15 recorded months, these four roosts were recaptured 4-5 times each to mark new individuals with a maximum interval of six months between recaptures. Capture and handling of animals was carried out with permission from the Autoridad Nacional del Ambiente in Panama with approval from the Institutional Animal Care and Use Committee of the Smithsonian Tropical Research Institute (2012-0505-2015).

Life cycle of *Molossus molossus*

Captures - We first determined the proportion of adults recaptured and checked if they switched roosts. We identified the pregnancy and the appearance of fledged juveniles using the capture data.

BaTLis - With the BaTLis, we were able to additionally monitor the timing of the increase of untranspondered individuals passing the entrance, indicative of a cohort of freshly fledged offspring. This allowed us to evaluate the timing of birth. Juveniles we caught and marked after fledging were used to determine the timing of juvenile dispersal as determined by the BaTLis.

Variation of group size

Captures - We calculated total group size (adults and juveniles) and adult group size at each capture to assess the temporal variation of group size [see Additional file 1 for raw data]. We also determined the proportion of adult males and adult females.

BaTLis – the BaTLis allowed a higher temporal resolution of changes in group size caused by death, immigration, or harem male replacement, but only over the shorter time period of 15 months when BaTLis were employed.

Estimates of female lifespan

Our capture data showed that *M. molossus* lives in harems with regular replacement of the harem male, but stable female social groups (see results). Based on the switch from pregnant to lactating females as well as calculating back roughly one month from the time we first caught freshly fledged offspring, we determined that the major birth peak occurs in May. From the disappearance of marked female subadults from groups, we were able to tell that all offspring disperse within 1-8 month of fledging. We then estimated lifespan by filtering the capture data

in the following way: for each unmarked bat we captured we determined if there had been a previous capture of the same roost where this individual had not been present. This meant that it had dispersed from its natal group and immigrated since the last capture. Thus, we started counting its lifespan from the previous May as a conservative minimum estimate. For example, a female marked in November was considered born in May of the same year, resulting in a lifespan correction of seven months. We found no adult females that changed group, therefore we assumed that adult females' disappearance from a group indicated their death.

Predictors of female lifespan and monthly survival

We used two different survival analyses, a first analysis based on the capture dataset and a Cox proportional hazard survival model (Cox PH) and a second analysis based on the BaTLi dataset and multi-state mark recapture survival models (MSMR).

Cox proportional hazard model using capture data - These survival analyses are less robust because they do not take into account detection probability and changes in group size but we included more females and over a longer time period in this dataset (n = 70 over a maximum of 2.5 years after filtering) and we could estimate lifespan. We used the estimates derived from the capture data: time between estimated birth and last capture as described above, i.e. lifespan [see Additional file 2 for raw data]. Survival data were right-censored when the female was still alive at the last capture event. We used the Cox PH model (Andersen & Gill 1982), based on continuous time and the assumption of perfect detection (100% probability of

capture) and available in the R package *survival* (Therneau 2015). We built two models combining the lifespan estimates and two predictor variables: total group size and adult group size. In these models, the individual survival probability at the recapture event [t] was based on the group size (total or adult) of the individual's group during the previous recapture event [t-1]. We tested the two models for the proportional hazard assumption of the predictor variables based on the scaled Schoenfeld residuals also using the R package *survival*. We also split the survival range into yearly categories (0-1, 1-2, 2-3 and 3-4) and determined the number and proportion of females for each of them.

Multistate mark recapture models using BaTLi data - In this second analysis, fewer females were analysed over a shorter time period (n = 63 over 15 months) but the models implemented detection probability and transitions in group size. We investigated predictors of monthly female survival using BaTLi data from the four groups (n = 63 adult females, see Additional file 3) and multistate mark recapture models (MSMR, Lebreton *et al.* 2009). These models are Markovian (conditional on the present state of the system, its past and future are independent) and rely on discrete time categories (e.g. calendar month) that we used to model temporal change in individual state (e.g. group size). With the MSMR models, we simultaneously estimated initial state (i.e. group size whenever first captured), survival probability, changes in group size, as well as detection probability for one or several predictor variables (e.g. social group, observation month). Detection probability (P) is a crucial parameter, often smaller than one and highly variable in natural populations, which can lead to flawed biological conclusions

when not considered in mark-recapture analyses (Gimenez et al. 2008).

Our dataset consisted of 63 rows (one for each adult female) and 15 columns (one for each month of the study period). Each cell of the matrix contained either a "0" when the focal individual was absent or recorded the adult group size, when the focal individual was present. We categorized group size into "small" (3-6 individuals), "medium" (7-9) and "large" (10-13) to obtain higher confidence in the survival estimates. We assigned an age (i.e. marking year) and social group to each female. Although we observed occasional brief visits of adult females into neighbouring roosts (n = 9 events), no adult female was ever observed to permanently change groups and we therefore assumed stable group identity.

We performed multisite goodness-of-fit tests on the adult females dataset (Pradel et al. 2003) using the software U-Care v. 2.3.2. (Choquet, Lebreton, et al. 2009). We modelled monthly survival based on this survival matrix and five MSMR models. Each model comprised the following parameters (see also Table 2): IS[gs] or initial state for adult group size (the percentage of individuals initially observed in "small", "medium" and "large" groups, the same in all models), Φ or survival probability (i.e. from one month to the next), ψ[.] or group size transition probability (implemented in one of the five models, see below) and a constant detection probability P[.]. Preliminary investigation showed that the use of more than one predictor variable caused high uncertainty in the estimates. Consequently, we only estimated survival (Φ) using a single predictor variable per model: 1) adult group size (gs), 2) social group

identity, 3) month of the study, 4) marking year, and 5) a null model without predictor variable. The first model, estimating the effect of group size, also incorporated a transition probability between group sizes ψ[gs]. In this model, an individual survival probability at month [t] was based on the size of the individual's group during the previous month [t-1]. Model selection was performed using the program E-SURGE (Choquet, Rouan, et al. 2009) with the Akaike Information Criterion corrected for small samples (AICc) as a measure of the trade-off between goodness of fit and complexity of the model. A threshold of 10 AIC units of difference was used to select the best-fitting model (Burnham & Anderson 2002).

Estimates of tenure length for harem males

We estimated potential tenure length of harem males by calculating the time interval they were observed in the roost, from the first capture to the last capture as an adult male.

Results

Life cycle of *Molossus molossus*

Captures - We collected data during 81 capture events of 14 social groups and a total of 490 individuals (adults and juveniles). We recaptured only a subset of the bats we marked as post-dispersal adults (121 females and 31 males) and none of them were observed switching roosts over the entire study period (maximum of 4.3 years). We caught pregnant females between March and August and observed a pregnancy peak in April. Anecdotal data indicate a second minor birth peak at the end of the year, but we cannot confirm this. Based on capture data, we

assume that females disperse from the social group to settle permanently in a social group. It also appears that they can start reproducing directly after natal dispersal. We cannot infer the complete life cycle of males based on the capture data but we suspect a bachelor phase between natal dispersal and tenure of harems as well as after the end of tenure (McCracken & Bradbury 1981, Dechmann et al. 2005).

BaTLis – Data from the BaTLis (automated transponder readers custom-made by the workshop of the University of Konstanz) are consistent with captures. In addition, activity of unmarked individuals on the BaTLis suddenly increased around June, and this activity peak corresponded with juvenile fledging. We monitored a subset of 24 juvenile females and 19 juvenile males with the BaTLis. They were marked between July and September and were last detected by the transponder readers between July of the same year and February of the following year indicating their death or natal dispersal. The life cycle of female *Molossus molossus* is illustrated in Fig. 3-1.

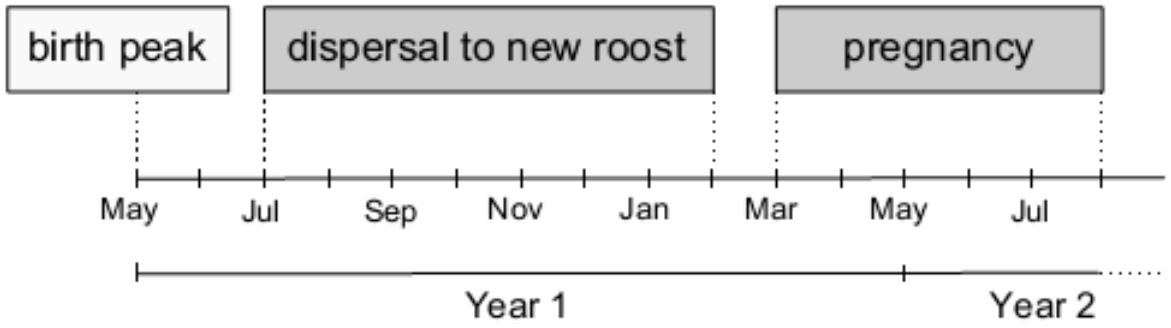

Figure 3-1. Life cycle of female *Molossus molossus*.

Variation of group size

Captures - We found that total size of groups we caught (adults and juveniles) was small compared to the know range of bat colonies, ranging from one to 32 individuals, with a median of eight and a mean of 9.6 ± 6.7. Single individuals were caught only on three occasions: the same adult male on two occasions (roost 164) and an adult female on one occasion (roost 152A). We suspect other individuals were inside the roost but did not emerge because we captured several individuals during other capture events of these groups. Adult group size varied between one and 25 individuals, with a median of seven and a mean of 8.1 ± 5.1 individuals. Adult sex-ratio was biased towards females with a median of 78% and mean of 71% ± 26%. The number of adult males was one or two in 75% of the capture events. Group size increased during July and August (Fig. 3-2), coincident with juvenile fledging.

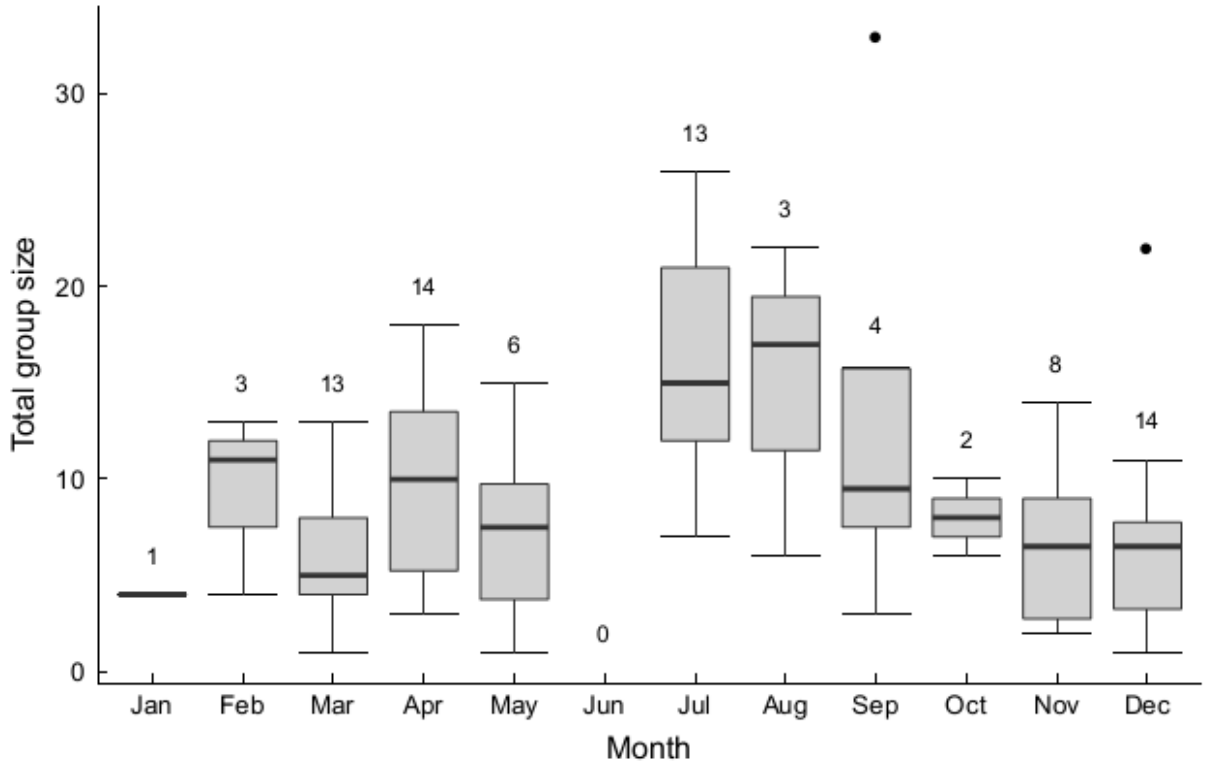

Figure 3-2. Temporal variation of total group size of *M. molossus* (including juveniles). Box plots represent from bottom to top: minimum, lower quartile, median, upper quartile and maximum. Dots indicate observations further than one SD away from the mean. The numbers of social groups caught per month are indicated above the boxplots.

BaTLis – Adult group size varied between three and 13 individuals. We found very similar mean and SD for group size: Group 1: 9.0 ± 2.3, Group 2: 10.0 ± 2.5, Group 3: 10.9 ± 3.9 and Group 4: 11.1 ± 2.6 individuals

Estimates of female lifespan

We followed the 14 social groups of the study between 0.5 and 4.7 years. We recaptured 30.2% of the 280 females marked as adults (n = 124, one to seven recaptures per individual). Lifespan of adult females from marking time ranged from five to 1709 days (4.7 years) with a median of 280 days (0.8 years). Corrected estimates from the closest birth peak expanded lifespan between 132 and 2044 days (0.4-5.6 years) with a median of 646 days (1.8 years).

Predictors of female lifespan and monthly survival

Cox proportional hazard model using capture data - We filtered out 44% of the captures when including only adult females that had immigrated since last capture. This resulted in 70 adult females with 114 recapture events. Corrected lifespan estimates (from potential birth to last capture) ranged here from 132 days (0.4 years) to 1210 days (3.3 years) with a median lifespan of 436 (1.2 years). The selected variables (adult or total group size) complied with the test of proportionality and had no statistical influence on survival estimates for both survival datasets (Table 3-1). 24 females survived less than a year (34.3%), 30 between one and two years (42.9%), 8 between two and three years (11.4%) and 8 other between three and four years (11.4%).

Table 3-1. Results from the two Cox proportional hazard survival models. The significance of the predictor variable and test of proportionality are based on scaled Schoenfeld residuals.

Survival dataset	Variable	Significance	Proportionality
Assumed birth to last capture	Total group size	0.37	0.08
Assumed birth to last capture	Adult group size	0.11	0.58

Multistate mark recapture models using BaTLi data - Four groups were randomly selected based on whether it was possible to install a BaTLi at the entrance. We used the transponder reader data to investigate the influence of a set of variables on adult female survival (n = 63). As we monitored these groups only for 15 months, the estimates here represent monthly survival rather than complete lifespans. Size of the four focal groups changed over time, ranging from three to 13 adult males and females but with very similar mean and SD for group size. We divided the range of group sizes into three categories to obtain higher confidence in our monthly survival estimates ("small": 3-6, "medium": 7-9, "large": 10-13). Multistate models were found to adequately fit the data for the four groups (Group 1: $\chi^2 = 4.348$, df = 3, P = 0.226; Group 2: $\chi^2 = 5.823$, df = 8, P = 0.667; Group 3: $\chi^2 = 3.083$, df = 2, P = 0.214; Group 4: $\chi^2 = 1.490$, df = 1, P = 0.222). The best fitting models, ordered by lower ΔQAICc, were 1) adult group size, 2) marking year, 3) the null model, 4) group ID (1-4), and 5) the month of first capture (Table 3-2). The best fitting model was strongly supported, with a ΔQAICc of 118 units in comparison to the next-best model (marking year). Note that while performing goodness-of-fit tests, we could not test for trap-dependence because of a lack of data. We reran the same set of models modified

to incorporate a trap-dependence effect. Specifically, we had a detection probability function of an individual covariate "captured" or "not captured" at the previous event. The ranking of the models and survival estimates from the best-fitting model here were similar to the analysis not incorporating trap-dependence on the detection probability, therefore we show only the results from the model without trap-dependence.

Table 3-2. Multistate mark-recapture models of survival for *M. molossus*. The survival estimates are based on 63 adult females from four social groups. The five models are ordered by the ΔQAICc where a lower value indicates a better fit of the model to the data. These models estimated initial state (IS), survival (Φ), transition probabilities (ψ) and constant detection probability (P) for the predictor variables adult group size (gs), marking year, social group and observation month.

Model	QAICc	ΔQAICc	Number of parameters	Deviance
(1) IS[gs]. Φ[gs]. ψ[gs]. P[.]	1380.0	0.0	12	1407.5
(2) IS[gs]. Φ[marking year]. ψ[.]. P[.]	1498.0	118.0	9	1480.0
(3) IS[gs]. Φ[.]. ψ[.]. P[.]	1509.4	129.4	5	1499.3
(4) IS[gs]. Φ[social group]. ψ[.]. P[.]	1513.5	133.5	8	1497.2
(5) IS[gs]. Φ[month]. ψ[.]. P[.]	1522.9	142.9	18	1485.6

Based on the best-fitting model (adult group size), we estimated survival for the three categories of group size. We found similar probabilities of monthly across categories (Fig. 3):

"small" groups (3-6 bats; 0.93, 95% Confidence Interval (CI): 0.85-0.97), "medium" groups (7-9 bats; 0.95, 95% CI: 0.91-0.97) and "large" groups (10-13 bats; 0.96, 95% CI: 0.93-0.98). The confidence in survival estimate is lower for "small" groups because we had only 10 group-month observations for this category, while we had 24 and 26 for "medium" and "large" groups, respectively. With the same model, we obtained a detection (or recapture) probability of P[.] of 0.95. We also obtained transition probabilities between group size ψ[gs] – the probability that the group size an individual occupies changes from one month to the other - ranging from 0.04 to 0.40 (Fig. 3-3). The most frequent group size transitions occurred between the two most frequent group sizes ("medium" and "large"), with transition probabilities per month of 0.40 (medium to large) and 0.39 (large to medium). There was no influence of temporal variation of group size on monthly survival probability.

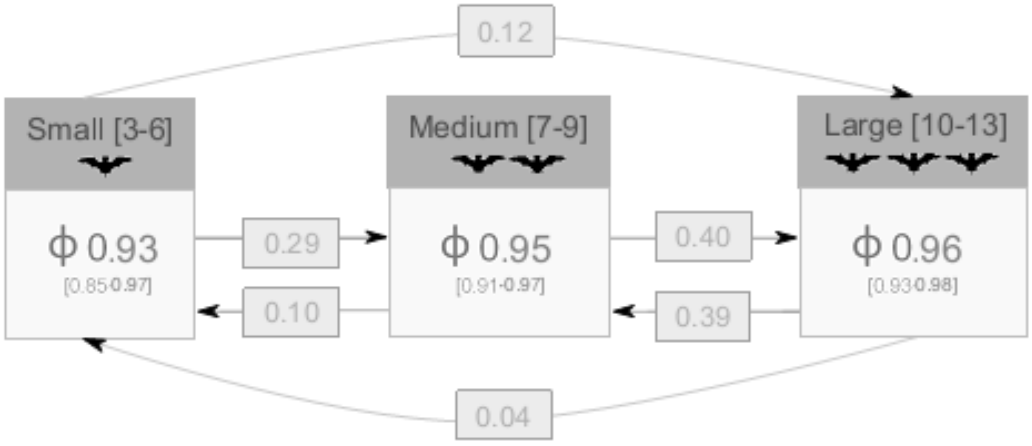

Figure 3-3. Multistate mark-recapture model for survival and group size in *Molossus molossus*. Survival estimates Φ for group size categories (small, medium and large) and

transition probabilities ψ between these categories are depicted. For example, the survival probability (from month t to t+1) in a small group is 0.93 (95% Confidence Interval: 0.85-0.97), and the probability that a small group will transition to a large group (from month t to t+1) is 0.12. These parameters were estimated with the multistate mark recapture model with group size including initial state of group size IS[gs] and detection probability P[.] in the model IS[gs]. Φ[gs]. ψ[gs]. P[.].

Estimates of tenure length for harem males

We recaptured 22.5% of the 107 males marked as adults (n = 31, one to four recaptures per individual). Time from marking to last recapture ranged from six to 1076 days (2.9 years) with a median of 228 days (0.6 years).

Discussion

Our data suggest that even though aggregations of bats can consist of up to several million individuals, there is strong selection for small group size (≤ 25 adults) in *Molossus molossus*. There was little variation of group size and short median female survival of 1.8 years (corrected estimates from all recaptured data). Furthermore, group size was not correlated with longevity as calculated from recaptures and monthly survival as calculated from automated monitoring in a subset of groups (i.e. BaTLis).

Thus, along with our predictions, *M. molossus* forms small groups, composed of a stable core of

adult females and one adult male in most groups (mean of 8.1 ± 5.1 adults). BaTLi data showed that, all juveniles of both sexes disperse from the natal group. Once they settled in a new group, adult females remained there longer than adult males (median of 0.8 years vs. 0.6 years, maximum of 4.7 years vs. 2.9 years). Thus, the cluster of adult females emerges as the stable and primary unit of *M. molossus* social organization. Stable clusters of females favour the evolution of a male strategy of female-defence polygamy (Bradbury & Vehrencamp 1977, Emlen & Oring 1977). Where females live in social groups, one single male is commonly responsible for most or all mating, as shown for instance in primates, antelopes or bats (Hrdy 1980, McCracken & Bradbury 1981, Ortega et al. 2003, Wirtz 2010). Our data suggest that maximum male tenure length (2.9 years) exceeds the sexual maturity of the sired daughters, driving natal dispersal of juvenile females to avoid inbreeding with their father (Clutton-Brock 1989). This phenomenon is observed in few other species of mammals (Greenwood 1980) but seemingly not uncommon in tropical bats living in stable groups (McCracken 1984, Nagy et al. 2013). Natal dispersal of juvenile males may result from an eviction by the harem male (potentially their father) and/or an attempt to find mating opportunities (Moore & Ali 1984, Moore 1984). We are unable to estimate lifespan for male *M. molossus* because we do not know the time from natal dispersal to harem take-over as well as the fate of replaced harem males. We suppose males remain solitary or join a bachelor group before and after harem tenure as already observed in other tropical bats (McCracken & Bradbury 1981, Dechmann et al. 2005), but the roost preferences of these males seem to differ from those of females as we did not find them during our exhaustive roost surveys in buildings. A challenge for future

studies will be to follow males throughout their life cycle to obtain realistic lifespan estimates. Similar to other polygynous mammals, we expect to find reduced longevity in adult males resulting from intense male-male competition and weaker selection for longevity (Clutton-Brock & Isvaran 2007).

Group fidelity of adult females was very strong as shown by one to seven recaptures of 121 females in the roosts where they were initially marked as adults. In the roost we monitored the longest time, a female was recaptured after 4.7 years with an estimated lifespan of 5.6 years. We expect that maximum lifespan records might still increase slightly with a longer study period, but we are confident that our mean estimates of 1.2 and 1.8 years are representative for the species. This is unexpected, because bats are famous for exceptionally long lifespans relative to their small body mass (i.e. allometry of lifespan), the record being held by a 8-gram insectivorous *Myotis brandtii* which was recaptured after 41 years in the wild. On average, lifespan of bats is around 3.5 times longer than in non-flying mammals after correcting for body mass (Wilkinson & South 2002). Maximum lifespan for two other molossid species with a similar ecological niche as *M. molossus* is 12 and 13 years, respectively for the much larger European *Tadarida teniotis* (mean mass of 30 g) and the American *T. brasiliensis* (mean mass of 12.5 g similar to our study species) (Barclay & Brigham 1991, Marques et al. 2004, Tacutu et al. 2013). The higher longevity observed in these two species may result from decreased predation associated with cave roosting (Wilkinson & South 2002) and/or a broader foraging niche in terms of prey as well as temporal and spatial food availability. Despite its relevance for

population dynamics, maximum lifespan represents only the upper limit of the survival curve. Better knowledge about average or median lifespans appears to be important, but often lacking life-history parameter in bat studies (but see (O'Shea et al. 2011) for the better understanding of the pace and shape of the survival curve (Baudisch 2011, Jones et al. 2014). Our data revealed a skewed survival curve for females, with median survival of 1.8 years and a maximum longevity of 5.6 years. Low values for median and maximum lifespan in *M. molossus* may result from life at the energetic edge due to a narrow foraging niche. This bat is an open-air forager, with long and narrow wings that result in high wing loading and high energetic costs of flight (Norberg & Rayner 1987, Voigt & Holderied 2012). This bat is also specialized to forage on insect swarms which are abundant when found but remain relatively unpredictable in space and time (Safi & Kerth 2007). The species shows a bimodal activity pattern, after sunset and before sunrise (Jones & Rydell 1994, Esbérard & Bergallo 2010). The predominant foraging activity occurs after sunset, sometimes for only a half an hour interval (Jones & Rydell 1994, Esbérard & Bergallo 2010). This limited burst of activity probably result from a peak in insect density (Voigt & Holderied 2012). Because insect patches can be dispersed by wind and rain, bats sometimes entirely skip a night of foraging. To limit the risk of starvation, this species can maximize energy intake by socially foraging (Dechmann et al. 2010) and minimize energy investment by lowering metabolism when roosting (Dechmann et al. 2011). However, due to the short foraging window, the unpredictability of the resource and the flight costs, these bats have a risk of starvation. Our data, suggesting that most females *M. molossus* only reproduce once or twice in their short lifetimes, is consistent with this hypothesis. Further investigation

will be necessary to determine how the small percentage of longer-lived individuals contribute to the maintenance of the species, what causes these enormous variations in lifespans and how they are linked to the ecology of species. In addition, it will be important to find other factors influencing variation in female lifespan, such as foraging efficiency, and also following up on anecdotal reports about twinning as well as an additional smaller reproductive peak to better understand how stable populations of this species can persist.

Social groups of *M. molossus* are stable year-round, implying that benefits of sociality permanently outweigh the costs (Silk 2007). Foraging benefits via information transfer about ephemeral resources have been postulated as a major reason for coloniality in seabirds (Beauchamp et al. 1997, Buckley 1997). And similarly, in *M. molossus* and other bats, the main benefit of group living is probably increased foraging efficiency through acoustic information transfer about ephemeral insect patches (Safi & Kerth 2007, Dechmann et al. 2009, 2010). The daily availability of *M. molossus*' food source is so short that information must be shared in the foraging arena on the wing, most likely via acoustic eavesdropping (Dechmann et al. 2009). This means that groups must coordinate flight and filter relevant information from the echolocation calls of their social partners. Even though we do not understand yet how this works in detail, such a network of signallers and receivers quickly becomes very complex and confusing. Our results confirm our hypothesis that this should exert strong selection pressure on small group size because the 14 groups were ranging between one and 25 adults. This is in contrast to opportunistically eavesdropping species that do not emerge in coordinated flight and maintain

group cohesion throughout their foraging period (Fenton & Morris 1976, Vaughan 1980).

While our expectations regarding small group size were confirmed, we did not find that individual lifespan was influenced by variation of group size. In both datasets (capture and BaTLi data), group size had no effect on lifespan or monthly survival despite group size being the most explanatory variable in the multistate mark recapture analysis. We postulate that selection on group size is so strong that the resulting variation, mainly caused by the brief appearance of pre-dispersing juveniles, is too small to have an effect on the adult females in the group. In fact, a closer look at previous work that found no or a negative relationship between group size and survival reveals consistently small group sizes in animals with complex social systems (≤ 33 individuals), e.g. 1-6 individuals in the Seychelles warbler (Brouwer et al. 2006), 2-12 adults in degus (Hayes et al. 2009), 2-10 adult females in the coati (Hass & Valenzuela 2002), 2-17 individuals in the wild dog (Gusset & Macdonald 2010) and 6-33 individuals in the leaf monkey (Borries et al. 2008). This suggests that selection for small group size in complex social systems may be fairly common. In other species, the limited variation of group size observed here (3 to 13 individuals) can still cause survival differences that can be detected. For example in the Seychelles' warbler, survival is negatively correlated with group size (one to six individuals) (Brouwer et al. 2006).

The apparent absence of relationship between group size and survival in *M. molossus* could have technical and/or biological explanations. Our estimates of monthly survival for the three

categories of group size were associated with narrow confidence intervals (respectively 0.85-0.97, 0.91-0.97 and 0.93-0.98). A dataset including more groups and individuals may lower confidence intervals and reveal survival differences that remained hidden so far. Alternatively, our results correspond to the reality and selection on ideal group size is so strong that the remaining low variation does not affect survival. In the framework of life history, the relationship between group size and reproduction remains to be investigated in future studies.

Bats are well known for roosting in large or even gigantic colonies, e.g. the closely related molossid species *Tadarida brasiliensis* that occurs in caves numbering up to tens of millions of individuals (McCracken et al. 1994). A confounding effect here may stem from the fact that many bat species are highly dependent on suitable, but limited roosts. *Molossus molossus*, too, may roost in such larger aggregations (300 individuals or more, (Reid 1998)), composed of several social groups at other study sites where roosts are more limited. Several bat species, such as *Myotis bechsteinii* (Kerth 2010), *Nyctalus lasiopterus* (Popa-Lisseanu et al. 2008) or *Eptesicus fuscus* (Willis & Brigham 2004), form fission-fusion societies. This allows animals to have access to the knowledge of a large pool of individuals but the daily subgroups are relatively small as a flexible reaction to social and environmental conditions. This may also mediate other detrimental effects of group living in bats such as parasite or disease transmission rates or competition (Côté & Poulin 1995, Krause & Ruxton 2002). Similar societies are found in a great diversity of taxa, e.g. house sparrows (Griesser et al. 2011), chimpanzees and leaf monkeys (Symington 1990), or spotted hyenas (Smith et al. 2008). However, most

socially complex bat species form smaller and more stable social groups similar to *M. molossus* (≤ 25 adults), e.g. the molossid *Tadarida pumila* (McWilliam 1988), other socially foraging bats (McCracken & Bradbury 1981, Dechmann et al. 2009), tent-making bats (Timm & Mortimer 1976, Storz et al. 2000, Chaverri et al. 2008) and other roost-making bats (Hodgkison et al. 2003, Dechmann et al. 2005). We expect that with future research this will be a consistent pattern, especially in species that are ecologically dependent on information transfer about the location of ephemeral resources.

Conclusions

In summary, this study suggests strong selection for small groups (≤ 25 adults) in a socially foraging bat. Our results are in agreement with models of recruitment on ephemeral resources suggesting a small and stable range of signalers in the groups optimizes individual uptake (Torney et al. 2011). In our *in situ* eavesdropping scenario, where every individual is a signaler and receiver at a same time, the same selection pressure seems to apply to optimize trade-off between foraging benefits from information transfer and acoustic confusion impairing prey detection performance (Cvikel et al. 2015). Our alternative survival analyses based on free-ranging animals independently found no effect of group size on survival, a pattern found in few similar studies and potentially resulting from life at the energetic edge due to a highly specialized diet. We expect similar results for future research conducted on species dependent on information transfer and ephemeral resources.

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Chapter 4 - Foraging patterns and efficiency in a socially foraging bat

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In preparation for submission

Chapter 4

Foraging patterns and efficiency in a socially foraging bat

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Abstract

Information transfer may enhance individual fitness in species relying on dispersed resources. Benefits of acoustic information transfer have been proposed as a reason for group living in echolocating bats specialized on patches of ephemeral insects. In the tropical bat *Molossus molossus*, members of each harem are known to forage together for very short time but the body mass gain and foraging efficiency associated remains unknown. We used automated recorders - integrating a PIT-tag reader and a scale – installed at the entrance of five roosts to determine foraging patterns and efficiency free-ranging bats. A single foraging bout at dusk, likely benefiting from improved prey searching with information transfer, ensures the required amount of energy for the majority of group members to fast until the following dusk. Few individuals, especially harem males, showed shorter foraging bouts, likely as a strategy to ensure harem maintenance and reproductive success. Dawn foraging appears as a strategy for these individuals facing higher energetic pressure to obtain enough energy to fast until foraging at dusk. This study constitutes an additional step to understand the different evolutionary pressures experienced by social bats.

Introduction

The transition from a solitary lifestyle to sociality occurred multiple times in evolutionary history (Shultz et al. 2011, Kapheim et al. 2015). The use of social information has been proposed as an important driving force in social evolution (Danchin et al. 2004, Dall et al. 2005). For example, individuals may respond to stress cues of others, resulting in anti-predator benefits, or observe others to discover new food patches, with foraging benefits. The use of social information appears especially beneficial in a foraging context, where resources are dispersed and clustered (Johnson et al. 2002). One important mechanism of information transfer is local enhancement, where individuals can detect and join conspecifics exploiting patchy resources (Buckley 1997). Local enhancement generally reduces the amount of time individuals need to locate food patches, resulting in increased foraging efficiency and/or reduced risk of starvation (Caraco 1981, Clark & Mangel 1984, Brown 1988, Beauchamp 1998). However, there is a point when the costs outweigh the benefits of social foraging (Janson 1988, Krause & Ruxton 2002). The balance of cost and benefits of social foraging is directly influenced by several key parameters including patch size, patch richness and group size (Clark & Mangel 1986, Beauchamp 1998). The relationship between foraging efficiency and group size has been studied in birds, large mammals, fish and invertebrates (Krause & Ruxton 2002). A comprehensive social foraging theory has emerged in the last decade, with models predicting the size of foraging groups and the use of food resources, both for individual members of a foraging group and for the whole group (Giraldeau & Caraco 2000). However, little empirical evidence is available in mammals despite the richness of mammalian social species. One

technical difficulty remains the automatic acquisition of foraging duration linked to body mass gain in free-ranging animals.

The recent development of automated scales combined with PIT-tag readers offer new perspectives (e.g. Hou et al. 2015). We used this methodology on the Pallas' free-tailed bat *Molossus molossus* to automatically record the foraging duration, body mass gain and therefore deduce foraging efficiency. The extreme specialization to insect patches shortly available at dusk and dawn (Jones & Rydell 2003) and the energetic costs of flight (Voigt & Holderied 2012) potentially make this species at the energetic edge. Social foraging, by eavesdropping on the echolocation calls of group members to find ephemeral food sources, appears as a reason for group living (Dechmann et al. 2010). Social groups of this species usually live in small and stable harems of ca. 10 adults in crevices from houses (Chapter 3). Because individuals access the roost by crawling (and not flying), we set the automated scales at the entrance of the roosts of several social groups of *M. molossus*. Our objectives were to characterize the patterns of foraging activity and identify the ecological parameters influencing foraging efficiency (i.e. foraging period, sex and group size). We predicted that the small group size occurring naturally optimize foraging efficiency, as a balance between benefits of improved prey search and conspecific interference (Chapter 3). To test this, we artificially reduced group size by retaining 25-50% of group members into captivity for a few days. Our unique approach, relying on automated recordings of foraging duration and body mass, allowed us to obtain unique information on the foraging strategy of naturally behaving animals.

Material and methods

Sampling and data acquisition

We collected data on 74 individuals from five groups of *Molossus molossus* in Gamboa (Panama, 09°07’ N 79°41’ W). The groups roosted in cavities in houses and were characterized by small harems with a stable core of adult females and one adult male in most groups (Chapter 3). We repeatedly captured these groups between 2009 and 2014 (2-9 capture events per group) with mist-nets (Ecotone, Gydnia, Poland) placed around the roost entrance to catch the groups at sunset emergence. During the first capture, we visually determined sex and age (pattern of fusion of phalange epiphysis, (Kunz & Edythe 1982). We also marked each bat with a transponder (Trovan ID-100, Euro ID, Weilerswist, Germany). We sampled wing membrane tissue with a dermatological biopsy punch (2 or 3 mm ⌀, Stiefel) for genotyping purposes. From September 2012 to December 2013, the five roost entrances were equipped with an automated reader (BaTLi, custom-made by the workshop of the University of Konstanz) consisting of a PIT-tag reader, a scale and directional lasers to detect entrance or exit of the bats. The passage of the bat triggered the recording of 60 body mass measurements over six seconds (i.e. 10 measurements per second) in combination with data on two directional lasers. Laser trigger patterns were useful to determine if bats were entering or exiting the roost.

Group size manipulation

Between November 2013 and December 2013, we automatically recorded body mass data of all transpondered individuals for the five roosts. We calibrated the automated scales on a daily

basis to guarantee precision of the body mass data. The experimental protocol for group size manipulation consisted of monitoring each group (non-simultaneously) i) with natural group size for five days, ii) reduced group size for five subsequent days and iii) natural group size five more days. Three automated scales had technical failure for one to three days of the second experimental phase, we ensured we collected data for a total of five days. We caught the different groups at emergence to artificially reduce group size. Our initial aim was to keep 50% of group members in captivity, except for the male responsible for harem tenure. The control of the transponder readers directly after the capture revealed that all transpondered bats did not emerge. We kept all bats that were captured at the exception of the male that was identified as the tenant of the harem male (higher corpulence and level of activity at the transponder readers). For each roost, the percentage of bats kept in captivity varied between 20% and 50% of the total number. The captive bats were kept in small tents and hand-fed each evening with mealworms. We released the captive bats directly into their roosts at the end of the manipulation. Over the short period of the experiment, all transpondered bats remained faithful to their group.

Foraging analyses

Extraction of the dependent variables

We extracted the data from the automated readers using the software *Batcontrol*, developed by Georg Heine from the workshop of the University of Konstanz (see example in Supplementary Figure 1). We manually extracted i) duration of foraging bout (min) and ii) body

mass at emergence and return from foraging (g). We obtained a sample size with 314 foraging bouts (41 females and 11 males, 6.0 ± 3.8 bouts per individual), including 123 associated with body mass data (39.2%). Duration of foraging bout could be identified based on the detection of the transponders in combination with the body mass and laser data. We selected only data where at least two measurements (out of the 60 for each recording) showed a mean body mass associated with a standard deviation inferior to 0.1 g. This stringent filter was necessary to exclude possible errors because the variation of body mass gain is small (between 0 and 2.5 g). We calculated body mass gain as the difference between body mass at return and body mass at emergence (g) and as a percentage relative to the body mass at emergence (%). We obtained the foraging efficiency by dividing the percentage of body mass gain by the duration of the foraging bout (e.g. 10% of body mass gain divided by 50 min). Based on previous work from Esbérard *et al.* on *M. molossus* (Esbérard & Bergallo 2010), we classified the foraging bouts into a dusk foraging period (before sunset to 420 min after sunset) and a dawn foraging period (between 564 minutes after sunset and sunrise). We obtained sunset and sunrise times for the study site from the R package maptools (Bivand & Lewin-Koh 2015).

Observational effects of foraging period and sex

To describe the data collected, we calculated mean ± SD for females and males for both foraging periods for the following parameters: emergence time (minute relative to sunset or sunrise), foraging duration (min), body mass at emergence (g), body mass gain during the foraging period (g), body mass gain during the foraging period relative to body mass at

emergence (%) and foraging efficiency (body mass gain relative to body at emergence divided by the foraging period). For each combination of foraging period and parameter, we compared the means between females and males using a t-test. We did not investigate here the effects of group size manipulation on foraging efficiency because only eight individuals showed reliable mass data over the three phases of the group size manipulation.

Results

Foraging analyses

Observational effects of foraging period and sex

Bats showed a bimodal pattern of foraging, with a first foraging bout after dusk and a second foraging bout before dawn. The majority of bats emerged only for the dusk foraging bout while only few emerged for the dawn foraging bout. Around dusk, bats emerged 15 ± 10 minutes after sunset and foraged for 71 ± 32 min (Table 1 and Figure 1). In average, females emerged later than males but foraged for longer, therefore gaining a higher mass. However, the foraging efficiency was the same between the two sexes. At dawn, bats foraged for shorter times and lower mass gain (Figure 2). Thus, bats emerged 60 minutes ± 21 minutes before sunrise and foraged 36 ± 16 min (Table 1 and Figure 1). The sexes differed only in foraging duration, with males foraging for longer than females. The sex-ratio was biased towards males, with six males and three females foraging at dawn. We only obtained data for both foraging periods of the same night for two males. Both males foraging at dawn showed long foraging periods during the previous dusk (89.5 and 86 min) associated with low body mass gain (6.8% and 3.6%).

Table 1. Mean ± SD for six parameters related to foraging duration and body mass gain.

Emergence time at dusk is relative to sunset while emergence time at dawn is relative to sunrise. Emergence time and foraging duration are based on 314 foraging bouts and the other parameters (associated with body mass) are based on 123 foraging bouts. The values in bold represent a significant statistical difference between males and females for the corresponding foraging period and parameter (see Supplementary Table 1).

		Emergence time	Foraging duration (min)	Body mass at emergence (g)	Body mass gain (g)	Body mass gain (%)	Foraging efficiency (%/min)
Dusk	Female	**17 ± 9**	**73 ± 32**	**9.5 ± 0.7**	**1.2 ± 0.5**	**12.7 ± 5.3**	0.19 ± 0.11
	Male	**9 ± 9**	**62 ± 32**	**11.7 ± 0.7**	**1.0 ± 0.5**	**8.4 ± 4**	0.15 ± 0.11
Dawn	Female	-59 ± 22	**24 ± 6**	8.7 ± 0.8	0.3 ± 0.1	3.7 ± 1.1	0.14 ± 0.06
	Male	-58 ± 21	**39 ± 16**	11.5 ± 0.8	0.4 ± 0.3	3.9 ± 2.9	0.13 ± 0.13

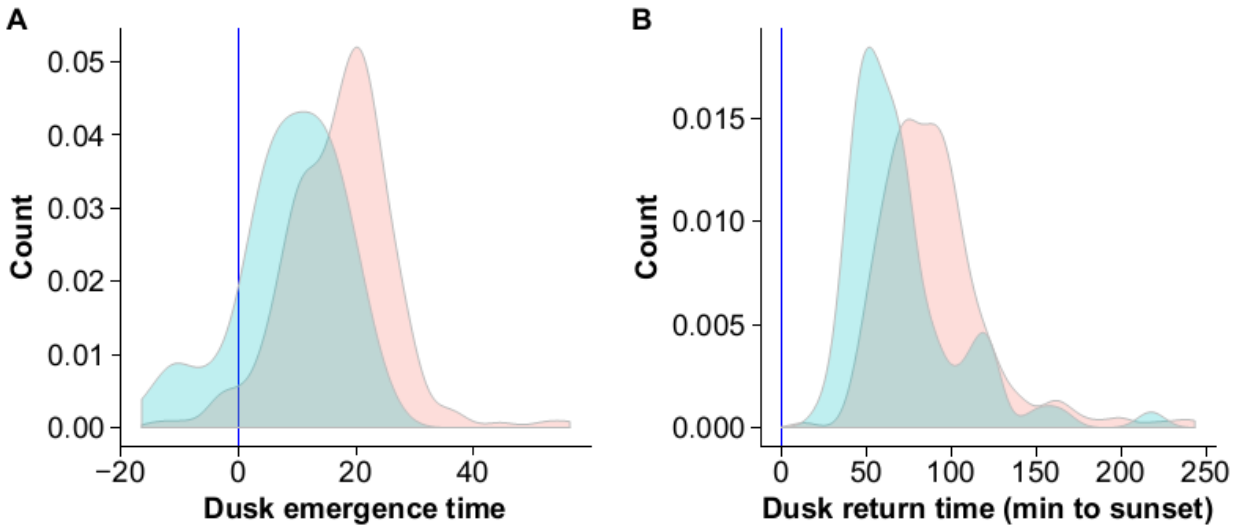

Figure 4-1. Smooth density estimates for the timing of foraging bouts of *Molossus molossus* in Gamboa, Panama (n = 298). For the dusk foraging bout, the emergence and return times are minutes relative to sunset (blue line). The density for females and males is respectively colored in pink and blue.

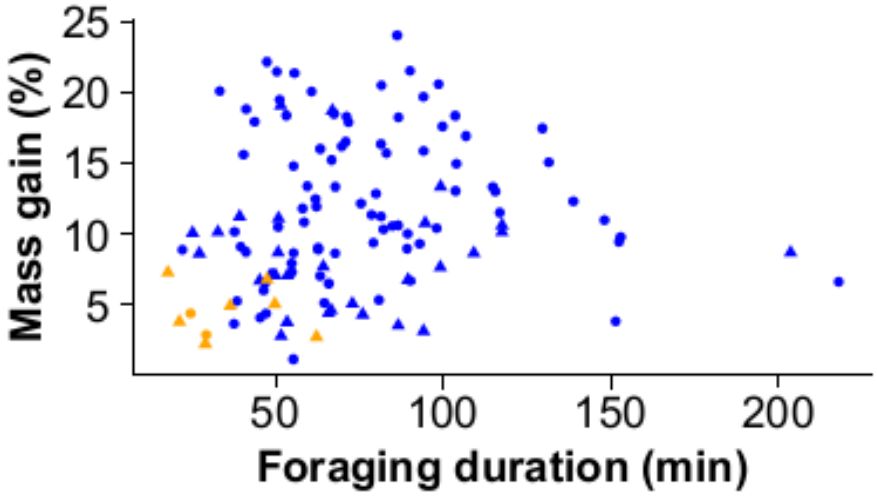

Figure 4-2. Relationship between body mass gain (% compared to body mass at emergence)

and foraging duration (min). The dataset consists of 123 foraging bouts associated with body mass data. The dark blue points represent the dusk foraging bout while the orange points represent the dawn foraging bout. The dots represent females while the triangles represent males.

Discussion

Our study provides unique insights into the foraging patterns and efficiency of a bat species foraging with group members. We found a bimodal pattern of foraging after dusk and before dawn, typical of bats exploiting insect patches. Social foraging seemed prevalent for the dusk foraging bout while dawn foraging seemed to be solitary foraging. Dawn foraging was biased toward males and appeared as a compensation mechanism for shorter foraging bouts at dusk.

The data supports well two foraging bouts, the first generally after dusk and the second before dawn. Other studies on *M. molossus* already reported these two foraging bouts (Chase et al. 1991, Dechmann et al. 2010, Esbérard & Bergallo 2010, Holland et al. 2011). This bimodal pattern of foraging is typical of insectivorous bats that exploits the patches of ephemeral insects, peaking in the upper strata after sunset and before sunrise (Jones & Rydell 2003). However, the foraging activity was clearly biased with the majority of individuals only foraging at dusk. Based on a previous telemetry study, social foraging between group members of *M. molossus* occurred after dusk (Dechmann et al. 2010). Reliance on acoustic information transfer to increase rates of discover of food patches would explain how these bats can forage for very

short foraging periods (73 ± 32 min for females and 62 ± 32min for males) associated with important gain of body mass (12.7 ± 5.4 % for females and 8.4 ± 4 % for females) to fast until the following dusk. In complement, low metabolic rates in the roost appear as a strategy to save energy for the rest of the night and the following day (Dechmann et al. 2011).

For most of the nights, only one individual went out foraging at dawn, suggesting social foraging is not occurring at dawn, or at least to a much lower extent. Dawn foraging was biased toward males. Interestingly, males showed shorter foraging bouts at dusk. Dawn foraging could therefore function as a compensation mechanism for lower energy intake at dusk. Shorter foraging bouts at dusk are potentially associated to the mating system and the fact that the males are responsible for harem tenure. These harem males would go back earlier to the roost to defend it against male competitors. Previous studies showed that the harem male reduce its foraging activity to insure the vigilance of the roost and defend the cluster of females against competitors (Alberts et al. 1996, Kunz et al. 1998) and therefore secure mating and paternity. We expect a similar strategy of dawn foraging for individuals at the energetic edge, including i) individuals affected by bad weather during the dusk foraging session and ii) pregnant and lactating females facing higher physiological costs.

In summary, our study provides novel information on foraging strategies in bats specialized on insect patches. A single foraging bout at dusk, likely benefiting from improved prey searching with information transfer, ensures the required amount of energy to fast until the following

dusk for the majority of group members. Few individuals, especially harem males, showed shorter foraging bouts, a strategy we suspect is used to ensure harem maintenance and reproductive success. Dawn foraging appears as a strategy for these individuals facing higher energetic pressure to obtain enough energy to fast until the subsequent foraging bout at dusk. This study constitutes an additional step to understand the different evolutionary pressures experienced by social bats.

Acknowledgements

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General discussion

In my thesis, I focused on information transfer as one potential reason for sociality in bats, an ideal system to study evolution of group living with more than 1300 species described ("1331 and counting" 2015). In **Chapter 1**, I reviewed the current literature about information transfer connected to sociality and foraging behavior in bats, and the potential implications on the evolution of sociality. In **Chapter 2**, I proposed a multi-method approach to differentiate three sympatric species of bats from the genus *Molossus*, including the socially foraging *M. molossus*. Using a set of social groups previously identified as *M. molossus*, I investigated group size and its relationship with survival and longevity in **Chapter 3** and foraging patterns and efficiency in **Chapter 4**.

Correct identification of species is a crucial basis for most research, especially with free-ranging animals from regions with high but poorly described species diversity. Most studies differentiate species based on commonly used single characters such as morphology, molecular markers or ecological data. However, all of these characters have their limitations, and the field of taxonomy is seeing a switch towards an integrative approach to help correct identification of species (Padial et al. 2010, Galimberti et al. 2012). In particular, we were interested to see how laboratory based methods such as molecular analyses, could inform the identification of species in the field via traits that can be quickly collected and analyzed on site. In **Chapter 2**, we used individuals of the bat genus *Molossus*, with a complex taxonomic history due to

morphologically similar species with a complex taxonomic history, to compare and evaluate the value of molecular (i.e. slower and lab-based, but presumably more reliable), and morphometric as well as acoustic information (commonly used information that can be collected and analyzed in the field). We found evidence for the sympatry of at least three species: *M. molossus*, *M. coibensis* and *M. bondae* at our study site: the village of Gamboa, Panama. All types of information we used were useful for species discrimination, but also showed limitations, such as morphological and acoustic trait overlap, potential admixed individuals or uncertainty in phylogenetic reconstructions. Our findings emphasize the importance of integrating morphological and molecular information to determine the best combination of traits in any given location to inform identification in the field. This original taxonomic research on sympatric species was the prerequisite reliably identify a set of social groups of *M. molossus*, a socially foraging species of bat (Dechmann et al. 2010).

In the context of the evolution of sociality, group size has broad effects on individual behavior and ecology and multiple ecological, demographic, and cognitive constraints on group size have been shown (Wrangham 1980, Mitani et al. 2002, Dávid-Barrett & Dunbar 2013). While most of the current work on the evolution of group living has assessed the effects of group size on reproductive success, the size of a group can also have profound effects on survival and longevity, but these effects are either underexplored or unclear. In **Chapter 3**, we tested the effect of group size on individual survival using mark-recapture and a novel transponder monitoring system in *Molossus molossus*, a bat species that relies on social foraging to find

ephemeral food patches. Because of their reliance on social group members to find food (Dechmann et al. 2010), we hypothesized that an increase in group size would benefit individual survival up to a point where cognitive constraints and communication interference limit the effectiveness of large groups. Our results suggest that even though lifespan is surprisingly short for these bats, individuals optimize survival in a tightly constrained range of group size, likely as the result of a cost-benefit trade-off of social foraging.

Survival and longevity are long-term currencies to investigate the evolution of sociality. In this context, foraging efficiency is another crucial currency, measurable on a shorter-term. Foraging efficiency can be influenced by many determinants including group size (Santema et al. 2009). Despite the high potential, this currency is seldom used because it remains technically difficult to collect automatically in the field. In **Chapter 4**, we used newly developed automated scales, set at the entrance of the roosts of the social groups, to obtain foraging efficiency. Our results clearly show two foraging bouts, after dusk and before dawn, well adapted to highest prey availability (Jones & Rydell 2003). We found similar foraging efficiency in both sexes but differences in the patterns of foraging activity, potentially due to the competition for harem tenure between males.

In this thesis, we provided novel information on the taxonomy, behavioral and evolutionary ecology of a socially foraging bat where many other aspects remain to be studied. First, GPS combined with acoustic microphones could be used to record feeding buzzes (Cvikel et al.

2015) and determine foraging success of group members directly on the wing. However, this method is not suitable because the species *M. molossus* is prone to removing devices, either glued to the back or attached with a collar (O'Mara, Wikelski, et al. 2014) and the GPS remain too heavy for such a light species. Second, genetic relatedness could be investigated to investigate if kin-selection is a factor either causing individuals to cluster, or in promoting the long-term associations between group members. Parallel analyses, based on the microsatellites developed in **Chapter 3,** show groups are mostly composed of non-related individuals (data not included in this thesis), hinting that kin selection is not a factor involved in the sociality of *M. molossus*. Finally, in the framework of the life history theory (Hirshfield & Tinkle 1975, Roff 1992), it would be crucial to identify the relationship between group size and reproductive success in different social groups of *M. molossus*. The set of microsatellites developed in **Chapter 3** could be used for inferring parentage analyses and determining reproductive success within and between social groups (Rossiter 2009).

To conclude, I would first like to highlight the advantage to investigate the evolution of sociality in bats. This mammalian Order shows a broad range of social systems, from a solitary lifestyle over seasonal aggregations to stable closed societies found along with morpho-ecological gradients over different habitats and climates. I would also like to highlight the importance of long-term monitoring on marked individuals from different social groups to understand the adaptive values of sociality.

References

1331 and counting (2015) *BATS magazine*. http://www.batcon.org/resources/media-education/bats-magazine/bat_article/1506

Adams RI, Brown KM, Hamilton MB (2004) The impact of microsatellite electromorph size homoplasy on multilocus population structure estimates in a tropical tree (*Corythophora alta*) and an anadromous fish (*Morone saxatilis*). *Molecular ecology* 13: 2579–88.

Alberdi A, Garin I, Aizpurua O, Aihartza J (2012) The foraging ecology of the mountain long-eared bat *Plecotus macrobullaris* revealed with DNA mini-barcodes. *PLoS ONE* 7: e35692.

Alberts SC, Altmann J, Wilson ML (1996) Mate guarding constrains foraging activity of male baboons. *Animal Behaviour* 51: 1269–1277.

Alcock J (2003) *The triumph of sociobiology* Oxford University Press, New-York

Ammerman LK, Lee DN, Tipps TM (2012) First molecular phylogenetic insights into the evolution of free-tailed bats in the subfamily Molossinae (Molossidae, Chiroptera). *Journal of Mammalogy* 93: 12–28.

Andersen PK, Gill RD (1982) Cox's regression model for counting processes: a large sample study. *The Annals of Statistics* 10: 1100–1120.

Andriollo T, Naciri Y, Ruedi M (2015) Two mitochondrial barcodes for one biological species: the case of European Kuhl's pipistrelles (Chiroptera). *PloS one* 10: e0134881.

Arlettaz R, Ruedi M, Hausser J (1991) Field morphological idenification of *Myotis myotis* and *Myotis blythii* (Chiroptera, Vespertilionidae): A multivariate approach. *Myotis* 29: 7–16.

Avilés L, Tufiño P (1998) Colony size and individual fitness in the social spider *Anelosimus eximius*. *The American Naturalist* 152: 403–418.

Axelrod R, Dion D (1988) The further evolution of cooperation. *Science* 242: 1385–1390.

Barataud M, Giosa S, Leblanc F, Rufray V, Disca T, Tillon L, Delaval M, Haquart A, Dewynter M (2013) Identification et écologie acoustique des chiroptères de Guyane française. *Le Rhinolophe* 19: 103–145.

Barclay RMR (1982) Interindividual use of echolocation calls: eavesdropping by bats. *Behavioral Ecology and Sociobiology* 10: 271–275.

Barclay RMR (1999) Bats are not birds - a cautionary note on using echolocation calls to identify bats: a comment. *Journal of Mammalogy* 80: 290–296.

Barclay RMR, Brigham RM (1991) Prey detection, dietary niche breadth, and body size in bats: why are aerial insectivorous bats so small? *The American Naturalist* 137: 693–703.

Barratt EM, Deaville R, Burland TM, Bruford MW, Jones G, Racey PA, Wayne RK (1997) DNA answers the call of pipistrelle bat species. *Nature* 387: 138–9.

Baudisch A (2011) The pace and shape of ageing. *Methods in Ecology and Evolution* 2: 375–382.

Beauchamp G (1998) The effect of group size on mean food intake rate in birds. *Biological Reviews* 73: 449–472.

Beauchamp G (1999) The evolution of communal roosting in birds: origin and secondary losses. *Behavioral Ecology* 10: 675–687.

Beauchamp G (2005) Does group foraging promote efficient exploitation of resources? *Oikos* 111: 403–407.

Beauchamp G (2010) Group-foraging is not associated with longevity in North American birds. *Biology letters* 6: 42–4.

Beauchamp G, Belisle M, Giraldeau L-A (1997) Influence of conspecific attraction on the spatial distribution of learning foragers in a patchy habitat. *Journal of Animal Ecology* 66: 671–682.

Belwood JJ, Fullard JH (1984) Echolocation and foraging behaviour in the Hawaiian hoary bat, *Lasiurus cinereus semotus*. *Canadian Journal of Zoology* 62: 2113–2120.

Berthier P, Excoffier L, Ruedi M (2006) Recurrent replacement of mtDNA and cryptic hybridization between two sibling bat species *Myotis myotis* and *Myotis blythii*. *Proceedings of the Royal Society B*: 3101–3109.

Bilde T, Coates KS, Birkhofer K, Bird T, Maklakov AA, Lubin Y, Avilés L (2007) Survival benefits select for group living in a social spider despite reproductive costs. *Journal of Evolutionary Biology* 20: 2412–2426.

Bivand R, Lewin-Koh N (2015) maptools: tools for reading and handling spatial objects.

Blumstein DT, Møller AP (2008) Is sociality associated with high longevity in North American birds? *Biology letters* 4: 146–8.

Bogdanowicz W, Hulva P, Černá Bolfíková B, Buś MM, Rychlicka E, Sztencel-Jabłonka A, Cistrone L, Russo D (2015) Cryptic diversity of Italian bats and the role of the Apennine refugium in the phylogeography of the western Palaearctic. *Zoological Journal of the Linnean Society* 174: 635–648.

Bohmann K, Monadjem A, Noer C, Rasmussen M, Zeale MRK, Clare E, Jones G, Willerslev E, Gilbert MTP (2011) Molecular diet analysis of two African free-tailed bats (Molossidae) using high throughput sequencing. *PLoS ONE* 6.

Bohn KM, Moss CF, Wilkinson GS (2009) Pup guarding by greater spear-nosed bats. *Behavioral Ecology and Sociobiology* 63: 1693–1703.

Borries C, Larney E, Lu A, Ossi K, Koenig A (2008) Costs of group size: lower developmental and

reproductive rates in larger groups of leaf monkeys. *Behavioral Ecology* 19: 1186–1191.

Boston ESM, Hanrahan N, Puechmaille SJ, Ruedi M, Buckley DJ, Lundy MG et al. (2011) A rapid PCR-based assay for identification of cryptic *Myotis* spp. (*M. mystacinus*, *M. brandtii* and *M. alcathoe*). *Conservation Genetics Resources* 3: 557–563.

Botero-Castro F, Delsuc F, Douzery EJP (2015) Thrice better than once: quality control guidelines to validate new mitogenomes. *Mitochondrial DNA*: 1–6.

Bradbury JW, Vehrencamp SL (1977) Social organization and foraging in emballonurid bats: III. Mating systems. *Behavioral Ecology and Sociobiology* 2: 1–17.

Brouwer L, Richardson DS, Eikenaar C, Komdeur J (2006) The role of group size and environmental factors on survival in a cooperatively breeding tropical passerine. *Journal of Animal Ecology* 75: 1321–1329.

Brown CR (1988) Social foraging in cliff swallows: local enhancement, risk sensitivity, competition and the avoidance of predators. *Animal Behaviour* 36: 780–792.

Brown CR, Brown MB (1996) *Coloniality in the cliff swallow: the effect of group size on social behavior* The University of Chicago Press, Chicago

Brown CR, Brown MB (2004) Group size and ectoparasitism affect daily survival probability in a colonial bird. *Behavioral Ecology and Sociobiology* 56: 498–511.

Brown CR, Covas R, Anderson MD, Brown MB (2003) Multistate estimates of survival and movement in relation to colony size in the sociable weaver. *Behavioral Ecology* 14: 463–471.

Brown RM, Nichols RA, Faulkes CG, Jones CG, Bugoni L, Tatayah V, Gottelli D, Jordan WC (2010) Range expansion and hybridization in Round Island petrels (*Pterodroma* spp.): evidence from microsatellite genotypes. *Molecular Ecology* 19: 3157–3170.

Buckley NJ (1996) Food finding and the influence of information, local enhancement, and communal roosting on foraging success of north american vultures. *The Auk* 113: 473–488.

Buckley NJ (1997) Spatial-concentration effects and the importance of local enhancement in the evolution of colonial breeding in seabirds. *The American Naturalist* 149: 1091–1112.

Budenz T, Heib S, Kusch J (2009) Functions of bat social calls: The influence of local abundance, interspecific interactions and season on the production of Pipistrelle (*Pipistrellus pipistrellus*) type D social calls. *Acta Chiropterologica* 11: 173–182.

Burnham KP, Anderson DR (2002) *Model selection and multimodel inference: a practical information-theoretic approach* Springer US, New-York

Caraco T (1981) Risk-sensitivity and foraging groups. *Ecology* 62: 527.

Caraco T, Uetz GW, Gillespie RG, Giraldeau L-A (1995) Resource consumption variance within and among individuals: On coloniality in spiders. *Ecology* 76: 196–205.

Caraco T, Wolf LL (1975) Ecological determinants of group sizes of foraging lions. *The American Naturalist* 109: 343–352.

Carter GG, Wilkinson GS (2013) Food sharing in vampire bats: reciprocal help predicts donations more than relatedness or harassment. *Proceedings of the Royal Society B: Biological Sciences* 280: 20122573–20122573.

Chase J, Small MY, Weiss EA, Sharma D, Sharma S (1991) Crepuscular activity of Molossus molossus. *Journal of Mammalogy* 72: 414–418.

Chaverri G, Schneider CJ, Kunz TH (2008) Mating system of the tent-making bat *Artibeus watsoni* (Chiroptera: Phyllostomidae). *Journal of Mammalogy* 89: 1361–1371.

Choquet R, Lebreton J-D, Gimenez O, Reboulet A-M, Pradel R (2009) U-CARE: Utilities for performing goodness of fit tests and manipulating CApture-REcapture data. *Ecography* 32: 1071–1074.

Choquet R, Rouan L, Pradel R (2009) Program E-SURGE : a software application for fitting multievent models. *Environmental and Ecological Statistics* 3: 845–865.

Clare EL, Lim BK, Fenton MB, Hebert PDN (2011) Neotropical bats: estimating species diversity with DNA barcodes. *PloS one* 6: e22648.

Clark CW, Mangel M (1984) Foraging and flocking strategies: information in an uncertain environment. *The American Naturalist* 123: 626–641.

Clark CW, Mangel M (1986) The evolutionary advantages of group foraging. *Theoretical Population Biology* 30: 45–75.

Clutton-Brock TH (1989) Female transfer and inbreeding avoidance in social mammals. *Nature* 337: 70–2.

Clutton-Brock TH, Gaynor D, McIlrath GM, Maccoll ADC, Kansky R, Chadwick P, Manser M, Skinner JD, Brotherton PNM (1999) Predation, group size and mortality in a cooperative mongoose, *Suricata suricatta*. *Journal of Animal Ecology* 68: 672–683.

Clutton-Brock TH, Isvaran K (2007) Sex differences in ageing in natural populations of vertebrates. *Proceedings of the Royal Society B: Biological Sciences* 274: 3097–104.

Conradt L, Roper TJ (2003) Group decision-making in animals. *Nature* 421: 155–158.

Correa da Costa LJ, Gonçalves de Andrade FA, Uieda W, Gregorin R, Barroncas Fernandes ME (2013) First record of *Molossus coibensis* (Chiroptera: Molossidae) in the Brazilian Amazon. *Mastozoologia Neotropical* 20: 143–147.

Côté IM, Poulin R (1995) Parasitism and group size in social animals: a meta-analysis. *Behavioral Ecology* 6: 159–165.

Crawley MJ (2007) *The R Book* John Wiley & Sons Ltd

Crowley PH (1996) Evolving cooperation: strategies as hierarchies of rules. *Biosystems* 37: 67–80.

Cvikel N, Egert Berg K, Levin E, Hurme E, Borissov I, Boonman A, Amichai E, Yovel Y (2015) Bats aggregate to improve prey search but might be impaired when their density becomes too high. *Current Biology* 25: 1–6.

Dąbrowski MJ, Bornelöv S, Kruczyk M, Baltzer N, Komorowski J (2015) "True" null allele detection in microsatellite loci: a comparison of methods, assessment of difficulties and survey of possible improvements. *Molecular ecology resources* 15: 477–88.

Dall SRX, Giraldeau L-A, Olsson O, McNamara JM, Stephens DW (2005) Information and its use by animals in evolutionary ecology. *Trends in Ecology & Evolution* 20: 187–193.

Danchin E, Giraldeau L-A, Valone TJ, Wagner RH (2004) Public information: from nosy neighbors to cultural evolution. *Science* 305: 487–491.

Darriba D, Taboada GL, Doallo R, Posada D (2012) jModelTest 2: more models, new heuristics and parallel computing. *Nature Methods* 9: 772.

Dávid-Barrett T, Dunbar RIM (2013) Processing power limits social group size: computational evidence for the cognitive costs of sociality. *Proceedings of the Royal Society B* 280: 20131151.

Dechmann DKN, Ehret S, Gaub A, Kranstauber B, Wikelski M (2011) Low metabolism in a tropical bat from lowland Panama measured using heart rate telemetry: an unexpected life in the slow lane. *The Journal of experimental biology* 214: 3605–3612.

Dechmann DKN, Heucke SL, Giuggioli L, Safi K, Voigt CC, Wikelski M (2009) Experimental evidence for group hunting via eavesdropping in echolocating bats. *Proceedings of the Royal Society B: Biological Sciences* 276: 2721–2728.

Dechmann DKN, Kalko EK V., König B, Kerth G (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 DKN, Kranstauber B, Gibbs D, Wikelski M (2010) Group hunting—a reason for sociality in Molossid bats? *PLoS ONE* 5: 1–7.

Delsuc F, Brinkmann H, Philippe H (2005) Phylogenomics and the reconstruction of the tree of life. *Nature reviews. Genetics* 6: 361–75.

Dietz C, Dietz I, Siemers BM (2006) Wing measurement variations in the five european horseshoe bat species (Chiroptera: Rhinolophidae). *Journal of Mammalogy* 87: 1241–1251.

Dietz C, von Helversen O (2004) *Illustrated identification key to the bats of Europe*

Dolan PG (1989) Systematics of Middle American mastiff bats of the genus *Molossus*. *Special Publications, The Museum Texas Tech University* 29: 1–71.

Dugatkin L a, Mesterton-Gibbons M (1996) Cooperation among unrelated individuals: reciprocal altruism, by-product mutualism and group selection in fishes. *Biosystems* 37: 19–30.

Edgar RC (2004) MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic acids research* 32: 1792–7.

Eger JL (2008) Family Molossidae. In: Gardner AL (ed) *Mammals of South America Marsupials, Xenarthrans, Shrews, and Bats, vol. 1.*, 399–440. The University of Chicago Press, Chicago and London.

Emlen S, Oring L (1977) Ecology, sexual selection, and the evolution of mating systems. *Science* 197: 215–223.

Esbérard CEL, Bergallo HG (2010) Foraging activity of the free-tailed bat *Molossus molossus* (Chiroptera; Molossidae) in southeastern Brazil. *Brazilian journal of biology* 70: 1011–4.

Falush D, Stephens M, Pritchard JK (2003) Inference of population structure using multilocus genotype data: linked loci and correlated allele frequencies. *Genetics* 164: 1567–87.

Fenton MB (2003) Eavesdropping on the echolocation and social calls of bats. *Mammal Review* 33: 193–204.

Fenton MB, Morris GK (1976) Opportunistic feeding by desert bats (*Myotis* spp.). *Canadian Journal of Zoology* 54: 526–530.

Fenton MB, Portfors C V, Rautenbach IL, Waterman JM (1998) Compromises: sound frequencies used in echolocation by aerial-feeding bats. *Canadian Journal of Zoology* 76: 1174–1182.

Fernández-Juricic E, Erichsen JT, Kacelnik A (2004) Visual perception and social foraging in birds. *Trends in Ecology & Evolution* 19: 25–31.

Flemming SP, Smith PC, Seymour NR, Bancroft RP (1992) Ospreys use local enhancement and flock foraging to locate prey. *The Auk* 109: 649–654.

Floyd R, Abebe E, Papert A, Blaxter M (2002) Molecular barcodes for soil nematode identification. *Molecular ecology* 11: 839–850.

Francis CM, Borisenko A V, Ivanova N V, Eger JL, Lim BK, Guillén-Servent A, Kruskop S V, Mackie I, Hebert PDN (2010) The role of DNA barcodes in understanding and conservation of mammal diversity in southeast Asia. *PloS one* 5: e12575.

Frosch C, Kraus RHS, Angst C, Allgöwer R, Michaux J, Teubner J, Nowak C (2014) The genetic legacy of multiple beaver reintroductions in Central Europe. *PloS one* 9: e97619.

Galef BG, Giraldeau L-A (2001) Social influences on foraging in vertebrates: causal mechanisms and adaptive functions. *Animal Behaviour* 61: 3–15.

Galimberti A, Spada M, Russo D, Mucedda M, Agnelli P, Crottini A, Ferri E, Martinoli A, Casiraghi M (2012) Integrated operational taxonomic units (IOTUs) in echolocating bats: a bridge

between molecular and traditional taxonomy. *PloS one* 7: e40122.

Geipel I, Kalko EKV, Wallmeyer K, Knörnschild M (2013) Postweaning maternal food provisioning in a bat with a complex hunting strategy. *Animal Behaviour* 85: 1435–1441.

Genome 10K Community of Scientists. (2009) Genome 10K: a proposal to obtain whole-genome sequence for 10,000 vertebrate species. *The Journal of heredity* 100: 659–74.

Gillam EH (2007) Eavesdropping by bats on the feeding buzzes of conspecifics. *Canadian Journal of Zoology* 85: 795–801.

Gimenez O, Viallefont A, Charmantier A, Pradel R, Cam E, Brown CR et al. (2008) The risk of flawed inference in evolutionary studies when detectability is less than one. *The American Naturalist* 172: 441–448.

Giraldeau L-A, Caraco T (2000) *Social Foraging Theory* Princeton University Press, Princeton

González-Ruiz N, Ramírez-Pulido J, Arroyo-Cabrales J (2011) A new species of mastiff bat (Chiroptera: Molossidae: *Molossus*) from Mexico. *Mammalian Biology - Zeitschrift für Säugetierkunde* 76: 461–469.

Götmark F, Winkler DW, Andersson M (1986) Flock-feeding on fish schools increases individual success in gulls. *Nature* 319: 589–91.

Gouy M, Guindon S, Gascuel O (2010) SeaView version 4: A multiplatform graphical user interface for sequence alignment and phylogenetic tree building. *Molecular biology and evolution* 27: 221–4.

Greenwood PJ (1980) Mating systems, philopatry and dispersal in birds and mammals. *Animal Behaviour* 28: 1140–1162.

Gregorin R, Tahara AS, Buzzato DF (2011) *Molossus aztecus* and other small *Molossus* (Chiroptera: Molossidae) in Brazil. *Acta Chiropterologica* 13: 311–317.

Griesser M, Ma Q, Webber S, Bowgen K, Sumpter DJT (2011) Understanding animal group-size distributions. *PLoS ONE* 6: e23438.

Griffin DR (1971) The importance of atmospheric attenuation for the echolocation of bats (Chiroptera). *Animal Behaviour* 19: 55–61.

Gusset M, Macdonald DW (2010) Group size effects in cooperatively breeding African wild dogs. *Animal Behaviour* 79: 425–428.

Hagan III JM, Walters JR (1990) Foraging behavior, reproductive success, and colonial nesting in Ospreys. *The Auk* 107: 506–521.

Hamilton WD (1964) The Genetical Evolution of Social Behaviour. Parts I, II. *Journal of Theoretical Biology* 7: 1–52.

Haney JC, Fristrup KM, Lee DS (1992) Geometry of visual recruitment by seabirds to ephemeral foraging flocks. *Ornis Scandinavica* 23: 49–62.

Hartl DL, Clark AG (2007) *Principles of population genetics* Cornell University

Hass CC, Valenzuela D (2002) Anti-predator benefits of group living in white-nosed coatis (*Nasua narica*). *Behavioral Ecology and Sociobiology* 51: 570–578.

Hatchwell B, Komdeur J (2000) Ecological constraints, life history traits and the evolution of cooperative breeding. *Animal behaviour* 59: 1079–1086.

Hayes LD, Chesh AS, Castro RA, Tolhuysen LO, Burger JR, Bhattacharjee J, Ebensperger LA (2009) Fitness consequences of group living in the degu *Octodon degus*, a plural breeder rodent with communal care. *Animal Behaviour* 78: 131–139.

Hebert PDN, Gregory TR (2005) The promise of DNA barcoding for taxonomy. *Systematic biology* 54: 852–859.

von Helversen O, Heller KG, Mayer F, Nemeth A, Volleth M, Gombkötö P (2001) Cryptic mammalian species: a new species of whiskered bat (*Myotis alcathoe* n. sp.) in Europe. *Naturwissenschaften* 88: 217–23.

Hirshfield MF, Tinkle DW (1975) Natural selection and the evolution of reproductive effort. *Proceedings of the National Academy of Sciences* 72: 2227–2231.

Hodgkison R, Balding ST, Akbar Z, Kunz TH (2003) Roosting ecology and social organization of the spotted-winged fruit bat, *Balionycteris maculata* (Chiroptera: Pteropodidae), in a Malaysian lowland dipterocarp forest. *Journal of Tropical Ecology* 19: 667–676.

Hoffman W, Heinemann D, Wiens JA (1981) The ecology of seabird feeding flocks in Alaska. *The Auk* 98: 437–456.

Holland RA, Meyer CFJ, Kalko EK V., Kays R, Wikelski M (2011) Emergence time and foraging activity in Pallas' mastiff bat, *Molossus molossus* (Chiroptera: Molossidae) in relation to sunset/sunrise and phase of the moon. *Acta Chiropterologica* 13: 399–404.

Hoogland JL (1995) *The black-tailed prairie dog: social life of a burrowing mammal* University of Chicago Press, Chicago

Hoppitt W, Laland KN (2013) *Social learning: an introduction to mechanisms, methods, and models* Princeton University Press, Princeton

Horn HS (1968) The adaptive significance of colonial nesting in the Brewer's blackbird (*Euphagus cyanocephalus*). *Ecology* 49: 682–694.

Hou L, Verdirame M, Welch KC (2015) Automated tracking of wild hummingbird mass and energetics over multiple time scales using radio frequency identification (RFID) technology. *Journal of Avian Biology* 46: 1–8.

Hrdy SB (1980) *The langurs of Abu: Female and male strategies of reproduction* Harvard University Press, Cambridge

Ibáñez C, García-Mudarra JL, Ruedi M, Stadelmann B, Juste J (2006) The Iberian contribution to cryptic diversity in European bats. *Acta Chiropterologica* 8: 277–297.

Jakobsson M, Rosenberg NA (2007) CLUMPP: a cluster matching and permutation program for dealing with label switching and multimodality in analysis of population structure. *Bioinformatics (Oxford, England)* 23: 1801–6.

Janson CH (1988) Intra-specific food competition and primate social structure: a synthesis. *Behaviour* 105: 1–17.

Johnson DDP, Kays R, Blackwell PG, Macdonald DW (2002) Does the resource dispersion hypothesis explain group living? *Trends in Ecology & Evolution* 17: 563–570.

Jombart T (2008) adegenet: a R package for the multivariate analysis of genetic markers. *Bioinformatics (Oxford, England)* 24: 1403–5.

Jombart T, Collins C (2015) *A tutorial for Discriminant Analysis of Principal Components (DAPC) using adegenet 2.0.0*

Jombart T, Devillard S, Balloux F (2010) Discriminant analysis of principal components: a new method for the analysis of genetically structured populations. *BMC genetics* 11: 94.

Jones G, Rydell J (1994) Foraging strategy and predation risk as factors influencing emergence time in echolocating bats. *Philosophical Transactions of the Royal Society B: Biological Sciences* 346: 445–455.

Jones G, Rydell J (2003) Bat-insect interactions. In: Kunz TH , In: Fenton MB (eds) *Bat ecology*, 301–345. University of Chicago Press.

Jones OR, Scheuerlein A, Salguero-Gómez R, Camarda CG, Schaible R, Casper BB et al. (2014) Diversity of ageing across the tree of life. *Nature* 505: 169–73.

Jones G, Siemers BM (2011) The communicative potential of bat echolocation pulses. *Journal Of Comparative Physiology A* 197: 447–457.

Jovani R, Mavor R, Oro D (2008) Hidden patterns of colony size variation in seabirds: a logarithmic point of view. *Oikos* 117: 1774–1781.

Jung K, Kalko EK V. (2011) Adaptability and vulnerability of high flying Neotropical aerial insectivorous bats to urbanization. *Diversity and Distributions* 17: 262–274.

Jung K, Molinari J, Kalko EK V (2014) Driving factors for the evolution of species-specific echolocation call design in new world free-tailed bats (Molossidae). *PloS one* 9: e85279.

Kalinowski ST, Taper ML (2006) Maximum likelihood estimation of the frequency of null alleles at microsatellite loci. *Conservation Genetics* 7: 991–995.

Kalinowski ST, Taper ML, Marshall TC (2007) Revising how the computer program CERVUS accommodates genotyping error increases success in paternity assignment. *Molecular ecology* 16: 1099–106.

Kamilar JM, Bribiescas RG, Bradley BJ (2010) Is group size related to longevity in mammals? *Biology letters* 6: 736–9.

Kapheim KM, Pan H, Li C, Salzberg SL, Puiu D, Magoc T et al. (2015) Genomic signatures of evolutionary transitions from solitary to group living. *Science* 348: 1139–1143.

Kerth G (2008) Causes and consequences of sociality in bats. *Bioscience* 58: 737–746.

Kerth G (2010) Group decision-making in fission-fusion societies. *Behavioural Processes* 84: 662–663.

Kiefer A, Mayer F, Kosuch J, Helversen O Von, Veith M (2002) Conflicting molecular phylogenies of European long-eared bats (*Plecotus*) can be explained by cryptic diversity. *Molecular Phylogenetics and Evolution* 25: 557–566.

Kingston T, Jones G, Zubaid A, Kunz T (2003) Alternation of echolocation calls in five species of aerial feeding insectivorous bats. *Journal of mammalogy* 84: 205–215.

Knornschild M, Jung K, Nagy M, Metz M, Kalko E (2012) Bat echolocation calls facilitate social communication. *Proceedings of the Royal Society B: Biological Sciences* 279: 4827–4835.

Koopman KF (1994) Chiroptera: Systematics. In: Niethammer J , In: Schliemann H , In: Starck D (eds) *Handbuch der Zoologie VIII*, 1–217. Walter de Gruyter, Berlin.

Kossl M, Mora E, Coro F, Vater M (1999) Two-toned echolocation calls from *Molossus molossus* in Cuba. *Journal of Mammalogy* 80: 929–932.

Kraus RHS, Wink M (2015) Avian genomics: fledging into the wild! *Journal of Ornithology*.

Krause J, Ruxton GD (2002) *Living in groups* Oxford University Press, Oxford

Krebs JR (1974) Colonial nesting and social feeding as strategies for exploiting food resources in the Great Blue Heron (*Ardea herodias*). *Behaviour* 51: 99–134.

Kunz TH, Edythe LPA (1982) Age estimation and post-natal growth in the bat *Myotis lucifugus*. *Journal of Mammalogy* 63: 23–32.

Kunz TH, Robson SK, Nagy KA (1998) Economy of harem maintenance in the greater spear-nosed bat, *Phyllostomus hastatus*. *Journal of Mammalogy* 79: 631–642.

Lack JB, Van Den Bussche RA (2010) Identifying the confounding factors in resolving phylogenetic relationships in Vespertilionidae. *Journal of Mammalogy* 91: 1435–1448.

Lebreton FJ, Nichols JD, Barker RJ, Pradel R, Spendelow JA (2009) Modeling individual animal histories with multistate capture - recapture models. In: Caswell H (ed) *Advances in Ecological*

Research, 87–173. Elsevier Inc., Burlington.

Lehmann L, Keller L (2006) The evolution of cooperation and altruism -a general framework and a classification of models. *Journal of Evolutionary Biology* 19: 1365–1376.

Levin E, Roll U, Dolev A, Yom-Tov Y, Kronfeld-Shcor N (2013) Bats of a Gender Flock Together: Sexual Segregation in a Subtropical Bat. *PLoS ONE* 8.

List C (2004) Democracy in animal groups: a political science perspective. *Trends in Ecology & Evolution* 19: 168–169.

Loeb SC, Britzke ER (2010) Intra- and interspecific responses to Rafinesque's big-eared bat (*Corynorhinus rafinesquii*) social calls. *Acta Chiropterologica* 12: 329–336.

Marques JT, Rainho A, Carapuço M, Oliveira P, Palmeirim JM (2004) Foraging behaviour and habitat use by the European free-tailed bat *Tadarida teniotis*. *Acta Chiropterologica* 6: 99–110.

Mayer F, Dietz C, Kiefer A (2007) Molecular species identification boosts bat diversity. *Frontiers in zoology* 4: 4.

McCracken GF (1984) Social dispersion and genetic variation in two species of emballonurid bats. *Zeitschrift für Tierpsychologie* 66: 55–69.

McCracken GF, Bradbury JW (1981) Social organization and kinship in the polygynous bat *Phyllostomus hastatus*. *Behavioral Ecology and Sociobiology* 8: 11–34.

McCracken GF, McCracken MK, Vawter AT (1994) Genetic structure in migratory populations of the bat *Tadarida brasiliensis mexicana*. *Journal of Mammalogy* 75: 500–514.

McCracken GF, Wilkinson GS (2000) Bat mating systems. In: Crichton EG , In: Krutzsch PH (eds) *Reproductive biology of bats*, 321–362. Academic Press, San Diego.

McDonough MM, Ferguson AW, Ammerman LK, Granja-Vizcaino C, Burneo SF, Baker RJ (2011) Molecular verification of bat species collected in Ecuador: results of a country-wide survey. *Occasional Papers, Museum of Texas Tech University* 301: 1–28.

McNamara JM, Dall SRX (2010) Information is a fitness enhancing resource. *Oikos* 119: 231–236.

McWilliam AN (1988) Social organisation of the bat *Tadarida* (*Chaerephon*) *pumila* (Chiroptera: Molossidae) in Ghana, West Africa. *Ethology* 77: 115–124.

Milinski M (1987) Tit-for-tat in sticklebacks and the evolution of cooperation. *Nature* 325: 433–435.

Miller GS (1913) Notes on bats of the genus *Molossus*. *Proceedings of the United States National Museum* 46: 85–92.

Miramontes O, Desouza O (1996) The nonlinear dynamics of survival and social facilitation in

termites. *Journal of Theoretical Biology* 181: 373–380.

Mitani JC, Watts DP, Pepper JW, Merriwether DA (2002) Demographic and social constraints on male chimpanzee behaviour. *Animal Behaviour* 64: 727–737.

Moore J (1984) Female transfer in primates. *International Journal of Primatology* 5: 537–589.

Moore J, Ali R (1984) Are dispersal and inbreeding avoidance related? *Animal Behaviour* 32: 94–112.

Mora EC, Macías S, Vater M, Coro F, Kössl M (2004) Specializations for aerial hawking in the echolocation system of *Molossus molossus* (Molossidae, Chiroptera). *Journal of comparative physiology. A* 190: 561–74.

Muir G, Schlötterer C (2005) Evidence for shared ancestral polymorphism rather than recurrent gene flow at microsatellite loci differentiating two hybridizing oaks (*Quercus* spp.). *Molecular ecology* 14: 549–61.

Murphy WJ, Pevzner PA, O'Brien SJ (2004) Mammalian phylogenomics comes of age. *Trends in genetics* 20: 631–9.

Nagy M, Günther L, Knörnschild M, Mayer F (2013) Female-biased dispersal in a bat with a female-defence mating strategy. *Molecular ecology* 22: 1733–45.

Nilsson RH, Ryberg M, Kristiansson E, Abarenkov K, Larsson K-H, Kõljalg U (2006) Taxonomic reliability of DNA sequences in public sequence databases: a fungal perspective. *PLoS ONE* 1: e59.

Norberg UM, Rayner JM V (1987) Ecological morphology and flight in bats (Mammalia; Chiroptera): wing adaptations, flight performance, foraging strategy and echolocation. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences* 316: 335–427.

Nowak RN (1994) *Walker's bats of the world* John Hopkins University Press

O'Farrell MJ, Miller BW (1999) Use of vocal signatures for the inventory of free-flying Neotropical bats. *Biotropica* 31: 507–516.

O'Farrell MJ, Miller BW, Gannon WL (1999) Qualitative identification of free-flying bats using the Anabat detector. *Journal of Mammalogy* 80: 11–23.

O'Mara MT, Dechmann DKN, Page RA (2014) Frugivorous bats evaluate the quality of social information when choosing novel foods. *Behavioral Ecology* 25: 1233–1239.

O'Mara MT, Wikelski M, Dechmann DKN (2014) 50 years of bat tracking: device attachment and future directions. *Methods in Ecology and Evolution* 5: 311–319.

O'Shea TJ, Ellison LE, Stanley TR (2011) Adult survival and population growth rate in Colorado big brown bats (*Eptesicus fuscus*). *Journal of Mammalogy* 92: 433–443.

Ortega J, Maldonado JE, Wilkinson GS, Arita HT, Fleischer RC (2003) Male dominance, paternity, and relatedness in the Jamaican fruit-eating bat (*Artibeus jamaicensis*). *Molecular Ecology* 12: 2409–2415.

Padial JM, Miralles A, De la Riva I, Vences M (2010) The integrative future of taxonomy. *Frontiers in Zoology* 7: 16.

Page RA, Ryan MJ (2006) Social transmission of novel foraging behavior in bats: frog calls and their referents. *Current Biology* 16: 1201–1205.

Pfalzer G, Kusch J (2003) Structure and variability of bat social calls: implications for specificity and individual recognition. *Journal of Zoology* 261: 21–33.

Pianka ER (1970) On r- and K-selection. *The American Naturalist* 104: 592–597.

Pimenta V, Fonseca BS, Hoppe JPM, Ditchfield AD (2014) First occurence of *Molossus coibensis* Allen, 1904 (Chiroptera,Molossidae) in Atlantic Forest. *Chiroptera Neotropical* 20: 1237–1242.

Pitcher TJ, Magurran AE, Winfield IJ (1982) Fish in larger shoals find food faster. *Behavioral Ecology and Sociobiology* 10: 149–151.

Popa-Lisseanu AG, Bontadina F, Mora O, Ibáñez C (2008) Highly structured fission-fusion societies in an aerial-hawking, carnivorous bat. *Animal Behaviour* 75: 471–482.

Pradel R, Wintrebert CMA, Gimenez O (2003) A proposal for a goodness-of-fit test to the Arnason-Schwarz multisite capture-recapture model. *Biometrics* 59: 43–53.

Pritchard JK, Stephens M, Donnelly P (2000) Inference of population structure using multilocus genotype data. *Genetics* 155: 945–959.

Puechmaille SJ, Allegrini B, Boston ESM, Dubourg-Savage M-J, Evin A, Knochel A et al. (2012) Genetic analyses reveal further cryptic lineages within the *Myotis nattereri* species complex. *Mammalian Biology - Zeitschrift für Säugetierkunde* 77: 224–228.

Puechmaille SJ, Hizem WM, Allegrini B, Abiadh A (2012) Bat fauna of Tunisia: Review of records and new records, morphometrics and echolocation data. *Vespertilio* 16: 211–239.

R Core Team (2015) R: A language and environment for statistical computing.

Racey PA, Swift SM (1985) Feeding Ecology of Pipistrellus pipistrellus (Chiroptera: Vespertilionidae) during Pregnancy and Lactation. I. Foraging Behaviour. *The Journal of Animal Ecology* 54: 205–215.

Randi E (2008) Detecting hybridization between wild species and their domesticated relatives. *Molecular ecology* 17: 285–93.

Ratcliffe JM, Ter Hofstede HM (2005) Roosts as information centres: social learning of food preferences in bats. *Biology Letters* 1: 72–74.

Ratnasingham S, Hebert PDN (2007) bold: The Barcode of Life Data System (http://www.barcodinglife.org). *Molecular Ecology Notes* 7: 355–364.

Raymond M, Rousset F (1995) GENEPOP (Version1.2): Population genetics software for exact tests and ecumenicism. *Journal of Heredity* 86: 248–249.

Reid FA (1998) *A field guide to the mammals of Central America and Southeast Mexico* Oxford University Press, Oxford

Robinette KW, Andelt WF, Burnham KP (1995) Effect of group-size on survival of relocated prairie dogs. *Journal of Wildlife Management* 59: 867–874.

Roff D (1992) *The evolution of life histories: theory and analysis* Chapman and Hall, New-York

Rossiter SJ (2009) Parentage and kinship analysis. In: Kunz TH , In: Parsons S (eds) *Ecological and Behavioral Methods for the Study of Bats*, 695–714. John Hopkins University Press, Baltimore.

Rossiter SJ, Benda P, Dietz C, Zhang S, Jones G (2007) Rangewide phylogeography in the greater horseshoe bat inferred from microsatellites: implications for population history, taxonomy and conservation. *Molecular Ecology* 16: 4699–4714.

Rousset F (2008) genepop'007: a complete re-implementation of the genepop software for Windows and Linux. *Molecular ecology resources* 8: 103–6.

Rubenstein D (2009) Social behavior and sociobiology. In: Ruse M , In: Travis J (eds) *Evolution: The First Four Billion Years*, 237–255. Harvard University Press, Cambridge.

Rubinoff D, Cameron S, Will K (2006) A genomic perspective on the shortcomings of mitochondrial DNA for "barcoding" identification. *The Journal of Heredity* 97: 581–594.

Ruedi M, Stadelmann B, Gager Y, Douzery EJP, Francis CM, Lin L-K, Guillén-Servent A, Cibois A (2013) Molecular phylogenetic reconstructions identify East Asia as the cradle for the evolution of the cosmopolitan genus *Myotis* (Mammalia, Chiroptera). *Molecular Phylogenetics and Evolution* 69: 437–449.

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

Santema P, Griffith SC, Langmore NE, Komdeur J, Magrath MJL (2009) Does foraging efficiency vary with colony size in the fairy martin *Petrochelidon ariel* ? *Journal of Avian Biology* 40: 57–66.

Schliep KP (2011) phangorn: phylogenetic analysis in R. *Bioinformatics* 27: 592–3.

Schmieder DA, Benítez HA, Borissov IM, Fruciano C (2015) Bat species comparisons based on external morphology: a test of traditional versus geometric morphometric approaches. *PloS one* 10: e0127043.

Schnitzler H, Moss CF, Denzinger A (2003) From spatial orientation to food acquisition in

echolocating bats. *Trends in Ecology & Evolution* 18: 386–394.

Serrano D, Oro D, Ursua E, Tella JL (2005) Colony size selection determines adult survival and dispersal preferences: Allee effects in a colonial bird. *The American Naturalist* 166: E22–E31.

Shohami D (2015) Elucidating animal foraging in heterogeneous landscapes: the importance of detailed quantification of resource distribution and movement, illustrated by the Egyptian fruit bat *4th International Bat Meeting, Berlin, Germany, 13-15 March 2015.*,

Shultz S, Opie C, Atkinson QD (2011) Stepwise evolution of stable sociality in primates. *Nature* 479: 219–222.

Sibly RM (1983) Optimal group size is unstable. *Animal Behaviour* 31: 947–948.

Silk JB (2007) The adaptive value of sociality in mammalian groups. *Philosophical Transactions of the Royal Society B: Biological Sciences* 362: 539–559.

Simmons NB (2005) Order Chiroptera. In: Wilson DE , In: Reeder DM (eds) *Mammal species of the world: a taxonomic and geographic reference*, 312–529. Johns Hopkins University Press.

Smith JE, Kolowski JM, Graham KE, Dawes SE, Holekamp KE (2008) Social and ecological determinants of fission–fusion dynamics in the spotted hyaena. *Animal Behaviour* 76: 619–636.

Song S, Liu L, Edwards S V, Wu S (2012) Resolving conflict in eutherian mammal phylogeny using phylogenomics and the multispecies coalescent model. *Proceedings of the National Academy of Sciences of the United States of America* 109: 14942–7.

Springer MS, Teeling EC, Madsen O, Stanhope MJ, De Jong WW (2001) Integrated fossil and molecular data reconstruct bat echolocation. *Proceedings of the National Academy of Sciences of the United States of America* 98: 6241–6246.

Stevens M, Párraga CA, Cuthill IC, Partridge JC, Troscianko TS (2007) Using digital photography to study animal coloration. *Biological Journal of the Linnean Society* 90: 211–237.

Storz JF (1999) Genetic consequences of mammalian social structure. *Journal of Mammalogy* 80: 553–569.

Storz JF, Balasing J, Bhat HR, Nathan PT, Doss DPS, Prakash AA, Kunz TH (2001) Clinal variation in body size and sexual dimorphism in an Indian fruit bat, *Cynopterus sphinx* (Chiroptera: Pteropodidae). *Biological Journal of the Linnean Society* 72: 17–31.

Storz JF, Balasingii J, Nathan PT, Emmanuel K, Kunz TH (2000) Dispersion and site fidelity in a tent-roosting population of the short-nosed fruit bat (*Cynopterus sphinx*) in southern India. *Journal of Tropical Ecology* 16: 117–131.

Swofford DL (2003) PAUP*. Phylogenetic Analysis Using Parsimony (*and Other Methods).

Symington MM (1990) Fission-fusion social organization in *Ateles* and *Pan*. *International Journal of Primatology* 11: 47–61.

Tacutu R, Craig T, Budovsky A, Wuttke D, Lehmann G, Taranukha D, Costa J, Fraifeld VE, de Magalhães JP (2013) Human Ageing Genomic Resources: integrated databases and tools for the biology and genetics of ageing. *Nucleic Acids Research* 41: D1027–33.

Tamura K, Dudley J, Nei M, Kumar S (2007) MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. *Molecular Biology and Evolution* 24: 1596–9.

Therneau T (2015) A package for survival analysis in S.

Thomas SP, Suthers RA (1972) The physiology and energetics of bat flight. *Journal of Experimental Biology* 57: 317–335.

Timm RM, Mortimer J (1976) Selection of roost sites by Honduran white bats *Ectophylla alba* (Chiroptera: Phyllostomatidae). *Ecology* 75: 385–389.

Torney CJ, Berdahl A, Couzin ID (2011) Signalling and the evolution of cooperative foraging in dynamic environments. *PLoS Computational Biology* 7: 1–10.

Vaughan TA (1980) Opportunistic feeding by two species of *Myotis*. *Journal of Mammalogy* 61: 118–119.

Velazco PM, Patterson BD (2013) Diversification of the yellow-shouldered bats, genus *Sturnira* (Chiroptera, Phyllostomidae), in the New World tropics. *Molecular Phylogenetics and Evolution* 68: 683–98.

Voigt CC, Holderied MW (2012) High manoeuvring costs force narrow-winged molossid bats to forage in open space. *Journal of comparative physiology. B* 182: 415–24.

Voigt-Heucke SL, Taborsky M, Dechmann DKN (2010) A dual function of echolocation: bats use echolocation calls to identify familiar and unfamiliar individuals. *Animal Behaviour* 80: 59–67.

Ward P, Zahavi A (1973) The importance of certain assemblages of birds as "information-centres" for food-finding. *The Ibis* 115: 517–534.

West SA, Griffin AS, Gardner A (2007) Social semantics: altruism, cooperation, mutualism, strong reciprocity and group selection. *Journal of Evolutionary Biology* 20: 415–432.

Wilkinson GS (1992a) Information transfer at evening bat colonies. *Animal Behaviour* 44: 501–518.

Wilkinson GS (1992b) Communal nursing in the evening bat, *Nycticeius humeralis*. *Behavioral Ecology and Sociobiology* 31: 225–235.

Wilkinson GS, Boughman JW (1998) Social calls coordinate foraging in greater spear-nosed bats. *Animal Behaviour* 55: 337–350.

Wilkinson GS, Mayer F, Kerth G, Petri B (1997) Evolution of repeated sequence arrays in the D-loop region of bat mitochondrial DNA. *Genetics* 146: 1035–48.

Wilkinson GS, South JM (2002) Life history, ecology and longevity in bats. *Aging Cell* 1: 124–131.

Willis CKR, Brigham RM (2004) Roost switching, roost sharing and social cohesion: forest-dwelling big brown bats, *Eptesicus fuscus*, conform to the fission–fusion model. *Animal Behaviour* 68: 495–505.

Wilson E (2000) *Sociobiology: The new synthesis* The Belknap Press of Harvard University Press, Cambridge, Massachusetts and London

Wilson J-J, Sing K-W, Halim MRA, Ramli R, Hashim R, Sofian-Azirun M (2014) Utility of DNA barcoding for rapid and accurate assessment of bat diversity in Malaysia in the absence of formally described species. *Genetics and Molecular Research* 13: 920–5.

Wirtz P (2010) Territory holders, satellite males and bachelor males in a high density population of waterbuck (*Kobus ellipsiprymnus*) and their associations with conspecifics. *Zeitschrift für Tierpsychologie* 58: 277–300.

Wittwer CT (2009) High-resolution DNA melting analysis: advancements and limitations. *Human mutation* 30: 857–9.

Worthington Wilmer J, Barrett E (1996) A non-lethal method of tissue sampling for genetic studies of chiropterans. *Bat Research News* 37: 1–3.

Wrangham RW (1980) An ecological model of female-bonded primate groups. *Behaviour* 75: 262–300.

Zhang G (2015) Genomics: Bird sequencing project takes off. *Nature* 522: 34–34.

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Author contributions

Chapter 1

Information transfer: a reason for sociality in bats?

Yann Gager wrote the manuscript.

Chapter 2

The value of molecular vs. morphometric and acoustic information for species identification using sympatric molossid bats

Yann Gager, Emilia Tarland, Arne Ludwig and Dina K.N. Dechmann designed the study. Yann Gager, Emilia Tarland, Matthieu Ménage and Dina K.N. Dechmann collected samples. Emilia Tarland and Dietmar Ludwig isolated the microsatellite loci from the first library, developed the corresponding marker systems and genotyped a subset of the samples. Yann Gager genotyped the remaining samples with the help of SJR. Robert H.S. Kraus supervised genetic data analysis. Fidel Botero-Castro ran the phylogenetic analyses. Matthieu Ménage measured the echolocation parameters from the acoustic recordings. Yann Gager analyzed data and wrote the manuscript with contributions from all co-authors. All authors read and approved the final manuscript.

Chapter 3

Group size, survival and surprisingly short lifespan in socially foraging bats

Yann Gager and Dina K.N. Dechmann designed the study. Yann Gager, M. Teague O'Mara and Dina K.N. Dechmann collected samples. Yann Gager and Olivier Gimenez analyzed data. Yann Gager wrote the manuscript with contributions from Olivier Gimenez, M. Teague O'mara and Dina K.N. Dechmann. All authors read and approved the final manuscript.

Chapter 4

Foraging patterns and efficiency in a socially foraging bat

Yann Gager and Dina K.N. Dechmann designed the study. Yann Gager, M. Teague O'Mara and Dina K.N. Dechmann collected samples. Yann Gager analyzed data. Robert H.S. Kraus supervised genetic data analysis. Yann Gager wrote the manuscript with contributions from all authors.