



DIPARTIMENTO DI BIOLOGIA E BIOTECNOLOGIE

CHARLES DARWIN

SAPIENZA UNIVERSITÀ DI ROMA

CORSO DI DOTTORATO IN BIOLOGIA ANIMALE

XXVI CICLO

(2010 – 2013)

Leonardo Ancillotto

PHD DISSERTATION

**EFFECTS OF EXPERIENCE ON THE DEVELOPMENT OF SOCIAL
BEHAVIOUR IN HOUSE-DWELLING BATS**

Tesi di dottorato di ricerca

***EFFETTI DELL'ESPERIENZA SULLO SVILUPPO DEL COMPORTAMENTO
SOCIALE NEI CHIROTTERI ANTROPOFILI***



Roma, 2014

CORSO DI DOTTORATO IN BIOLOGIA ANIMALE
DIPARTIMENTO DI BIOLOGIA E BIOTECNOLOGIE "CHARLES DARWIN"
SAPIENZA UNIVERSITÀ DI ROMA

XXVI CICLO
(2010-2013)

EFFECTS OF EXPERIENCE ON THE DEVELOPMENT OF SOCIAL BEHAVIOUR IN HOUSE-DWELLING
BATS

EFFETTI DELL'ESPERIENZA SULLO SVILUPPO DEL COMPORTAMENTO SOCIALE NEI CHIROTTERI
ANTROPOFILI

Leonardo Ancillotto

Roma, 2014

Table of Contents

Table of Contents	3
Index of Tables	5
Index of Figures	6
Index of Appendices	7
Abstract	8
Riassunto	11
Introduction	15
<i>Bats' social systems</i>	15
<i>Recognition mechanisms in bats</i>	17
<i>Aims of the study and thesis outline</i>	18
Materials and methods	27
<i>Study species</i>	27
European free-tailed bat (<i>Tadarida teniotis</i>)	28
Kuhl's pipistrelle (<i>Pipistrellus kuhlii</i>)	30
Savi's bat (<i>Hypsugo savii</i>)	31
<i>Study design and data analysis</i>	33
Factors affecting social behaviour of bats	33
Effects of early experience on social bonding	39
Mechanisms of interspecific social cohesion	45
Results	49
<i>Factors affecting social interactions of bats</i>	49
Effects of early experience on social bonding	50

<i>Mechanisms of interspecific social cohesion</i>	56
Discussion	64
<i>Factors affecting social interactions of bats</i>	64
<i>Effects of early experience on social bonding</i>	67
<i>Mechanisms of interspecific social cohesion</i>	71
Final remarks	74
<i>Integrating individual-based mechanisms in the study of bat social systems</i>	74
Appendices	77
<i>Supplementary figures</i>	77
References	85

Index of Tables

Table 1. Behavioural categories for captive European free-tailed bats <i>T. teniotis</i> recorded during dyadic arena tests and their description.	36
Table 2. Categories used to record behavioural data from captive Kuhl's pipistrelle <i>P. kuhlii</i> and their meaning.	43
Table 3. Network descriptors calculated from association data (contact between bats in bat boxes) recorded from captive juveniles <i>Pipistrellus kuhlii</i> ; in the 'bat code' column, bats from the same rearing group are labelled with the same letter.	54
Table 4. Factors influencing the frequency of three behaviours by a mixed-species group of captive bats (<i>P. kuhlii</i> and <i>H. savii</i>) explained by Generalized Linear Models (minimal adequate models).	57
Table 5. Individual attributes (sex, species and body condition index) and network descriptive metrics of a captive mixed-species group of bats. Network metrics are presented separately for each network derived from different social behaviours. Sex: m = male, f = female; Sp = species: Hs = <i>H. savii</i> , Pk = <i>P. kuhlii</i> . The letter in individuals' ID indicates group membership. CC = clustering coefficient; BT = betweenness; DG = degree; IDG = indegree; ODG = outdegree.	58
Table 6. ANOVA density models for variable homophily of networks derived from physical contacts, huddling and allogrooming interactions of a captive mixed-species group of bats, based on 5000 permutations.	63

Index of Figures

- Figure 1. Adult female of European free-tailed bat (*Tadarida teniotis*). Photo by L. Ancillotto. **29**
- Figure 2. Adult captive male of Kuhl's pipistrelle (*Pipistrellus kuhlii*). Photo by L. Ancillotto. **30**
- Figure 3. Adult captive female of Savi's bat (*Hypsugo savii*). Photo by L. Ancillotto. **31**
- Figure 4. Spectrograms of *T. teniotis* social calls (a= contact call; b= aggressive call). Contact call emitted by subadult female; aggressive call emitted by adult male. **37**
- Figure 5. Means±standard deviations of Affiliation Index for captive *T. teniotis* during dyadic different and same sex encounters. AI score= Affiliation index scores; Black bars: male in familiar encounters; dark-grey bars: males in unfamiliar encounters; light-grey: females in familiar encounters; white: females in unfamiliar encounters. Significant differences among conditions as well as their interactions are indicated: **= $p < 0.001$; * = $p < 0.05$. **49**
- Figure 6. Mean individual frequencies of same-sex and different-sex interactions for huddling (N=205), allogrooming (N=51) and aggression (N=45). For all interactions sexes of bats involved were recorded; interactions were classified as either "same-sex" or "different-sex" according to the sex of individuals involved. Different-sex interactions were further classified as "male" or "female" based on the sex of the instigating individual. Black = male; grey = female. Between-group ANOVA levels of significance are shown in central labels; within-group sexual differences are shown near columns. * < 0.05 ; ** < 0.01 **51**
- Figure 7. Sociogram associating juvenile *P. kuhlii* roosting in mutual contact; symbols used to indicate nodes show different rearing-group membership; black nodes= males, white nodes= females; the thickness of linking segments is proportional to tie strength. **53**
- Figure 8. Clustering-dendrogram made from association data of captive juvenile *P. kuhlii* roosting in mutual contact; first two letters of individuals' names indicate group membership. Cophenetic correlation coefficient for the dendrogram= 0.760. Individuals' symbols as in Figure 4. **55**
- Figure 9. Social networks of a mixed-species group of captive bats, based on four different social behaviours; a: physical contacts inside roost; b: huddling; c: allogrooming; d: aggressive behaviour. Node shape indicates individual's group of origin. Black nodes = males, white nodes = females. Large nodes = *H. savii*, small nodes = *P. kuhlii*. Tie strength between nodes is proportional to line thickness. A spring-embedding algorithm derives distances between nodes. **61**

Index of Appendices

- Figure S 1. Schematic representation of the arena used in Experiment 1. A= side view; b= top view. Drawing by L. Ancillotto. **77**
- Figure S 2. Video camera mounted on tripod beneath an experimental bat box. Photo by L. Ancillotto. **78**
- Figure S 3. Newborn (age: 1-3 days) Kuhl's pipistrelle (*P. kuhlii*) on hand. Photo by L. Ancillotto. **79**
- Figure S 4. Flight-room used for Experiment 2 and Experiment 3. Experimental bat boxes and net-roosts are visible on the walls. Photo by L. Ancillotto. **80**
- Figure S 5. Two adult *T. teniotis* engaging in an aggressive display: the bat on the left is emitting an aggressive social call; the bat on the right is going to perform a chase towards the other individual. Photo by L. Ancillotto. **81**
- Figure S 6. Modified experimental bat box during inspection for data collection (Experiment 3). Coloured plastic split-rings are visible on bats' forearms for individual identification. Photo by L. Ancillotto. **82**
- Figure S 7. Examples of intra-group (i.e. bats raised in the same experimental group: see colour of plastic split-ring on individuals' right forearm) heterospecific physical contacts among young captive *H. savii* (individual on the right, in all pictures) and *P. kuhlii* (individuals on the top and left, in all pictures) raised in mixed-species groups. Photo by L. Ancillotto. **83**
- Figure S 8. Social interactions among young bats during captive rearing. a) Interspecific huddling between young *P. kuhlii* (down) and *H. savii* (up and left), b) intraspecific reciprocal grooming between young captive *P. kuhlii*; c) interspecific reciprocal grooming between young captive *P. kuhlii* (left) and *H. savii* (right). Photos by L. Ancillotto. **84**

Abstract

This study aims at clarifying the mechanisms underlying the formation and maintenance of stable social units in bats (Chiroptera), a characteristic shared by most species of this group of mammals, by investigating the effects of the early social environment, i.e. pursuing the hypothesis that imprinting-like influences on the development of social behaviour exist and may have a profound impact on the social lives of bats.

First I present an overview on bats' social behaviour and systems: these mammals comprise a high number of species and thus constitute an excellent group for testing general hypotheses about evolution and development of social behaviour. Bat social systems in fact range from solitary species to others aggregating in conspicuous groups of up to millions individuals. Such complexity leads to a variety of social behaviours rarely found in other taxonomic groups: there is increasing evidence that bats are able of cooperative social behaviours such as allogrooming, communal nursing, group hunting and social learning, all interactions that require high-level cognitive skills.

Investigating such a complex system needs a multi-disciplinary approach, fundamental for disentangling the mechanisms through which bat sociality develops. I performed a series of experiments and used classical ethological and statistical methods (ethogram composition, general linear models) together with social network analysis (SNA), developing the analyses of social interactions on an individual-based approach.

Experiment 1 deals with the ability of bats to modulate their behaviour (e.g. aggressiveness) according to intrinsic (e.g. age, sex) and extrinsic (familiarity) factors during a social interaction. I performed dyadic arena-encounters where two bats per test were allowed to freely interact. I recorded aggressive and affiliative behaviours and measured the degree of affiliation towards familiar and unfamiliar individuals of captive European free-tailed bats

(*Tadarida teniotis*). By testing individuals from different captive colonies and of different age, I measured the effects of familiarity, sex and age on the aggressive behaviour of this species, using aggressiveness as a proxy for xenophobia. I found significant effects of all the selected factors upon the degree of affiliation among individuals: familiar bats were more prone to perform affiliative behaviours, behaving xenophobically towards non-group members. This xenophobic attitude was lower in females and almost null towards juveniles. These results indicate that adult bats' behaviour is influenced by previous social experiences, also suggesting that social bonds formed inside colonies are long lasting regardless of genetic relatedness existing between individuals.

With experiment 2 I investigate the mechanisms leading to the formation of social subunits in groups bats, using *Pipistrellus kuhlii* as a model species. By manipulating the early social environment of young bats and describing their pattern of association inside artificial roost as well as measuring their rates of interaction, I demonstrate that spatial proximity inside roosts promotes social cohesion. These associations are maintained by bats throughout adulthood by means of cooperative behaviours such as allogrooming and social thermoregulation. Both classical approach and social network analysis of interacting bats indicate that physical contacts and cooperative behaviours among bats inside a colony are non-random and are more frequently performed between individuals that already had contacts at a very young age.

Following the same approach and techniques of Experiment 2, with Experiment 3 I test the hypothesis that the same mechanisms that produce group cohesion inside roosts can lead to the formation of multi-specific associations of bats. Such multi-specific groups are widespread among mammals, and in bats they are assumed to form due to eco-physiological reasons (i.e. species sharing micro-climatic requirements). I manipulated the early social environment of two species that naturally occur inside the same roosts; very young Kuhl's (*P. kuhlii*) and Savi's (*Hypsugo savii*) bats were exposed to artificial multi-specific social contacts

in captivity. I demonstrate that early social experience does influence social bonding also beyond the species' boundaries. Independent young bats in fact selected previous group-members for social thermoregulation and reciprocal grooming, regardless of species membership.

Results from all experiment clearly indicate a strong effect of early social environment on the interaction and association patterns in bats, both at short (Experiments 2-3) and long (Experiment 1) time scales, suggesting the existence of imprinting-like mechanisms. Such mechanisms lead to the formation of cryptic social subunits within bat colonies and probably enhance the cohesion of the entire social structure, with obvious and strong consequences on behavioural and ecological (e.g. demographic and epidemiological) scales.

Riassunto

Scopo di questa ricerca è chiarire alcuni aspetti alla base della formazione di gruppi sociali stabili e coesi, e del loro mantenimento, in un ordine di Mammiferi altamente sociale, i chiroterri, con particolare riferimento agli effetti dell'esperienza individuale e dell'ambiente sociale durante le prime fasi dello sviluppo.

La prima parte della presente tesi introduce il tema dello sviluppo comportamentale nei pipistrelli e dei loro sistemi sociali. I chiroterri costituiscono il secondo ordine di mammiferi per numerosità, con oltre 1230 specie attualmente classificate, la maggior parte delle quali vive in gruppi sociali almeno durante parte del proprio ciclo biologico; data la loro grande variabilità ecologica e comportamentale, i chiroterri costituiscono un eccellente gruppo per testare ipotesi generali sull'evoluzione e sullo sviluppo del comportamento sociale nei mammiferi.

Le strutture sociali dei pipistrelli, infatti, variano da sistemi molto semplici (specie solitarie) a complessi raggruppamenti composti da milioni d'individui. Tale complessità comporta una varietà molto ampia di differenti comportamenti sociali difficilmente rilevabili in altri gruppi tassonomici: l'avanzamento delle tecniche d'indagine comportamentale dirette e indirette ha consentito in tempi recenti di rilevare comportamenti quali grooming reciproco, condivisione delle cure parentali, foraggiamento di gruppo e apprendimento sociale, tutte interazioni che necessitano di capacità cognitive di alto livello.

Lo studio di un sistema così complesso richiede necessariamente un approccio multidisciplinare per isolare i singoli meccanismi attraverso cui si sviluppa il comportamento sociale in questi animali. Pertanto per questa ricerca ho condotto una serie di esperimenti, usando sia metodologie classiche di analisi comportamentale (composizione di etogrammi,

modelli lineari) sia un approccio di tipo network (social network analysis), seguendo sempre un'impostazione "individual-based".

L'Esperimento 1 è stato sviluppato per verificare l'effetto di fattori intrinseci (età e sesso) ed estrinseci (familiarità/esperienza) durante le interazioni sociali di chiroterri adulti. Ho pertanto promosso degli incontri diadici tra individui adulti di chiroterri in arene neutre, registrando comportamenti agonistici ed affiliativi e calcolando in seguito un indice di affiliazione per ciascun individuo in ciascun incontro, in modo da testare ogni soggetto in tutte le condizioni di fattori possibili. Utilizzando come soggetti sperimentali individui di molosso di Cestoni (*Tadarida teniotis*) provenienti da differenti colonie in cattività e con diverse età, ho potuto testare gli effetti di familiarità, sesso ed età sul comportamento sociale di questa specie, usando l'aggressività come indice di xenofobia. Tutti i fattori considerati si sono rivelati significativi: durante gli incontri con individui provenienti da colonie differenti, i pipistrelli si rivelano significativamente più aggressivi rispetto ad incontri tra membri dello stesso gruppo sociale. Questa tendenza xenofobica è meno evidente per le femmine rispetto ai maschi, e viene quasi del tutto soppressa durante incontri tra adulti ed individui giovani. Questi risultati indicano una plasticità comportamentale nei chiroterri, che sono in grado di modulare il proprio comportamento durante l'interazione con altri individui anche in seguito all'esperienza individuale precedente.

L'Esperimento 2 è stato costruito per evidenziare i meccanismi che portano alla formazione dei legami sociali nei chiroterri, in particolare utilizzando individui di pipistrello albolimbato (*Pipistrellus kuhlii*). Manipolando l'ambiente sociale durante le prime fasi di sviluppo dei giovani soggetti sperimentali e descrivendone in seguito i pattern di associazione tra individui all'interno di rifugi artificiali, dimostro che il contatto fisico durante le prime fasi di sviluppo di un pipistrello promuove la formazione di legami sociali durevoli. I legami sociali tra individui vengono inoltre mantenuti e rinsaldati dai chiroterri in età adulta tramite interazioni

affiliative e cooperative non casuali, che vengono effettuate selettivamente tra individui tra loro familiari. Questo risultato dimostra un forte effetto dell'esperienza precoce sul comportamento di questi mammiferi, indicando la presenza di meccanismi d'imprinting sociale.

Seguendo lo stesso approccio e le stesse tecniche dell'Esperimento 2, con l'Esperimento 3 testo l'ipotesi che gli stessi meccanismi di imprinting sociale che producono la formazione di gruppi coesi all'interno delle colonie siano alla base dei fenomeni di aggregazioni multispecifiche tra chiroteri, spesso rilevate nei rifugi in natura. Gruppi multispecifici sono diffusi tra i mammiferi, e nel caso dei chiroteri la loro rilevanza adattativa viene generalmente indicata nel risparmio energetico dovuto alla termoregolazione facilitata. La registrazione di associazioni multispecifiche preferenziali tra alcune specie di chiroteri in natura e l'ampia disponibilità di spazio in molti rifugi dove queste associazioni si ritrovano lasciano però supporre la presenza di meccanismi più complessi all'origine della socialità interspecifica nei pipistrelli. Pertanto, ho manipolato l'ambiente sociale precoce in due specie di chiroteri che naturalmente formano colonie multispecifiche (*P. kuhlii* e *Hypsugo savii*), esponendo giovani individui a contatti multispecifici controllati, in cattività. L'analisi comportamentale (come per l'Esperimento 2) ha rivelato che l'esperienza sociale precoce influenza la formazione di legami tra individui indipendentemente dalla loro specie di appartenenza: individui di specie diverse ma cresciuti negli stessi gruppi sperimentali tendono, infatti, a rimanere associati e ad interagire selettivamente anche se viene offerta loro l'opportunità di associarsi a conspecifici (non familiari).

Analizzati nel loro insieme, i risultati di questi tre esperimenti indicano un significativo effetto dell'ambiente precoce e dell'esperienza individuale nello sviluppo del comportamento sociale nei chiroteri, in particolare suggerendo la presenza di meccanismi di imprinting sociale, con effetti sia sul breve che sul lungo termine sulla vita sociale dei singoli individui.

La scoperta di questi meccanismi e dei loro effetti contribuisce alla comprensione delle dinamiche sociali ed ecologiche dei chiroteri sia a livello individuale sia di comunità, con importanti conseguenze dal punto di vista etologico, ecologico, evolutivo ed epidemiologico.

Introduction

Bats' social systems

Bats are a numerous and diverse mammal order numbering about 1230 different species (IUCN 2013), characterized by high sociality, with hundreds of species featuring a wide range of body sizes, ecological preferences and trophic niches and showing very different social systems (McCracken & Wilkinson 2000). While very few species of bat are solitary (e.g. *Lasiurus* spp.), most temperate and tropical bats live in groups at least during part of their biological cycle, forming colonies that number from a few to several hundred individuals, sometimes showing complex social structures (Wilkinson 1984, 1985; Kerth 2008a).

Different hypotheses have been developed in order to explain the strong tendency of bats to engage in social groups, all concerning key-aspects of their ecology and biology. First, bats are generally long-lived mammals, clearly falling out of the linear relationship between size and longevity found for other mammals (Prothero & Jürgens 1987). The cause of their longevity is being the focus of considerable research effort with implications for the development of human medical applications (Brunet-Rossinni & Austad 2004). One of the main factors deemed important for the unusual longevity of bats is that these heterothermic mammals make a large use of torpor during winter (at least in temperate areas). However, heterothermy cannot be the only factor affecting bats' longevity, as also species which do not undergo torpor (e.g. those living at tropical latitudes) are unusually long living (Prothero & Jürgens 1987). Longevity, paired by the mammalian tendency to philopatry - mostly for females (Wolff 1994; Burland et al. 2001), - allow different generations of bats to overlap both in time and space, and thus to form unevenly-aged, long-lived groups of familiar individuals (Kerth 2008a). The consequent stability of these groups is considered among the main drivers of the evolution of complex social behaviours (Emlen 1994).

Being mainly small mammals, bats are characterized by a very high physiological energy demand (Kurta et al. 1989; Willis & Brigham 2007). Active flight is a very expensive locomotion modality in terms of energy, thus most bat species, particularly the echolocators (Hutson & Mickleburgh 2001), are characterized by facultative heterothermy, i.e. they can regulate body temperature (and consequently energy expenditure) during the 24h cycle (e.g. daily torpor) and during the year (i.e. hibernating). During breeding (e.g. pregnancy and nursing of the offspring) bats though have to balance the need of saving energy with the ease of breeding, i.e. foetus development and milk production (Kurta et al. 1989) are both processes that need higher body temperatures. Bats can accomplish this by aggregating, and thus engaging in social thermoregulation (Willis & Brigham 2007).

Bats are also usually constrained by their particular ecological needs, such as roost availability. Only a few species of bats, so called “tent-making” bats from the family Phyllostomidae (Sagot & Stevens 2011), are able to “build” autonomously their own roost; otherwise, most species are dependent on discretely distributed existing habitat elements (Kunz & Fenton 2006). A limited resource item such as an optimal roost (e.g. a cave or a senescent tree) can exert a strong attraction on a number of individuals, forcing bats to aggregate and thus leading to the evolution of mechanisms for physical tolerance and eventually of social behaviours (Kerth 2008a).

However, limited roost availability alone cannot explain sociality in bats, as many species use very common and largely available structures (Kunz & Fenton 2006), e.g. buildings, foliage and rock crevices. Such roosts can be so widespread that many species typically use a network of available roosts (i.e. changing roost frequently), a behaviour known as “roost-switching” (Willis & Brigham 2004; Russo et al. 2005). Moreover, the same species can use different types of roosts according to sex (Angell et al. 2013; Levin et al. 2013), while species

that use the same roosts can have significantly different social systems (McCracken & Wilkinson 2000).

The state of the art of the knowledge of bat sociality is clearly far from being exhaustive, and much is still to be investigated, particularly on the evolutionary origin and the ecological correlates of aggregation and colony stability, as well as the mechanisms underlying the establishment of group cohesion.

There is increasing evidence from studies on wild and captive colonies that bats form long-lasting relationships with their colony mates (Kerth et al. 2011), and that these associations increase individual fitness by means of cooperating activities such as social thermoregulation (Willis & Brigham 2007), communal nursing (Bohn et al. 2009), food sharing (Paolucci et al. 2006; Carter & Wilkinson 2013) and cooperative hunting (Dechmann et al. 2010). A well-known example of a complex cooperation system among bats is found in vampire bats (Wilkinson 1984, 1986): reciprocal food-sharing and social allogrooming are frequent in the common vampire *Desmodus rotundus*, and seem to be directed both towards kin and non-kin familiar mates (Wilkinson 1984, 1985), relying on individual recognition mechanisms mediated by odour (Carter & Wilkinson 2013) and hearing of social calls (as for *Diaemus youngi*: Carter et al. 2012).

Recognition mechanisms in bats

Group cohesion among bats relies on the occurrence of group and individual recognition mechanisms mediated by a range of sensorial cues such as olfactory (De Fanis & Jones 1995a; Safi & Kerth 2003; Englert & Greene 2009) and acoustic signals, including both echolocation and social calls (Balcombe 1990; Balcombe & McCracken 1992; Siemers & Kerth 2006; Carter et al. 2008; Voigt-Heucke et al. 2010; Mann et al. 2011).

Female bats are known to perform relatively prolonged parental care towards their offspring (Raghuram & Marimuthu 2007; Bohn et al. 2009; Geipel et al. 2013), thus the evolution of recognition mechanisms for maintaining social cohesion (e.g. between mother and pup) probably had a main role in shaping bat social evolution. Recognition between mother and pup has been deeply investigated, as it is a fundamental ability for bats, e.g. inside large aggregations at nursery sites (Balcombe 1990). Particularly, in *T. brasiliensis*, a largely studied species in USA, there is evidence of reciprocal recognition between mother and pup by means of olfactory (Gustin & McCracken 1987) and acoustic communication (Balcombe 1990).

As most bats leave their offspring alone inside the roost as newborns while going out to forage (Dietz et al. 2009), it is highly probable that acoustic and olfactory imprinting between mother and pup occur in the first hours (or days) of a bat's life. Imprinting is a widespread process of memorization of other individuals' identity, which has great importance upon a variety of aspects in different species, ranging from social to sexual individual preferences (Bateson 1979; Slagsvold et al. 2002; Verzijden & Ten Cate 2007). This memorization process is usually limited in time during the early stages of an individual's life, generally called "sensitive period", but it is too persistent to affect individual's behaviour throughout its life (Bateson 1979; Slagsvold et al. 2002; Verzijden & Ten Cate 2007).

Aims of the study and thesis outline

The main aim of this study is to clarify the mechanisms underlying the formation and maintenance of cohesive groups among bats. Particularly, I will try to answer the following questions:

1. Do bats change social relationships according to previous experience? I.e. can group

membership be determined by experience rather than mere genetic relatedness between individuals?

2. Do early experiences influence social behaviour of bats? I.e. do bats form social bonds through spatial proximity between individuals at an early age?
3. Do mechanisms investigated under point 2) also have a role in the formation of mixed-species colonies of bats?

As working hypothesis, I suggest that social imprinting-like mechanisms might play a major role in shaping the development of social relationship in bats, highlighting the role of experience and early social environment in structuring the social lives of these animals.

To test this hypothesis, between years 2009 and 2012 I conducted a series of experiments in order to disentangle the development of social bonding and its behavioural consequences in captive bats. The use of captive colonies is fundamental to investigate intimate aspects of bat behaviour, as most social interactions among colony-mates occur inside roosts, a condition which strongly limits the chances to record or observe direct behaviours and thus conduct in-field studies (Siemers & Page 2009).

The first experiment (Experiment 1) deals with the ability of bats to modulate their behaviour (e.g. aggressiveness) according to intrinsic (e.g. age, sex) and extrinsic (previous social experience) factors during a social interaction. I recorded aggressive and affiliative behaviours and measured the degree of affiliation towards familiar and unfamiliar bats during dyadic arena-encounters where two bats per test were allowed to freely interact. Aggression, i.e. the set of behaviours used with the intent to cause direct physical harm (Hinde 1974) or indirect detrimental effects (Wingfield et al. 1987) to an opponent, is an important determinant in the characterization of mating and social systems of animals (Trivers 1971).

Most social species evolved ritualized displays and non-physical communication (e.g. posturing and acoustic signals) to channel aggression during conflictual situations (Lorenz 1963). For social species, i.e. those living in groups with high frequencies of interactions among individuals, aggressive behaviours and tolerance between group-members are two diverging forces which play a major role in shaping the social structure of the group, as well as of their mating system (Trivers 1971; McCracken & Wilkinson 2000). Such forces are also shaped by a species' ecological and biological traits, as demonstrated by comparative studies on rodents (Patris et al. 2002). The degree of aggressiveness exhibited or elicited by an individual is, in general, also influenced by intrinsic factors such as individual's age and sex. Juvenile animals in fact are known to elicit and perform fewer aggressive interactions, thus being more tolerated (e.g. Scott et al. 2004 and Hirsch 2007). Females of many species also tend to be less aggressive than conspecific males, particularly towards same-sex individuals (but see von Engelhard et al. 2000), who report female intrasexual high aggressiveness). It has been suggested that this difference in the levels of aggressiveness between sexes is due to physiology, but the links among hormone levels, social status and aggressiveness in females of social species are still unclear (Christiansen 2001; Goymann et al. 2001).

In group-forming species, the aversion towards non-group members - named xenophobia (from Greek: *xènos*, stranger and *phobos*, fear) - is common (Wilson 1975) and generally expressed by more frequent aggressive interactions and/or less frequent amicable ones directed towards non-group members. Such selective aggressiveness relies on mechanisms of individual or group-membership recognition which are widespread in social animals and are considered as a basis for the development of complex behaviours in a wide range of species, including invertebrates (lobsters: Karavanich & Atema 1998; octopuses: Tricarico et al. 2011), amphibians (Morais et al. 2012), reptiles (LaDage & Ferkin 2006), birds (D'Eath & Stone 1999; Berg et al. 2011) and mammals (Safi & Kerth 2003; Proops et al. 2009;

Townsend et al. 2012).

Conducting this experiment, I formulated the following hypotheses:

1) The degree of aggressiveness exhibited in interactions between group members will differ from that shown between non-group members as a consequence of group membership recognition, i.e. bats will exhibit xenophobia. Specifically, I predict that fewer aggressive events and more amicable ones will be recorded in interactions between colony mates than in those involving strangers.

2) Because female bats are often more sociable (Ortega & Maldonado 2006; Ortega et al. 2008) and tend to be more gregarious (Kerth 2008a) than males, I predict that interactions between females will involve lower rates of aggressive events and higher rates of amicable ones than those between males.

3) Because parental care in bats is usually prolonged even after weaning (Wilkinson 1984; Raghuram & Marimuthu 2007; Geipel et al. 2013) and thus juveniles tend to remain in their natal roost for some time also after becoming volant, I finally hypothesize that juveniles will be more tolerated by adults and predict that lower aggressiveness and higher rates of amicable behaviour will occur in juvenile vs. adult interactions than in those between adults.

With the second experiment (Experiment 2) I investigate the mechanisms leading to the formation of the above-mentioned social subunits inside bat colonies. Adult bats may recognise colony mates and maintain stable social relationships influencing group activities such as roost choice, roost switching or communal foraging (Wilkinson & J 1998; Kerth & König 1999; Kerth et al. 2001, 2011; Garroway & Broders 2007).

Although the existence of social subunits and cryptic social structures in bat colonies is known (Patriquin et al. 2010; Kerth et al. 2011), the potential influence of early social experiences on the establishment of such preferences has not yet been explored.

In most echolocating bats, the young are left in the roost when their mothers leave to forage (Altringham 1996; McCracken & Wilkinson 2000). Because young bats show poor thermoregulation, to reduce the cost of homeothermy they form clusters, sometimes numbering thousand individuals, preserving body heat by establishing a warm and thermally stable microclimate (Kurta et al. 1989; Kunz & Fenton 2006; Kerth 2008a). In several species, females roost separate from their young except when suckling it (Altringham 1996). Being altricial (Baptista et al. 2000), young bats remain in such clusters for long, a situation that may offer important opportunities to interact with neighbouring subjects and establish early social relationships. However, no study has so far addressed the effects of such spatial proximity on the development of preferential associations among individuals.

Developing and retaining inter-individual bonds among social animals is known to have important consequences for survival and individual fitness (Cameron et al. 2009; Silk et al. 2010). In most species living in groups, mothers and siblings are the first conspecifics newborns may encounter and interact with, and thus constitute their earliest social context (Spencer-Booth 1971).

The social environment experienced during early development may largely influence adult social behaviour (Laviola & Terranova 1998). The effects on the individual of this close social environment have been widely investigated in vertebrates (Bekoff 1972; Bateson 1979; Margulis et al. 2005; Arnold & Taborsky 2010).

Sexual choice and aggregation in several social species are mediated by kin recognition achieved through comparison of known versus unknown stimuli experienced in the first stages of the individuals' lives (Mateo 2009). Such recognition mechanisms may result fundamental for group cohesion, influencing group choice (Van Horn et al. 2007) or communal movement patterns in bats (Kerth 2008b).

Following the same approach and techniques of the second experiment, with Experiment 3 I test the hypothesis that the same mechanisms that produce group cohesion among individuals inside a roost can lead to the formation of multi-specific associations of bats. Advantages of group living are obvious for those species that form stable monospecific groups, in which an increase of inclusive fitness is evident, e.g. by cooperating with kin (Grafen 1990) or by reciprocal altruism (Seyfarth & Cheney 1984). Some adaptive consequences of social grouping may be shared by mono- and multi-specific animal aggregations, e.g. increased foraging efficiency, dilution of predation risk and more efficient thermoregulation, however mixed species groups (MSGs) lack all the advantages derived from cooperation with kin and thus different explanations are needed for their formation and maintenance (Farine et al. 2012).

Living in MSG offers advantages such as increased foraging efficiency and dilution of predation risk (Stensland et al. 2003; Sridhar et al. 2009), as well as improved information transfer (Goodale et al. 2010) and thermoregulation (Bogdanowicz 1983). Associating with heterospecifics may provide more benefits than those offered by intraspecific associations if the latter result in strong competition among subjects sharing an identical ecological niche (Goodale et al. 2010). For the same reason, MSGs are expected to be more frequent between species having distinct ecological niches but sharing signalling mechanisms and/or the need to counter common potential predators to minimize interspecific competition and optimize anti-predatory information transfer.

A number of social species are involved in the formation of MSGs including insects (Menzel et al. 2008), fishes (Ward et al. 2002), birds (Sridhar et al. 2009) and mammals (Stensland et al. 2003). Among the latter, multi-specific associations mostly occur in primates (Heymann & Buchanan-Smith 2000; Chapman et al. 2002), cetaceans (Frantzis & Herzing 2002) and ungulates (Fitzgibbons 1990). Besides, associations may also occur between different

mammalian orders (Whitesides 1989; Desbiez et al. 2010; Koda 2012) or between mammals and birds (Ruggiero 1996; Ruggiero & Eves 1998; Coetzee & Province 2010).

In all cases, interspecific associations require increased heterospecific spatial tolerance. An aspect of how such groups form and maintain cohesion so far overlooked is whether experience, especially that acquired early in individual life, may play a role in facilitating the development of interspecific associations. Bats often associate in colonies consisting of two or more species (Dietz et al. 2009). The existence of such interspecific colonies is mostly explained in terms of thermoregulatory benefits acquired by clustering together (Bogdanowicz 1983; Rodriguez-Duran 1998); yet the occurrence of preferred associations between particular pairs of species may not be simply driven by common thermal requirements, and hypotheses about the existence of more complex factors influencing such associations may be formulated.

In colonies made of multiple species this proximity may also involve heterospecific newborns clustering together or roosting close to each other. Thus, I hypothesize that the formation of MSGs in bats may be favoured by close proximity of heterospecific in early development through the onset of imprinting-like mechanisms similar to those that occur between conspecifics (see Experiment 2). Thus, I manipulated the early social environment of two species that naturally occur inside the same roosts and exposed individuals to artificial multi-specific social contacts.

Given the different methodologies both in the study-design and data analyses adopted through these three experiments, as well as the different questions addressed, the following chapters are organized maintaining these different aspects of the study separated, in order to make it easier to scroll and track different themes and experiments.

Overall, I adopted two fundamental approaches to explore all these hypotheses.

I used an individual-based approach, e.g. using classical observation protocols and linear models for data analyses, to establish whether and how individuals' attributes determine patterns of association and interaction among bats (all experiments). This allowed me to determine which factors influence and shape rates of social interactions within a given social group, i.e. whether attributes of interacting individuals, including growing up in the same social group or not, will lead to behavioural biases.

To examine the differences in extent and intensity of social connections between subjects, as well as their positions in the social structure, I also adopted a network-based approach (Experiment 2 and 3), testing whether individuals from the same group selectively established social bonds, i.e. showed homophily towards former group-mates (McPherson et al. 2001). Network analysis also makes it possible to test whether bats differing in species and sex occupy different positions and play different roles within the social group.

Social network analysis (SNA) is a powerful set of data analyses and measures that takes into account the role and relative position of individuals (considered “nodes”) inside a network of individual relationships (i.e. the “links” or “ties” between different nodes), being also able to “weigh” different kinds (or intensities) of ties (Hanneman & Riddle 1998; Whitehead 2008).

SNA is recently receiving wide attention for its use in ecological and behavioural studies (Krause et al. 2007; Makagon et al. 2012; Vanderwaal et al. 2013), and analyses to increase the robustness of these techniques have been developed in order to make it suitable for addressing questions more complex than simple structuring of the network (Wey et al. 2008; Lusseau et al. 2008; James et al. 2009; Makagon et al. 2012).

When using linear models (glms, glm anova, glmm) for testing data, I used a frequentist approach (instead of other methods, e.g. information-theoretic approach), considering significant all results with $p < 0.05$, as this is the most commonly used approach in behavioural studies, thus making my results comparable to those from other studies (see

References list).

Materials and methods

Study species

I focused on three species of house-dwelling bats (Kunz & Fenton 2006), i.e. species that frequently use artificial human-made structures (e.g. roofs, coverings, drain pipes, bridges) as roost, for three main reasons.

First, the social structure of such species is often poorly understood, as well as their behaviour, apart from their spatial and temporal use of roosts (Dietz et al. 2009). Most house-dwelling species apparently seem to share very similar social systems, i.e. groups mainly composed by females and juveniles, with occasional males. This situation is common to many other bat species so social processes occurring in house-dwelling bats may confidently be generalized to much of the order Chiroptera.

Second, these species are also of utmost importance due to their proximity with humans. Understanding the social dynamics of species strongly linked to human settlements is fundamental for predicting (and managing) patterns of disease spreading (Langwig et al. 2012), particularly in the case of house-dwelling species, as these may be or become vectors of pathogens of medical or veterinary concern (e.g. Kite 1966).

Third, house-dwelling species are those more often admitted at wildlife rescue centres in peninsular Italy (Ancillotto et al. 2013), so that access to a high number of healthy captive individuals as experimental subjects is a relatively easy task and does not imply to disturbance of wild colonies nor to the capture of wild individuals.

For this study I selected three species often recorded at the LIPU's wildlife rescue centre of Rome (<http://www.crfslipuroma.it>), which provided experimental subjects and logistic for the experiments.

Despite they belong to different families (Molossidae and Vespertilionidae) and occupying slightly different ecological niches, these species all roost in human-made structures, which are used year-round and host both sexes and appeared suitable for testing my general hypothesis.

All such species are abundant in the Mediterranean region and are sympatric in much of south and southwest Europe, including the Balkans and the Mediterranean islands. Kuhl's pipistrelle (*Pipistrellus kuhlii*) and Savi's bat (*Hypsugo savii*) are also both currently expanding their range in northeastern Europe (Spitzenberger 1997; Sachanowicz et al. 2006), and have been recently found to share the same roosting sites in certain cases and form mixed-species reproductive colonies (Ancillotto et al. 2013).

European free-tailed bat (*Tadarida teniotis*)

For Experiment 1 I selected the European free-tailed bat *Tadarida teniotis* (Rafinesque, 1814). This is a large molossid (forearm length = 56-64 mm) forming numerous (> hundreds of individuals; Dietz et al. 2009) aggregations; non-reproductive colonies usually include both sexes (Balmori 2003), while during the nursery period males usually roost separately (Dietz et al. 2009). *T. teniotis* (Figure 1) frequently roosts in cliff crevices or human-made structures such as buildings whose narrow, vertically oriented spaces are used (Dietz et al. 2009). During the mating season, i.e. in late spring and early autumn (Dietz et al. 2009), harems of up to nine females are formed and actively defended by males (Balmori 2003). Balmori (2003) also reports that subadult individuals of *T. teniotis* form large and noisy aggregations at the end of the breeding season, but nothing is known about the duration or nature of such aggregation.



Figure 1. Adult female of European free-tailed bat (*Tadarida teniotis*). Photo by L. Ancillotto.

Molossid bats are known to form the largest colonies among bats (Davis et al. 1962) and should thus be expected to lack complex social structures; instead, bats from this family show a variety of social systems, from large aggregations (e.g. in *T. brasiliensis*: Davis et al. 1962) to small groups characterized by strong fission-fusion dynamics (e.g. in *T. australis*: Rhodes 2007), and different species proved to be able to discriminate between familiar and unfamiliar individuals (Bouchard 2001; Englert & Greene 2009).

Kuhl's pipistrelle (*Pipistrellus kuhlii*)

I selected individuals of Kuhl's (*P. kuhlii*; experiments 2 and 3) pipistrelles, a small vespertilionid (body mass: 5–10 g; forearm length: 30–37 mm (Dietz et al. 2009).

This bat frequently roosts in human-made structures, particularly behind drain-pipes and under roof tiles and coverings (Dietz et al. 2009). Kuhl's pipistrelle (Figure 2) is a very adaptable species, able to persist in deeply modified habitats and to quickly respond to large-scale environmental changes, e.g. urbanization (Tomassini et al. 2013).



Figure 2. Adult captive male of Kuhl's pipistrelle (*Pipistrellus kuhlii*). Photo by L. Ancillotto.

P. kuhlii is a sedentary species that forms reproductive colonies numbering 10–50 females and that generally use the same roost year-round (Bogdanowicz 2004). Such colonies may also include adult males. It is a sociable species, sometimes forming mixed-species colonies with the congeneric *P. pipistrellus*, *P. nathusii* and also with *Nyctalus noctula* (Dietz et al. 2009)

and *Hypsugo savii* (Ancillotto et al. 2013).

Savi's bat (*Hypsugo savii*)

Savi's bat (Figure 3) is another small (body mass: 7–12 g; forearm length: 31–38 mm (Dietz et al. 2009) house-dwelling bat, with roosting habits and ecology similar to *P. kuhlii*. Unlike the latter, *H. savii* usually forms somewhat smaller colonies (pers. obs.) and seems to be more closely linked to warmer and xeric areas (Dietz et al. 2009), yet it also occurs frequently in mountainous woodlands such as beech formations in the Appennines.



Figure 3. Adult captive female of Savi's bat (*Hypsugo savii*). Photo by L. Ancillotto.

This bat is usually considered a very common and widespread species in urban areas (Dietz et al. 2009) as also confirmed by data from wildlife rescue centres (Ancillotto et al. 2013). Little is known about the social behaviour and system of this highly variable (Arlettaz et al. 1993) species, but nursery roosts and roosting habits probably resemble those of *P. kuhlii*, except that colonies generally host smaller numbers of individuals (pers. obs.). *H. savii* seems to not form mixed-species colonies, except with *P. kuhlii* (Ancillotto et al. 2013).

Study design and data analysis

Factors affecting social behaviour of bats

In this experiment free-tailed bats belonging to the same captive colony are defined as "colony members". For the experiment, the colonies of adult bats were made of individuals that had joined the group at least two years before the experiments took place. I recognised each bat individually by examining its particular colour shade and/or pattern (e.g. light spots that sometimes occur in this species: Dietz et al. 2009) and by the occurrence of natural markings (scars, missing tip of the tail etc.). I selected 18 adults (>1 year old) of *T. teniotis* from two captive colonies, one (4 males and 4 females) held at the LIPU wildlife rescue centre of Rome and the other (3 males and 7 females) at the Bioparco Foundation (formerly Rome Zoo); I also included in the experiment 15 subadult bats temporarily kept at the LIPU centre. Only bats with no behavioural/motor disabilities were included in the experiments.

All individuals came from the same natural colony, numbering about 600 adults (pers. obs.), so that any potential influence determined by a different origin of the study subjects was controlled for and the observed effects could be safely attributed to social experience. Bats were rescued as young juveniles (about 2 weeks old) in different years so the chance that they had already developed social bonds in the wild was negligible.

On the days preceding each trial, the experimental subjects were allowed to explore alone the trial set for 30 minutes to minimize the potential effect of testing them in an unfamiliar environment. Each bat was allowed to explore the arena twice during one week preceding the tests. I performed the experiments in the early evening, i.e. when bats were naturally active. Those involving adults took place in April and May 2012, while those between adult and subadult bats were performed in September 2012. The reproductive activity of *T. teniotis*

occurs both in spring and early autumn (Dietz et al. 2009), thus adult bats in both testing seasons were in the same physiological condition.

Bats were allowed to interact in dyadic encounters in an 80x45x40h cm customized arena (Ligout et al. 2011) made of transparent Perspex. The latter featured two adjoining small rooms (15 x 10 cm) where subjects were introduced (at the same time) to initiate the experiment (see Figure S1 in Appendices for a scheme of the experimental setting). The arena's floor was covered with paper towels. After each encounter, I replaced the towels with clean ones and the tray was cleaned with 90% alcohol in order to eliminate odour cues otherwise potentially affecting the next encounter. I allowed two bats at a time to enter the experimental arena from opposite sides and freely interact for 15 minutes, after which subjects were removed from the arena and housed in travelling fauna-boxes.

I assigned bats to experimental pairs at random, in order to cover all “sex x group of origin” pairings. Each bat was tested two or three times in encounters involving different individuals, thus each dyad was unique. I recorded all encounters with a Sony Handycam SR501 camcorder using the nightshot mode. The video camera was positioned one meter above the experimental setup with a 90° angle. The use of artificial lighting during the experiment was avoided. The experimenter remained quiet in the room during the tests, ca. two meters from the testing arena. To check that the device would not produce ultrasound potentially interfering with natural bat behaviour and record social calls emitted by the experimental subjects, I sampled sound continuously in the real-time mode (sampling rate 384000 Hz) with a D1000X bat detector (Pettersson Elektronik AB, Uppsala, Sweden). Recordings were saved as wav files onto 4 Gb flashcards.

I employed preliminary dyadic encounters involving subjects not used for subsequent experiments to compile an ethogram of all behaviours exhibited. I classified observed behaviours into 18 different categories (Table 1). Only social (categories 1-8) behaviours

were included in the analyses. Social interactions were classified as aggressive (categories 1-6) or amicable (categories 7-8). Video-recordings were analysed through focal-animal sampling for both experimental subjects involved in each encounter (Martin & Bateson 1993): I recorded all occurrences of selected behaviours (Table 1) during such encounters (see Figure S5 in Appendices).

Table 1. Behavioural categories for captive European free-tailed bats *T. teniotis* recorded during dyadic arena tests and their description.

Behaviour	Description	Category
1 Bite	Actor bat bites the receiver or pushes its open mouth onto the receiver's body	Social - Aggressive
2 Punch	Actor bat hits the receiver with its wrist and thumb	Social - Aggressive
3 Chase	Actor bat insistently follows receiver through the arena, trying to reach it	Social - Aggressive
4 Push	Actor bat hits the receiver with its head, walking towards it	Social - Aggressive
5 Aggressive Call	(spectrogram in Figure 4b)	Social - Aggressive
6 Social Mount	Actor bat mounts receiver, pushing it to the ground and keeping it still	Social - Aggressive
7 Contact Call	(spectrogram in Figure 4a)	Social - Amicable
8 Huddling	Actor bat comes in physical contact next to receiver, vibrating its body softly	Social - Amicable
9 Mating	Actor bat mounts the receiver and tries to copulate (pelvic movements visible)	Sexual
10 Inspection	Actor bat actively smelling the receiver, getting in close proximity	Neutral
11 Touch	Casual physical contact between bats involving any part of their bodies	Neutral
12 Crawling	Bat moving on the floor	Non-social
13 Stretching	Bat extending one or both wings for a few seconds	Non-social
14 Yawn	Self-explanatory	Non-social
15 Self Grooming	A bat chews, licks or scratches its own hair, wings, feet	Non-social
16 Resting	Bat resting motionless	Non-social
17 Alert	Bat resting with ears in movement	Non-social
18 Jump	A bat jumps on legs, sometimes briefly opening wings	Non-social

I followed Freitas et al. (2008) to distinguish between aggressive and amicable behaviours as follows. I classified as aggressive behaviour those that (a) increased distance between subjects, or (b) could inflict physical injuries to, or avoid physical injuries by, the opponent. Amicable behaviour either increased or maintained proximity between subjects without leading to the infliction of physical injuries to the opponent. When social calls were produced, I distinguished between “aggression” calls (category 5) and “contact” calls (category 7), whose spectrograms were markedly different between each other (Figure 4).

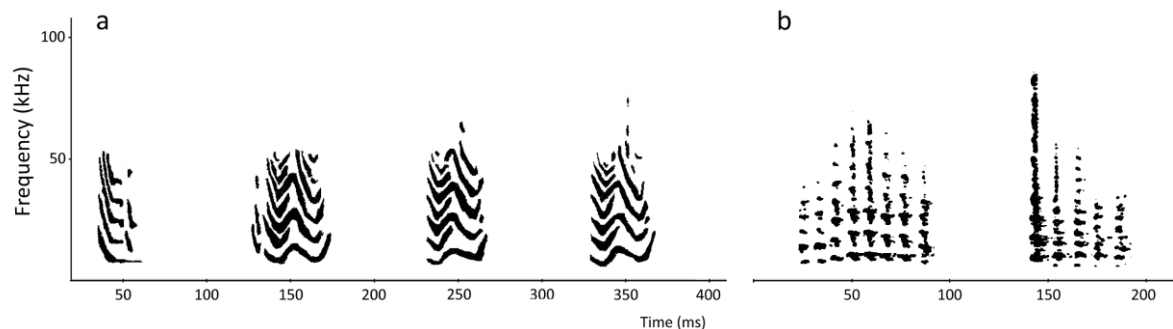


Figure 4. Spectrograms of *T. teniotis* social calls (a= contact call; b= aggressive call). Contact call emitted by subadult female; aggressive call emitted by adult male.

Social calls are species-specific sound emissions produced by bats for communication (Yovel et al. 2009; Carter & Wilkinson 2013) during social contexts such as aggressions, mate attraction or individual recognition (Pfalzer & Kusch 2003). Social calls in my experiments were audible to the unaided ear and the two types easily distinguished during all tests, thus I considered them as additional behavioural categories.

I evaluated an affiliation index (AI) for each individual during each encounter, calculated as the ratio of the total number of amicable (categories 7-8) behavioural events performed by an individual during an encounter and the total number of “aggressive + amicable” (categories 1-8) ones performed by the same bat during the whole encounter (Freitas et al. 2008). AI ranges

between 0 (only aggressive behaviour performed) and 1 (only amicable behaviour performed).

As each individual was tested two or three times, each time forming a new unique dyad, I performed a General Linear Mixed Model (GLMM) using AI values as response variable, measuring the effects of colony membership (i.e. familiarity between interacting bats), individual sex (male or female), sex condition (same-sex and different-sex dyads), individual age (adult or subadult) and age condition (two adults or one adult and one subadult) as independent variables. The lack of independence across tests due to the usage of the same bats in some of the trials was controlled for by entering individual identity as a random factor in the model, in order to take into account potential individual ‘tendencies’ (Pinheiro & Bates 2000). When GLMMs provided significant results I tested the occurrence of differences between categories by contrasts (Boik 1979).

No subadult bats sharing the same colony of the adults used for the experiments were available so I could not test the “age x colony membership” interaction. Although newborn bats may quickly develop preferential social relationships in the early stages of their life, I refrained from testing dyads made of juveniles because all those used for the experiments had to be released into the wild at latest by early autumn; consequently, the short time available did not allow juveniles to be split into separate captive groups for a time long enough to test the effect of familiarity. All tested juveniles had been kept in the same group. My specific aim was to test adults vs. juveniles to explore whether the latter’s young age could mitigate any xenophobic reaction by adults.

Effects of early experience on social bonding

Behavioural analysis

Twenty newborn *P. kuhlii* were admitted at the wildlife rescue centre of Rome and used for my experiment. Bats were aged according to centre staff's experience and published information (Barnard 2009), e.g. by the observation of remains of the umbilical cord, and sexed by observing external genitalia, which are clearly visible in bats since the first day from birth. To minimize the effect of social experience acquired in the original colonies before bats were rescued, for this experiment I only considered newborn bats of 1-3 days of age (Figure S3 in Appendices). Care was also taken in selecting newborns each from a different natural colony, in order to prevent potential effects of kin. Bats were initially housed in 25x18x30cm cardboard boxes. The box walls were holed to allow sufficient airflow. The boxes were also fitted with strings and fabric strips on which bats could hang. A section of the box was warmed by placing it on a *Zoo Med* heat cable for reptile terrariums (15 Watt voltage, supplied by Zoo Med Europa, Ekeren Antwerpen, Belgium).

Bats were first fed on First Age-Royal Canine powdered milk replacement for puppies until they were three to four weeks old (Kelly et al. 2008) using a syringe with a plastic cannula (Catheter Radiopaque Jelco®). After this period bats were weaned with mealworms (Barnard 2009). By the end of the weaning period, juveniles had learned to feed independently. Water was available *ad libitum* in small steel bowls.

Bats were kept in boxes for about six weeks in four separate groups, three made of subjects from different roosts, one including colony mates. That time was somewhat longer than that taken in the wild for weaning (e.g. Altringham 1996): the rearing protocols allowed sufficient time to compensate for the slower growth rate observed in captive *P. kuhlii* and achieve full development of young. In this phase, no contact was allowed between bats from different groups; to avoid indirect olfactory contact, different keepers took care of each group.

Bats were banded with two coloured plastic split rings (Ecotone, Gdynia, Poland) purposely modified for bats (Mitchel-Jones & McLeish 2004), one on each forearm, identifying each individual and its group membership.

In the second phase of the experiments all bats – now able to fly – were released in an 8x5x3 m flight room (see Figure S4 in Appendices). Feeding and drinking stations were made available, and two windows exposed bats to the natural photoperiod. Mosquito nets and four bat boxes were placed on walls for roosting bats and labelled with a code for data collection. Bats did not use nets for roosting during the experiment so all data collection refers to bat boxes. To determine the composition of roosting groups and record individual position, bat boxes were modified to be quickly and easily inspected.

The observations in the flight room were carried out for ten consecutive days between 10th and 20th August. Further observations were not allowed because bats then had to be released according to rehabilitation and animal welfare protocols.

To analyse association patterns, I only considered the physical contact occurring during day roosting between neighbouring bats, which was recorded twice a day (at 10 am and 7 pm). In this case directionality of behaviour (i.e. the identification of the individual bat initiating the interaction) was not assessed because I determined contacts from photographs. To reduce the observer's permanence in the flight room and minimise disturbance, I photographed roosting groups and subsequently examined photos for individual identification (see Figure S6 in Appendices).

To record social interactions, I also filmed bats with a Sony Handycam SR501 camcorder with nightshot function. Bats were filmed during two 90-min recording sessions respectively in the morning post-flight phase (since 1100 am) – during daytime roosting – and in the evening pre-flight phase (since 0800 pm, i.e. 48.1 ± 4.6 min before sunset) – during pre/post-

flight activity (Winchell & Kunz 1993). At such times bats are especially active inside roosts and more likely to show mutual interaction (Winchell & Kunz 1996).

The video camera was mounted on a tripod and directed towards a cluster of roosting individuals or placed beneath a bat box hosting roosting bats (see Figure S2 in Appendices). On each session the group to be filmed was chosen at random. To minimize disturbance, the video camera was left unattended during recording.

To analyse contacts between roosting bats, I organized the association data into a symmetric sociometric matrix. To examine the patterning of physical contacts among individuals in a way that is independent from marginal effects, i.e. that assumes each bat has exactly the same number of potential contacts as others, I normalised the data by the fitting of homogeneous margins to the association matrix (Bishop et al. 2007).

I also developed a binary matrix (group index) that indicates for each pair of bats whether they belong to the same rearing group (indexed by “1”) or to different ones (indexed by “0”). A Mantel test was used to test whether rearing group membership influenced the frequency of inter-individual contact. Pearson's product-moment's r resulting from the test gives a measure of the correlation between two different matrices and ranges from -1 (complete negative correlation) to +1 (complete positive correlation), with the value 0 indicating absence of any correlation (Conover 1980).

Video recordings were examined in the laboratory and watched several times, each selecting a different focal subject (Martin & Bateson 1993) from which I recorded the number of occurrences of the behavioural categories selected (Altmann 1974). The observed behaviours were classified according to an ethogram of captive *P. kuhlii* (Table 2) compiled during preliminary observations (Martin & Bateson 1993; Munoz-Romo 2006; Ortega & Maldonado 2006) as follows: 1) resting; 2) alert; 3) self-grooming; 4) crawling; 5) huddling; 6)

allogrooming; and 7) aggressive interaction (Table 2). For analyses, I only used social interactions, i.e. behavioural categories 5 – 7.

Table 2. Categories used to record behavioural data from captive Kuhl’s pipistrelle *P. kuhlii* and their meaning.

Behaviour	Description
Resting	Bat resting motionless, with eyes closed
Alert	Bat hanging, but eyes opened and head raised; comfort activities (yawning, urinating, defecating, stretching) may be shown
Self-grooming	The bat chews, licks or scratches hair, wings, feet
Crawling	Bat moves on roost floor or other surfaces
Huddling	Two bats in close contact, their bodies softly vibrate
Allogrooming	Bat grooms mate’s body (generally muzzle and/or ears)
Aggression	Bat shows teeth to an opponent, emits audible vocalizations or pushes the opponent with its muzzle and wrists

For each interaction I distinguished a performer (the bat which initiated the action) and a receiver. I calculated frequencies of occurrence of such behaviours for all bats and classified interactions. For all interactions the sexes of bats involved were recorded; interactions were thus classified as either “same-sex” or “different-sex” according to the sex of individuals involved (hereafter indicated respectively as ss/ds interactions). Different-sex interactions were further classified as “male” or “female” based on the sex of the instigating individual. I classified interactions recorded as “within-group” if they involved bats reared in the same group, “between-group” if bats were from different rearing groups.

A General Linear Model 2-way ANOVA followed by Tukey’s post-hoc tests was employed to detect the influence of sex, group and “sex x group” interaction on these frequencies. Non-significant terms were removed from models. The analysis was carried out with Minitab 13.1.

For bats in the group 4, all originating from the same colony, I hypothesized that their behaviour might be influenced by possible kinship or previous contacts occurred in the original roost before being rescued and admitted to the centre. Therefore, I ran all statistical

analyses both on the entire dataset and after removing group 4 data. No difference was found, so I used all groups for final analyses. For all tests, significance was set at $p < 0.05$.

Social network analysis

Based on the association in the bat-boxes, I also developed a network of bats' social links (Krause et al. 2009). Network construction and statistics were obtained with UCINET software package (Borgatti et al. 2002). I calculated descriptive statistics to quantify classical measures of social networks analysis as follows: 1) the *node degree* is a measure of the number of ties each bat had with others and represents the likelihood of an individual bat interacting with others (Krause et al. 2007); 2) *betweenness* represents the number of shortest paths between two individuals that pass through a particular one, so it may indicate the importance of a single individual to connect different parts of the network and 3) the *clustering coefficient* expresses the degree to which the individual's immediate neighbours are close to being a clique, that is an exclusive group (Croft et al. 2004). Network modularity, i.e. the fraction of the edges that fall within given groups minus the expected such fraction if edges were distributed at random (Newman 2006) was also calculated as a measure of strength division into subunits.

A hierarchical cluster analysis was successively adopted in order to detect spontaneous substructures of the network using average-linkage algorithm, as it is often considered optimal (Milligan & Cooper 1987), and calculating the relative cophenetic correlation coefficient as a measure of distance between sections of the clustering tree.

Mechanisms of interspecific social cohesion

Behavioural analysis

I selected 23 newborn bats (*P. kuhlii* n=12; *H. savii* n=11) admitted to LIPU's wildlife rescue centre in Rome for the experiment. Ageing and selection of experimental subjects followed the same protocols adopted in Experiment 2.

Following methods from Experiment 2, I raised bats in 4 different groups, each composed by six individuals, evenly distributed between species (only one of the groups comprised two rather than three *H. savii*). Each group was raised in a different container in order to avoid acoustic, tactile or olfactory contacts between bats from different groups. Further details on rearing and weaning methods are given in materials and methods of Experiment 2.

After weaning, I banded the study subjects with two coloured plastic split-rings each (Ecotone, Gdynia, Poland), purposely modified for bats (Mitchel-Jones & McLeish 2004), in order to identify each individual and its group membership. For each bat after weaning I recorded sex, species, group membership, forearm length and scaled mass index (as indicator of body conditions; (Peig & Green 2009)). Scaled mass index is derived from the simple body condition index (BCI), i.e. the ratio between body mass (g) and forearm length (mm), a commonly used parameter in the study of bats, but it is a better indicator of body fat reserves in small animals (Peig & Green 2009). All bats were then released together in the same flight room used for Experiment 2.

I carried out observations every day between 23th August and 6th September; at the end of the experiments, all bats were released according to wildlife rehabilitation and animal welfare protocols (Serangeli et al. 2012). I recorded individuals' positions inside the room and actual physical contacts (see Figure S4 in Appendices) between bats twice a day (at 11 am and 7 pm). I thus recorded a total of 30 positions and relative combinations of physical contacts for each individual bat. In order to minimize disturbance and to determine the exact position of

each bat inside the bat boxes, one operator briefly opened the bat box and rapidly took photographs of roosting bats. Individual positions and mutual contacts were determined by examining photographs.

To record social interactions, I also filmed bats with two Sony Handycam SR501 camcorders with nightshot function. Bats were filmed during two 90-min recording sessions in the morning (since 1100 am) and in the evening (since 0800 pm, i.e. 42.3 ± 5.1 min before sunset) respectively. At such times bats are especially active inside roosts and more likely to show mutual interactions (Winchell & Kunz 1996). I mounted cameras on tripods beneath occupied bat boxes and directed towards clusters of roosting individuals. On each session, the groups to be filmed were chosen at random. To minimize disturbance, I left the video cameras unattended during recording (see Figure S2 in Appendices). Video recordings were examined in the laboratory and watched several times, each time selecting a different focal subject (Martin & Bateson 1993), from which I recorded the number of occurrences of the behavioural categories selected (Altmann 1974). I classified the observed behaviours according to the ethogram of captive *P. kuhlii* from Experiment 2 (Table 2), as preliminary observations carried out on *H. savii* showed the occurrence of the same behavioural modules. For analyses, I only used social interactions, i.e. huddling, allogrooming and aggressive interactions. For each behavioural occurrence, I distinguished a performer (the bat that initiated the action) and a receiver. I calculated frequencies of occurrence of such behaviours for all bats and classified interactions.

To analyse contacts between roosting bats, I organized the association data into a symmetric socio-metric matrix. To examine the patterning of physical contacts among individuals in a way that is independent from marginal effects, i.e. that assumes each bat has exactly the same number of potential contacts as others, I normalized the data by the fitting of homogeneous margins to the association matrix (Bishop et al. 2007). I developed two binary matrices that

indicate for each pair of bats whether they belong to the same rearing group (indexed by '1') or to different ones (indexed by '0') and whether they belong to the same species (1) or different ones (0), respectively. Mantel tests were used to determine whether rearing group membership and species influenced the frequency of inter-individual physical contacts.

Each video-recorded behavioural occurrence was characterized according to the sex of interacting bats (Sex Condition: same-sex versus different-sex), species (Species Condition: same-species versus different-species) and rearing group (Group Condition: same-group versus different-group). I used Generalized Linear Mixed Models (GLMMs) to test the influence of actors' attributes (sex, species and body condition index), the conditions associated with the behavioural event (Sex Condition, Species Condition, Group Condition) and their interactions on the observed frequencies of behaviours, using individual identity as a random factor. I adopted a backward step-wise approach in selecting significant variables from a first full model, until only significant explaining variables were present in the final one (minimal adequate model). All models and tests were run in R 2.13.2 (R Core Team 2005).

Social network analysis

I used Ucinet software package (Borgatti et al. 2002) to build four different networks based on the occurrence of physical contacts and on the three types of social interactions. For each network I calculated the value of Q-modularity (Newman 2006), i.e. a measure of network division in subgroups, calculated as the difference between fraction of nodes that fall into a given group and the expected such fraction if nodes were distributed at random. I tested homophily, i.e. the tendency of individuals to form network ties with other ones sharing the same characteristic or attribute (McPherson et al. 2001), using the UciNet function for ANOVA density models of variable homophily, i.e. testing a block model in which within-group ties differ from between-group ones.

For each individual, in each of the four networks I separately calculated the following metrics: weighted *in-* and *out-degree centrality*, i.e. the number of interactions initiated and received by a bat, respectively (pooled together in the case of non-directional behaviour such as physical contact); *clustering coefficient*, i.e. the degree to which the individual's immediate neighbours in the network are close to be a closed group (Croft et al. 2004); normalized *betweenness*, i.e. the number of shortest paths between two individuals that pass through the focal one, indicating the importance of a single bat in connecting different parts of the network. I tested the effects of individual attributes (sex, species, SMI) on these metrics of each individual for each network by running generalized linear models (GLMs). Network metrics were first tested for independence by running Pearson correlation tests; as no strong correlation was detected ($r < 0.5$), all metrics were used in the GLMs.

Results

Factors affecting social interactions of bats

I recorded 901 social interactions in 65 encounters (45 between adult bats, 20 between adults and subadults). AI values were significantly influenced by individual sex, age and familiarity with the interacting bat (Figure 2).

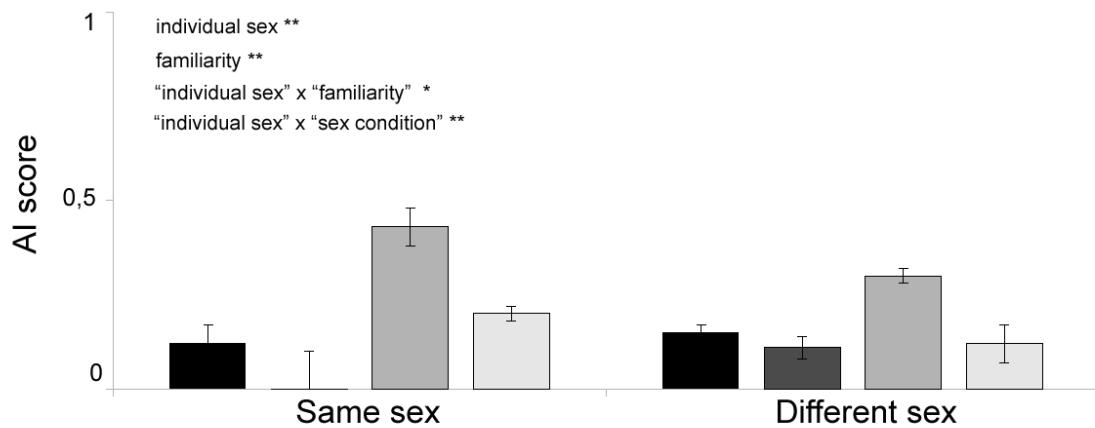


Figure 5. Means \pm standard deviations of Affiliation Index for captive *T. teniotis* during dyadic different and same sex encounters. AI score= Affiliation index scores; Black bars: male in familiar encounters; dark-grey bars: males in unfamiliar encounters; light-grey: females in familiar encounters; white: females in unfamiliar encounters. Significant differences among conditions as well as their interactions are indicated: **= $p < 0.001$; * = $p < 0.05$.

Bats involved in encounters within the same social group (i.e. between familiar subjects) had significantly higher AI values than those in unfamiliar encounters (0.26 and 0.09, respectively; $F_{1,101} = 18.56$, $p < 0.001$; contrast test: $t = 3.47$, $p < 0.01$). The effect of sex on the number of aggressive and amicable behaviours was more complex: females showed higher mean AI values (0.60) than males (0.46), and this difference ($F_{1,101} = 10.48$, $p < 0.001$,

contrast test: $t = 2.47$, $p < 0.05$) was more evident during same sex encounters, with male showing lowest and females highest AI values (Sex*Sex Condition: $F_{1,101} = 17.78$, $p < 0.001$). Familiarity also influenced the effect of sex (Figure 5): females were more amicable towards familiar females and males more xenophobic towards unfamiliar males (Sex*Familiarity: $F_{1,101} = 4.03$, $p < 0.05$). Adult bats involved in encounters with subadults also showed significantly higher AI values ($F_{1,101} = 8.54$, $p < 0.01$; contrast test: $t = 2.81$, $p < 0.05$).

Effects of early experience on social bonding

Behavioural analysis

Day-roosting bats reared in the same group established mutual physical contact (individual association) significantly more often than did those from different groups. The row-wise matrix correlation analysis between physical contacts made during day-roosting (recorded by photographs) and rearing groups resulted in a Mantel's $r = 0.548$, $p < 0.0001$ (based on 10,000 permutations).

Overall, I analysed 301 social interactions from categories 5-7. Huddling was the most frequent behaviour performed ($N = 205$), followed by allogrooming ($N = 51$) and aggression ($N = 45$) (Figure 3).

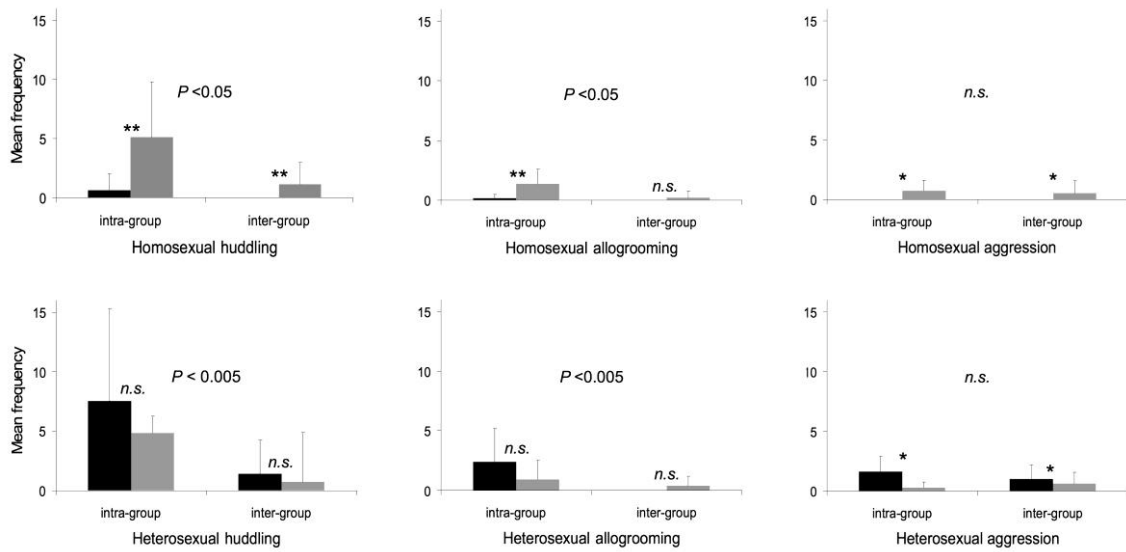


Figure 6. Mean individual frequencies of same-sex and different-sex interactions for huddling (N=205), allogrooming (N=51) and aggression (N=45). For all interactions sexes of bats involved were recorded; interactions were classified as either “same-sex” or “different-sex” according to the sex of individuals involved. Different-sex interactions were further classified as “male” or “female” based on the sex of the instigating individual. Black = male; grey = female. Between-group ANOVA levels of significance are shown in central labels; within-group sexual differences are shown near columns. * <math>P < 0.05</math>; ** <math>P < 0.01</math>

Unlike all amicable interaction categories considered, aggression rates were not influenced by rearing group membership ($F_{1,34} = 0.14$ and 0.16 , *n.s.*, respectively for SS and DS interactions) but only by sex: females engaged more often in SS ($F_{1,34}=6.74$ $p<0.05$) aggressive events, males in DS ones ($F_{1,34}= 7.11$ $p<0.05$; Figure 3). Both SS ($F_{1,34}= 6.15$, $p<0.02$) and DS ($F_{1,34}= 12.23$, $p<0.005$) huddling involved more frequently members of the same rearing group than bats reared in different groups (Figure 3); sex influenced significantly SS huddling ($F_{1,34}= 8.88$, $p<0.01$), which was more frequent between females, but not DS huddling ($F_{1,34}= 1.3$, *n.s.*).

Finally, both SS ($F_{1,34}= 7.06$, $p<0.05$) and DS ($F_{1,36}= 21.66$, $p<0.001$) allogrooming occurred more frequently within groups (Figure 3). Sex influenced SS ($F_{1,34}= 8.34$, $p<0.01$) – but not

DS ($F_{1,34} = 1.07$, *n.s.*) – allogrooming: female – female allogrooming was more frequent than male – male allogrooming. A significant “group x sex” interaction emerged for SS allogrooming ($F_{1,34} = 4.61$, $p < 0.05$), reflecting the fact that the difference between sexes was mainly observed for within-group allogrooming (females, $n = 11$, 2.54 ± 1.75 interactions; males, $n = 8$, 0.37 ± 0.52 interactions); between-group allogrooming was either very rare or absent.

Social network analysis

Both a visual assessment of the network (Figure 7) and the values of corresponding statistical descriptors suggest a preferential association between former members of the same rearing group. Individual and mean values of node degree, clustering coefficient and betweenness are presented in Table 3.

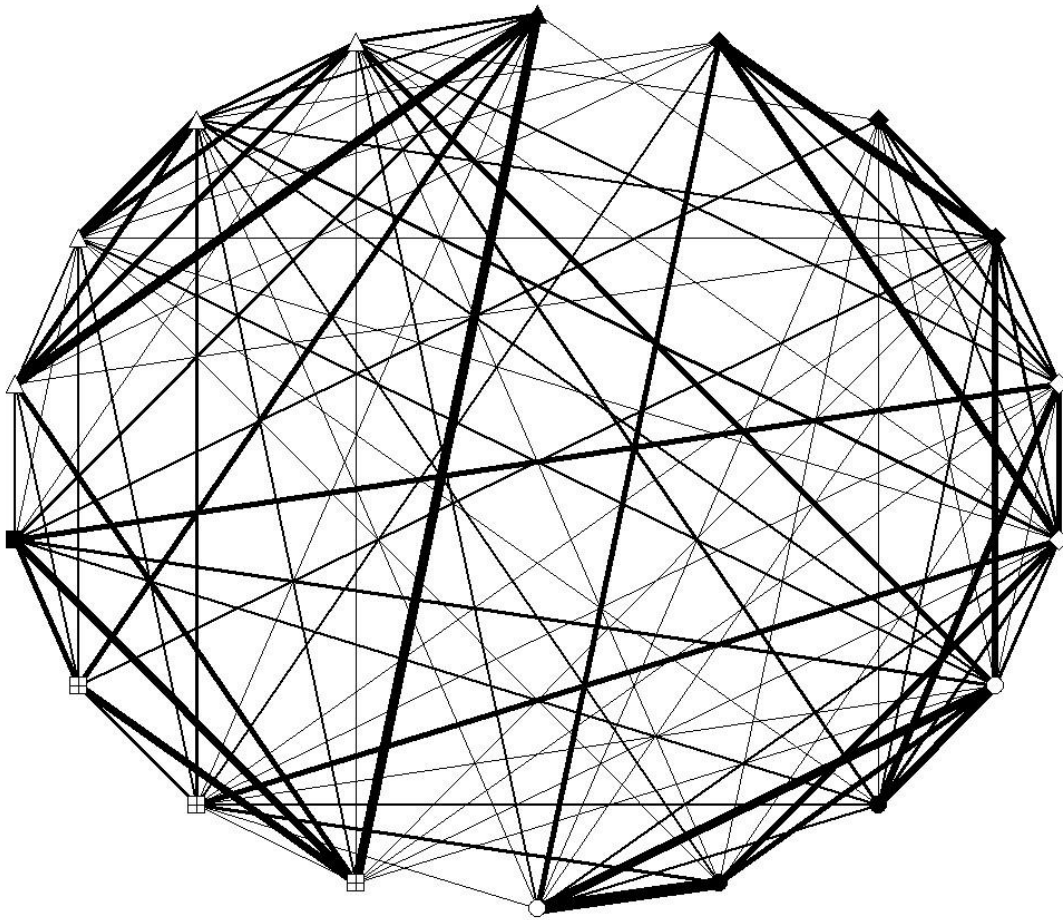


Figure 7. Sociogram associating juvenile *P. kuhlii* roosting in mutual contact; symbols used to indicate nodes show different rearing-group membership; black nodes= males, white nodes= females; the thickness of linking segments is proportional to tie strength.

Table 3. Network descriptors calculated from association data (contact between bats in bat boxes) recorded from captive juveniles *Pipistrellus kuhlii*; in the ‘bat code’ column, bats from the same rearing group are labelled with the same letter.

Bat code	Node degree	Clustering coefficient	Node betweenness
R01	13	0.58	6.28
R02	9	0.67	2.12
R03	13	0.62	5.56
R04	12	0.67	3.76
R05	9	0.65	1.60
Y01	13	0.61	6.12
Y02	8	0.71	1.60
Y03	12	0.61	4.46
Y04	9	0.78	1.18
Y05	13	0.71	3.28
W01	10	0.73	1.80
W02	13	0.71	3.25
W03	9	0.78	0.99
W04	10	0.76	1.47
P01	11	0.66	3.00
P02	13	0.62	5.75
P03	10	0.62	3.60
P04	7	0.76	0.65
Mean (SD)	10.8 (2.0)	0.68 (0.1)	3.11 (1.88)

Although the high values of node degree indicate a moderately structured network (mean value: 10.8, i.e. bats interacted on average with 60% of available individuals), clustering coefficients do show a relatively high 'cliquishness' suggesting the existence of cohesive subgroups in the network.

The clustering tree obtained from associations also permits to recognize cohesive subgroups, with at least two main clusters of close individuals, with a cophenetic correlation coefficient of 0.760 indicating a good representation of the actual distances between individuals (Bridge 1993). As shown in Figure 8, bats from the same group tend to cluster together, with only one outlier (the individual labelled as “P02”) that clusters anyway in the main subgroup of bats from the same rearing-group.

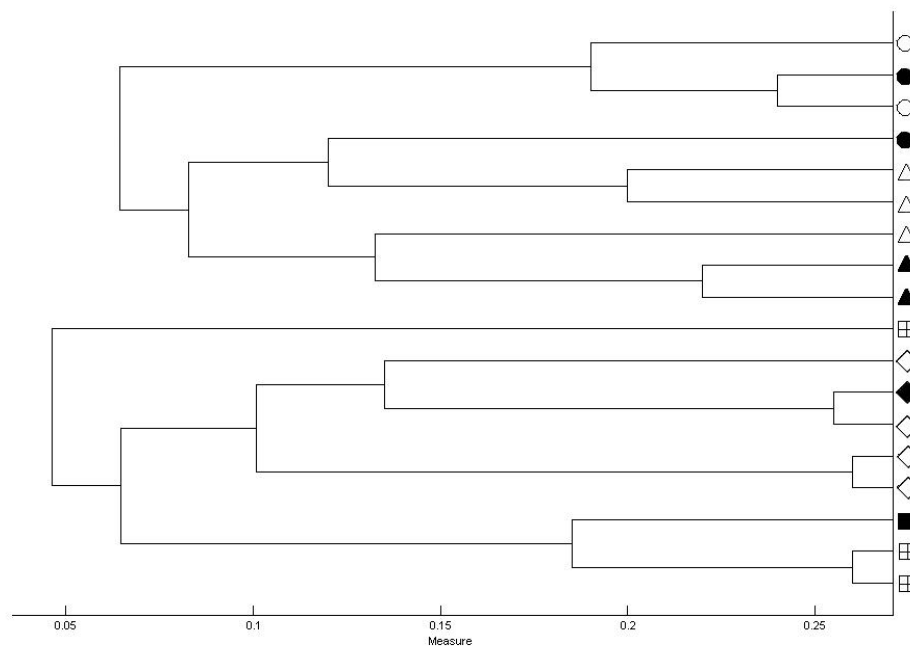


Figure 8. Clustering-dendrogram made from association data of captive juvenile *P. kuhlii* roosting in mutual contact; first two letters of individuals' names indicate group membership. Cophenetic correlation coefficient for the dendrogram= 0.760. Individuals' symbols as in Figure 7.

The Q-modularity test resulted in a value of $Q = 0.26$ indicating that number of edges within groups exceeds that expected by chance (Clauset et al. 2004).

Mechanisms of interspecific social cohesion

Behavioural analyses

Bats day-roosted in mutual physical contact significantly more often with individuals from the same rearing group ($r = 0.51$, $p < 0.0001$, based on 10000 permutations) while no effect of species was found ($r = -0.05$, $p = 0.25$, based on 10000 permutations), i.e. mutual contact was not more frequent between conspecifics. I recorded 295 social interactions (allogrooming = 107, huddling = 168, aggression = 20). Group condition (i.e. the membership to the same rearing group) was the most important variable explaining the patterns of social behaviours within MSGs (huddling: $F_{1,174} = 8.11$, $p < 0.0001$; allogrooming: $F_{1,174} = 6.13$, $p < 0.0001$; aggression: $F_{1,174} = 7.35$, $p < 0.0001$, Table 4;). Group membership showed significant interactions with body conditions ($F_{1,174} = 5.45$, $p < 0.05$) and sex ($F_{1,174} = 2.36$, $p < 0.01$) of the bat initiating the interaction: in same-group huddling (i.e. huddling between individuals raised in the same group), most interactions were initiated by males and by bats with lower body conditions.

Table 4. Factors influencing the frequency of three behaviours by a mixed-species group of captive bats (*P. kuhlii* and *H. savii*) explained by Generalized Linear Models (minimal adequate models).

Behaviour	Factor	P
Huddling	Group condition	< 0.0001
	Group condition * SMI	0.015
	Group condition * Sex	0.005
	SMI * Sex	0.014
	Sex * Species	< 0.001
	Sex * Species condition	< 0.0001
	Species condition * Sex condition	0.025
Allogrooming	Group condition	< 0.001
	Sex condition	0.003
	Sex * Species condition	0.007
Aggressive	Group condition	< 0.0001
	Species condition	0.0005

Body conditions did not feature in the final models for allogrooming and aggression, as its influence was not significant. Sex of the initiating bat did not directly influence the occurrence of social behaviours, but significant interactions were recorded for both huddling and allogrooming, with females being more often engaged in allogrooming and males in huddling, in both cases with individuals from the same rearing group. Sex condition, i.e. same-sex or different-sex interactions, only influenced allogrooming ($F_{1,173} = 9.98$, $p = 0.003$), with same-sex interactions being significantly more frequent than different-sex ones (Table 4).

Social network analysis

The values of different network metrics differed greatly among bats forming the artificial MSG (Table 5).

Table 5. Individual attributes (sex, species and body condition index) and network descriptive metrics of a captive mixed-species group of bats. Network metrics are presented separately for each network derived from different social behaviours. Sex: m = male, f = female; Sp = species: Hs = *H. savii*, Pk = *P. kuhlii*. The letter in individuals' ID indicates group membership. CC = clustering coefficient; BT = betweenness; DG = degree; IDG = indegree; ODG = outdegree.

ID	Sex	Sp	BCI	Physical contact			Huddling				Allogrooming				Aggression			
				CC	BT	DG	CC	BT	IDG	ODG	CC	BT	IDG	ODG	CC	BT	IDG	ODG
B01	f	Hs	0.20	0.72	1.5	19	0.26	19.5	16	11	0.67	0.3	6	5	0.00	3.5	2	5
B02	m	Hs	0.18	0.67	0.1	7	0.25	11.3	17	17	-	0.0	7	5	0.00	0.0	0	2
B03	m	Hs	0.18	0.60	0.3	7	1.00	0.0	18	11	0.00	4.1	7	8	0.00	0.4	2	1
B04	f	Pk	0.15	0.70	2.0	14	0.48	4.0	13	7	0.50	10.7	5	6	0.00	0.9	1	2
B05	f	Pk	0.18	0.52	5.5	10	0.67	0.2	2	7	0.33	11.9	1	7	0.00	0.4	1	1
B06	f	Pk	0.20	0.56	8.6	17	0.25	25.1	11	11	0.33	7.4	6	4	-	0.0	0	0
A01	f	Hs	0.19	0.68	2.9	17	0.34	15.6	11	18	0.42	10.8	5	5	-	0.0	3	0
A02	m	Hs	0.19	0.69	1.4	8	0.83	0.1	8	6	0.75	0.0	2	5	-	0.0	1	0
A03	f	Pk	0.19	0.79	1.0	19	0.70	1.0	7	3	0.42	20.6	4	7	0.00	1.1	3	0
A04	m	Pk	0.17	0.68	3.0	15	0.48	5.3	13	7	0.45	0.9	13	5	0.00	2.3	3	3
A05	m	Pk	0.18	0.76	1.3	15	0.50	0.9	10	8	0.23	24.9	13	10	0.00	2.2	1	3
V01	f	Hs	0.18	0.67	1.0	7	-	0.0	0	2	0.83	0.0	2	4	-	0.0	0	0

V02	m	Hs	0.19	0.42	3.7	4	0.10	7.8	6	3	-	0.0	0	0	-	0.4	1	2
V03	f	Hs	0.18	0.57	1.1	5	0.00	0.0	0	4	0.50	0.0	0	3	-	0.0	0	0
V04	f	Pk	0.18	0.62	1.5	8	0.33	0.0	9	0	0.50	0.9	5	7	-	0.0	0	0
V05	m	Pk	0.20	0.62	3.9	11	0.15	3.5	1	6	0.30	9.7	5	3	-	0.0	0	0
V06	f	Pk	0.17	0.44	5.3	10	0.33	0.0	1	7	0.50	0.2	6	3	-	0.0	1	0
G01	m	Hs	0.24	0.39	3.3	5	0.20	3.9	4	3	0.00	4.1	4	1	-	0.0	0.0	0.0
G02	f	Hs	0.25	0.78	1.2	18	0.45	11.6	13	5	0.17	18.7	8	4	-	0.0	0.0	0.0
G03	m	Hs	0.14	0.70	1.1	6	0.58	0.9	4	1	0.50	0.0	3	0	-	0.0	1.5	0.0
G04	f	Pk	0.15	0.60	3.7	13	0.20	5.6	1	8	0.25	9.6	2	7	-	0.0	3.0	0.0
G05	m	Pk	0.16	0.50	0.3	2	0.50	0.0	2	2	-	0.0	1	2	-	0.0	1.5	0.0
G06	f	Pk	0.16	0.70	2.3	16	0.38	8.7	5	15	0.25	2.4	2	6	-	0.0	1.5	0.0
Mean				0.63 ±	11.3 ±	11.2 ±	0.39 ±	5.4 ±	7.3 ±	7.3 ±	0.39 ±	5.9 ±	4.6 ±	4.6 ±	0.00 ±	0.5 ±	1.4 ±	1.4 ±
± SD				0.11	9.2	5	0.25	6.8	5.4	5.0	0.22	7.3	3.4	2.4	0.00	0.9	1.4	2.0

The network of physical contacts was poorly structured (Q-modularity: -0.01, Figure 9a): bats established contacts on average with ca. 50% of the individuals in the group (mean \pm s.d.; degree centrality: 11.2 ± 5.0); females generally established more contacts than males ($F_{1,7} = 11.632$, $p = 0.01$). Clustering coefficients (0.63 ± 0.11) indicate a tendency to form closed subgroups, but this index was influenced neither by individuals' species nor sex.

Huddling network was only moderately structured (Q-modularity: 0.40, Figure 9b), but individuals occupied similar relative positions inside the network as no metric differed between sexes or species (Table 4). Aggression network was similarly structured (Q-modularity: 0.44, Figure 9d) and no particular trend was detected.

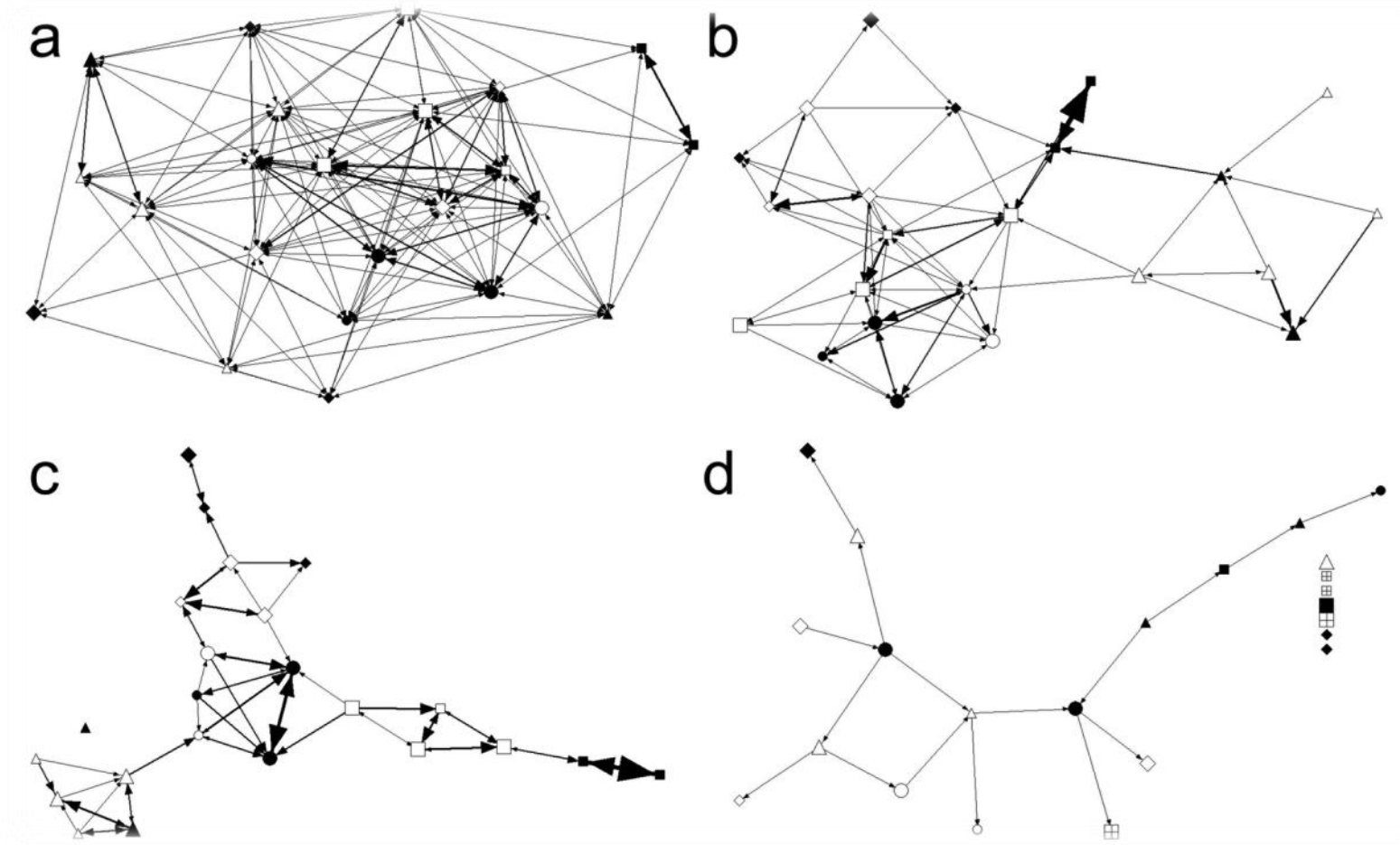


Figure 9. Social networks of a mixed-species group of captive bats, based on four different social behaviours; a: physical contacts inside roost; b: huddling; c: allogrooming; d: aggressive behaviour. Node shape indicates individual's group of origin. Black nodes = males, white nodes = females. Large nodes = *H. savii*, small nodes = *P. kuhlii*. Tie strength between nodes is proportional to line thickness. A spring-embedding algorithm derives distances between nodes.

The allogrooming network showed different trends, e.g. it was highly structured (Q-modularity = 0.64, Figure 9c): bats groomed in relatively closed subgroups (clustering coefficient: 0.34 ± 0.24), with females showing a stronger tendency to form closed cliques ($F_{1,7} = 4.631$, $p = 0.05$). In this network, *P. kuhlii* had higher betweenness values ($F_{1,7} = 6.207$, $p = 0.04$), i.e. bats of this species were more important in connecting different parts of the group. *P. kuhlii* also initiated more often an allogrooming event ($F_{1,7} = 7.50$, $p = 0.02$), particularly in the case of individuals in lower health conditions (SMI*species: $F_{1,7} = 6.01$, $p = 0.04$). No difference was evident in the numbers of received allogrooming events among bats of different species or sex, but both in- and out-degree indicate that bats interacted with a reduced number of individuals, i.e. ca. 25%, among those available in the group (indegree centrality: 4.6 ± 3.4 ; outdegree centrality: 4.6 ± 2.4).

Tests for homophily towards former group-members proved significant for all considered behaviours (Table 6), i.e. bats engaged in behavioural interactions selectively with familiar individuals (see Figure S7 in Appendices). Interestingly, this trend was more evident for cooperative behaviours (i.e. huddling and allogrooming) than for simple physical contacts, for which it was also not significant for all experimental groups.

Table 6. ANOVA density models for variable homophily of networks derived from physical contacts, huddling and allogrooming interactions of a captive mixed-species group of bats, based on 5000 permutations.

Physical contact			Huddling		Allogrooming	
R-square	P-value		R-square	P-value	R-square	P-value
0.07	0.012		0.21	< 0.001	0.29	< 0.001
	Coefficient	Significance	Coefficient	Significance	Coefficient	Significance
Intercept	5.56	< 0.001	0.09	0.999	0.02	0.999
Group B	-2.89	0.049	1.67	< 0.001	1.04	< 0.001
Group A	-5.56	< 0.001	1.60	0.001	1.48	< 0.001
Group V	-2.22	0.111	0.47	0.071	0.58	0.005
Group G	-1.56	0.188	0.70	0.031	0.58	0.005

Discussion

Factors affecting social interactions of bats

This experiment shows that in *T. teniotis* familiarity plays a key role in determining the degree of aggressiveness during social interactions, but also that intrinsic factors characterizing individual status such as sex and age play a role in influencing the rates at which aggressive or amicable behaviours are expressed.

Amicable behaviour is known to be of prime importance to reinforce existing social bonds and individual ranks (De Villiers et al. 2003) and the higher levels of affiliation recorded between colony members in my experiment support my first hypothesis and confirm what is largely known for a wide range of other group-forming species (e.g. crustaceans: Tøth & Duffy 2005; birds: D'Eath & Stone 1999; rodents: Szenczi et al. 2012). Familiarity is built on the experiences made since the early stages of a bat's life, leading to the formation of bonds, which will persist during adulthood (Willis & Brigham 2004). The two remaining hypotheses, i.e. that sex and age too influence the levels of affiliation, were also supported. The values of AI recorded during encounters were clearly influenced by the sex and age of interacting bats, variables of prime importance to most bat species from temperate areas, which congregate in summer roosts forming nursery colonies (Dietz et al. 2009). In roosting bats, the advantages of actively engaging in physical contact between individuals, exhibiting social tolerance and performing amicable interactions are primarily energetic: huddling helps females and juveniles to reduce the energetic cost of homoeothermy, needed during pregnancy and lactation to reproduce successfully (Chruszcz & Barclay 2002; Speakman 2008). In bat species other than that I studied, social tolerance and physical contacts also allow females to perform cooperative behaviour such as allogrooming (Kerth et al. 2003) – which besides reducing parasite load in the colony also pacifies the grooming partner –, food sharing

(Wilkinson 1984; Elizalde-Arellano et al. 2000; Paolucci et al. 2006; Carter & Wilkinson 2013) and non-parental nursing (McCracken 1984a; Eales et al. 1988; Wilkinson 1992; Bohn et al. 2009). On the other hand, the solitary tendency of adult males observed in many bats, as well as the competitive role of males in social and mating systems (McCracken & Wilkinson 2000) may explain both the low rate of amicable interactions and the very high frequencies of aggressive behaviour in encounters where only males were present; bat social and mating systems in fact often comprise the expression of agonistic behaviour as well as the formation of harems guarded and defended by single males (Safi 2008). Noticeably, although *T. teniotis* is known to form mixed colonies at least during part of the year (Balmori 2003; Dietz et al. 2009), males still proved less tolerant, especially towards other males, suggesting that the persistence of aggressiveness must have a prime adaptive value in shaping male's agonistic behaviour even in species where sexes do not constantly segregate and males are not solitary. It is unknown whether behavioural processes such as spatial segregation within colonies or clustering of individuals (and thus the formation of cryptic sub-units like in (Garraway & Broders 2007) may play a role in mitigating aggressive behaviour.

As predicted, subadult individuals were less frequently targeted by adult aggressions. Juvenile bats usually associate with their mothers for long periods after weaning, thus remaining in the same roost for long (Burland et al. 2001; Rossiter et al. 2002; Kerth et al. 2002), and this may explain the higher levels of tolerance adults expressed towards juveniles in my experiment, as also found by (Ortega et al. 2008) in *Artibeus jamaicensis*. In some species the prolonged presence of juveniles in colonies may favour active information transfer from adults, e.g. regarding the development of foraging skills (Brigham & Brigham 1989; Geipel et al. 2013), or mother-to-offspring transmission of echolocation call features (Jones & Ransome 1993), and even direct food provisioning after weaning, i.e. females bring partially consumed prey items to juveniles (Delpietro & Russo 2002; Raghuram & Marimuthu 2007; Geipel et al.

2013). This high tolerance also explains, for instance, why naive juveniles of *P. kuhlii* released ex situ were quickly accepted in stranger colonies (Serangeli et al. 2012). In most mammals, juveniles are the dispersing individuals (Wolff 1994), and this also holds true for several bat species (McCracken 1984b; Dechmann et al. 2007; Nagy et al. 2013). The tolerance towards juveniles is probably so hard-wired into a bat's behavioural repertoire that it also extends to juveniles from colonies other than their own, i.e. the "juvenile status" cues may override those signalling colony membership. However, infanticide by adult female bats is also known and has a xenophobic basis, being perpetrated towards pups from a different social group (Bohn et al. 2009), albeit very rare. Although I did not deal with the cues used by *T. teniotis* to recognize individual status, as in other bats these are likely to include personal odour, which may also differ according to age and thus be useful to distinguish between adults and juveniles (Nielsen et al. 2006). In *T. brasiliensis*, personal odour represents a crucial cue for individual recognition (Englert & Greene 2009).

In conclusion, although my study shows the existence of xenophobia in bats it also illustrates how factors characterizing individual status such as sex and age may influence it, revealing the multifaceted nature of this behaviour.

Although I only focused on *T. teniotis*, these results are likely to offer a general picture valid for many other bat species exhibiting similar roosting ecology and social behaviour. Of course my experiment was also constrained by the typical limitations of captive studies on behaviour (Siemers & Page 2009), so observational studies in nature confirming my findings are desirable. On the other hand, captivity offers an effective way to control social variables which would be otherwise extremely difficult to deal with in a natural environment (Siemers & Page 2009).

Effects of early experience on social bonding

Despite the well-known sociality of bats, limited attention has been paid to the quantity and quality of social interactions among colony mates, aspects potentially of great significance for a better understanding of the evolution of social behaviour and the value of group formation (Kerth et al. 2003). Within this general context, the ontogeny of social ties has been even more neglected.

By experimentally manipulating a bat's early social context, this study showed for the first time that frequent mutual interactions between newborns, such as those likely to occur in colony clusters, lead to preferred associations between emancipated subjects, as suggested by the observed rates of amicable interactions shown once they are able to fly and feed autonomously and by the high 'cliquishness' (Croft et al. 2004), the effective representation of the resulting clustering dendrogram (Bridge 1993) and the Q-modularity value of the network (Newman 2006). Since I could only test the resulting social preferences on a short term, whether they are persistent is unknown, albeit likely. In fact, the frequent occurrence of amicable interactions may help cement social bonds (Kerth et al. 2003) originally acquired during early association in rearing groups. Preferred association of bats in fission-fusion societies have been found to persist across years despite movements between summer roosts and hibernacula (Patriquin et al. 2010; Kerth et al. 2011) Given the very early age of bats associated in rearing groups, the resulting social preferences might also be at least partly influenced by an imprinting-like process (Bateson 1979).

The approach I followed to measure the strength of affiliative social ties established through earlier group membership, i.e. recording patterns of amicable behaviour, has been largely used for the study of other highly social species besides bats (Kerth et al. 2003), such as e.g. primates (Di Bitetti 1996; Barrett & Henzi 2001), rodents (Manno 2008), carnivores (Murdoch et al. 2008), and cetaceans (Gibson & Mann 2008). Noticeably, rearing group

membership influenced amicable interactions, whereas aggressive behaviour was only influenced by sex.

Although in several bat species the role of mother-newborn association has been thoroughly analysed (Balcombe 1990; Balcombe & McCracken 1992; De Fanis & Jones 1995a, 1995b; Bohn et al. 2009), to my best knowledge this study is the first to determine the possible influence of neighbours on a bat's social development and adds a further dimension to sociality in bats. These results suggest that for the establishment of social dynamics not only is the general association of bats at roost important (Whitehead & Dufault 1999; Kerth et al. 2011), but also the individual positions they occupy within clusters (Kerth et al. 2003).

I acknowledge that the situation found in roosting newborns forming different clusters (or occupying different cluster sectors) may somewhat differ from that I created experimentally. Hence, some caution is advisable in extrapolating my results to natural conditions. However, I clearly highlighted a phenomenon potentially leading to the development of cohesive subgroups within colonies, determining cryptic associative patterns and likely to play a major, so far ignored role in bat sociality, leading to preferential social ties.

One of the consequences of common group membership was that, once able to fly, former group mates actively searched for mutual contact, a fact that may have consequences for the foundation of new colonies, roost switching patterns and information transfer. In general, same-sex amicable interactions were more frequent in females. For same-sex allogrooming, this bias only occurred between bats reared in the same group, whereas it was apparent in both within-group and between-groups homosexual huddling. Allogrooming is also more frequent between females of the common vampire bat *Desmodus rotundus* (Wilkinson 1986). This suggests that the affiliative patterns arising from early experience may be influenced by the more pronounced female sociality, needed to establish nursery colonies, whose aggregation is of great benefit to communal breeding. The advantages of female sociality

include minimizing torpor during lactation by social thermoregulation, which would otherwise affect milk production and offspring survival (Racey 1973; Swift & Racey 1983; Willis & Brigham 2007; Speakman 2008; Patriquin et al. 2010) as well as performing alloparental care of young (McCracken 1984a; Eales et al. 1988; Wilkinson 1992; Bohn et al. 2009). The lower tendency in male bats to perform affiliative behaviour and in general the less gregarious habits might also explain the higher rate of aggressions performed by males over females I recorded.

Huddling – the most frequent interaction recorded – is a form of physical contact which may facilitate thermoregulation (Kotze et al. 2008); however, like other social vertebrates, bats too may adopt it for reconciliation (McGowan et al. 2006; Willis & Brigham 2007; Kotze et al. 2008). As in other mammals (e.g. Hart & Hart 1992; Borries et al. 1994), in bats too allogrooming plays a dual role, i.e. hygienic as well as social, reinforcing social bonds and increasing confidence with other individuals' odours and skin secretions (McCracken & Bradbury 1980; Wilkinson 1986; Kerth et al. 2003; Safi & Kerth 2003).

One of my experimental groups originated from the same colony, where, however, given the very young age of rescued bats (≤ 3 days of age), the time spent together before rescue had probably been too short to elicit the association phenomenon I observed in captivity. In fact, the common origin of those bats did not influence the association patterning I observed after rescue. On the other hand, factors such as possible kinship or cues such as “colony odour” (Safi & Kerth 2003) must have had negligible influence, or may have been overcome by the effect of the prolonged social experience gained in captivity.

A limit of this study is given by the fact that I only focused on *P. kuhlii*. However, I argue that this phenomenon is linked with the particular gregarious roosting behaviour of many bat species, so it is unlikely to occur only in the one I studied. One of the most dynamic social structures known for bats is observed in forest species, whose small groups are spread over

different roosting areas, switch roosts frequently and are subject to fission-fusion patterns (Kerth & König 1999; Willis & Brigham 2004; Russo et al. 2005; Popa-Lisseanu et al. 2008). In such species, non-random, preferred associations of individuals – i.e. cohesive subgroups within a meta-colonial system – have been noticed (Willis & Brigham 2004; Garroway & Broders 2007; Patriquin et al. 2010). Affiliation mechanisms such as the one I describe might have a role in influencing these association patterns, albeit testing this in nature is most challenging. Besides, they might induce the development of cryptic social subunits also within large colonies of bats faithful to their roost, such as cave-dwelling or house-dwelling species (Lewis 1996).

This study may also have some consequences for the improvement of protocols for the release of rescued bats in nature. Specifically, I recommend planning the selection of bats to be released in the wild so that once emancipated those reared together will be released at the same site, a practice increasing the likelihood of cohesion following release and the onset of e.g. communal roosting which might increase survival likelihood by favouring social thermoregulation and increasing antipredatory vigilance.

Mechanisms of interspecific social cohesion

My results indicate a strong effect of early social environment on the patterns of association and interaction among bats of different species, highlighting that imprinting-like mechanisms may lead to interspecific social aggregation and cooperation and overcome species behavioural barriers. Interspecific associations of bats, commonly reported in field studies, are generally explained in terms of social thermoregulation or as a consequence of limited roost availability (McFarlane 1989; Arita & Vargas 1995; Rodriguez-Duran 1998). The species I tested usually roost in buildings so their roosts are unlikely to be limiting (Dietz et al. 2009): their MSGs formed in natural conditions (Ancillotto et al. 2013) must then be explained otherwise.

Associations between different bat species are frequent and may be recorded year round (Dietz et al. 2009). So far, direct amicable social interactions between species involved in MSGs have not been recorded in natural conditions (Swift & Racey 1983; Wohlgenant 1994), but clustering of individuals from different species is not rare (Serra-Cobo et al. 2002; Boratyński & Kokurewicz 2012). I found that such associations are not restricted to relatively passive behaviours such as physical contact, but can also lead to cooperative behaviours (e.g. reciprocal grooming). Noticeably, such cooperative behaviours appear at a very early age in bats (e.g. Figure S8 in Appendices).

Among the advantages of interspecific social relationships, familiarity with heterospecifics may enhance the chances of social learning (Clarín et al. 2014) between individuals sharing the same roost, thus increasing information transfer and its quality (Goodale et al. 2010).

Surprisingly, for all affiliative behaviours I found strong effects of individual's previous social experience (bats selectively established social interactions with familiar individuals) but no effect of species. For most aspects, bats of the two species showed a similar tendency to heterospecific interactions, occupied equivalent positions and played similar roles within the

social structure. When grooming mutually, bats from one species (*P. kuhlii*) were more often the initiator of a behavioural event – perhaps this reflect the tendency of *P. kuhlii* to live in large colonies (pers. obs.) and thus be more social –, particularly when body condition was low. Bats showing lower body condition were also more prone to initiate huddling with other individuals. Perhaps these bats had more urgent thermoregulation needs or their tendency to establish body contact may have a social explanation.

The degree of clustering changed according to the behaviour recorded: it was loose for general physical contacts and huddling, much tighter for allogrooming. Because allogrooming implies spatial tolerance as well as coordinated movements between interacting individuals (Grueter et al. 2013) bats strictly selected members from the same original raising group to perform it. There is evidence from primates that social grooming is a social- rather than hygiene- related interaction (Dunbar 1991), thus the occurrence of this behaviour between different species confirms my prediction that true social bonding may occur in MSGs of bats.

Obvious advantages of reciprocal social imprinting between two (or more) different bat species may be improved thermoregulation and higher anti-predatory vigilance, as well as effective information transfer, e.g. when searching for new suitable roosts. Interspecific eavesdropping on echolocation, social (Dorado-Correa et al. 2013) and distress calls (Russ et al. 2004) may occur to obtain information about the location of potential roosts, foraging areas or predators (Dorado-Correa et al. 2013). Although in some cases passive information transfer may have undesired consequences for the emitter so that private communication bandwidth may have evolved to minimize unintended heterospecific communication (Russo et al. 2007), in MSG formation this may convey mutual benefits to both the emitter and the receiver. The ability to recognize heterospecific calls may be an early-acquired skill learnt by bats born in mixed species colonies.

Social imprinting may also be among the possible mechanisms favouring hybridization between bat species (e.g. between *Pipistrellus* ssp.: Sztencel-Jablonka & Bogdanowicz 2012; *Myotis* ssp.: Berthier et al. 2006). Familiarity with a different species during a bat's early life may influence individual sexual preferences, as demonstrated for birds (Kruijt & Meeuwissen 1991; Slagsvold et al. 2002), fishes (Verzijden & Ten Cate 2007) and spiders (Hebets 2003). This work represents a step forward in unravelling the mechanisms underlying the phenomenon of interspecific sociality in bats. This is fundamental for understanding their behavioural and ecological interactions at the community scale, as mixed-species colonies are important priorities for the simultaneous conservation of different bat species (Rainho & Palmeirim 2013). Moreover, patterns of social behaviours and association among bats can have relevant consequences in the spread of diseases (Serra-Cobo et al. 2002; Langwig et al. 2012), thus potentially having strong impact on different population- and community- scale processes.

Final remarks

Integrating individual-based mechanisms in the study of bat social systems

Results from this work confirm that social behaviour in bats is a complex issue (Kerth 2008a), influenced both by species natural history (i.e. intrinsic factors that innately influence individuals' behaviour) and individual experience. This strong plasticity of bat sociality makes it important to adopt a multidisciplinary approach for the study of bat behaviour and social systems, as both individual- and ecological/evolutionary-based approaches may provide important insights to this complex biological issue.

The present work provides insights onto different aspects of bats' behavioural patterns, indicating that 1) ontogenetic factors (i.e. experience) are among the first acting forces upon individuals' social lives, including the preference for familiar individuals (e.g. independently from kinship), that 2) these preferences developed at a young age in bats are persistent and can be modulated, according to intrinsic factors (e.g. sex and age) and that 3) such experiential mechanisms of association can act across the species boundaries.

Such results add clear evidences to the debate upon the relative importance of kin and group cohesion in the evolution of cooperation among animals (Clutton-Brock 2002), indicating that albeit kin selection may have a significant role in shaping social dynamics among animals (Foster et al. 2006), the social behaviour of animal societies is also strongly shaped by experience (Beach & Jaynes 1954) and thus both individual ontogeny and kinship act at the same time on animal groups.

The importance of ontogenetic factors in determining cohesion and cooperation in bats is suggested by the different genetic structures found in bat colonies, which can be composed by individuals not-strictly related to each other, e.g. as a consequence of fission-fusion dynamics, male-biased dispersal and breeding at swarming sites (Burland et al. 2001; Willis & Brigham

2004; Senior et al. 2005), all mechanisms that converge towards decreasing relatedness and increase genetic diversity of colonies and populations (Burland et al. 2001; Kerth et al. 2002, 2011; Carter & Wilkinson 2013).

The ability to form cohesive groups and interact cooperatively with heterospecifics, driven by early experience, also indicates a major role of social environment in shaping bat societies and communities, also highlighting that mixed-species associations found in natural colonies are the product of more complex mechanisms than simple aggregations for thermoregulatory benefits (Bogdanowicz 1983; Stensland et al. 2003).

My findings offer a new and innovative insight into the investigation of bat sociality, with important ethological and sociobiological consequences. The forces that affect individuals engaging in social interactions may in fact influence rate and quality of information transfer within and between colonies, as well as modify patterns of disease spreading, having a fundamental role upon individuals' and populations' health and fitness, thus being relevant not only at behavioural and ecological levels, but also for the conservation of these species (Langwig et al. 2012; Rainho & Palmeirim 2013); the social dimension of behaviour is in fact a key aspect for animal ecology, potentially influencing species and communities conservation planning (Caro 1999).

Studies on a fast-spreading lethal disease in North American bat population, the White Nose Syndrome (WNS: Blehert et al. 2009) indicates a strong correlation between pathogen transmission probability and the frequency of social contacts, with solitary species being far less impacted by this syndrome respect highly social species (Langwig et al. 2012). The use of social network analysis is fundamental in highlighting the potential role of single individuals and their relative positions inside the social structure in spreading diseases within and across social species. Studies on rodents (Perkins et al. 2009) and large herbivores (Vanderwaal et al. 2013) indicate a strong relevance of an individual-based approach in

predicting disease spreading; particularly, interactions and social associations networks better overlap with pathogen spreading networks when compared to larger-scale methods, e.g. home-range overlap among individuals (Perkins et al. 2009; Vanderwaal et al. 2013).

Within this perspective, my results indicate that bats inside a colony do not all have the same probability of contact or of interacting in the same way, e.g. aggressively or cooperatively, with strong differences between sexes and age classes. Females in particular seem to be more prone to actively interact with each other, particularly inside familiar subgroups inside colonies. Thus, health monitoring of wild bat populations should start right from nurseries in order to more efficiently detect possible pathogens. As additional result, the identification of true social interactions besides physical contacts inside mixed-species groups of bats highlights the role of this kind of associations as disease spreading centres across different species, at least potentially; consequently, monitoring effort should concentrate on such type of colonies.

Consequently, studies on the social behaviour of common and widespread bat species such as those I studied may help design future research on endangered species, contributing to the conservation of these still relatively poorly known mammals.

Appendices

Supplementary figures

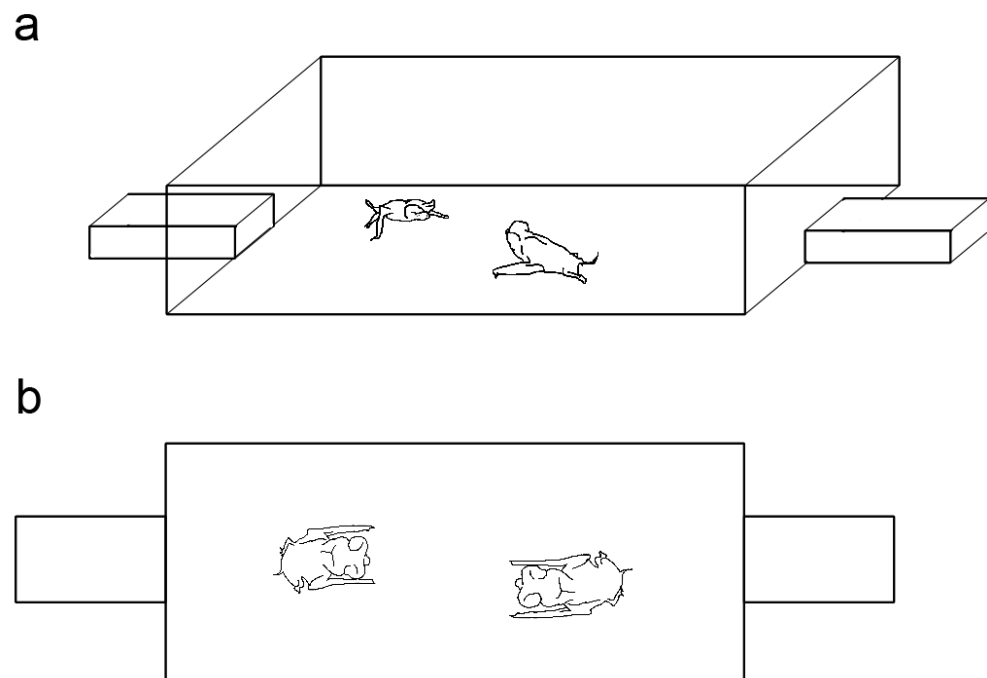


Figure S 1. Schematic representation of the arena used in Experiment 1. A= side view; b= top view.
Drawing by L. Ancillotto.



Figure S 2. Video camera mounted on tripod beneath an experimental bat box. Photo by L. Ancillotto.



Figure S 3. Newborn (age: 1-3 days) Kuhl's pipistrelle (*P. kuhlii*) on hand. Photo by L. Ancillotto.



Figure S 4. Flight-room used for Experiment 2 and Experiment 3. Experimental bat boxes and net-roosts are visible on the walls. Photo by L. Ancillotto.



Figure S 5. Two adult *T. teniotis* engaging in an aggressive display: the bat on the left is emitting an aggressive social call; the bat on the right is going to perform a chase towards the other individual. Photo by L. Ancillotto.



Figure S 6. Modified experimental bat box during inspection for data collection (Experiment 3). Coloured plastic split-rings are visible on bats' forearms for individual identification. Photo by L. Ancillotto.



Figure S 7. Examples of intra-group (i.e. bats raised in the same experimental group: see colour of plastic split-ring on individuals' right forearm) heterospecific physical contacts among young captive *H. savii* (individual on the right, in all pictures) and *P. kuhlii* (individuals on the top and left, in all pictures) raised in mixed-species groups. Photo by L. Ancillotto.

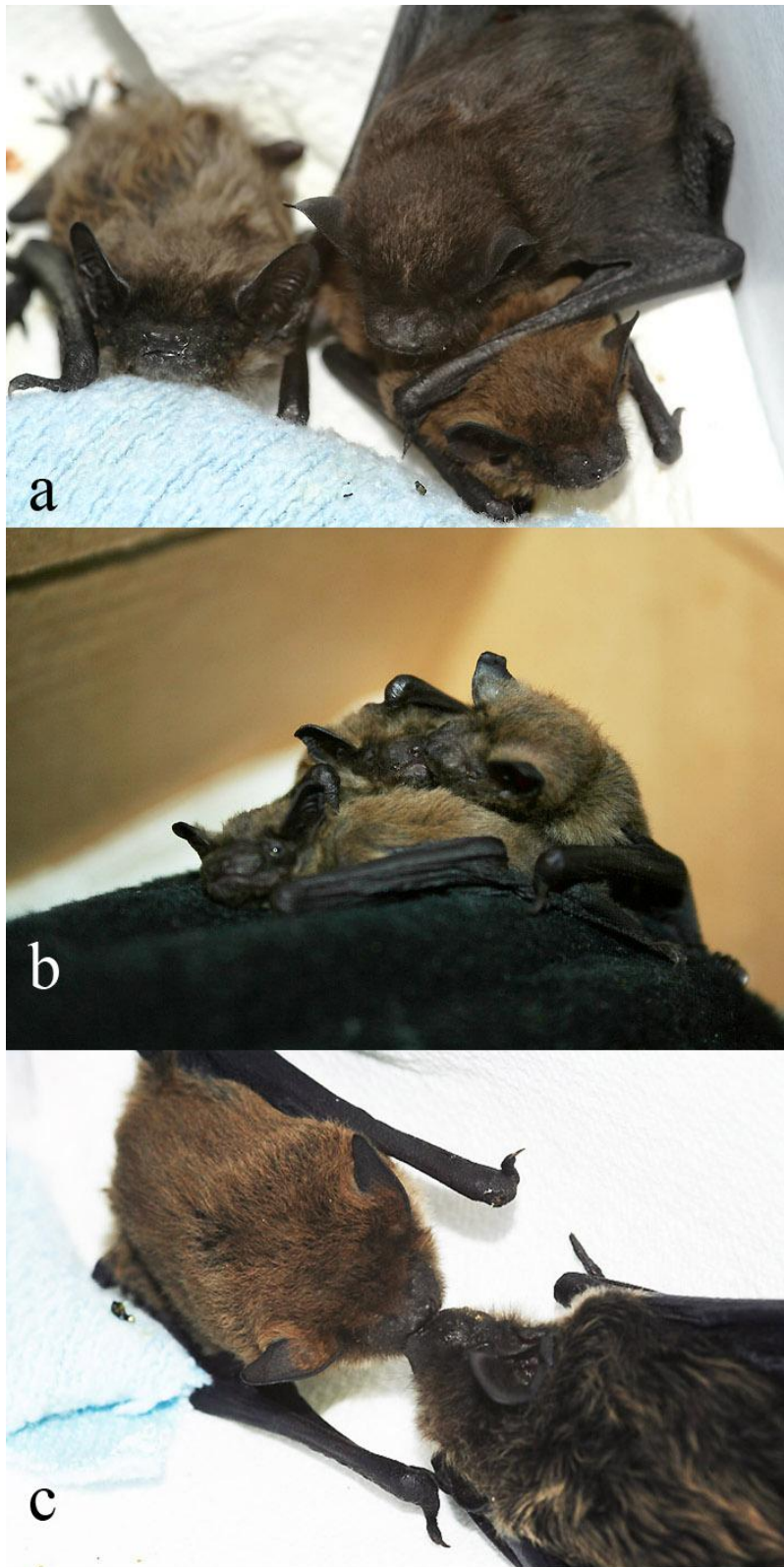


Figure S 8. Social interactions among young bats during captive rearing. a) Interspecific huddling between young *P. kuhlii* (down) and *H. savii* (up and left), b) intraspecific reciprocal grooming between young captive *P. kuhlii*; c) interspecific reciprocal grooming between young captive *P. kuhlii* (left) and *H. savii* (right). Photos by L. Ancillotto.

References

- Altmann, J.** 1974. Observational study of behavior: sampling methods. *Behaviour*, **49**, 227–267.
- Altringham, J. D.** 1996. *Bats' biology and behaviour*. Oxford, UK: Oxford University Press.
- Ancillotto, L., Serangeli, M. T. & Russo, D.** 2013. Curiosity killed the bat: domestic cats as bat predators. *Mammalian Biology - Zeitschrift für Säugetierkunde*, **78**, 369–373.
- Angell, R. L., Butlin, R. K. & Altringham, J. D.** 2013. Sexual segregation and flexible mating patterns in temperate bats. *PloS one*, **8**, e54194.
- Arita, H. T. & Vargas, J. A.** 1995. Natural-history, interspecific association, and incidence of the cave bats of Yucatan, Mexico. *Southwestern naturalist*, **40**, 29–37.
- Arlettaz, R., Guibert, E., Lugon, A., Médard, P. & Sierro, A.** 1993. Variability of fur coloration in Savi's bat *Hypsugo savii* (Bonaparte, 1837). *Bonner Zoologische Beiträge*, **44**, 293–297.
- Arnold, C. & Taborsky, B.** 2010. Social experience in early ontogeny has lasting effects on social skills in cooperatively breeding cichlids. *Behavioral Ecology and Sociobiology*, **79**, 621–630.
- Balcombe, J. P.** 1990. Vocal recognition of pups by mother Mexican free-tailed bats, *Tadarida brasiliensis mexicana*. *Animal Behaviour*, **39**, 79–87.
- Balcombe, J. P. & McCracken, G. F.** 1992. Vocal recognition in Mexican free-tailed bats: do pups recognize mothers? *Animal Behaviour*, **43**, 79–87.

- Balmori, A.** 2003. Contribution to the knowledge of the biology and social behaviour of the free-tailed bat (*Tadarida teniotis*). *Galemys*, **15**, 37–53.
- Baptista, T. L., Richardson, C. S. & Kunz, T. H.** 2000. Postnatal growth and age estimation in free-ranging bats: a comparison of longitudinal and cross-sectional sampling methods. *Journal of Mammalogy*, **81**, 709–718.
- Barnard, S.** 2009. Maintaining bats for captive studies. In: *Ecological and behavioral methods for the study of bats*, (Ed. by T. H. Kunz), pp. 329–372. Baltimore, Maryland: Johns Hopkins University Press.
- Barrett, L. & Henzi, S. P.** 2001. The utility of grooming in baboon troops. In: *Economics in nature*, (Ed. by V. Hooffjaram & P. Hammerstein), pp. 119–145. Cambridge: Cambridge University Press.
- Bateson, P. P. G.** 1979. How do sensitive periods arise and what are they for? *Animal Behaviour*, **27**, 470–486.
- Beach, F. A. & Jaynes, J.** 1954. Effects of early experience upon the behavior of animals. *Psychological Bulletin*, **51**, 239–263.
- Bekoff, M.** 1972. The development of social interaction, play and metacommunication in mammals: an ethological perspective. *The Quarterly Review of Biology*, **47**,
- Berg, K. S., Delgado, S., Okawa, R., Bessinger, S. R. & Bradbury, J. W.** 2011. Contact calls are used for individual mate recognition in free-ranging green-rumped parrotlets, *Forpus passerinus*. *Animal Behaviour*, **81**, 241–248.

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 Biological Sciences*, **273**, 3101–9.

Bishop, J. M. M., Fienberg, S. E. & Holland, P. W. 2007. *Discrete multivariate analysis*. Springer-Verlag.

Blehert, D. S., Hicks, A. C., Behr, M., Meteyer, C. U., Berlowski-zier, B. M., Buckles, E. L., Coleman, J. T. H., Darling, S. R., Gargas, A., Niver, R., Okoniewski, J. C., Rudd, R. J. & Stone, W. B. 2009. Bat White-Nose Syndrome : an emerging fungal pathogen? *Science*, **323**, 227.

Bogdanowicz, W. 1983. Community structure and interspecific interactions in bats hibernating in Poznan. *Acta Theriologica*, **28**, 357–370.

Bogdanowicz, W. 2004. *Pipistrellus kuhlii* (Kuhl, 1817) - Weissrandfledermaus. In: *Handbuch der Säugetiere Europas 4*, Aula Verla edn. (Ed. by J. Niethammer & F. Krapp), pp. 875–908. Wiesbaden.

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

Boik, R. J. 1979. Interactions, partial interactions and interaction contrasts in the analysis of variance. *Psychological Bulletin*, **86**, 1084–1089.

Boratyński, J. & Kokurewicz, T. 2012. Współwystępowanie gatunków nietoperzy w skupieniach podczas zimowania w podziemiach Centralnego Odcinka Frontu Umocnionego Łuku Odry i Warty. *Nietoperze*, **2**, 1–2.

- Borgatti, S. P., Everett, M. G. & Freeman, L. C.** 2002. Ucinet for Windows: Software for social network analysis.
- Borries, C., Sommer, V. & Srivastava, A.** 1994. Weaving a tight social net: allogrooming in free-ranging female langurs (*Presbytis entellus*). *International journal of primatology*, **15**, 421–443.
- Bouchard, S.** 2001. Sex discrimination and roostmate recognition by olfactory cues in the African bats, *Mops condylurus* and *Chaerephon pumilus* (Chiroptera: Molossidae). *Journal of Zoology (London)*, **254**, 109–117.
- Bridge, P. D.** 1993. Classification. In: *Biological data analysis*, (Ed. by J. C. Fry), pp. 219–242. Oxford, UK: Oxford University Press.
- Brigham, R. M. & Brigham, A. C.** 1989. Evidence for association between a mother bat and its young during and after foraging. *American Middle Naturalist*, **121**, 205–207.
- Brunet-Rossinni, A. K. & Austad, S. N.** 2004. Ageing studies on bats: a review. *Biogerontology*, **5**, 211–222.
- Burland, T. M., Barratt, E. M., Nichols, R. a & Racey, P. A.** 2001. Mating patterns, relatedness and the basis of natal philopatry in the brown long-eared bat, *Plecotus auritus*. *Molecular ecology*, **10**, 1309–21.
- Cameron, E. Z., Setsaas, T. H. & Linklater, W. L.** 2009. Social bonds between unrelated females increase reproductive success in feral horses. *Proceedings of the National Academy of Sciences*, **106**, 13850–13853.

Caro, T. 1999. The behaviour-conservation interface. *Trends in ecology & evolution*, **14**, 366–369.

Carter, G. G. & Wilkinson, G. S. 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.

Carter, G. G., Skowronski, M. D., Faure, P. A. & Fenton, M. B. 2008. Antiphonal calling allows individual discrimination in white-winged vampire bats. *Animal Behaviour*, **76**, 1343–1355.

Carter, G. G., Logsdon, R., Arnold, B. D., Menchaca, A. & Medellín, R. A. 2012. Adult vampire bats produce contact calls when isolated: acoustic variation by species, population, colony and individual. *PloS one*, **7**, e38791.

Chapman, C. A., Chapman, L. J. & Gillespie, T. R. 2002. Scale issues in the study of primate foraging: red colobus of Kibale National Park. *American journal of physical anthropology*, **117**, 349–63.

Christiansen, K. 2001. Hormones and sport: behavioural effects of androgen in men and women. *Journal of Endocrinology*, **170**, 39–48.

Chruszcz, B. J. & Barclay, R. M. R. 2002. Thermoregulatory ecology of a solitary bat, *Myotis evotis*, roosting in rock crevices. *Functional Ecology*, **16**, 18–26.

Clarín, T. M. A., Borissov, I., Page, R. A., Ratcliffe, J. M. & Siemers, B. M. 2014. Social learning within and across species: information transfer in mouse-eared bats. *Canadian Journal of Zoology*, **92**, 129–139.

- Clauset, A., Newman, M. & Moore, C.** 2004. Finding community structure in very large networks. *Physical Review E*, **70**, 066111.
- Clutton-Brock, T.** 2002. Breeding together: kin selection and mutualism in cooperative vertebrates. *Science*, **296**, 69–72.
- Coetzee, H. C. & Province, N. W.** 2010. Observations of southern ground-hornbill *Bucorvus leadbeateri* grooming common warthog *Phacocoerus africanus*. *African Journal of Ecology*, **48**, 1131–1133.
- Conover, W. J.** 1980. *Practical non-parametric statistics*. 2nd edn. New York, US: John Wiley and Sons.
- Croft, D. P., Krause, J. & James, R.** 2004. Social networks in the guppy (*Poecilia reticulata*). *Proceedings of the Royal Society B Biological Sciences*, **271 Suppl**, S516–9.
- D'Eath, R. B. & Stone, R. J.** 1999. Chickens use visual cues in social discrimination: an experiment with coloured lighting. *Applied animal behaviour science*, **62**, 233–242.
- Davis, R. B., Herreid, C. F. & Short, H. L.** 1962. Mexican free-tailed bats in Texas. *Ecological Monographs*, **32**, 311–346.
- De Fanis, E. & Jones, G.** 1995a. The role of odour in the discrimination of conspecifics by pipistrelle bats. *Animal Behaviour*, **49**, 835–839.
- De Fanis, E. & Jones, G.** 1995b. Postnatal growth, mother-infant interactions and development of vocalizations in the vespertilionid bat *Plecotus auritus*. *Journal of Zoology (London)*, **235**, 85–97.

- De Villiers, M. S., Richardson, P. R. & van Jaarsveld, A. S.** 2003. Patterns of coalition formation and spatial association in a social carnivore, the African wild dog (*Lycaon pictus*). *Journal of Zoology (London)*, **260**, 377–389.
- Dechmann, D. K. N., Kalko, E. K. V & Kerth, G.** 2007. All-offspring dispersal in a tropical mammal with resource defense polygyny. *Behavioral Ecology and Sociobiology*, **61**, 1219–1228.
- Dechmann, D. K. N., Kranstauber, B., Gibbs, D. & Wikelski, M.** 2010. Group hunting—a reason for sociality in molossid bats? *PloS one*, **5**, e9012.
- Delpietro, V. A. & Russo, R. G.** 2002. Observations of the common vampire bat (*Desmodus rotundus*) and the hairy-legged vampire bat (*Diphylla ecaudata*) in captivity. *Mammalian Biology*, **67**, 65–78.
- Desbiez, A. L. J., Rocha, F. L. & Keuroghlian, A.** 2010. Interspecific association between an ungulate and a carnivore or a primate. *Acta Ethologica*, **13**, 137–139.
- Di Bitetti, M. S.** 1996. Evidence for an important social role of allogrooming in a platyrrhine primate. *Animal Behaviour*, **54**, 199–211.
- Dietz, C., von Helversen, O. & Nill, D.** 2009. *Bats of Britain, Europe and Northwest Africa*. London: A & C Black.
- Dorado-Correa, A. M., Goerlitz, H. R. & Siemers, B. M.** 2013. Interspecific acoustic recognition in two European bat communities. *Frontiers in physiology*, **4**, 192.
- Dunbar, R. I. M.** 1991. Functional significance of social grooming in primates. *Folia Primatologica*, **57**, 121–131.

- Eales, L. A., Bullock, D. J. & Slater, P. J. B.** 1988. Shared nursing in captive pipistrelles (*Pipistrellus pipistrellus*). *Journal of Zoology (London)*, **216**, 584–587.
- Elizalde-Arellano, C., Lopez-Vidal, J. C., Arroyo-Cabrales, J., Medellín, R. A. & Laundre, J. W.** 2000. Food sharing behavior in the hairy-legged vampire bat *Diphylla ecaudata*. 314–322.
- Emlen, S. T.** 1994. Benefits, constraints and the evolution of the family. *Trends in ecology & evolution*, **9**, 282–285.
- Englert, A. C. & Greene, M. J.** 2009. Chemically-mediated roostmate recognition and roost selection by Brazilian free-tailed bats (*Tadarida brasiliensis*). *PloS one*, e7781.
- Farine, D. R., Garroway, C. J. & Sheldon, B. C.** 2012. Social network analysis of mixed-species flocks: exploring the structure and evolution of interspecific social behaviour. *Animal Behaviour*, **84**, 1271–1277.
- Fitzgibbons, C.** 1990. Mixed-species grouping in Thomson's and Grant's gazelles: the antipredator benefits. *Animal Behaviour*, **39**, 1116–1126.
- Foster, K. R., Wenseleers, T. & Ratnieks, F. L. W.** 2006. Kin selection is the key to altruism. *Trends in ecology & evolution*, **21**, 57–60.
- Frantzis, A. & Herzing, D. L.** 2002. Mixed-species associations of striped dolphins (*Stenella coeruleoalba*), short-beaked common dolphins (*Delphinus delphis*), and Risso's dolphins (*Grampus griseus*) in the Gulf of Corinth (Greece, Mediterranean Sea). *Aquatic mammals*, **28**, 188–197.

- Freitas, J. N., ElHani, C. N. & Da Rocha, P. L.** 2008. Affiliation in four echimyid rodent species based on intrasexual dyadic encounters: evolutionary implications. *Ethology*, **114**, 389–397.
- Garroway, C. J. & Broders, H. G.** 2007. Nonrandom association patterns at northern long-eared bat maternity roosts. *Canadian Journal of Zoology*, **85**, 956–964.
- Geipel, I., Kalko, E. K. V, Wallmeyer, K. & Knörnschild, M.** 2013. Postweaning maternal food provisioning in a bat with a complex hunting strategy. *Animal Behaviour*, **85**, 1435–1441.
- Gibson, Q. A. & Mann, J.** 2008. Early social development in wild bottlenose dolphins: sex differences, individual variation and maternal influence. *Animal Behaviour*, **76**, 375–387.
- Goodale, E., Beauchamp, G., Magrath, R. D., Nieh, J. C. & Ruxton, G. D.** 2010. Interspecific information transfer influences animal community structure. *Trends in ecology & evolution*, **25**, 354–61.
- Goymann, W., East, M. L. & Hofer, H.** 2001. Androgens and the role of female “hyperaggressiveness” in spotted hyenas (*Crocuta crocuta*). *Hormones and behavior*, **39**, 83–92.
- Grafen, A.** 1990. Do animals really recognize kin? *Animal Behaviour*, **39**, 42–54.
- Grueter, C. C., Bissonnette, A., Isler, K. & Schaik, C. P. Van.** 2013. Grooming and group cohesion in primates: implications for the evolution of language. *Evolution and Human Behavior*, **34**, 61–68.

- Gustin, M. K. & McCracken, G. F.** 1987. Scent recognition between females and pups in *Tadarida brasiliensis mexicana*. *Animal Behaviour*, **35**, 13–19.
- Hanneman, R. A. & Riddle, M.** 1998. Introduction to social network methods. University of California, Riverside.
- Hart, B. & Hart, L. A.** 1992. Reciprocal allogrooming in impala, *Aepyceros melampus*. *Animal Behaviour*, **44**, 1073–1083.
- Hebets, E.** 2003. Subadult experience influences adult mate choice in an arthropod: exposed female wolf spiders prefer males of a familiar phenotype. *Proceedings of the National Academy of Sciences of the United States of America*, **100**, 13390–5.
- Heymann, E. W. & Buchanan-Smith, H. M.** 2000. The behavioural ecology of mixed-species troops of callitrichine primates. *Biological reviews of the Cambridge Philosophical Society*, **75**, 169–90.
- Hinde, R. A.** 1974. *Biological bases of human social behaviour*. New York, US: McGraw-Hill.
- Hirsch, B. T.** 2007. Spoiled brats: is extreme juvenile agonism in ring-tailed coatis (*Nasua nasua*) dominance or tolerated aggression? *Ethology*, **113**, 446–456.
- Hutson, A. M. & Mickleburgh, Si. P. Eds.** 2001. *Microchiropteran bats: global status survey and conservation action plan*. IUCN.
- IUCN.** 2013. IUCN red list of threatened species. Version 2013.2.
- James, R., Croft, D. P. & Krause, J.** 2009. Potential banana skins in animal social network analysis. *Behavioral Ecology and Sociobiology*, **63**, 989–997.

- Jones, G. & Ransome, R. D.** 1993. Echolocation calls of bats are influenced by maternal effects and change over a lifetime. *Proceedings of the Royal Society B Biological Sciences*, **252**, 125–128.
- Karavanich, C. & Atema, J.** 1998. Individual recognition and memory in lobster dominance. *Animal Behaviour*, **56**, 1553–1560.
- Kelly, A., Goodwin, S., Grogan, A. & Mathews, F.** 2008. Post release survival of hand-reared pipistrelle bats (*Pipistrellus* spp.). *Animal Welfare*, **17**, 375–382.
- Kerth, G.** 2008a. Causes and consequences of sociality in bats. *Bioscience*, **58**, 737–746.
- Kerth, G.** 2008b. Animal sociality: bat colonies are founded by relatives. *Current Biology*, **18**,
- Kerth, G. & König, B.** 1999. Fission, fusion and nonrandom associations in female Bechstein's bats (*Myotis bechsteinii*). *Behaviour*, **136**, 1187–1202.
- Kerth, G., Wagner, M. & König, B.** 2001. Roosting together, foraging apart: information transfer about food is unlikely to explain sociality in female Bechstein's bats (*Myotis bechsteinii*). *Behavioral Ecology and Sociobiology*, **50**, 283–291.
- Kerth, G., Safi, K. & König, B.** 2002. Mean colony relatedness is a poor predictor of colony structure and female philopatry in the communally breeding Bechstein's bat (*Myotis bechsteinii*). *Behavioral Ecology and Sociobiology*, **52**, 203–210.
- Kerth, G., Almasi, B., Ribí, N., Thiel, D. & Lupold, S.** 2003. Social interactions among wild female Bechstein's bats (*Myotis bechsteinii*) living in a maternity colony. *Acta Ethologica*, **5**, 107–114.

- Kerth, G., Perony, N. & Schweitzer, F.** 2011. Bats are able to maintain long-term social relationships despite the high fission-fusion dynamics of their groups. *Proceedings of the Royal Society B Biological Sciences*, **278**, 2761–7.
- Kite, P. D.** 1966. The focal occurrence of histoplasmosis in house-dwelling bats on the Isthmus of Panama. *Medical mycology*, **4**, 158–163.
- Koda, H.** 2012. Possible use of heterospecific food-associated calls of macaques by sika deer for foraging efficiency. *Behavioural processes*, **91**, 30–4.
- Kotze, J., Bennett, N. C. & Scantlebury, M.** 2008. The energetics of huddling in two species of mole-rat (Rodentia: Bathyergidae). *Physiology & behavior*, **93**, 215–221.
- Krause, J., Croft, D. P. & James, R.** 2007. Social network theory in the behavioural sciences: potential applications. *Behavioral Ecology and Sociobiology*, **62**, 15–27.
- Krause, J., Lusseau, D. & James, R.** 2009. Animal social networks: an introduction. *Behavioral Ecology and Sociobiology*, **63**, 967–973.
- Kruijt, J. P. & Meeuwissen, G. P.** 1991. Sexual preferences of male zebra finches: effects of early and adult experience. *Animal Behaviour*, **42**, 91–102.
- Kunz, T. H. & Fenton, M. B.** 2006. *Bat ecology*. Chicago: University of Chicago Press.
- Kurta, A., Bell, G. P., Nagy, K. A. & Kunz, T. H.** 1989. Energetics of pregnancy and lactation in free-ranging little brown bats (*Myotis lucifugus*). *Physiological Zoology*, **62**, 804–818.
- LaDage, L. D. & Ferkin, M. H.** 2006. Male leopard geckos (*Eublepharis macularius*) can discriminate between two familiar females. *Behaviour*, **141**, 1033–1049.

- Langwig, K. E., Frick, W. F., Bried, J. T., Hicks, A. C., Kunz, T. H. & Kilpatrick, M. A.** 2012. Sociality, density-dependence and microclimates determine the persistence of populations suffering from a novel fungal disease, white-nose syndrome. *Ecology letters*, **15**, 1050–7.
- Laviola, G. & Terranova, M. L.** 1998. The developmental psychobiology of behavioural plasticity in mice: the role of social experiences in the family unit. *Neuroscience and behavioural reviews*, **23**, 197–213.
- 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**, e54987.
- Lewis, S. E.** 1996. Low roost-site fidelity in pallid bats: associated factors and effect of group stability. *Behavioral Ecology and Sociobiology*, **39**, 335–344.
- Ligout, S., Foulquière, D., Sèbe, F., Bouix, J. & Boissy, A.** 2011. Assessment of sociability in farm animals: the use of arena test in lambs. *Applied animal behaviour science*, **135**, 57–62.
- Lorenz, K.** 1963. *On aggression*. New York: Mariner Books.
- Lusseau, D., Whitehead, H. & Gero, S.** 2008. Incorporating uncertainty into the study of animal social networks. *Animal Behaviour*, **75**, 1809–1815.
- Makagon, M. M., McCowan, B. & Mench, J. a.** 2012. How can social network analysis contribute to social behavior research in applied ethology? *Applied animal behaviour science*, **138**, 152–161.
- Mann, O., Lieberman, V., Köhler, A., Korine, C., Hedworth, H. E. & Voigt-Heucke, S. L.** 2011. Finding your friends at a densely populated roosting place: male Egyptian fruit bats

(*Rousettus aegyptiacus*) distinguish between familiar and unfamiliar conspecifics. *Acta Chiropterologica*, **13**, 411–417.

Manno, T. G. 2008. Social networking in the Columbian ground squirrel, *Spermophilus columbianus*. *Animal Behaviour*, **75**, 1221–1228.

Margulis, S. W., Nabong, M., Alaks, G., Walsh, A. & Lacy, R. C. 2005. Effects of early experience on subsequent parental behaviour and reproductive success in oldfield mice, *Peromyscus polionotus*. *Animal Behaviour*, **69**, 627–634.

Martin, P. & Bateson, P. P. G. 1993. *Measuring behaviour: an introductory guide*. Cambridge: Cambridge University Press.

Mateo, J. M. 2009. The causal role of odour in the development of recognition templates and social preferences. *Animal Behaviour*, **77**, 115–121.

McCracken, G. F. 1984a. Communal nursing in Mexican free-tailed bat maternity colonies. *Science*, **223**, 1090–1109.

McCracken, G. F. 1984b. Social dispersion and genetic variation in two species of emballonurid bats. *Zoologischer Tierpsychologie*, **66**, 55–69.

McCracken, G. F. & Bradbury, J. W. 1980. Social organisation and kinship in the polygynous bat *Phyllostomus hastatus*. *Behavioral Ecology and Sociobiology*, **8**, 11–34.

McCracken, G. F. & Wilkinson, G. S. 2000. Bat mating systems. In: *Reproductive biology of bats*, (Ed. by E. G. Crichton & P. H. Krutzsch), pp. 321–362. New York, US: Academic Press.

- McFarlane, D. A.** 1989. Patterns of species co-occurrence in the Antillean bat fauna. *Mammalia*, **53**, 59–66.
- McGowan, A., Sharp, S. P., Simeoni, M. & Hatchwell, B. J.** 2006. Competing for position in the communal roosts of long-tailed tits. *Animal Behaviour*, **72**, 1035–1043.
- McPherson, M., Smith-lovin, L. & Cook, J. M.** 2001. Birds of a feather: homophily in social networks. *Annual review of sociology*, **27**, 415–444.
- Menzel, F., Linsenmair, K. E. & Blüthgen, N.** 2008. Selective interspecific tolerance in tropical *Crematogaster–Camponotus* associations. *Animal Behaviour*, **75**, 837–846.
- Milligan, G. W. & Cooper, M. C.** 1987. Methodology review: clustering methods. *Applied Psychological Measurement*, **11**, 329–354.
- Mitchel-Jones, A. J. & McLeish, A. P.** 2004. *Bat worker's manual*. Peterborough, UK: Joint Nature Conservation Committee.
- Morais, A. R., Batista, V. G., Gambale, P. G., Signorelli, L. & Bastos, R. P.** 2012. Acoustic communication in a Neotropical frog (*Dendropsophus minutus*): vocal repertoire, variability and individual discrimination. *Herpetology journal*, **22**, 249–257.
- Munoz-Romo, M.** 2006. Ethogram and diurnal activities of a colony of *Artibeus lituratus* (Phyllostomidae: Stenodermatinae). *Acta Chiropterologica*, **8**, 231–238.
- Murdoch, J., Ralls, K., Cypher, B. & Reading, R.** 2008. Social interactions among San Joaquin kit foxes before, during and after the mating season. *Journal of Mammalogy*, **89**, 1087–1093.

- Nagy, M., Günther, L., Knörnschild, M. & Mayer, F.** 2013. Female-biased dispersal in a bat with a female-defence mating strategy. *Molecular ecology*, **22**, 1733–1745.
- Newman, M. E. J.** 2006. Modularity and community structure in networks. *Proceedings of the National Academy of Sciences*, **103**, 8577–8582.
- Nielsen, L. T., Eaton, D. K., Wright, D. W. & Schmidt-French, B.** 2006. Characteristic odors of *Tadarida brasiliensis mexicana* Chiroptera: Molossidae. *Journal of cave and karst studies*, **68**, 27–31.
- Ortega, J. & Maldonado, J. E.** 2006. Female interactions in harem groups of the Jamaican fruit-eating bat *Artibeus jamaicensis* (Chiroptera: Phyllostomidae). *Acta Chiropterologica*, **8**, 485–495.
- Ortega, J., Guerrero, J. A. & Maldonado, J. E.** 2008. Aggression and tolerance by dominant males of *Artibeus jamaicensis*: strategies to maximize fitness in harem groups. *Acta Chiropterologica*, **89**, 1372–1378.
- Paolucci, M., Conte, R. & Tosto, G. D.** 2006. A model of social organization and evolution of food sharing in vampire bats. *Adaptive behaviour*, **14**, 223–238.
- Patriquin, K. J., Leonard, M. L., Broders, H. G. & Garroway, C. J.** 2010. Do social networks of female northern long-eared bats vary with reproductive period and age? *Behavioral Ecology and Sociobiology*, **64**, 899–913.
- Patris, B., Gouat, P., Jacquot, C., Christophe, N. & Baudoin, C.** 2002. Agonistic and sociable behaviors in the mound-building mice, *Mus spicilegus*: a comparative study with *Mus musculus domesticus*. *Aggressive behavior*, **28**, 75–84.

- Peig, J. & Green, A. J.** 2009. New perspectives for estimating body condition from mass/length data: the scaled mass index as an alternative method. *Oikos*, **118**, 1883–1891.
- Perkins, S. E., Cagnacci, F., Stradiotto, A., Arnoldi, D. & Hudson, P. J.** 2009. Comparison of social networks derived from ecological data: implications for inferring infectious disease dynamics. *The Journal of animal ecology*, **78**, 1015–22.
- Pfalzer, G. & Kusch, J.** 2003. Structure and variability of bat social calls: implications for specificity and individual recognition. *Journal of Zoology*, **261**, 21–33.
- Pinheiro, J. C. & Bates, D. M.** 2000. *Linear mixed-effects models: basic concepts and examples*. New York, US: Springer-Verlag.
- Popa-Lisseanu, A. G., Bontadina, F., Mora, O. & Ibanez, C.** 2008. Highly structured fission-fusion societies in an aerial-hawking, carnivorous bat. *Animal Behaviour*, **75**, 471–482.
- Proops, L., McComb, K. & Reby, D.** 2009. Cross-modal individual recognition in domestic horses (*Equus caballus*). *Proceedings of the National Academy of Sciences*, **106**, 947–951.
- Prothero, J. & Jürgens, K. D.** 1987. Scaling of maximal lifespan in mammals. In: *Evolution of longevity in animals*, pp. 49–74. Springer US.
- R Core Team.** 2005. R: A language and environment for statistical computing.
- Racey, P. A.** 1973. Environmental factors affecting the length of gestation in heterothermic bats. *Journal of Reproduction and Fertility*, **19**, 175–189.
- Raghuram, H. & Marimuthu, G.** 2007. Maternal feeding of offspring with vertebrate prey in captive Indian false vampire bat, *Megaderma lyra*. *Acta Chiropterologica*, **9**, 437–443.

- Rainho, A. & Palmeirim, J. M.** 2013. Prioritizing conservation areas around multispecies bat colonies using spatial modeling. *Animal Conservation*, **16**, 438–448.
- Rhodes, M.** 2007. Roost fidelity and fission-fusion dynamics of white-striped free-tailed bats (*Tadarida australis*). *Journal of Mammalogy*, **88**, 1252–1260.
- Rodriguez-Duran, A.** 1998. Nonrandom aggregations and distribution of cave-dwelling bats in Puerto Rico. *Journal of Mammalogy*, **79**, 141–146.
- Rossiter, S. J., Jones, G., Ransome, R. D. & Barratt, E. M.** 2002. Relatedness structure and kin-biased foraging in the greater horseshoe bat (*Rhinolophus ferrumequinum*). *Behavioral Ecology and Sociobiology*, **51**, 510–518.
- Ruggiero, R. G.** 1996. Interspecific feeding associations: mutualism and semi-parasitism between Hippopotami Hippopotamus amphibius and African Jacanas Actophilornis africanus. *Ibis*, **138**, 346–348.
- Ruggiero, R. G. & Eves, H. É.** 1998. Bird-mammal associations in forest openings of northern Congo (Brazzaville). *African Journal of Ecology*, **36**, 183–193.
- Russ, J. M., Jones, G., Mackie, I. J. & Racey, P. A.** 2004. Interspecific responses to distress calls in bats (Chiroptera: Vespertilionidae): a function for convergence in call design? *Animal Behaviour*, **67**, 1005–1014.
- Russo, D., Cistrone, L. & Jones, G.** 2005. Spatial and temporal patterns of roost use by tree-dwelling barbastelle bats *Barbastella barbastellus*. *Ecography*, **28**, 769–776.

- Russo, D., Mucedda, M., Bello, M., Biscardi, S., Pidinchedda, E. & Jones, G.** 2007. Divergent echolocation call frequencies in insular rhinolophids (Chiroptera): a case of character displacement? *Journal of Biogeography*, **34**, 2129–2138.
- Sachanowicz, K., Wower, A. & Bashta, A.-T.** 2006. Further range extension of *Pipistrellus kuhlii* (Kuhl, 1817) in central and eastern Europe. *Acta Chiropterologica*, **8**, 543–548.
- Safi, K.** 2008. Social bats: the males' perspective. *Journal of Mammalogy*, **89**, 1342–1350.
- Safi, K. & Kerth, G.** 2003. Secretions of the interaural gland contain information about individuality and colony membership in the Bechstein's bat. *Animal Behaviour*, **65**, 363–369.
- Sagot, M. & Stevens, R. D.** 2011. The evolution of group stability and roost lifespan : perspectives from tent-roosting bats. *Biotropica*, 1–7.
- Scott, E. M., Mann, J., Watson-capps, J. J., Sargeant, B. L. & Connor, R. C.** 2004. Aggression in bottlenose dolphins : evidence for sexual coercion , male-male competition , and female tolerance through analysis of tooth-rake marks and behaviour. *Behaviour*, 21–44.
- Senior, P., Butlin, R. K. & Altringham, J. D.** 2005. Sex and segregation in temperate bats. *Proceedings of the Royal Society B Biological Sciences*, **272**, 2467–73.
- Serangeli, M. T., Cistrone, L., Ancillotto, L., Tomassini, A. & Russo, D.** 2012. The post-release fate of hand-reared orphaned bats: survival and habitat. *Animal Welfare*, **21**, 9–18.
- Serra-Cobo, J., Amengual, B., Abellán, C. & Bourhy, H.** 2002. European bat lyssavirus infection in Spanish bat populations. *Emerging infectious diseases*, **8**, 413–20.
- Seyfarth, R. M. & Cheney, D. L.** 1984. Grooming, alliances and reciprocal altruism in vervet monkeys. *Nature*, **308**, 541–543.

Siemers, B. M. & Kerth, G. 2006. Do echolocation calls of wild colony-living Bechstein's bats (*Myotis bechsteinii*) provide individual-specific signatures? *Behavioral Ecology and Sociobiology*, **59**, 443–454.

Siemers, B. M. & Page, R. 2009. Behavioural studies of bats in captivity: methodology, training, and experimental design. In: *Ecological and behavioural methods for the study of bats*, 2nd edn. (Ed. by T. H. Kunz & S. Parsons), pp. 373–392. Baltimore: The John Hopkins University Press.

Silk, J. B., Beehner, J. C., Bergman, T. J., Wittig, C. R. M., Seyfarth, R. M. & Cheney, D. L. 2010. Strong and consistent social bonds enhance the longevity of female baboons. *Current Biology*, **20**, 1359–1361.

Slagsvold, T., Hansen, B. T., Johannessen, L. E. & Lifjeld, J. T. 2002. Mate choice and imprinting in birds studied by cross-fostering in the wild. *Proceedings of the Royal Society B Biological Sciences*, **269**, 1449–55.

Speakman, J. R. 2008. The physiological costs of reproduction in small mammals. *Philosophical Transactions of the Royal Society: Biological Science*, **363**, 375–398.

Spencer-Booth, Y. 1971. The relationship between mammalian young and conspecifics other than mothers and peers: a review. *Advances in the study of behavior*, **3**, 119–194.

Spitzenberger, F. 1997. Distribution and range expansion of Savi's bat (*Hypsugo savii*) in Austria. *Zeitschrift für saugtierkunde*, **62**, 179–181.

Sridhar, H., Beauchamp, G. & Shanker, K. 2009. Why do birds participate in mixed-species foraging flocks? A large-scale synthesis. *Animal Behaviour*, **78**, 337–347.

- Stensland, E. V. A., Angerbjörn, A. & Berggren, P. E. R.** 2003. Mixed species groups in mammals. *Mammal Review*, **33**, 205–223.
- Swift, S. M. & Racey, P. A.** 1983. Resource partitioning in two species of vespertilionid bats (Chiroptera) occupying the same roost. *Journal of Zoology (London)*, **200**, 249–259.
- Szenczi, P. M., Bànszegi, O., Groò, Z. & Altbäcker, V.** 2012. Development of the social behavior of two mice species with contrasting social systems. *Aggressive behavior*, **38**, 288–297.
- Sztencel-Jablonka, A. & Bogdanowicz, W.** 2012. Population genetics study of common (*Pipistrellus pipistrellus*) and soprano (*Pipistrellus pygmaeus*) pipistrelle bats from central Europe suggests interspecific hybridization. *Canadian Journal of Zoology*, **90**, 1251–1260.
- Tomassini, A., Colangelo, P., Agnelli, P., Jones, G. & Russo, D.** 2013. Cranial size has increased over 133 years in a common bat, *Pipistrellus kuhlii*: a response to changing climate or urbanization? *Journal of Biogeography*,
- Tòth, E. & Duffy, J. E.** 2005. Coordinated group response to nest intruders in social shrimp. *Biology letters*, **1**, 49–52.
- Townsend, S. W., Allen, C. & Manser, M. B.** 2012. A simple test of vocal individual recognition in wild meerkats. *Biology letters*, **8**, 179–182.
- Tricarico, E., Borrelli, L., Gherardi, F. & Fiorito, G.** 2011. I know my neighbour: individual recognition on *Octopus vulgaris*. *PloS one*, **6**, e18710.
- Trivers, R. L.** 1971. The evolution of reciprocal altruism. *The Quarterly Review of Biology*, **46**, 35–57.

- Van Horn, R. C., Buchan, J. C., Altmann, J. & Alberts, S. C.** 2007. Divided destinies: group choice by female savannah baboons during social group fission. *Behavioral Ecology and Sociobiology*, **61**, 1823–1837.
- Vanderwaal, K. L., Atwill, E. R., Isbell, L. a & McCowan, B.** 2013. Linking social and pathogen transmission networks using microbial genetics in giraffe (*Giraffa camelopardalis*). *The Journal of animal ecology*,
- Verzijden, M. N. & Ten Cate, C.** 2007. Early learning influences species assortative mating preferences in Lake Victoria cichlid fish. *Biology letters*, **3**, 134–6.
- Voigt-Heucke, S. L., Taborsky, M. & Dechmann, D. K. N.** 2010. A dual function of echolocation: bats use echolocation calls to identify familiar and unfamiliar individuals. *Animal Behaviour*, **80**, 59–67.
- Von Engelhard, N., Kappeler, P. M. & Heistermann, M.** 2000. Androgen levels and female social dominance in *Lemur catta*. *Proceedings. Biological sciences / The Royal Society*, **267**, 1533–1539.
- Ward, A., Axford, S. & Krause, J.** 2002. Mixed-species shoaling in fish: the sensory mechanisms and costs of shoal choice. *Behavioral Ecology and Sociobiology*, **52**, 182–187.
- Wey, T., Blumstein, D. T., Shen, W. & Jordán, F.** 2008. Social network analysis of animal behaviour: a promising tool for the study of sociality. *Animal Behaviour*, **75**, 333–344.
- Whitehead, H.** 2008. *Analyzing animal societies: quantitative methods for vertebrate social analysis*. Chicago: University of Chicago Press.

- Whitehead, H. & Dufault, S.** 1999. Techniques for analyzing vertebrate social structure using identified individuals: review and recommendations. *Advances in the study of behavior*, **28**, 33–74.
- Whitesides, G.** 1989. Interspecific associations of Diana monkeys, *Cercopithecus diana*, in Sierra Leone, West Africa: biological significance or chance? *Animal Behaviour*, **37**, 760–776.
- Wilkinson, G. S.** 1984. Reciprocal food sharing in the vampire bat. *Nature*, **308**, 181–184.
- Wilkinson, G. S.** 1985. The social organization of the common vampire bat. *Behavioral Ecology and Sociobiology*, **17**, 123–134.
- Wilkinson, G. S.** 1986. Social grooming in the common vampire bat *Desmodus rotundus*. *Animal Behaviour*, **34**, 1880–1889.
- Wilkinson, G. S.** 1992. Communal nursing in the evening bat *Nycticeius humeralis*. *Behaviour*, **31**, 225–235.
- Wilkinson, G. S. & J, W. B.** 1998. Social calls coordinate foraging in greater spear-nosed bats. *Animal behaviour*, **55**, 337–50.
- Willis, C. K. R. & Brigham, R. M.** 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.
- Willis, C. K. R. & Brigham, R. M.** 2007. Social thermoregulation exerts more influence than microclimate on forest roost preferences by a cavity-dwelling bat. *Behavioral Ecology and Sociobiology*, **62**, 97–108.

Wilson, E. O. 1975. *Sociobiology, the new synthesis*. Cambridge: The Belknap Press of Harvard University Press.

Winchell, J. M. & Kunz, T. H. 1993. Sampling protocols for estimating time budgets of roosting bats. *Canadian Journal of Zoology*, **71**, 2244–2249.

Winchell, J. M. & Kunz, T. H. 1996. Day-roosting activity budgets of the eastern pipistrelle bat, *Pipistrellus subflavus* (Chiroptera: Vespertilionidae). *Canadian Journal of Zoology*, **74**, 431–441.

Wingfield, J. C., Ball, G. F., Dufty, A. M., Hegner, R. E. & Ramenofsky, M. 1987. Testosterone and aggression in birds. *American scientist*, **75**, 602–608.

Wohlgenant, T. J. 1994. Roost interactions between the common vampire bat (*Desmodus rotundus*) and two frugivorous bats (*Phyllostomus discolor* and *Sturnira lilium*) in Guanacaste, Costa Rica. *Biotropica*, **26**, 344–348.

Wolff, J. O. 1994. More on juvenile dispersal in mammals. *Oikos*, **71**, 349–352.

Yovel, Y., Melcon, M. L., Franz, M. O., Denzinger, A. & Schnitzler, H. U. 2009. The voice of bats: how greater mouse-eared bats recognize individuals based on their echolocation calls. *PLoS computational biology*, **5**, e1000400.