

**Social system and astrovirus transmission in bats  
of the European temperate zone**

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Veronika Maria Zeus

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Dekan: Prof. Dr. Gerald Kerth

1. Gutachter: Prof. Dr. Gerald Kerth

2. Gutachter: Prof. Dr. Barbara König

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# ABSTRACT



## ABSTRACT

Bats belong to the most gregarious and diverse mammals with highly complex social behaviors. Despite extensive research on their ecology and social behavior in some bat species, gained insights are restricted to only few of the more than 1300 species. In the recent past, bats have also become a central topic of a different branch of research: Since the 1990s bats came to the fore of virologists and immunologists due to the bats' apparent importance as reservoir hosts and vectors of several (mostly tropical) diseases. While this research is focused mainly on emerging infectious diseases linked to bats, and their zoonotic potential, little has been invested regarding the link between disease transmission and bat social systems.

In my work, I aim at filling this gap by merging automated daily roosting observations, social network analysis, and a virological screening in Natterer's bats (*Myotis nattereri*). In a collaborative approach, my co-workers and I analyzed the social structure of individually marked Natterer's bats, their astrovirus detection rate and transmission pathways within their colony, as well as roosting interactions between different co-occurring con- and heterospecific bat colonies.

We discovered Natterer's bats to display a very divergent social network structure that contradicts the findings of previous studies on large fission-fusion groups. Contrary to the modular social network structure found in e.g. primates or other bats species, the social network of Natterer's bats consists of only one highly interconnected community. Moreover, although the close proximity between bat hosts in the colony should strongly promote direct transmission, we found indications that astrovirus infections follow at least partly an indirect transmission pathway via contaminated roost use. Lastly, our results prove that co-occurring con- and heterospecific bat colonies, e.g. as in this study Natterer's bats, brown long-eared bats and Bechstein's bats, can influence each other in their roost use by avoiding conspecific roosts and by being attracted towards those of heterospecifics. This holds implication for the transmission of parasites and pathogens within and between different colonies with opportunities for spillovers. To conclude, this multidisciplinary study led to valuable insights in the hitherto hidden mechanisms within and among bat colonies.

## ZUSAMMENFASSUNG

Fledermäuse weisen unter allen Säugetieren eine besonders hohe Vielfalt an Sozialsystemen und hochkomplexe sozialen Verhaltensweisen auf. Trotz umfangreicher Forschung bezüglich ihrer Ökologie und ihres Sozialverhaltens sind die bisher gewonnenen Erkenntnisse auf nur wenige der über 1300 Arten beschränkt. Seit den 1990er Jahren sind Fledermäuse aufgrund ihrer potentiellen Bedeutung als Reservoirwirte und Krankheitsüberträger zudem in den Fokus von Virologen und Immunologen gerückt. Ihr Forschungsschwerpunkt liegt dabei hauptsächlich auf neu auftretenden (tropischen) Infektionskrankheiten welche mit Fledermäusen in Verbindung gebracht wurden, und deren zoonotischem Potential. Die interdisziplinären Zusammenhänge zwischen Krankheitsübertragungen und den sozialen Systemen der Fledermäuse sind jedoch bisher wenig erforscht.

Ziel meiner Arbeit war es durch die Zusammenführung eines automatisierten täglichen Quartiermonitorings, der Analyse des sozialen Netzwerks, sowie eines virologisches Screening diese Wissenslücken für Fransenfledermäuse zu schließen. In einem kooperativen Ansatz untersuchten meine Kollegen und ich die soziale Struktur der Fransenfledermäuse, ihre Astrovirenprävalenz und die Übertragungswege der Viren innerhalb der Kolonie, sowie die unterschiedlichen Wechselwirkungen zwischen verschiedenen Kolonien der gleichen sowie unterschiedlichen Arten bezüglich ihrer Quartiernutzung.

Wir haben festgestellt, dass Fransenfledermauskolonien eine sehr spezifische soziale Struktur aufweisen, welche den Ergebnissen anderer Studien bezüglich großer sozialer Gruppen mit Fission-Fusion-Verhalten widerspricht. Im Gegensatz zu dem modularen Netzwerk welches in einigen untersuchten Primaten- und Fledermausarten gefunden wurde, besteht das soziale Netzwerk der Fransenfledermäuse aus einer einzigen, stark vernetzten Einheit.

Darüber hinaus fanden wir Hinweise darauf, dass Astrovirusinfektionen in Fransenfledermäusen zumindest teilweise einem indirekten Übertragungsweg über kontaminierte Quartiere folgen, obgleich die räumliche und soziale Nähe zwischen den Kolonienmitgliedern eine direkte Virenübertragung naheliegen würde. Unsere Ergebnisse beweisen zudem, dass gemeinsam vorkommende Kolonien der gleichen oder unterschiedlicher Arten sich gegenseitig beeinflussen können, indem sie Quartiere artgleicher Kolonien vermeiden und von solchen spezifischer anderer Arten angezogen werden. Dies kann sich auf die Übertragung von Parasiten und Krankheitserregern, sowohl innerhalb einer Kolonie als

auch zwischen verschiedenen Kolonien, auswirken und gegebenenfalls zu einer Überschreitung der Artbarriere führen. Zusammenfassend wurden durch diese multidisziplinäre Studie wertvolle Erkenntnisse über die bisher verborgenen Mechanismen innerhalb und zwischen Fledermauskolonien gewonnen.



# 1 INTRODUCTION



# 1 INTRODUCTION

## 1.1 Social systems and roosting dynamics in bats

Bats belong to the most gregarious mammals. Thereby, they show a high diversity in social systems ranging from monogamous pairs of individuals (e.g. the spectral bat *Vampyrum spectrum* and lesser sac-winged bat *Saccopteryx leptura*) and harems with more or less stable composition (e.g. the greater spear-nosed bat *Phyllostomus hastatus* or the common noctule *Nyctalus noctula* during mating season) to large mixed-sex mating colonies (e.g. the Mexican free-tailed bat *Tadarida brasiliensis* or the greater mouse-eared bat *Myotis myotis*) (McCracken & Wilkinson, 2000). In Europe, social composition may vary between seasons (see for example *Nyctalus noctula* as mentioned before) but all species form maternity colonies during summer where several females - that are often related due to high philopatry (Kerth, 2008b; Rivers et al., 2005) - raise their offspring communally. Colony sizes of European temperate bat species typically range from only a few individuals (e.g. in some colonies of the brown long-eared bat *Plecotus auritus* or the lesser horseshoe bat *Rhinolophus hipposideros*) up to a thousand or more (e.g. in some colonies of the greater horseshoe bat *Rhinolophus ferrumequinum* or the greater mouse-eared bat *Myotis myotis*) (Dietz & Kiefer, 2014).

In many bat species of the temperate zone, especially forest-living ones that regularly switch communal roosts, colonies show high social dynamics. They frequently split into temporary subgroups - with a varying member composition - that use different day roosts. A behavior called “fission-fusion” (Garroway & Broders, 2007; Kerth & König, 1999; Popa-Lisseanu et al., 2008) that has also been reported in many other social mammals such as primates or cetaceans (see review by Aureli et al., 2008). The reason behind this highly dynamic roosting behavior is not completely understood yet. While it is assumed that roost switching enables to select optimal roosting conditions and reduces the risk of predation and parasite infestation (Kerth et al., 2001; Lewis, 1995; Patterson et al., 2007), fission-fusion might promote information transfer within the colony and allows to balance individual needs and group preferences (Fleischmann & Kerth, 2014; Kerth et al., 2006; Kerth & Reckardt, 2003; Popa-Lisseanu et al., 2008; Pretzlaff et al., 2010).

Despite these high fission-fusion dynamics, in several bat species individuals have been shown to maintain long-term relationships to some of their colony members (Baigger et al., 2013; Kerth et al., 2011; Patriquin et al., 2010). However, as reported for various mammalian species,

the number of close social relationships between group members seems to be limited, possibly due to cognitive constraints (Dunbar, 1991, 1992b; Kerth et al., 2011; Kudo & Dunbar, 2001; Lehmann et al., 2007; Wilkinson, 2003) or time constraints (Dunbar, 1991, 1992a; Lehmann et al., 2007). Alternatively, the limitation of close associations might be a strategy to reduce disadvantages of living in large groups such as competition for resources, infanticide risk, or vulnerability to diseases (Kashima et al., 2013; Smith et al., 2008). In general, it has been described that group size predicts group modularity in fission-fusion societies, as social links between group members become more differentiated with increasing group size (Baigger et al., 2013; Kerth et al., 2011; Kudo & Dunbar, 2001; Lehmann & Boesch, 2004). Thereby, in many mammals, the strength of associations between pairs of group members seems to be influenced by kinship, with stronger links between closer related individuals (Archie et al., 2006; Gompper et al., 1997; Kerth et al., 2003, 2011; Wilkinson, 1984). Moreover, group members that share individual traits such as age, breeding status, or sex have been described to generally form closer social links (Kerth et al., 2011; Ramos-Fernández et al., 2009; Wittemyer et al., 2005), possibly due to similar needs and demands (assortative mixing, Newman, 2003).

Despite the widespread fission-fusion behavior in bat species and the complex mechanisms behind social network dynamics which have been examined for different mammalian species (see Aureli et al., 2008), to this day detailed information on the colony structure in bats are still scarce (see reviews by Johnson, Kropczynski, & Lacki, 2013; Kerth, 2008b).

## **1.2 Bats and viruses**

Interestingly, for a long time, bats had been largely overlooked concerning their virome (Calisher, 2015). This started to change in the recent past when researchers found several novel emerging diseases of zoonotic importance to be apparently linked to bats (e.g. Hendra and Nipah virus in the 1990s; Chua et al., 2000; Murray et al., 1995). Nowadays, bats - especially from the tropics and subtropics – are in the spotlight of numerous studies on their potential importance as reservoir hosts and vectors for a variety of viruses (Calisher et al., 2006; Chan et al., 2013; Klimpel & Mehlhorn, 2014; Luis et al., 2013; Omatsu et al., 2007). These studies indicated that bats, although they rarely display any clinical symptoms (Baker et al., 2013), can harbor a variety of viruses with high zoonotic potential. Considering the sociability and mobility of bats in general, these findings may appear obvious.

While living in large social groups has its benefits (Krause & Ruxton, 2002), group members also face a higher risk of exposure to diseases - among other possible disadvantages (Krause & Ruxton, 2002) - as the presence of pathogens in a group is known to increase with its number of members (Loehle, 1995). The close proximity between colony members, the direct contact to excrements of their roost mates, as well as the possible participation in social interactions such as allogrooming (Kerth et al., 2003; Ortega & Maldonado, 2006; Wilkinson, 1986) suggests a high potential of pathogen transmission within bat colonies. Moreover, the frequent switching between a large number of roosts, which is particularly pronounced in species with high fission-fusion behavior, is likely to result in the contamination of many of the available roosts in the colony's home range.

However, while many recent studies have focused on their potential of spreading diseases to other animals and to humans (Plowright et al., 2015; Wood et al., 2012), to our knowledge, virus transmission networks within bat colonies remain largely unexplored and the sparse knowledge is based on simulated transmission data.

### 1.3 Study subjects

#### Bat species

This study was focused on three forest-dwelling bat species of the European temperate zone, namely the Natterer's bat (*Myotis nattereri*) as well as - to a lesser extent - the Bechstein's bat (*Myotis bechsteinii*) and the brown long-eared bat (*Plecotus auritus*). All three species have a wide range of distribution across Europe (Dietz & Kiefer, 2014), can be frequently found co-occurring in the same forest habitats (Kaňuch et al., 2008; Schöner et al., 2010), and are ecologically similar: These medium-sized, insectivorous bat species are highly philopatric to their natal colony (Burland et al., 2001; Kerth et al., 2002; Rivers et al., 2005). As typical for forest-dwelling bat species, adult females raise their offspring in tree roosts during the maternity season in summer (Kaňuch et al., 2008), though bat boxes are also frequently accepted and buildings can be used as well (Dietz & Kiefer, 2014). During the breeding season, adult males are typically solitary but may occasionally be found in the colonies (Safi, 2008). After fledging of the young in late summer, the colonies disperse and the individuals move to swarming sites for mating (Burland et al., 2001; Kerth & Morf, 2004; Parsons et al., 2003; Rivers et al., 2005) and subsequently hibernate there until spring before returning to their natal colonies.

All three species studied in my work display fission-fusion behavior (Červený & Horáček, 1981; Heise, 1988, p.; Kerth & König, 1999), and have been shown to react to social calls of con- and – to a certain extent – heterospecifics when searching for new roosts (Schöner et al., 2010). However, while comprehensive knowledge on the social structure and roosting behavior concerning Natterer's bats is still limited - there is evidence for distinct interspecific differences regarding these aspects (Fleischmann & Kerth, 2014; Schöner et al., 2010).

### Astroviruses

Astroviruses are single-stranded RNA viruses that commonly infect various avian and mammalian species including humans (De Benedictis et al., 2011; Moser & Schultz-Cherry, 2005) and bats (Fischer et al., 2016, 2017; Halczok et al., 2017), with a remarkably high detection rate and diversity in the latter (Chu et al., 2008; Drexler et al., 2011; Fischer et al., 2016, 2017). Mammalian astroviruses are transmitted via the fecal-oral route (Mendenhall et al., 2015) and typically cause diarrhea (Moser & Schultz-Cherry, 2005). In bats, however, astrovirus positive individuals have been reported to appear clinically healthy (Fischer et al., 2017). So far, little is known about astrovirus infection routes in bats. However, as astrovirus infections have been described to be especially common in settings with a high density closely connected potential hosts (Glass et al., 1996; Grellet et al., 2012; Shan et al., 2011), bat individuals can be assumed to face the risk of infection when they closely associate with infected colony members. Moreover, as astroviruses have been shown to be able to persist for several days in the environment (human astrovirus, Abad et al. 2001), bats could potentially get infected when using contaminated roosts even if the virus shedding animals are no longer present. Though, as bat astroviruses seem to be highly species-specific (Fischer et al., 2016), the spillover risk to other co-occurring bat species might be limited.

## **1.4 Study aim and hypotheses**

The **aim of my work** was to analyze virus transmission within a Natterer's bat colony with regards to the colony's social network, and to assess the implications of roosting behavior and roosting interactions between colonies.

Astroviruses are well suited for analyzing such virus transmission networks as they have a remarkably high detection rate and diversity in bats (Chu et al., 2008; Drexler et al., 2011; Fischer et al., 2016, 2017). The focal bat species of this study is the Natterer's bat (*Myotis nattereri*) as it has an especially high astrovirus detection rate and diversity (Fischer et al., 2016). Moreover, this species is known to display highly dynamic roosting behavior though information on their social network - hitherto solely based on ringing data - is scarce and deficient (August et al., 2014; Park et al., 1998).

In order to fill these gaps and to ensure a sound foundation for the subsequent analysis of virus transmission, we first assessed the social network structure in a free-ranging colony of Natterer's bats with regard to individual parameters; namely sex, age and breeding status. Next, astrovirus detection rate and transmission pathways within the colony were examined based on their individual roost use, individual astrovirus samples, and previously gained insights on their social network - a link that had not been researched before. Finally, to place our findings within a bigger context, the roosting dynamics between co-occurring colonies of Natterer's bats (*Myotis nattereri*), Bechstein's bats (*Myotis bechsteinii*), and brown long-eared bats (*Plecotus auritus*) were investigated; a research topic that might be especially interesting in the context of pathogen transmission. Although all three species have been shown to be attracted to roosts with a play-back set-up emitting social calls of con- and heterospecifics (Schöner et al., 2010), almost nothing is known about how different con- and heterospecific colonies influence each other in their roost use.

We hypothesized that:

- 1.) The social network of Natterer's bats closely resembles those of other ecologically similar bat species e.g. the Bechstein's bat (Kerth et al., 2011).
- 2.) Individuals that are strongly connected to their roost mates (high node strength in a weighted network) or switch between only few roosts are more exposed to astroviruses and thus have a higher infection risk. Moreover, given the gregariousness and close contact between colony mates in bats (Kerth, 2008a), the colony's social network is likely to significantly influence astrovirus transmission within the colony. Alternatively, if astrovirus transmission in Natterer's bats follows an indirect pathway via

contaminated roosts, we expect individuals that frequently used the same roost in close temporal succession to carry the same astrovirus sequence variants.

- 3.) As parasites and pathogens are often species-specific (Fischer et al., 2016; Rupp et al., 2004), colonies should avoid roosts previously used by foreign conspecifics (compare Reckardt & Kerth, 2007). In contrast, roosts of any heterospecific colony might be attractive (Schöner et al., 2010).

## 2 RESULTS AND DISCUSSION



## 2 RESULTS AND DISCUSSION

### 2.1 The social structure of Natterer's bats

→Manuscript 1: Zeus et al. 2018

In order to get insight into the social structure of a colony of Natterer's bats, we monitored occupied roosting (bat) boxes with an automatic RFID-system across three breeding seasons (Zeus et al., 2017). Moreover, in both spring and autumn, the colony was captured to gain information on sex, age class, breeding status and relative relatedness of its members. Subsequently, the resulting daily roosting associations and the abovementioned individual parameters were analyzed to gain information on the network structure and on the temporal stability of associations. In addition, we assessed the influence of the individual parameters on an individual's node strength (the summed association strength), examined the influence of relatedness to pairwise association strength, and tested for assortative mixing.

Despite the very frequent roost switching and extremely high fission-fusion behavior (Zeus et al., 2017), the social network was almost fully connected with very low modularity and thus only consisted of one community. This observed structure is contrary to findings in many other large fission-fusion societies, e.g. in the ecologically similar Bechstein's bat (Baigger et al., 2013; Kerth et al., 2011), where large groups consist of a modular network with several communities. In these species, group members maintain weak bonds between and strong ones within these communities. In contrast, in our colony of Natterer's bats the associations between colony members - while non-random - were less differentiated and pairs of individuals maintained an overall lower rate of association.

Nevertheless, despite the high fission-fusion dynamics and comparably weak social bonds, Natterer's bats were able to maintain relatively stable associations during the breeding season as well as across several years, indicating that social bonds were revived after the disintegration of the colony for several months during hibernation. While such long-term social bonds have been described in species with a very differentiated social structure (Archie et al., 2006; Kerth et al., 2011; Patriquin et al., 2010), it is unclear how Natterer's bats are able to maintain these stable associations in their highly dynamic unimodular network.

The analysis of the collected individual parameters revealed that all of them - namely sex, breeding status and age class - influenced the overall association strength of an individual within its colony. In summary, adults, breeding bats, and in particular the females showed the strongest bonds to their colony mates. As all analyzed attributes are highly dependent of each other, these findings may not be surprising in a maternity colony where reproductive active females communally raise their young (McCracken & Wilkinson, 2000). Contrary, there are no reports in temperate bats of males and non-breeding females contributing actively to the rearing of the young (Kunz & Hood, 2000), which likely results in different roost requirements and demands compared to their breeding female colony members (e.g. roost temperature; Hoying & Kunz, 1998; Kunz & Hood, 2000; Wilde, Knight, & Racey, 1999). We found stronger pairwise associations not only between closely related individuals (similar to Archie et al., 2006; Gompper et al., 1997; Kerth et al., 2003, 2011; Wilkinson, 1984) but also between those with similar traits (assortative mixing, Newman, 2003), which both might have also influenced the temporal stability between individuals within and across years as mentioned before.

To conclude, while the social organization in Natterer's bats follows many patterns found in other species with distinct fission-fusion behavior, their underlying social structures differ fundamentally.

## 2.2 Astrovirus transmission pathways in Natterer's bats

→Manuscript 2: Zeus et al. submitted

So far, research on transmission pathways within the social network of bat colonies is scarce and primarily based on simulated data (Kashima et al., 2013; Webber et al., 2016). This study was aimed to gain new insights by collecting and combining different field data sets. We intended to assess the presence of astroviruses in a colony of Natterer's bats, and whether astrovirus transmission follows a direct route (social contact) or indirect route (roost use). To do so, we captured data on the social associations (Zeus et al., 2017, 2018) as well as on the individual roost use in a colony of Natterer's bats during one breeding period. This was done via an automatic monitoring of occupied roost boxes with an RFID-system. We further gained information on the astrovirus detection rate and occurring astrovirus sequence groups from individual fecal and urine samples that were collected during capture events in spring and summer. Subsequently we used GLM and MRQAP models to analyze our data.

Our data revealed a very high diversity and detection rate of astroviruses in the colony, which coincides with the findings of previous studies (Fischer et al., 2016, 2017; Halczok et al., 2017; Kemenesi et al., 2014, 2016). However, although astrovirus infections typically occur in settings with a high density of closely connected hosts (e.g. Glass et al., 1996; Grellet et al., 2012; Shan et al., 2011), the detected astrovirus presence neither correlated with individual node strength nor with the number of different roosts used by an individual.

As astroviruses are typically transmitted via the fecal-oral route (Mendenhall et al., 2015), a potential host might get infected through direct contact to a virus shedding individual, as well as via indirect transmission (Taylor et al., 2001), e.g. by using contaminated roosts. Interestingly, the strength of pairwise associations was not correlated with the presence of shared sequence groups in the respective pair of individuals. Prima facie, this contradicts a direct transmission pathway, although the generally close contact between colony mates in bat maternity colonies would have suggested otherwise (Kerth, 2008a). However, an effect of association strength on astrovirus transmission should not be completely disregarded as the MRQAP model which included both subsequent roost sharing and association strength was our best fit. It is conceivable that due to the unusual unimodular, and almost fully connected network structure in Natterer's bats (Zeus et al., 2018), with comparably less differentiated links

between individuals (compared to e.g. Bechstein's bats: Baigger et al., 2013; Kerth et al., 2011), any apparent effect of association on disease transmission might be diluted. Our results further indicate that astroviruses are at least partially transmitted indirectly via contaminated roosts as we found individuals that frequently used the same roosts in short succession to carry the same astrovirus sequence groups. While frequent roost switching – as also distinctly observed in Natterer's bats (Zeus et al., 2018) – is used by several bat species to avoid parasites and pathogens (Kashima et al., 2013; Lewis, 1995; Reckardt & Kerth, 2007), our findings suggest that the extensive fission-fusion behavior in Natterer's bats could even facilitate the spreading of pathogens via roost contamination. This is also supported by comparably lower astrovirus detection rates (Fischer et al., 2016) in bat species with less prominent fission-fusion behavior (Zeus et al., 2017, 2018).

However, the MRQAP models used here only partly explained our data, which might be due to unavoidable constraints in our field data. Nevertheless, the gained results still constitute valuable insights for future studies.

To conclude, while the underlying factors that influence the presence of astroviruses in bats still need further examination, we found indications for an indirect transmission route of astroviruses via contaminated roosts. Therefore, this study provides first-time insight in the transmission pathways within the social network of bat colonies.

### **2.3 Roosting dynamics between co-occurring con- and heterospecific bat colonies**

→Manuscript 3: Zeus et al. 2017

To investigate roosting dynamics between con- and heterospecific bat colonies, we assessed the roosting behavior and roost usage of five co-occurring bat colonies – a colony of Natterer’s bats, a colony of Bechstein’s bats, as well as three colonies of brown long-eared bats. Occupied roosting boxes were automatically monitored via an RFID system across three breeding seasons to observe roosting and prospecting behavior. Moreover, we ran simulations to test whether colonies significantly preferred using roosts of certain other con- or heterospecific colonies.

As expected, different colonies never used the same roost simultaneously, as in many forest-living bat species different colonies typically do not mix (August et al., 2014; Kerth et al., 2000; Kerth & Van Schaik, 2012). However, roosting ranges of all heterospecific colonies overlapped to a great extent, and the same roosts were often occupied by different species in very short succession. Conversely, the three conspecific colonies of brown long-eared bats showed strict spatial separation during each breeding season. Avoiding roosts of other conspecific colonies likely reduces the risk of acquiring species-specific parasites (Rupp et al., 2004) or pathogens (Fischer et al., 2016; Zeus et al., submitted).

However, although the colonies of brown long-eared bats never occupied the roosts of their conspecifics, they were distinctly attracted to these roosts and visited them during nightly prospecting significantly more often than roosts of heterospecifics. As these findings undermine the aforementioned hypotheses of parasite and pathogen avoidance in this species, the observed prospecting and roosting behavior in the brown-long eared bats might indicate alternative causes e.g. the assessment (Doligez et al., 1999; Valone & Templeton, 2002) and the avoidance (Amarasekare, 2002; Ambrosio & Baeza, 2016; Goss-Custard, Durell, McGrorty, & Reading, 1982) of competition respectively. While the Bechstein’s bat colony did not display any interest in occupied roosts of the other colonies, we found the Natterer’s bats to be significantly attracted to roosts of brown long-eared bats. Natterer’s bats prospected roosts of brown long-eared bats significantly more often than those of any other colony.

Moreover, our simulations showed that - given its roosting behavior and preferences for individual roost boxes - the Natterer's bat colony also occupied roosts that were previously used by brown long-eared bats significantly more often than expected, sometimes apparently evicting brown long-eared bats in the process. Bats have been shown to avoid roosts that pose a possible health risk and instead occupy new roosts (Reckardt & Kerth, 2007). However, searching for roosts is costly, especially during maternity (Henry et al., 2002; Kunz & Hood, 2000) and species are known to differ in their abilities of finding new roosts (Fleischmann & Kerth, 2014). Thus, taking roost choices of other co-occurring bat species into account might be cost efficient, depending on the own demand and capabilities.

Out of all five colonies, the Natterer's bats colony showed the highest demand for roosts due to their colony size, extensive fission-fusion behavior, and the number of consecutive days a roost was used. Thus, for the Natterer's bats, taking over roosts of heterospecifics might have been a cost efficient alternative to finding new roosts on their own and less risky than re-occupying their own roosts given the species specificity of many parasites and pathogens (Fischer et al., 2016; Rupp et al., 2004). Roosts of Bechstein's bats might have been less attractive given the larger body size and aggressive behavior of this species towards intruders (Kerth et al., 2002). In contrast to the Natterer's bats, the other two bat species might either not have the need to take over foreign roosts (Bechstein's bat; Fleischmann & Kerth, 2014) or not the means to do so.

To conclude, we found that co-occurring bat colonies can influence each other in their roost use by avoiding roosts of conspecific, as well as by being attracted to those of heterospecifics. Both observed behaviors might serve the avoidance of species-specific parasites and pathogens, although other explanations have to be taken into account as well. Ultimately, the observed attraction towards roosts of heterospecifics might also facilitate the spillover of less specific parasites and pathogens, which underlines the importance of this study as an effort to entangle the complex and dynamic processes between co-occurring social groups.

## 2.4 Conclusion

The present thesis sheds light on hitherto largely unknown dynamics within colonies of Natterer's bats and their interactions with co-occurring heterospecifics:

It became apparent that the Natterer's bat, one of many forest-dwelling bat species with distinct fission-fusion behavior, differs drastically in their social structure from other ecologically similar species or large social fission-fusion societies in general, as they consist of only one highly connected community instead of a modular network.

Moreover, we found evidence that astroviruses, which are extremely common and diverse among bats, are rather transmitted via contaminated roosts than via direct contact although the close-knit social networks of bat colonies and their roosting behavior would have suggested otherwise.

Finally, we gained insights in the complex roosting dynamics between co-occurring con- and heterospecific bat colonies. Our results showed that different colonies can influence each other in their roosting behavior by avoiding or being attracted to each other's roosts. This might serve the containment of species-specific parasites and pathogens, but could also facilitate the spreading of more generalistic ones.

While there is still much research to be done on the interlinkage of bat social systems and virus transmission, this thesis provided essential new insights on the interactions within and among bat colonies regarding their roosting ecology and astrovirus transmission.



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## 4 PUBLICATION LIST



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### **4.1 The social structure of Natterer's bats**

#### **Manuscript 1**

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## ORIGINAL ARTICLE



# Long-term roosting data reveal a unimodular social network in large fission-fusion society of the colony-living Natterer's bat (*Myotis nattereri*)

Veronika M. Zeus<sup>1</sup> · Christine Reusch<sup>1</sup> · Gerald Kerth<sup>1</sup>

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## Abstract

In many social animals, groups recurrently split into subgroups that regularly re-merge. Such fission-fusion behavior allows individuals to better balance the cost and benefits of group living. However, maintaining a large number of close social links in groups with fission-fusion dynamics may be difficult. It has been suggested that this is the reason why in several species, large groups show more subunits (higher modularity) than do small ones. Many bat species exhibit fission-fusion dynamics in their colonies. This makes them well suited to investigate the proposed link between group size, stability of social links, and group modularity. We studied the daily roosting associations of a Natterer's bat colony (*Myotis nattereri*), where up to 80 members carried individual RFID-tags. Based on more than 10,000 individual recordings, we analyzed the influence of relatedness, age, sex, and breeding status on the colony's social network structure during three breeding seasons. We found an almost fully connected social network with very low modularity and generally weak pairwise associations. Nevertheless, the relative strengths of associations between individuals remained stable across years. Sex, age, and breeding status significantly influenced the strength of an individual's associations and determined the influence of individuals in the network. In general, associations between bats that were similar in all abovementioned traits were stronger than those between dissimilar individuals. Our results show that despite high fission-fusion dynamics, large colony sizes, and low modularity of their social network, Natterer's bats were able to maintain stable long-term associations.

## Significance statement

For a variety of social and ecological reasons, large social groups often consist of several communities with stronger individual bonds within and weaker individual bonds between such social subunits. Unlike that predicted for its relatively large size, the studied Natterer's bat colony that consisted of up to 80 individually marked bats was not subdivided into communities. Despite the fully connected network, the individual associations were not random. Instead, their strength was mainly driven by relatedness and similarity in age and breeding status of the colony members. Moreover, we found stable long-term pairwise relationships between individuals across several years. Our study shows that despite the strong fission-fusion behavior and large size of their colony, Natterer's bats formed a fully connected, unimodal social network.

**Keywords** Bats · Social network · Association · Assortativity · Fission-fusion · Modularity

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✉ Veronika M. Zeus  
[veronika.zeus@gmail.com](mailto:veronika.zeus@gmail.com)

<sup>1</sup> Zoological Institute and Museum, Greifswald University, Loitzerstrasse 26, 17489 Greifswald, Germany

## Introduction

In social animals, the strength of individual associations between group members often show a non-random pattern (Krause and Ruxton 2002; Krause et al. 2007). In general, animals may be passively associated with each other in space and time, for example because they share strategies for use of habitat and resources (Mitani et al. 1991; Chaverri et al. 2007; Aplin et al. 2013). Alternatively, they may be actively attracted to—or repelled by—certain other individuals. In this

case, their associations reflect individual preferences for each other (Gompper et al. 1997; Krützen et al. 2003; Archie et al. 2006; Mitani 2009). The strengths of individual associations are often influenced by individual preferences or demands that arise from differences in individual traits such as age, kinship, breeding status, or sex (Kerth and König 1999; Wolf et al. 2005; Mitani 2009; Patriquin et al. 2010; Wey and Blumstein 2010). Consequently, such traits often also determine the position and connectivity of individuals in the social network of a group, which reflects individual associations among group members (Wittemyer et al. 2005; Ramos-Fernández et al. 2009; Kerth et al. 2011). In many social networks, individuals show assortative mixing and form subunits with individuals that are similar to themselves with regard to traits such as those mentioned above (Newman 2003).

In order to balance individual needs and group preferences, groups can temporarily split into subgroups that later fuse again, a phenomenon called “fission-fusion” behavior (Aureli et al. 2008). In recent years, various studies investigated the fission-fusion dynamics of social groups in animals (e.g., Lehmann and Boesch 2004; Archie et al. 2006; Popa-Lisseanu et al. 2008; Ramos-Fernández et al. 2009; Kerth et al. 2011). Several of these studies show that despite the high fission-fusion dynamics of their groups, at least some individuals are able to maintain long-term associations (Archie et al. 2006; Patriquin et al. 2010; Kerth et al. 2011). Furthermore, in species with fission-fusion behavior, group size seems to predict group modularity as social links between individuals become more differentiated with increasing numbers of group members (Kudo and Dunbar 2001; Lehmann and Boesch 2004; Kerth et al. 2011; Baigger et al. 2013). It has been discussed that a limited number of close social relationships arising from the modular structure of groups might be a strategy to reduce disadvantages of large groups such as intra-group competition, vulnerability to diseases, or infanticide risk (Smith et al. 2008; Kashima et al. 2013). Alternatively, it could be a consequence of time constraints (Dunbar 1991, 1992a; Lehmann et al. 2007) or cognitive constraints (Dunbar 1992b; Kudo and Dunbar 2001; Wilkinson 2003; Lehmann et al. 2007; Kerth et al. 2011) that limit the number of close relationships that can be maintained in the long term.

Fission-fusion behavior is widespread among bats, but only a minority of species have been studied in enough detail to characterize their colony structure (see reviews by Kerth 2008; Johnson et al. 2013). For Bechstein’s bats, a strong link between the size and the modularity of colonies has been found and it has been suggested that individuals can maintain long-term relationships with only a limited number ( $\leq 20$ ) of colony members (Kerth et al. 2011; Baigger et al. 2013). In the present study, we investigated the colony structure of Natterer’s bats (*Myotis nattereri*), a species which is widespread throughout Europe and beyond (Dietz and Kiefer 2014). During summer, Natterer’s

bats typically roost in tree cavities or bat boxes in forests, although occasionally, buildings are occupied as well (Smith and Racey 2005). Both sexes are highly philopatric (Rivers et al. 2005, 2006; Halczok et al. 2017). Females typically return to their natal colony, where they give birth and raise their young during the maternity season in summer. While some males can be found in these colonies, males have also reported to roost solitary or in all-male groups in the vicinity of their natal colony (Červený and Horáček 1981; Park et al. 1998; Rivers et al. 2006). During autumn, maternity colonies dissolve and the bats migrate to hibernation sites, which are often dozens of kilometers away from the summer habitat (Rivers et al. 2005; Dietz et al. 2007).

As they form colonies of up to 80 individuals, which show a strong fission-fusion behavior (Zeus et al. 2017), Natterer’s bats are well suited for a study that aims to assess the social network structure in large and highly dynamic social groups. So far, however, very little is known about the fine scale social structure of Natterer’s bats. To our knowledge, previously only two ringing studies addressed this issue (Park et al. 1998; August et al. 2014). The available data suggest that, unlike in many other European bat species, mixed sex groups may be common, with females having a higher number of associates (degree centrality; August et al. 2014). There is also some evidence for the existence of long-lasting but weak individual roosting associations between colony members (Park et al. 1998; August et al. 2014). The caveat of ringing studies is that they depend on capturing the bats from their roosts, and thus do not allow for monitoring roosting associations without disturbing the colony. In an effort to minimize stress on the animals, intervals between data collection in these studies were very long, resulting in very low numbers of recaptures per individual and year (August et al. 2014). Consequently, these studies did not allow for a detailed analysis of the daily fission-fusion behavior of the bats, group modularity, and the stability of social links within colonies over different time periods. Moreover, to our knowledge, no studies on Natterer’s bats investigated the influence of kinship, age, and breeding status on the strength of roosting associations among colony members and on their position in the colony’s social network.

During three consecutive years, between May and September, we daily monitored 134 bat boxes that were regularly used by one large colony of Natterer’s bats as day roosts. In this colony, up to 80 colony members carried individual radio-frequency identification (RFID)-tags. Using automatic RFID-loggers attached to the bats’ communal day roosts (bat boxes), we were able to quantify the individual roosting associations without disturbing the bats (Zeus et al. 2017). Based on these individual roost association data, we created social networks and assessed the influence of age, sex,

and individual relatedness on the strength of the roosting associations between colony members.

Due to the large size of the colony, we hypothesized that its social network shows a high modularity and comprises several communities, as has been reported for Bechstein's bats that use the same type of roosts (Kerth et al. 2011). We also expected membership within communities to be stable over time with colony members maintaining long-term individual associations (Kerth et al. 2011; Baigger et al. 2013; August et al. 2014). We further assumed that individual traits, namely sex, age class, relatedness, and breeding status, influence the summed strength of an individual's associations (node strength in the colony's network). Finally, we expected assortative mixing between individuals that share the same individual traits and are, therefore, likely to have similar preferences and demands.

## Methods

### Data collection

The study took place each May to September in the years 2013 to 2015 in a forest near the city of Würzburg, Germany. The area is home to one large colony of Natterer's bats (*Myotis nattereri* sensu stricto; Puechmaile et al. 2012). Every year before the 20th of May, when the females became visibly pregnant, and after the fledging of the offspring in late July and August, all bats were captured from their day roosts by hand (about two to three times per year). All unmarked individuals, adults and juveniles, were marked with RFID-tags that were inserted via subcutaneous injection, and DNA samples from the wing membrane were taken for subsequent analysis of relatedness (Kerth et al. 2002).

At the same capture events, we assessed the sex, age, and breeding status of the bats: As the colony members were first marked in 2011, it was not possible to determine the exact age of individuals that had already been adult in 2011. Therefore, we defined three distinct age classes: "juvenile" (born in the respective year of capture), "yearlings" (adult bats that had been captured as juveniles in the previous year) and "older bats" (adult bats that are two or more years old). We distinguished between juveniles (only present in July to September) and adult bats (yearlings and older bats) by examining the epiphyseal gap of the fourth metacarpal (Anthony 1988). Juveniles were not included in subsequent analyses because we expected them to still depend strongly on the presence of their lactating mother and thus not to be able to independently choose their day roosts. If bats were specified as adult in the year of their first capture, these individuals were labeled with age "unknown" for that year, as it was not possible to discriminate between yearlings and older without knowing the year of birth. In the following year, such bats were labeled as older

because then they had been at least 2 years old. Adult females were regarded as "breeding" if the bats showed worn patches around the nipples (Racey 1988) or "non-breeding" in the absence of such patches. We classified the breeding status of adult females as "unknown" if they were not captured during that time period or only so late in summer that signs of lactation were no longer explicit. All males that were present in the maternity colonies were classified as non-breeding as males of temperate bat species do not participate in the rearing of the young (Kunz and Hood 2000).

In previous years, 134 bat boxes (type 2FN, Schwegler, Germany) had been installed in the study area (Zeus et al. 2017). These boxes have been regularly used as communal day roosts by the colony during the study period. Each year, data recording started after the last marking event mid of May and stopped in September, when the colony started to dissolve. Roost associations were recorded for a total of 341 days (117 days in 2013, 114 days in 2014, and 110 days in 2015) with a radio-frequency identification (RFID) system that is well established for the automatic monitoring of bats in our study site (Kerth and König 1999; Kerth et al. 2011; Zeus et al. 2017). Every day, all boxes were checked visually without opening them, using an established monitoring protocol (Kerth and Reckardt 2003; Kerth et al. 2011). Occupied boxes were equipped with automatic RFID-tag readers (reader type "LID 650" by EURO I.D.) to monitor the individual group composition in the day roosts. The logger antennae were placed in the boxes' entrances in a way that bats that passed the entrance were recorded by their RFID-tag numbers together with the respective date and time. This set-up has proved to be very reliable in a study by Kerth and Reckardt (2003), where 97% of the bats passing the antenna had been recorded. Bats that were recorded during the emergence from the bat box in the evening were assigned as having spent the day associated with each other in the respective box (Kerth and Reckardt 2003; Kerth et al. 2011). As all association data were recorded automatically, there was little need for blinded methods. The rate of logger malfunctions due to technical problems such as battery failure or antennae damage was very low (3.7% of all monitored day roosts) and the respective days were excluded from the dataset.

### Genetic analysis

Following the approach of Halczok et al. (2017), we used 15 nuclear microsatellite markers to assess relatedness between individuals (see supplement for more information). Markers were tested for Hardy-Weinberg equilibrium with Genepop version 4.5.1 (Raymond and Rousset 1995) and for the presence of Null-Alleles with Micro-Checker version 2.2.3 (van Oosterhout et al. 2004) with 10,000 iterations and a 95% confidence interval. In order to confirm natal philopatry in the colony, we identified mother-offspring pairs with Cervus

version 3.0.7 (Kalinowski et al. 2007). The relative relatedness between pairs of individuals was calculated with TrioML (Wang 2007) using Coancestry version 1.0.1.5 (Wang 2011).

### Network analysis

In social network analyses, animals with fewer than a certain number of observations are often excluded because they may cause misinterpretations of the social structure (Whitehead 2008; Farine and Whitehead 2015). The determination of a threshold of minimum number of observations, however, has been handled in various ways in different studies. Many studies have excluded all data below a more or less arbitrarily selected number of observations. In our study, we followed the approach of Aplin et al. (2013) and tested for a relationship between the number of observations and the number of observed association partners. We analyzed each year separately and plotted the number of observations against the number of different association partners (binary degree), which resulted in a saturation curve. We excluded all individuals that did not reach the plateau of the curve. This resulted in the omission of all individuals below 12 observations in 2013 (3 individuals; 5.8% of all analyzed bats in that year), all individuals below 10 observations in 2014 (9 individuals; 11.0%), and all individuals below 20 observations in 2015 (6 individuals; 7.0%). We do not expect any bias in the properties of the network by excluding such a limited number of bats with few observations, as according to Silk et al. (2015), as little as 30% of individuals can be enough to produce a conclusive network.

All network analyses were performed in “R” version 3.3.1 (R Core Team 2016). From our observed roosting data, we generated a weighted, undirected association network by using the R package “asnipe” (Farine 2016a) with values ranging between 0 (never found roosting together) and 1 (always found roosting together). There is a wide variety of different association indices to compute associations between individuals, most of which correct for potential observation biases in the obtained data by giving certain observations a higher or lower weight (Whitehead 2008). However, such weightings are often somewhat arbitrary, generally do not result in a better performance, and can even impede the interpretability of results (Hoppitt and Farine 2017). Therefore, we decided to use the “Simple Ratio Index” (SRI) (Cairns and Schwager 1987; Ginsberg and Young 1992) that needs no such corrections.

Due to the high reliability of the automatic RFID-monitoring, we are highly confident concerning the collected data on the presence or absence of individuals in our monitored boxes at any given day. Nevertheless, we only recorded on average 47% of all colony members per day (minimum 1%, maximum 98%). This can be mainly attributed to two factors: The presence of unmonitored, natural roosting opportunities (tree

cavities) at the study site and the departure of individuals at the end of summer. For both reasons, the probability of failing to record two individuals when they are together is the same as failing to record them when they are apart and in such a case, the SRI is unbiased (Hoppitt and Farine 2017).

In order to assess whether the observed network consisted of several subunits (communities), we used the “infomap” algorithm (Rosvall and Bergstrom 2008) as, according to Fortunato and Lancichinetti (2009), it is the best performing algorithm for undirected, weighted networks. Based on this output, we then calculated the modularity of the network. We used the R package “igraph” (Csardi and Nepusz 2006) for both the community detection via “infomap” and the subsequent computation of modularity.

To test whether the pairwise associations in the observed network are non-random, we conducted 100,000 data stream permutations with one random swap per permutation (Farine and Whitehead 2015), as implemented in the “asnipe” package. Subsequently, we computed a new association matrix after every 100 swaps, which resulted in 1000 permuted random networks. In the following, we calculated the proportion of times the coefficients of variation (CVs) of the 1000 permuted random networks were larger than the CV of the observed network (Farine and Whitehead 2015). When 95% of the CVs from the random data were smaller than the CV of the observed data, the test was declared significant at the 5% level. This method was also used for all other analyses that required permutation tests as mentioned below.

In order to test whether the parameters sex, age class, and breeding status influence an individual’s node strength, we conducted models of both the observed data and the random networks (Farine and Whitehead 2015). We applied restricted maximum likelihood linear-mixed effect models (LMMs) with normal errors to assess differences in node strength between reproductive classes and between age classes. Since individuals were repeatedly sampled across the years, we included the individual ID as a random intercept in our model as individual association behavior might not be independent between years (see our analyses on temporal stability of associations) despite the complete disintegration of the colony during hibernation. We used a linear model (LM) for the influence of sex on node strength, as this analysis was only possible for 2013 due to the absence of males in the other 2 years. The influence of breeding status and age class was also tested for each year separately via LM and the results thereof can be found in Table S1 in the supplement. For each individual, we calculated the weighted degree to measure node strength which was used as the response variable in the linear models. The attributes sex, age class, and breeding status that were determined during the captures were set as the predictors in our model. *P* values were computed from the regression coefficients of the observed and random networks and corrected via Holm’s Sequential Bonferroni Procedure (Holm 1979;

Bretz et al. 2016) in order to avoid Alpha errors due to multiple comparisons. We also conducted the same analysis with eigenvector centrality—a measure of influence (Borgatti 2005)—as the response variable. However, as both network parameters were highly correlated for each year (Kendall rank correlation coefficient (Kendall 1938) 2013: 0.961; 2014: 0.972; 2015: 0.979), the results concerning eigenvector centrality can be found in the supplement (Tables S2 and S3).

To test for a relationship between the association indices and pairwise relatedness, we used the multiple regression quadratic assignment procedure (MRQAP) that is implemented in the “asnipe” package. More specifically, we used the MRQAP with custom permutation networks from the data stream permutations. The association rate was set as the dependent variable and pairwise relatedness as the independent variable.

Moreover, we used the R package “assortnet” (Farine 2016b) to test for assortative mixing of the colony, a weighted measure to assess the extent to which individuals are associated with others that are similar to themselves. Thereby, sex, age, and breeding status were taken into account. The weighted assortativity coefficients of the observed data and the permuted networks were used to compute the *p* value. Moreover, the output allowed deeper insights into the distribution of association values (edge weights). As there might be a bias for classes that contain proportionally more individuals, we tested via permutation tests if the edge weights between and within classes were higher or lower than expected by chance.

### Temporal analysis

To assess the temporal stability of the observed pairwise associations during the course of one maternity season, we calculated the lagged association rates (LARs) (Whitehead 2008), which is the probability of re-association after a certain period of time. To do so, we used the “asnipe” package and set the step length to 1 day. For this analysis, we used all non-juvenile individuals of the colony, including those that were previously excluded for the network analysis because of their low number of observations in some years. This was done in order to avoid positively biasing the LARs by only restricting the dataset to frequently observed individuals (Baird and Whitehead 2000; Whitehead 2008). To facilitate the interpretation of the LARs, we further determined the lagged rate of association null, which represents animals associated randomly. This rate was calculated by the mean group size experienced by an individual divided by the mean number of total associates of each individual (mean binary degree) (Farine 2013). We also calculated the lagged identification rate (LIR) which estimates the probability of re-identification after a certain time lag (Whitehead 2001). This measure provides information on demographic effects such as survival or emigration (Whitehead 2001) which might have influenced the

LARs. The standard errors of the LARs and LIRs were computed via jackknifing.

Finally, in order to test whether associations between individuals remain stable across years, we recalculated the association matrices from the observed data by only including individuals that were present in both years. These matrices were then compared to each other. To do so, we ran a MRQAP analyses by setting the association network in the later year as the dependent variable, generating 1000 permutation networks thereof via data stream permutation as described above, and using the network of the earlier year as the independent variable. Thereby, we were able to test if pairwise associations between years are interdependent and remain stable.

**Data availability statement** The datasets generated and analyzed during the current study are available from the corresponding author on reasonable request.

## Results

### Network structure

During the 341 days of the study, we recorded a total of 10,557 individual roosting associations (3028 in 2013, 3307 in 2014, 4221 in 2015). On average, each individual used between 30 and 32 different roost boxes per year (for yearly average with range see Table 1). Thereby, boxes were switched almost daily (see “mean number of consecutive days/box,” Table 1). A plot for each year with the number of different roosts against the number of days a bat was present can be found in the supplement (Fig. S1). Regarding the roost use of two associated individuals, a pair of individuals spent on average between 14 and 20 days together per year (for yearly average with range, see Table 1). Thereby, a pair spent on average 1.4 consecutive days in their most frequented roost (Table 1). Thus, the most preferred roost accounted for roughly 20% of a pair’s shared roost use (Table 1). A plot for each year with this proportion against the number of days spent together can be found in the supplement (Fig. S2).

In all 3 years, the colony showed high fission-fusion dynamics as can be seen by the percentage of days on which the colony occupied more than one box (2013: 80.3%; 2014: 61.4%; 2015: 77.2%; Zeus et al. 2017). Nevertheless, in each year, the network—that was based on the individual roosting associations (SRI values)—was almost fully connected (see “Network density,” Table 2) with very low modularity and thus consisted of only one community (see Fig. 1 for the year 2015). However, associations were non-random ( $CV_{2013}$ : 62.756, mean random  $CV_{S2013}$ : 52.189,  $p_{2013} < 0.001$ ;  $CV_{2014}$ : 59.627, mean random  $CV_{S2014}$ : 52.896,  $p_{2014} < 0.001$ ;  $CV_{2015}$ : 44.584, mean random  $CV_{S2015}$ : 33.327,  $p_{2015}$

**Table 1** Average roost use of individual bats and associated pairs per year. The range is given in parentheses

Year	2013	2014	2015
Mean number of different boxes per individual	32.2 (5.0–46.0)	27.5 (7.0–45.0)	29.6 (11.0–43.0)
Mean number of consecutive days per box	1.4 (1.1–1.9)	1.4 (1.0–1.8)	1.4 (1.2–1.7)
Mean number of days a pair spent together	20.1 (1.0–62.0)	14.3 (1.0–54.0)	17.1 (1.0–53.0)
Mean number of days spent together in most frequented roost	1.4 (1.0–12.0)	1.3 (1.0–9.0)	1.3 (1.0–7.0)
% of days spent together in most frequented roost	20.7 (5.6–100.0)	22.2 (4.8–100.0)	17.2 (5.6–100.0)

<0.001). SRI values indicated that in each year, pairs of individuals shared the same roost only about 20% of the monitoring days on average (see “mean SRI values” Table 2; Figs. 1, S3, and S4 in the Supplementary). Furthermore, individuals only associated with a relatively small proportion of colony members each day (mean per year 32–34%, compare “number of marked individuals” and “mean group size,” Table 2).

### Temporal stability of associations

Within each year, the LARs started at a value of around 0.5 and further decreased, albeit very gradually, with time. This suggests that in all 3 years, only half of the individual associations persisted after 1 day but remained relatively stable across an extended period of time. In 2015, this time frame is roughly limited to 25 days after which the LAR dropped below the lagged rate of association null, indicating that after this time lag, the probability of re-associations was less likely than expected by random associations. For 2013 and 2014, this was only the case at the end of the respective seasons when the LARs became increasingly unstable. In the same timeframe, the slopes of the LIRs alternate between increasing and decreasing which can be an indication that the bats used the monitored bat boxes less consistently.

The colony members were able to maintain long-term associations across years, as the association networks were significantly positively correlated between successive years (MRQAP, 2013–2014:  $n = 34$  individuals, regression coefficient = 0.423,  $p < 0.001$ ; 2014–2015:  $n = 57$  individuals, regression coefficient = 0.238,  $p < 0.001$ ) as well as between 2013 and 2015 ( $n = 28$  individuals, regression coefficient = 0.864,  $p < 0.001$ ).

### Influence of attributes on node strength

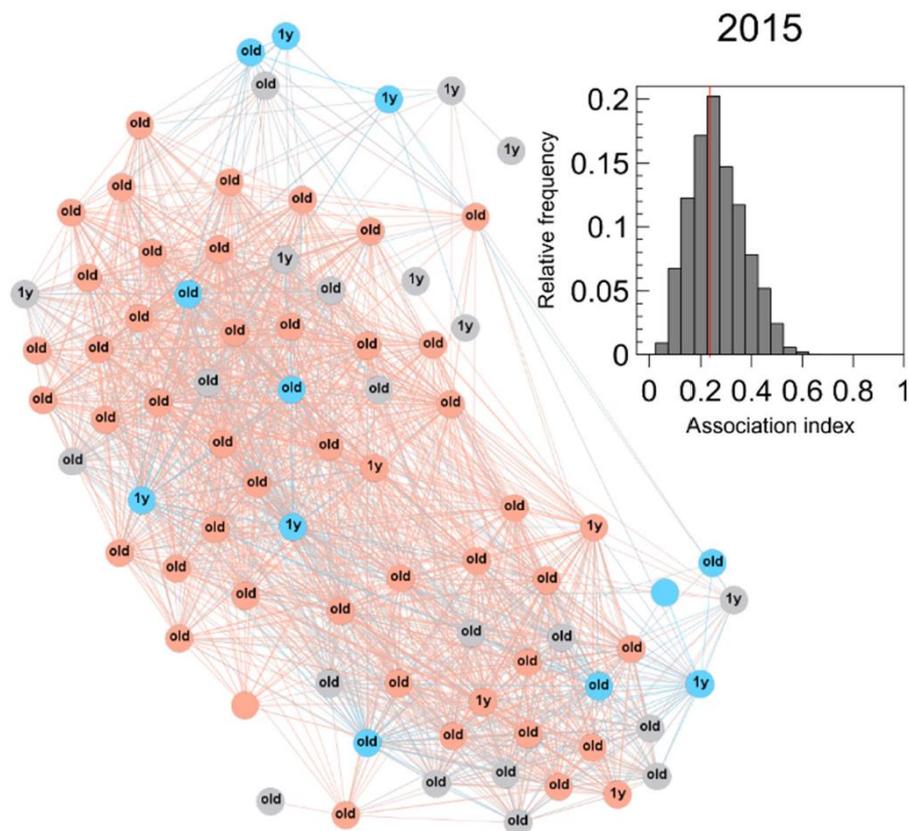
Assessing the influence of sex on node strength was only possible for 2013 due to the absence of males in the other 2 years. The LM *Strength~Sex* revealed that all six male individuals were significantly weaker associated with their association partners compared to the female individuals and their corresponding association partners (Table 3).

The LMM *Strength~Breeding status* across all 3 years further suggested that females that reproduced in a given year have higher node strength (Table 3) than non-breeding bats (in 2014 and 2015 only non-breeding females; in 2013: non-breeding females and males). Thus, they seem to have stronger links with their association partners. Conversely, according to the LMM *Strength~Age* across all 3 years,

**Table 2** Attributes of individuals and network parameters in all 3 years. “Mean binary degree” is the average number of association partners within the respective year. “Mean group size” is the average number of colony members in a roost. “Network density” is the proportion of all possible links between individuals. “Mean SRI value  $\pm$  CV” signifies the

average association index between pairs of individuals and also stating the coefficients of variation in percent. “Modularity” expresses whether a network is divided into several sub-communities; the value of modularity can either be positive or negative with positive values hinting the presence of community structure

Year	2013	2014	2015
Number of marked individuals	49	73	80
Sex: female/male	43/6	73/0	80/0
Age: older/yearling/n.a.	32/7/10	56/10/7	62/16/2
Breeding status: breeding/non-breeding/n.a.	25/7/17	51/7/15	47/12/21
Mean binary degree	47.6 (44–48)	70.7 (63–72)	79 (78–79)
Mean group size	16.432 (11.5–20.3)	23.267 (16.5–29.2)	27.074 (19.7–36.4)
Network density	99.150	98.136	99.968
Mean SRI value $\pm$ CV	0.224 $\pm$ 60.447	0.199 $\pm$ 58.048	0.243 $\pm$ 42.870
Modularity	– 3.108624e–15	3.552714e–15	– 4.884981e–15



**Fig. 1** The network structure and strength of pairwise associations in 2015, when 80 individuals had been analyzed: The right graph shows the frequency distribution of the strength of pairwise associations with the red vertical line indicating the mean association strength. The left graph presents the network structure that was produced with Gephi 0.9.2. (Bastian et al. 2009) using a force-directed algorithm where short edges between two nodes represent stronger associations between these two

individuals. While the network is fully connected, for a better visualization, this graph only shows social links that are above average strength. The breeding status of the individuals is color coded (red = breeding, blue = non-breeding, gray = no information available) and the age of the individuals can be found in the node label (“1y” = yearlings, “old” = older bats). Respective figures for the years 2013 and 2014 can be found in the supplementary

yearlings had significantly weaker associations than older bats (Table 3).

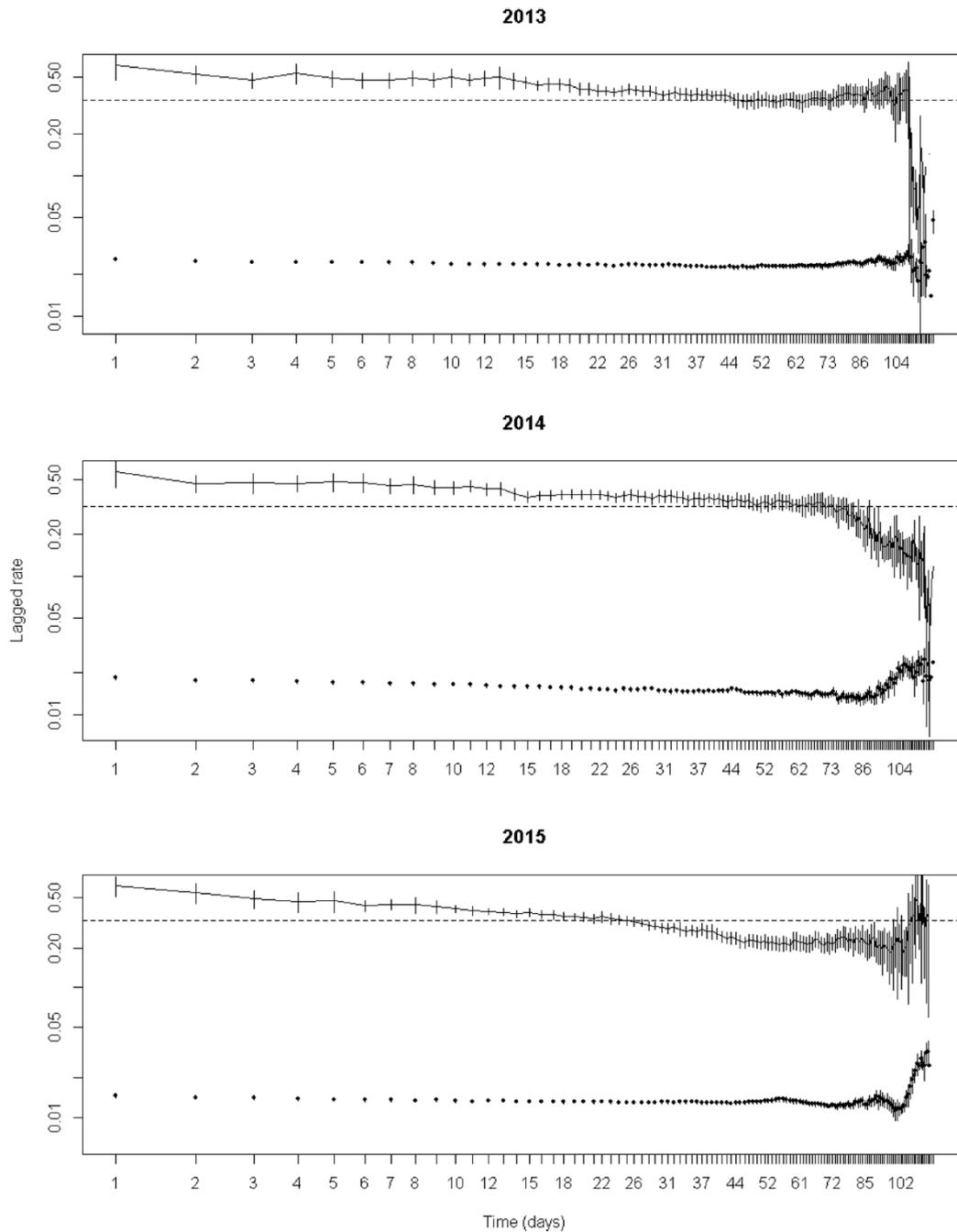
### Assortative mixing

Even though the colony consisted of only one community, we found significant assortative mixing for all traits that were taken into account, namely sex (significant in all years; 2013 by permutation test, and in 2014 and 2015 by the absence of males), age (significant in 2013 and 2014), and breeding status (significant in 2013 and 2015, Table 4). This shows that associations were significantly stronger between individuals that were similar in the measured traits.

Having a closer look at the discrete attributes in years with significant assortative mixing (Table 4), data of 2013 revealed that, with a mean edge weight of 0.263 (95% CI = 0.254 to 0.271), associations between females accounted for 90% of the sum of all edge weights in the network. This effect was not

a byproduct of their large class size as the  $p$  value of the permutation test was significant ( $p = 0.001$ ). This indicates that females did associate with each other stronger than expected by chance. In contrast, edge weights between males with a mean of 0.204 (95% CI = 0.161 to 0.248, proportion of all edge weights = 1.2%) were not significantly higher than expected by chance ( $p = 0.137$ ), while those between sexes were significantly lower (proportion of all edge weights = 8.8%,  $p = 0.001$ ) with an average value of 0.090 (95% CI = 0.083 to 0.097). This suggests that female individuals prefer the presence of their own sex, while males might even avoid females. This evidence for social sexual segregation is also supported by the low proportion of males in the colony in 2013 and their total absence in 2014 and 2015.

For the attributes of age and breeding status, we had to deal with a considerable proportion of individuals with unavailable data (“n.a.” in Table 2) ranging between 4 and 24% concerning the age and between 27 and 57% concerning the



**Fig. 2** Lagged rates: The solid line is the LAR, the dashed line is the lagged rate of association null, and the dotted line the LIR. Vertical lines signify the standard error

breeding status. Thus, for better comprehension, in the following paragraph, 100% reflects the number of individuals with known status only.

In 2013 and in 2014, the years with significant assortative mixing concerning age class, associations between older bats were significantly stronger than expected from random (2013:

mean edge weight = 0.284, 95% CI = 0.272 to 0.296, proportion of all edge weights = 70.2%,  $p < 0.001$ ; 2014: mean edge weight = 0.218, 95% CI = 0.212 to 0.223, proportion of all edge weights = 77.1%,  $p = 0.021$ ). Even though, only small proportions of the total edge weights occurred between yearlings (2013: 3.5%; 2014: 1.3%), these were significantly

**Table 3** Results of the LM (*Strength~Sex*, 2013 only) and the LMMs (across all 3 years) assessing relationships between node strength and attribute sex (basic level: female), breeding status (basic level: non-breeding), and age (basic level: older bats). The estimate ( $\text{coef}_{\text{observed}}$ ) is given with standard error, degrees of freedom, and  $t$  value, as well as the

mean estimate of the permuted networks (mean  $\text{coef}_{\text{random}}$ ), and the computed  $p$  value from the observed and permuted data (corrected via Holm's sequential Bonferroni procedure). Significant  $p$  values are shown in italics

Model	$\text{coef}_{\text{observed}}$	SE	df	$t$	mean $\text{coef}_{\text{random}}$	$p$
<i>Node strength~Sex</i>	-6.684	1.189	47	-5.623	-6.175	<i>0.001</i>
<i>Node strength~Breeding status</i>	4.075	1.008	53	4.042	3.157	<i>&lt; 0.001</i>
<i>Node strength~Age</i>	-2.635	0.922	81	-2.858	-1.557	<i>&lt; 0.001</i>

stronger than expected by chance in 2013 (mean edge weight = 0.335, 95% CI = 0.294 to 0.367,  $p < 0.001$ ). In contrast, individuals of different age classes were significantly weaker associated than expected from the permuted data in both years (2013: mean edge weight = 0.235, 95% CI = 0.220 to 0.251, proportion of all edge weights = 26.3%,  $p < 0.001$ ; 2014: mean edge weight = 0.160, 95% CI = 0.151 to 0.170, proportion of all edge weights = 20.6%,  $p < 0.001$ ).

Concerning the breeding status, the largest proportions of the total edge weights were found between breeding females (2013: 81.4%, mean edge weight = 0.307, 95% CI = 0.294 to 0.320; 2015: 68.0%, mean edge weight = 0.281, 95% CI = 0.276 to 0.287). These associations were significantly stronger than expected by chance in both 2013 ( $p < 0.001$ ) and 2015 ( $p < 0.001$ ). Edge weights were significantly lower than expected from random between bats that did not breed in 2015 (mean edge weight = 0.212, 95% CI = 0.190 to 0.235, proportion of all edge weights = 3.1%,  $p = 0.009$ ). Breeding and non-breeding bats also associated significantly weaker than expected from the permuted data in 2013 and 2015 (2013: mean edge weight = 0.099, 95% CI = 0.085 to 0.112, proportion of all edge weights = 15.2%,  $p < 0.001$ ; 2015: mean edge weight = 0.228, 95% CI = 0.220 to 0.237, proportion of all edge weights = 28.8%,  $p < 0.001$ ).

### Influence of relatedness on pairwise associations

Pairwise relatedness was significantly positively correlated with the strength of pairwise association between individuals in all 3 years (pairwise relatedness: regression coefficient<sub>2013</sub> = 0.129,  $p$  value<sub>2013</sub> < 0.001; regression coefficient<sub>2014</sub> = 0.041,  $p$  value<sub>2014</sub> = 0.001; regression coefficient<sub>2015</sub> = 0.074,  $p$  value<sub>2015</sub> < 0.001).

### Discussion

In large social groups, individuals often maintain a few strong individual associations and many weak associations to other members of their group which typically results in a modular network with several communities. Such modular social groups have been described for instance in primates

(Lehmann and Boesch 2004) and in bat species such as the Bechstein's bat (Kerth et al. 2011; Baigger et al. 2013). The modularity of large animal groups has been interpreted as a strategy to reduce disadvantages of living in large groups such as increased intra-group competition, infanticide risk, or vulnerability to diseases (Smith et al. 2008; Kashima et al. 2013). Alternatively, it might be a result of time and/or cognitive constraints that may prevent individuals from keeping close bonds with many individuals (Dunbar 1991, 1992a; Kudo and Dunbar 2001; Wilkinson 2003; Lehmann et al. 2007; Kerth et al. 2011) or simply a byproduct of how individuals use space and resources. Interestingly, such group modularity did not occur in the large colony of Natterer's bats (up to 80 adult colony members) during this study, although its roost-switching and fission-fusion behavior was similar to that of Bechstein's bat colonies (Zeus et al. 2017). For comparison, strong modularity occurred in Bechstein's bat colonies that compromised between 30 and 40 individuals while it was absent in small colonies of less than 20 bats (Kerth et al. 2011; Baigger et al. 2013).

One notable factor that determined the strength of an individual's associations to other colony members was their relative relatedness with stronger associations between closely related individuals. Besides in our study species, which is known to show high natal philopatry (Rivers et al. 2005, 2006; Halczok et al. 2017), such a positive influence of kinship on association strength has been described in several other species with fission-fusion behavior, e.g., African elephant (Archie et al. 2006), white-nosed coati (Gompper et al. 1997), common vampire bat (Wilkinson 1984), and Bechstein's bat (Kerth et al. 2003, 2011). In all of the aforementioned species, including Natterer's bats (Rivers et al. 2005), mating takes place outside the natal group. With such a breeding strategy, closely related individuals do not run the risk of inbreeding (Godfrey et al. 2014).

Aside from kinship, we found several individual traits to also influence the strength of an individual's associations (node strength). Although we analyzed each parameter separately, some of them might not be independent of each other as for example breeding individuals in maternity colonies are always female (Kunz and Hood 2000; Rivers et al. 2005), and yearlings might have a lower breeding rate than older bats

**Table 4** The assortativity of the network with respect to sex, age, and breeding status in all 3 years. The observed assortativity coefficient is given with standard error, as well as the mean assortativity coefficientof the permuted networks (mean coef<sub>random</sub>) and the computed *p* value from the observed and permuted data. Significant *p* values are shown in italics

	Year	Assortativity coefficient	SE	Mean coef <sub>random</sub>	<i>p</i>
Sex	2013	0.163	0.034	0.123	< 0.001
Age	2013	0.245	0.012	0.219	< 0.001
	2014	0.247	0.008	0.238	0.002
	2015	0.072	0.011	0.067	0.077
	2013	0.253	0.007	0.230	< 0.001
Breeding status	2014	0.298	0.006	0.296	0.126
	2015	0.229	0.005	0.219	< 0.001

(Gaisler 1979). Given that in many bat species, including Natterer's bats, females form maternity colonies with the purpose to give birth and communally rear their young (McCracken and Wilkinson 2000), the observed closer bonds between breeding females may not be surprising. While in most bats of the temperate zone maternity colonies also comprise non-breeding females and—depending on the species—occasionally males, there are no reports that these individuals contribute actively in the rearing of the young (Kunz and Hood 2000). As a consequence, breeding and non-breeding colony members are not expected to share the same roosting requirements. For instance, while torpor (reduction of body temperature) is generally seen as beneficial for adult bats to optimize their energy budget at times of low food availability, it can slow down the development of the pups (Hoying and Kunz 1998; Kunz and Hood 2000) and interfere with lactation in breeding females (Wilde et al. 1999). Thus, stronger associations between individuals that have similar traits—the observed assortative mixing—might be due to similar needs and shared preferences for certain roost conditions. Yet, it is surprising and requires further studies to answer why the significant assortative mixing in the colony did not result in a modular network.

Despite the high fission-fusion dynamics in the colony, we found associations between individuals to be relatively stable up to a timeframe of around 80 days (see LARs), which equals roughly the duration of pregnancy and lactation period (Swift 2001). Afterwards, until the bats started to leave the study area in September, individuals were less present in the monitored boxes (data not shown) which can also be seen in the slopes of the LIRs. Bats might have used natural tree cavities more often at this time (compare (Kerth et al. 2001) or temporally may have left the study site for mating at so-called swarming sites (Rivers et al. 2005). Both behaviors could explain the observed breakdown of individual association in late summer.

We also found evidence that individual associations persist across several years. This could be explained with individual social bonds being revived after long periods of separation as the maternity colonies disintegrate in autumn and individuals

spend the subsequent hibernation period, which lasts several months, mostly solitary (Beaucourmu 1958; Hanák et al. 1962; Gaisler and Hanák 1969). However, most of the parameter that potentially influenced the strength of individual associations within each year, namely relatedness, sex, breeding status, and age class—once the individual is older than one year—did not differ (much) between years. For example, of those individuals with sufficient data, 43 individuals out of 53 (87%) did not differ in their breeding status in two successive years, and 8 out of 9 that were included in the analysis had the same breeding status in 2013 and 2015 (not shown in results). Thus, we cannot fully distinguish whether for Natterer's bats the observed long-term associations are the consequence of long-term individual social bonds—as (Kerth et al. 2011) suggested for Bechstein's bats, a species that also shows fission-fusion behavior (Zeus et al. 2017)—or also reflect assortativity due to shared roosting requirements that remain largely unchanged across several years. Nevertheless, as a pair's most frequented roost only accounted for one quarter of its shared roost use on average, it seems highly unlikely that shared roost preferences alone explain the observed individual roosting associations.

We are aware that the results presented in this paper reflect the social network of one single bat colony. As it has been shown for Bechstein's bats, the social network structure might differ between colonies of the same species (Kerth et al. 2011) or even change in the same colony over time (Baigger et al. 2013). However, in both mentioned studies, group size predicted modularity. Thus, our finding of a non-modular network in such a large colony of Natterer's bats shows that bat species with a very similar roosting and fission-fusion behavior can differ drastically in their network structure, as Bechstein's bats already showed modularity at much smaller colony sizes.

To conclude, while the ecologically similar bat species Bechstein's bat and Natterer's bat both form stable long-term associations with stronger links between closely related individuals (Kerth et al. 2003, 2011), they differ considerably in their network structure: Previous studies have shown—in

accordance with our findings—that extensive fission-fusion behavior, as present in our study species (Zeus et al. 2017), seems to come at the expense of close individual bonds that may be too costly to maintain with many individuals (Dunbar 1991, 1992b; Kudo and Dunbar 2001; Lehmann et al. 2007). A counter-measure that has been observed in many other species, including the Bechstein's bat, is the segregation of the group into communities with strong bonds within and weak bonds between these social units (Lehmann and Boesch 2004; Kerth et al. 2011). In contrast, Natterer's bats maintained full connectivity with overall weak associations. At the same time, pairwise associations were significantly stronger between individuals that were closely related or similar in age and breeding status (assortativity). It remains puzzling, why and how Natterer's bats were able to maintain stable long-term individual associations despite large colony sizes, high fission-fusion dynamics, and low modularity of their social network.

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### Compliance with ethical standards

**Conflict of interest** The authors declare that they have no competing interests.

**Ethical approval** All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. Handling and tagging of the bats were conducted under the permits for species protection (55.1-8642.01-2/00) and animal welfare (55.2-2531.01-47/11 and 55.2-2532-2-20) that had been issued by the government of Lower Franconia.

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## SUPPLEMENT

### DNA Extraction and genetic markers

The DNA from the collected wing tissue was extracted with an ammonium acetate precipitation method (Nicholls et al. 2000). The subsequent amplification via PCR was performed following Halczok et al. (2017).

The following microsatellite markers were used:

A2-Mluc, A13-Mluc, EF15-Mluc, G6-Mluc, G30-Mluc, G31-Mluc, H23-Mluc (Jan et al. 2012);  
b22 (Kerth et al. 2002);

D15, H29 (Castella and Ruedi 2000);

Mnatt1, Mnatt2 (Boston et al. 2012);

Mnatt11 (Scott et al. 2013);

FV5AP (O' Donnell et al. 2016);

GZBYR (Halczok et al. 2017).

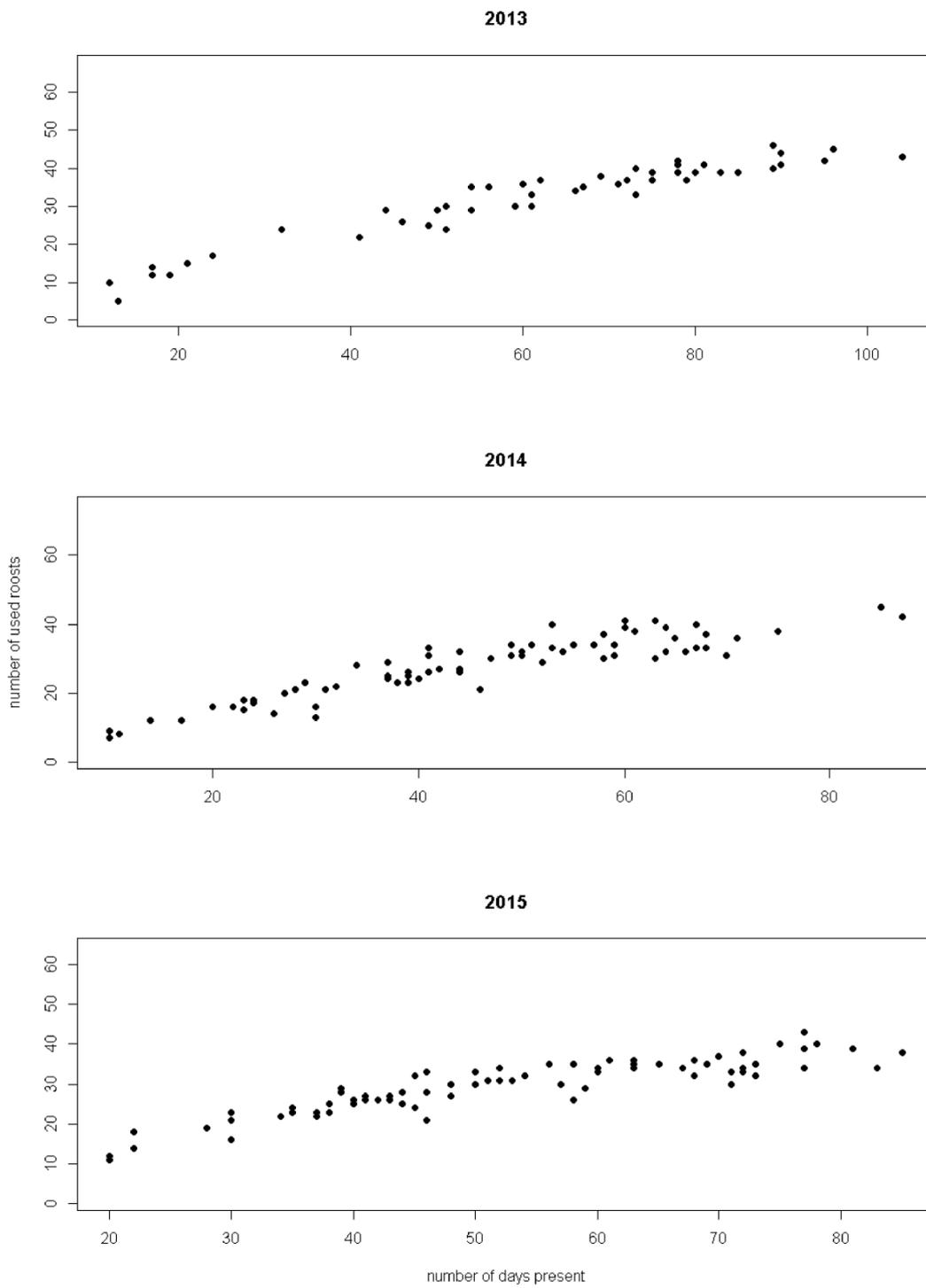
### Analysis of mother-offspring pairs

For the yearlings, which spent their first summer as independent bats in the colony, we additionally aimed on directly identifying their mothers. Mother-offspring pairs were assigned with Cervus version 3.0.7 (Kalinowski et al. 2007). This program uses a likelihood-based approach calculated from the genotypes of all sampled individuals in a population, the estimated proportion of the population sampled, and the probability of typing errors. As potential mothers, we took all marked adult females ("yearlings" and "older" bats) into account that were present in the year the offspring was born (e.g. in 2012 for the yearlings in the network of 2013). This resulted in 38 potential mothers and 28 offspring for the network of 2013, 71 potential mothers and 35 offspring for the network of 2014, and 82 potential mothers and 41 offspring for the network of 2015.

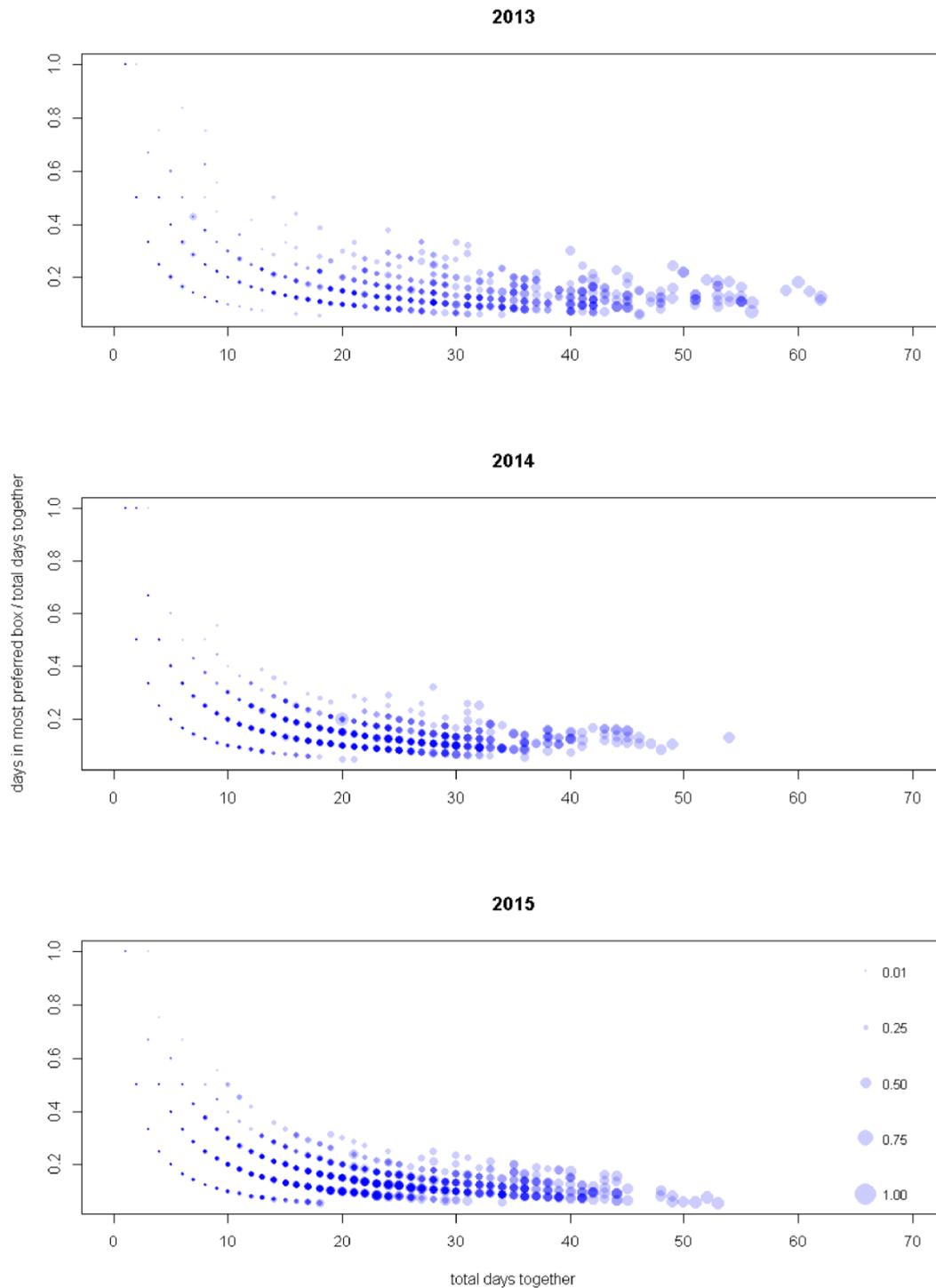
To assess the confidence in assigned mother-offspring pairs, we first ran a simulation of parentage analysis. Therefore, we set the number of offspring to be simulated at 100000. The proportion of candidate mothers sampled in a certain year was estimated from field data by the proportion of all older females that were caught unmarked during the capture events in the following spring and thus could have been missed as potential mothers for the year in question. Based on these calculations we set the proportion of candidate mothers at 56% for 2012 (due to the high number of bats that were still unmarked in spring 2013) and to 85% for both 2013 and 2014. The number of candidate mothers was calculated accordingly from the

number of mothers in the field data and the estimated proportion of mothers sampled. The proportion of mistyped loci was set at the default of 1% and the confidence level, which we set at 95%, was calculated using the delta values. We ran the simulations several times to select one simulation for the subsequent parental analysis of the field data. A simulation was considered useable if all critical delta values of the strict confidence level were within 10% of the mean critical delta value across all simulations. By using the field data and the simulation, mother-offspring pairs were assigned at a confidence level of 95%. The number of assigned mother-offspring pairs was 4 for the network of 2013, 7 for the network of 2014, and 13 for the network of 2015 (i.e. both were present in the colony in the respective year — the offspring as yearling, the mother as older bat).

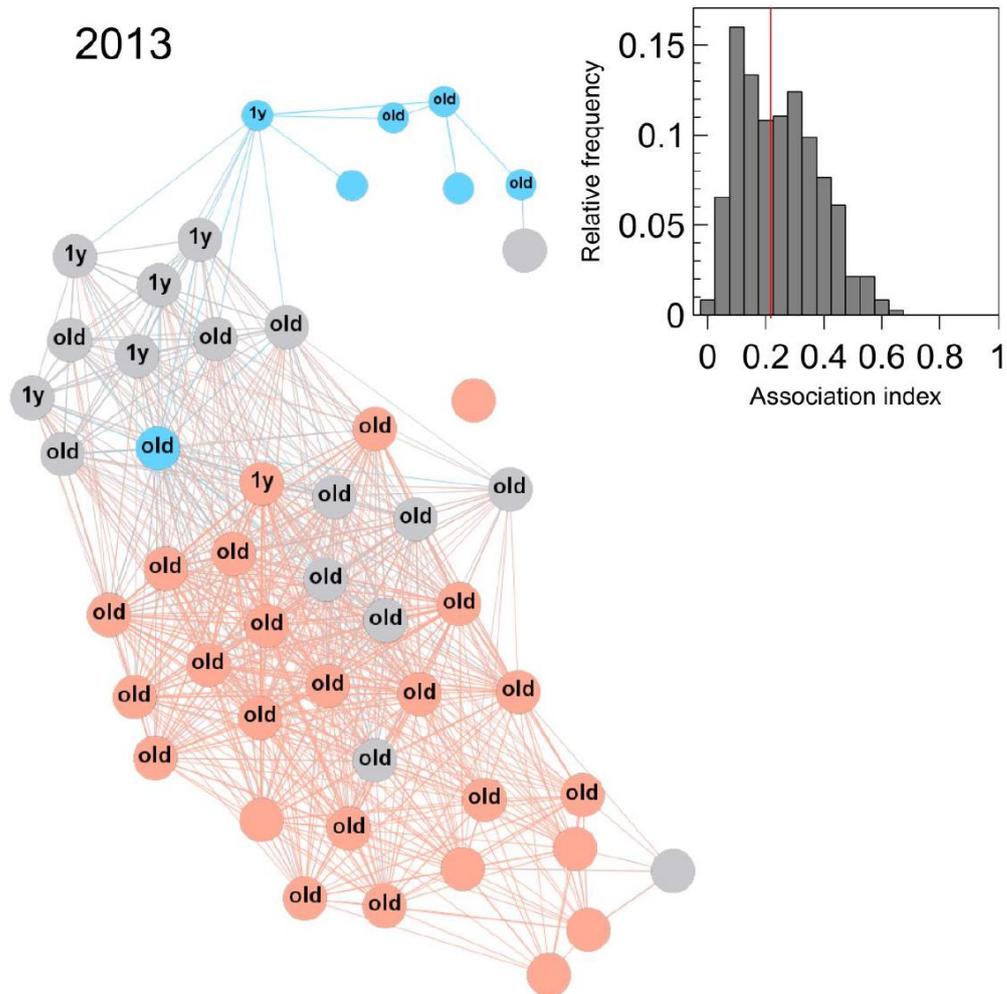
Additional Figures



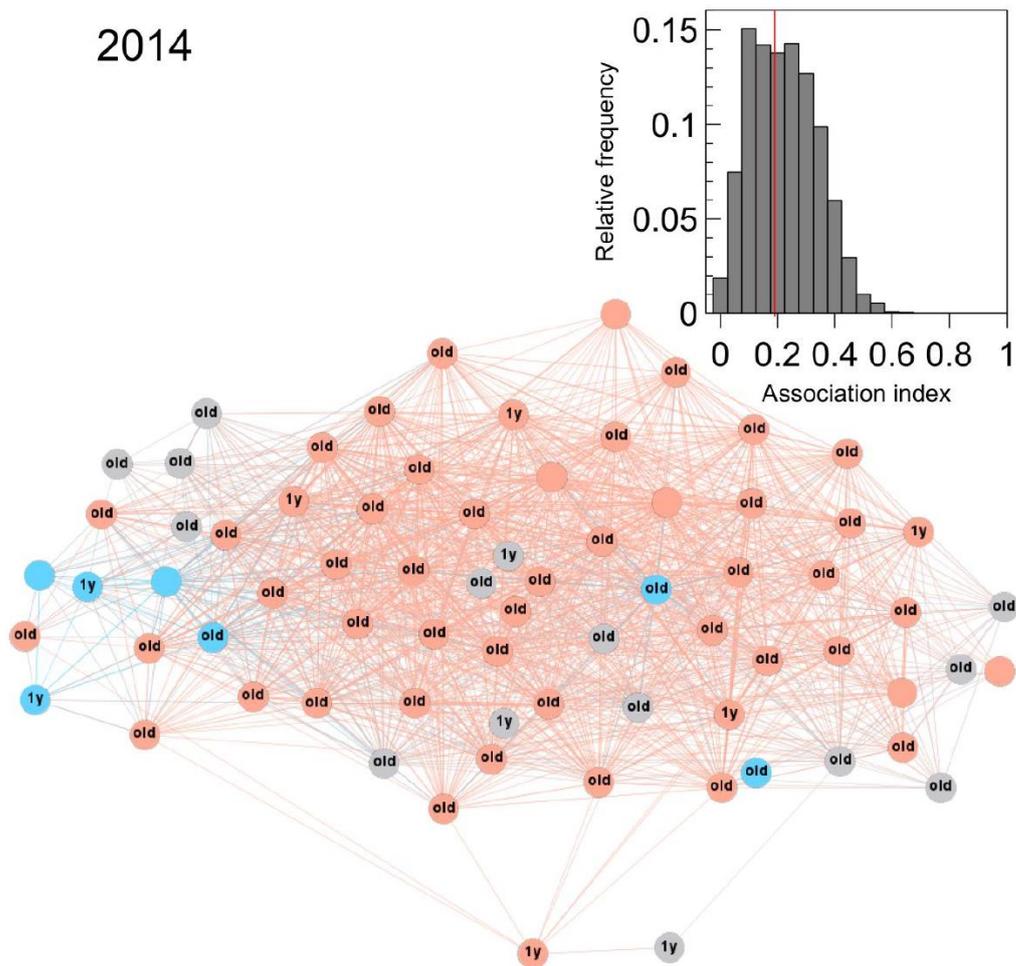
**Fig. S1** The number of different roosts plotted against the number of days an individual was present in the study area (number of observations). Each data point is a bat.



**Fig. S2** For each year, the proportion of days a pair spent in its most frequented roost to the total number of days spent together plotted against the total number of days a pair of individuals spent together. Each data point is a pair of individuals. The diameter of each point represents the strength of association between each pair with larger points signifying stronger associations; see legend in the lower right corner. The difference in color is indicating the number of bat pairs/dyads sharing the same combination of values. The darker a point appears, the more points are plotted in layers above each other



**Fig. S3** The network structure and strength of pairwise associations in 2013, when 49 individuals had been analysed: The right graph shows the frequency distribution of the strength of pairwise associations with the red vertical line indicating the mean association strength. The left graph presents the network structure that was produced with Gephi 0.9.2. (Bastian et al. 2009) using a force-directed algorithm where short edges between two nodes represent stronger associations between these two individuals. While the network is fully connected, for a better visualization this graph only shows social links that are above average strength. The reproductive status of the individuals is colour coded (red = breeding, blue = non-breeding, grey = no information available), the age of the individuals can be found in the node label (“1y” = yearlings, “old” = older bats) and smaller nodes represent male individuals



**Fig. S4** The network structure and strength of pairwise associations in 2014, when 73 individuals had been analysed: The right graph shows the frequency distribution of the strength of pairwise associations with the red vertical line indicating the mean association strength. The left graph presents the network structure that was produced with Gephi 0.9.2. (Bastian et al. 2009) using a force-directed algorithm where short edges between two nodes represent stronger associations between these two individuals. While the network is fully connected, for a better visualization this graph only shows social links that are above average strength. The reproductive status of the individuals is colour coded (red = breeding, blue = non-breeding, grey = no information available), and the age of the individuals can be found in the node label (“1y” = yearlings, “old” = older bats)

Additional tables

Table S1 Results of the linear models assessing relationships between node strength and attributes reproductive status (basic level: non-breeding), and age (basic level: older bats) in all three years separately. The estimate ( $\text{coef}_{\text{observed}}$ ) is given with standard error and t value as well as the mean estimate of the permuted networks ( $\text{mean coef}_{\text{random}}$ ) and the computed p-value from the observed and permuted data (corrected via Holm's Sequential Bonferroni Procedure). Significant p values are shown in bold

Year	lm	$\text{coef}_{\text{observed}}$	SE	t	$\text{mean coef}_{\text{random}}$	p
2013	<i>Strength ~ Reproductive status</i>	5.912	1.266	4.671	5.280	<b>&lt; 0.001</b>
2014	<i>Strength ~ Reproductive status</i>	4.189	1.401	2.991	4.068	0.347
2015	<i>Strength ~ Reproductive status</i>	3.154	0.901	3.499	1.548	<b>&lt; 0.001</b>
2013	<i>Strength ~ Age</i>	-1.328	1.186	-1.119	-0.229	<b>&lt; 0.001</b>
2014	<i>Strength ~ Age</i>	-3.761	1.467	-2.564	-3.180	<b>0.014</b>
2015	<i>Strength ~ Age</i>	-3.251	0.957	-3.397	-1.870	<b>&lt; 0.001</b>

Table S2 Results of the linear models assessing relationships between eigenvector centrality and attributes sex (basic level: female), breeding status (basic level: non-breeding), and age (basic level: older bats) in all three years separately. The estimate ( $\text{coef}_{\text{observed}}$ ) is given with standard error and t value as well as the mean estimate of the permuted networks ( $\text{mean coef}_{\text{random}}$ ) and the computed p-value from the observed and permuted data (corrected via Holm's Sequential Bonferroni Procedure). Significant p values are shown in bold

Year	lm	$\text{coef}_{\text{observed}}$	SE	t	$\text{mean coef}_{\text{random}}$	p
2013	<i>Eigenvector ~ Sex</i>	-0.471	0.082	-5.760	-0.441	<b>0.010</b>
2013	<i>Eigenvector ~ Reproductive status</i>	0.423	0.086	4.917	0.379	<b>0.002</b>
2014	<i>Eigenvector ~ Reproductive status</i>	0.214	0.072	2.978	0.203	0.221
2015	<i>Eigenvector ~ Reproductive status</i>	0.142	0.039	3.640	0.069	<b>0.000</b>
2013	<i>Eigenvector ~ Age</i>	-0.109	0.084	-1.294	-0.016	<b>0.000</b>
2014	<i>Eigenvector ~ Age</i>	-0.190	0.074	-2.558	-0.156	<b>0.002</b>
2015	<i>Eigenvector ~ Age</i>	-0.144	0.042	-3.460	-0.082	<b>0.000</b>

Table S3 Results of the LMMs (across all three years) assessing relationships between eigenvector centrality and the attributes breeding status (basic level: non-breeding), and age (basic level: older bats). The estimate ( $\text{coef}_{\text{observed}}$ ) is given with standard error, degrees of freedom and t value as well as the mean estimate of the permuted networks ( $\text{mean coef}_{\text{random}}$ ) and the computed p-value from the observed and permuted data (corrected via Holm's Sequential Bonferroni Procedure). Significant p values are shown in bold

model	$\text{coef}_{\text{observed}}$	SE	df	t	mean $\text{coef}_{\text{random}}$	P
<i>Eigenvector ~ Breeding status</i>	0.231	0.038	53	6.046	0.180	<b>&lt;0.001</b>
<i>Eigenvector ~ Age</i>	-0.144	0.0368	81	-3.930	-0.083	<b>&lt;0.001</b>

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## 4.2 Astrovirus transmission pathways in Natterer's bats

### Manuscript 2

**Zeus, V. M.**, Köhler, A., Reusch, C., Fischer, K., Balkema-Buschmann, A. & Kerth, G. (submitted). Analysis of astrovirus transmission pathways in a free-ranging fission-fusion colony of Natterer's bats (*Myotis nattereri*).

## Behavioral Ecology and Sociobiology

### Analysis of astrovirus transmission pathways in a free-ranging fission-fusion colony of Natterer's bats (*Myotis nattereri*)

--Manuscript Draft--

<b>Manuscript Number:</b>	BEAS-D-20-00080	
<b>Full Title:</b>	Analysis of astrovirus transmission pathways in a free-ranging fission-fusion colony of Natterer's bats ( <i>Myotis nattereri</i> )	
<b>Article Type:</b>	Original Article	
<b>Corresponding Author:</b>	Veronika Maria Zeus Universität Greifswald GERMANY	
<b>Order of Authors:</b>	Veronika Maria Zeus Arian Köhler Christine Reusch Kerstin Fischer Anne Balkema-Buschmann Gerald Kerth	
<b>Corresponding Author Secondary Information:</b>		
<b>Corresponding Author's Institution:</b>	Universität Greifswald	
<b>Corresponding Author's Secondary Institution:</b>		
<b>First Author:</b>	Veronika Maria Zeus	
<b>First Author Secondary Information:</b>		
<b>Order of Authors Secondary Information:</b>		
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	Deutsche Forschungsgemeinschaft (RTG2010)	Dr. Gerald Kerth
<b>Abstract:</b>	<p>Bats have recently reported to harbor a variety of viruses, some of which may have a zoonotic potential. Consequently, many recent studies have focused on the potential of bats to spread diseases to other animals and to humans. However, virus transmission networks within bat colonies remain largely unexplored. We studied the detection rate and transmission pathway of astroviruses in a free-ranging colony of Natterer's bats (<i>Myotis nattereri</i>) that showed a high fission-fusion dynamic. Based on automatic roost monitoring data of RFID-tagged bats, we assessed the impact of the strength of roosting associations among colony members (node strength) and the number of roosts an individual used – both being proxies for individual exposure risk - on the detected presence of astrovirus related nucleic acid in individual swab samples. Moreover, we tested whether astrovirus sequence types were shared between individuals that frequently roosted together, as a proxy for direct transmission risk, or between bats sharing the same roosts in close temporal succession, as a proxy for indirect transmission risk. Neither node strength nor the number of different roosts used had an effect on detected virus presence in individual bats. Transmission network data suggest that astroviruses are at least partially transmitted via indirect contact, implying that roosts pose a risk of astrovirus infection for several days after the bats left them. Our study offers novel insights in the presence and transmission of viruses within social networks of bat colonies.</p>	
<b>Suggested Reviewers:</b>	Raina Plowright Pennsylvania State University University Park : Penn State	

	rplowright@gmail.com
	Gareth Jones University of Bristol gareth.jones@bristol.ac.uk
	Paul Racey University of Exeter P.A.Racey@exeter.ac.uk
	Kimberly VanderWaal University of Minnesota kvw@umn.edu
	Mark Brigham University of Regina Mark.Brigham@uregina.ca
	Damien Farine Max-Planck-Institut für Verhaltensbiologie dfarine@ab.mpg.de
<b>Opposed Reviewers:</b>	

1 Analysis of astrovirus transmission pathways in a free-ranging  
2 fission-fusion colony of Natterer's bats (*Myotis nattereri*)

3 Veronika M. Zeus<sup>1\*</sup>, Arian Köhler<sup>2</sup>, Christine Reusch<sup>1</sup>, Kerstin Fischer<sup>2</sup>, Anne  
4 Balkema-Buschmann<sup>2</sup>, Gerald Kerth<sup>1</sup>

<sup>1</sup>Zoological Institute and Museum, Greifswald University, Greifswald, Germany.

<sup>2</sup>Friedrich-Loeffler-Institut, Institute of Novel and Emerging Infectious Diseases, Greifswald-  
Insel Riems, Germany

5

6

7 \*Loitzerstrasse 26,

D-17489, Greifswald,

Germany.

veronika.zeus@gmail.com

+0049 17643395401

8 Orcid: 0000-0002-3936-1343

9

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11

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16

## 17 ABSTRACT

18           Bats have recently reported to harbor a variety of viruses, some of which may have a  
19 zoonotic potential. Consequently, many recent studies have focused on the potential of bats  
20 to spread diseases to other animals and to humans. However, virus transmission networks  
21 within bat colonies remain largely unexplored. We studied the detection rate and transmission  
22 pathway of astroviruses in a free-ranging colony of Natterer's bats (*Myotis nattereri*) that  
23 showed a high fission-fusion dynamic. Based on automatic roost monitoring data of RFID-  
24 tagged bats, we assessed the impact of the strength of roosting associations among colony  
25 members (node strength) and the number of roosts an individual used – both being proxies for  
26 individual exposure risk - on the detected presence of astrovirus related nucleic acid in  
27 individual swab samples. Moreover, we tested whether astrovirus sequence types were shared  
28 between individuals that frequently roosted together, as a proxy for direct transmission risk, or  
29 between bats sharing the same roosts in close temporal succession, as a proxy for indirect  
30 transmission risk. Neither node strength nor the number of different roosts used had an effect  
31 on detected virus presence in individual bats. Transmission network data suggest that  
32 astroviruses are at least partially transmitted via indirect contact, implying that roosts pose a  
33 risk of astrovirus infection for several days after the bats left them. Our study offers novel  
34 insights in the presence and transmission of viruses within social networks of bat colonies.  
35

## 36 SIGNIFICANCE STATEMENT

37 Bats have recently moved into the focus of virological research as potential carriers of disease  
38 agents. However, little is known about how viruses are transmitted within bat colonies, and the  
39 sparse information available is based on simulated transmission data. In this field study, we  
40 examined the daily roosting behavior in a wild bat colony in relation to the presence of viruses  
41 in individual colony members. Our findings suggest that astroviruses are at least partly  
42 transmitted indirectly via contaminated roosts. It has been shown previously that astroviruses  
43 can endure in feces for several days, and bats are known to typically defecate in their roosts.  
44 Thus, our findings imply that bats can contract astroviruses even if they use contaminated  
45 roosts days after infected individuals have left. This study provides first-time insights in the  
46 transmission of astroviruses within bat colonies in the wild.

47

48

## 49 INTRODUCTION

50 Living in social groups provides multiple benefits to the individual group members  
51 (Krause and Ruxton 2002), but at the same time increases the risk of exposure to diseases,  
52 as large groups will typically harbor more pathogens than small groups or solitary individuals  
53 (Loehle 1995). Pathogens such as viruses can be transmitted within the group via physical  
54 contact (direct transmission) and/or contaminated, shared resources (indirect transmission)  
55 (Taylor et al. 2001). Thus, individual exposure risk may depend on both, the association  
56 behavior of group members and on joint resource use, even if individuals do not meet directly  
57 because they use the resource at different times.

58 Bats belong to the most gregarious mammals and often form colonies where many  
59 individuals roost in close body contact (Kerth 2008). In many bat species, the colonies often  
60 split temporarily into subgroups that use a number of separate roosts (Kerth and König 1999;

61 O'Donnell 2000; Garroway and Broders 2007; Popa-Lisseanu et al. 2008). This "fission-fusion"  
62 behavior also occurs in many other social mammals (Aureli et al. 2008). Bats are also known  
63 as vectors and reservoir hosts for a number of viruses, including those with zoonotic potential  
64 (Calisher et al. 2006; Omatsu et al. 2007; Luis et al. 2013; Chan et al. 2013; Klimpel and  
65 Mehlhorn 2014). However, while many recent studies have focused on their possible role of  
66 spreading diseases to other animals and to humans (Wood et al. 2012; Plowright et al. 2015),  
67 virus transmission networks within bat colonies remain largely unexplored with only few studies  
68 available that are relying on simulated transmission data (Kashima et al. 2013; Webber et al.  
69 2016).

70         Astrovirus infections are common in birds and mammals including humans (Moser and  
71 Schultz-Cherry 2005; De Benedictis et al. 2011) and bats (Fischer et al. 2016, 2017; Halczok  
72 et al. 2017). Mammalian astroviruses typically cause diarrhea (Moser and Schultz-Cherry 2005)  
73 and are transmitted via the fecal-oral route (Mendenhall et al. 2015). In bats, however,  
74 individuals carrying astrovirus related RNA appear healthy (Fischer et al. 2017). While little is  
75 known about astrovirus infection routes in bats, individuals can be assumed to be at infection  
76 risk when associating with infected colony members. Moreover, as (human) astroviruses can  
77 persist for several days in the environment (Abad et al. 2001), bats may get infected when  
78 using contaminated roosts even if the virus shedding animals are no longer present. The high  
79 detection rate and diversity of bat astroviruses (Chu et al. 2008; Drexler et al. 2011; Fischer et  
80 al. 2016, 2017) makes them well suited for analyzing virus transmission networks in bat  
81 colonies.

82         The aim of this study was to examine astrovirus transmission pathways in the social  
83 networks of bat colonies. Therefore, we compared individual association behavior and roost  
84 use of a free-ranging maternity colony of Natterer's bats (*Myotis nattereri*) to data of the bats'  
85 detected individual astrovirus infection status (positive/negative).

86         Bat astrovirus sequences have been found in feces and urine (Fischer et al. 2017),  
87 which are excreted during roost use and remain there after the colony moves to a new  
88 communal roost. During the breeding season in summer, female Natterer's bats form fission-

89 fusion colonies, with subgroups switching day roosts almost daily (Zeus et al. 2017). Thus, if  
90 astroviruses circulate among colony members, their highly dynamic roosting behavior likely  
91 results in the contamination of many of the colony's roosts. Despite the fission-fusion dynamics,  
92 the colony network of Natterer's bats is almost fully connected with low modularity and many  
93 links per individual (Zeus et al. 2018). Such a social organization, characterized by many social  
94 contacts, is presumed to facilitate the spread of pathogens throughout the colony (Keeling  
95 2005; Griffin and Nunn 2012).

96 We created an undirected, weighted association network based on daily roosting  
97 observations (Zeus et al. 2018). Additionally, individual fecal and urine samples were collected  
98 and subsequently tested for the presence of astrovirus related RNA (Fischer et al. 2016). We  
99 examined whether an individual's node strength and its roost repertoire (number of different  
100 roosts) influence its astrovirus infection status detected by PCR-based methods. We expected  
101 individuals that are closely connected to many other colony members in their social network  
102 (high node strength) - and thus more exposed to pathogens carried by other bats - to have a  
103 higher infection risk than individuals with weaker social bonds. Moreover, we expected that  
104 individual bats that switch between a low number of roosts face an increased infection risk, as  
105 they have a higher probability to re-use contaminated roosts while the astroviruses are still  
106 infectious. Such a pattern has been found for an ectoparasite that reproduces in bat roosts  
107 (Reckardt and Kerth 2006, 2007). Finally, we examined possible astrovirus transmission routes  
108 between colony members. If astroviruses were mainly transmitted via direct contact, closely  
109 associated individuals should share the same virus sequence types more often. Conversely, if  
110 virus transmission occurred indirectly via contaminated roosts, we expected more shared  
111 sequence types between individuals that used the same roosts in short temporal succession.

112

## 113 METHODS

### 114 **Data collection and preparation**

#### 115 Social network data

116 We studied a colony of 73 RFID-tagged female Natterer's bats in a forest near the city  
117 of Würzburg, Germany between May and September 2014. The colony roosts in bat boxes  
118 (type 2FN, Schwegler, Germany) that have been monitored with (radio-frequency identification)  
119 RFID-loggers in previous studies to collect data on roost use (Zeus et al. 2017) and association  
120 behavior (Zeus et al. 2018). Daily roosting associations between adult bats (simultaneous  
121 presence in the roost) were analyzed using the R package "asnipe" (Butts 2016). Individual  
122 node strength and pairwise associations (hereinafter referred to as "association strength")  
123 were calculated using the "Simple Ratio Index" (SRI) (Cairns and Schwager 1987; Ginsberg  
124 and Young 1992). Only bats that were already marked at the beginning of the study in May  
125 were included in this analysis to ensure sufficient data on their social network (for further details  
126 see (Zeus et al. 2018)).

127

#### 128 Subsequent roost sharing

129 Based on the RFID-data we assessed each individual's roost repertoire. Moreover, we  
130 calculated for each pair of individuals how often they separately occupied the same roost within  
131 four days after one another (hereinafter referred to as "subsequent roost sharing"). The  
132 timeframe was chosen as the stability of infectious astroviruses in the environment is likely to  
133 decline after four days (Abad et al. 2001). For example, for a given pair consisting of an  
134 individual A and an individual B, we counted each time individual A was using a roost within 4  
135 days after individual B had left that roost to move to a new day roost. If during this time frame  
136 the bat B returned to the roost where A was still present - resulting in a direct contact between  
137 the two bats - the census was cancelled and started again after one of the two bats had moved  
138 to another roost. This was done to exclude periods with direct contact between the respective  
139 bats from this roost sharing dataset. We proceeded accordingly for individual B using roosts  
140 after individual A, and subsequently summed the results of both individual A following individual  
141 B in its roost use and vice versa.

142 This yielded an undirected roosting index for each pair of individuals that represents  
143 how often they shared the same roost within four days, albeit not simultaneously. The R script

144 for these calculations is available on demand. We repeated this analysis for time spans of two  
145 and three days, to test for the sensitivity of our analyses with respect to the time span between  
146 subsequent roost sharing events.

147

148 Detection of astrovirus sequences and definition of sequence type groups

149         During the capture events in spring and summer, individual fecal and urine samples  
150 were collected in 500µl RNA $\text{later}^{\text{TM}}$  buffer (Thermo Fisher Scientific). The samples were stored  
151 frozen at -20°C until analysis. RNA was extracted using the QIAamp Viral RNA Mini Kit  
152 (QIAGEN) following the standard protocol. A hemi-nested RT-PCR following the protocol  
153 published by Chu et al. (2008) targeting the *RNA-dependent-RNA-polymerase* gene was used.  
154 Samples showing a single band in the expected size of around 400 nt were purified using the  
155 GeneJET PCR Purification Kit (Thermo Fisher Scientific). Sequencing of purified RNA  
156 fragments was performed via tube read service by Eurofins genomics. A forward read and a  
157 reverse read were used to build a consensus sequence and unambiguous sequence areas  
158 were used to identify sequence variants by aligning and comparing them with known bat  
159 astrovirus sequences from NCBI GenBank. All PCR analyses were performed in three  
160 individual experiments to minimize the rate of ambiguous sequences. Afterwards a consensus  
161 sequences of all alignments was built for each sample. Due to the relatively low stability of viral  
162 RNA in field samples, the triplicate PCR assays did not reveal positive results in all runs. Only  
163 samples that were tested positive at least two out of three times were counted as positive.  
164 Individuals, for which several samples had been collected across different capture events were  
165 counted astrovirus positive, if at least one of their samples was tested positive.

166         Next, we defined sequence type groups for the identification of variations in the  
167 amplified sequences. This was necessary since the we did not have access to the full-length  
168 genome data of the detected astrovirus variants, as the applied PCR protocol leads to the  
169 amplification of a 400bp fragment within the ORF1 (*RdRP* gene). The relatively poor RNA  
170 quality in the collected fecal and urine samples did not allow for the generation of additional  
171 sequence data. We therefore had to restrict our analysis to this short and comparably highly

172 conserved fragment, taking into consideration that we may be missing additional variations  
173 outside the analyzed area. We thus analyzed this target region of 60 amino acids (aa), and  
174 defined all variants with an identical sequence in this area as one type group (TG). To identify  
175 this region, we used the specific 32 aa sequence “WYxxxxxxxxxxPxGExxxxxxGNPSGQxST”  
176 which was present in all of our detected astrovirus fragments for a reliable orientation, and  
177 added 5 aa positions upstream and 23 aa positions downstream of this sequence, thus adding  
178 up to 60 aa.

179

180

### 181 **Data Analysis**

182 Data analysis was performed in R version 3.6.2 (R Core Team 2016). Only bats that  
183 were successfully sampled for virus data were included to avoid an influence by false negative  
184 individuals.

185 In order to assess if an individual's node strength or its roost repertoire influenced  
186 whether that individual was astrovirus positive, we used a generalized linear model (GLM). For  
187 each individual, the presence/absence of astrovirus RNA were set as a response variable,  
188 while the individual's node strength and roost repertoire were set as predictor.

189 Although a pair's association strength and their index of sharing the same roost after  
190 each other (but not simultaneously) were moderately positively correlated (Spearman's rank  
191 correlation,  $\rho=0.543$ ,  $p<0.001$ ), the scatter plot (see Fig. 1S in the Supplement) revealed a  
192 broad distribution of data points. Thus, we further tested whether association strength (proxy  
193 for direct transmission) or subsequent roost sharing within two, three or four days (proxy for  
194 indirect transmission) better predicted virus transmission between pairs of individuals. This  
195 was calculated via MRQAP with double Dekker semipartialling (Dekker et al. 2007) and 1000  
196 permutations using the 'sna' package in R (Butts 2016) – similar to the paper by VanderWaal  
197 et al. on social and pathogen transmission networks in giraffes (VanderWaal et al. 2014). If a  
198 pair of individuals shared at least one TG, we assumed that an astrovirus variant was  
199 transmitted between these bats. Virus transmission was tested against each covariate

200 separately and both in combination by using logistic regression models. The selection of the  
201 best model was determined via the Akaike information criterion (AIC).

202

203 RESULTS

#### 204 **Virus detection rate and type groups**

205       During our capture events, we were able to collect fecal or urine samples from 45 of  
206 the 72 colony members, 33 of which were subsequently tested astrovirus positive. Among  
207 those 33 bats, we found 10 different TGs with the most common TG being present in 70% (23)  
208 of the positive bats, while 6 TGs were only recovered from one bat each (Figure 1). In 15% (5)  
209 of the positive individuals, we detected more than one TG on a given sampling day. Across the  
210 study period, individuals harbored a variety of TGs with up to three different TGs found in one  
211 individual albeit not simultaneously.

212

#### 213 **Influence on individual infection status**

214       The GLM assessing relationships between virus detection, and the attributes node  
215 strength (ranging between 3.95 and 20.32, mean node strength: 14.32) and roost repertoire  
216 (ranging between 7 and 45 different roosts, mean number of roosts per individual: 27.5)  
217 revealed no significant influence of either parameter (Table 1).

218

219

#### 220 **Virus transmission**

221       We found a significant influence of subsequent roost sharing within four days on virus  
222 transmission (Table 3, Figure 2). Although pairwise association strength per se was not  
223 significant, the model including subsequent roost sharing and association strength was slightly  
224 better ( $\Delta AIC = 2$ ) to predict virus transmission than the one taking solely the subsequent roost  
225 sharing into account (Table 2). However, the large step in the AIC ( $\Delta AIC = 17$ ) as soon as

226 roost sharing is added to the model indicates that this variable is clearly the more relevant one  
227 (Table 2). The other tested time spans of 2 and 3 days between subsequent roost sharing  
228 events revealed very similar results with slightly higher AICs (data not shown).

229

## 230 DISCUSSION

231       Astrovirus infections are widespread and diverse in mammals (Moser and Schultz-  
232 Cherry 2005; De Benedictis et al. 2011), including Natterer's bats (Kemenesi et al. 2014, 2016;  
233 Fischer et al. 2016, 2017; Halczok et al. 2017). This was confirmed in our study, as we found  
234 an astrovirus detection rate of 73% among all sampled individuals. Furthermore, we observed  
235 10 different astrovirus sequence groups (TGs) among 33 positive tested bats. Although the  
236 analysis of TGs based on a 60 aa fragment cannot completely replace a full genome analysis,  
237 we consider this approach a good proxy for our application, given the fact that a full genome  
238 analysis was not possible with the available fecal and urine samples. In humans and domestic  
239 animals, astrovirus infections are especially common in settings with a high density of hosts,  
240 e.g. in schools and nursery homes or in breeding kennels and farms, respectively (Glass et al.  
241 1996; Shan et al. 2011; Grellet et al. 2012). Interestingly, in the highly interconnected colony  
242 of Natterer's bats studied here, neither the overall strength of an individual's associations with  
243 all other colony members (node strength) nor its repertoire of used roosts correlated with the  
244 detected presence of astrovirus in the respective individual.

245       Astroviruses are typically transmitted between hosts via the fecal-oral route  
246 (Mendenhall et al. 2015). The results of our MRQAP models showed no clear indication for a  
247 direct astrovirus transmission in Natterer's bats via association. This is surprising, given the  
248 close contact between roost mates in bat maternity colonies in general (Kerth 2008), but might  
249 be the result of the unimodular network structure in Natterer's bats (Zeus et al. 2018): In  
250 comparison to bat species with a similar roosting behavior (Bechstein's bat, Kerth et al. 2011;  
251 Baigger et al. 2013), their almost fully connected network with comparably less differentiated  
252 links between individuals might dilute any effect of association on disease transmission.  
253 However, albeit not significant, an effect of association strength should not be completely

254 disregarded as the model which included both subsequent roost sharing and association  
255 strength was our best fit.

256           Regarding the p-values of the MRQAP analysis, our data suggest that in Natterer's bats  
257 indirect transmission via contaminated roosts might play a significant role in astrovirus  
258 infections, with the virus likely to persist for several days in the environment (Abad et al. 2001).  
259 Bats display two main behavioral mechanisms of parasite and pathogen avoidance: roost  
260 selection and roost switching (Lewis 1995; Reckardt and Kerth 2007). Bechstein's bats have  
261 been shown to prefer new roosts to previously occupied ones, which are potentially infected  
262 with parasites and / or other pathogens- (Reckardt and Kerth 2007). Moreover, in both,  
263 Bechstein's bats (Kerth et al. 2000) and brown long-eared bats (Zeus et al. 2017), different  
264 colonies do not share roosts, which likely reduces the exposure risk of species-specific  
265 ectoparasites. Many forest-living bat species (Kerth and König 1999; O'Donnell 2000;  
266 Garroway and Broders 2007; Popa-Lisseanu et al. 2008), including Natterer's bats (Zeus et al.  
267 2017, 2018), switch roosts almost daily and frequently split into subgroups. This "fission-fusion"  
268 behavior has been assumed as a mechanism to avoid parasites and other pathogens that may  
269 be present in the roosts (Reckardt and Kerth 2007; Kashima et al. 2013; Webber et al. 2016).  
270 On the other hand, the frequent switching between many roosts might also facilitate pathogen  
271 transmission, as it would result in the contamination of many roosts.

272           I must be noted that according to the low Pseudo-R<sup>2</sup> values, none of our three models  
273 explained our data fully. This might be due to certain constraints of our field data - e.g. lack of  
274 temporal resolution of the virus data, relatively small percentage of individuals sampled for  
275 virus data, and the clear predominance of one TG in the colony. Such caveats are common  
276 when working with elusive, protected, and free-ranging animals. However, we are convinced  
277 that such data sets collected in the field are invaluable for future research both for the design  
278 of follow-up field studies and for setting refined parameters in simulation studies exploring virus  
279 transmission in bat colonies.

280           To conclude, the precise factors influencing the presence of astroviruses in Natterer's  
281 bats still need further evaluation, as neither an individual's node strength nor the number of

282 different roosts it used during the season played a significant role in our study. At the same  
283 time, we found implications for indirect transmission routes via previously occupied roosts,  
284 although more - yet unknown - factors also seem to be involved. Overall, our study provides  
285 first insights into the transmission of astroviruses within the social network of free-ranging bats.  
286

## 287 **ETHICAL STATEMENT**

### 288 Funding

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291 the DFG Research training Group "Biological Responses to Novel and Changing  
292 Environments" (RTG 2010).

293

### 294 Conflict of interest

295 The authors declare that they have no competing interests.

296

### 297 Ethical approval

298 All applicable international, national, and/or institutional guidelines for the care and use of  
299 animals were followed. Handling and tagging of the bats were conducted under the permits for  
300 species protection (55.1-8642.01-2/00) and animal welfare (55.2-2531.01-47/11 and 55.2-  
301 2532-2-20) that had been issued by the government of Lower Franconia.

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434

435

#### 436 FIGURE CAPTIONS

437 Figure 1: Detection rate of the different virus type groups (TGs) in Natterer's bats from the same colony.

438 Figure 2: Relationship between subsequent roost sharing within 4 days and the probability of sharing at least one

439 TG in our best fitted model (Model C, Table 2). The value of the covariate 'association strength' is set as mean

440 association value 0.24. Shading indicates a 95% confidence interval around the regression line.

441

442

#### 443 TABLES

444

445 Table 1: Results of the GLM. The estimate is given with standard error, z value, and the p-value.

Model	Estimate	SE	z value	p-value
<i>Detected infection status ~ Node strength</i>	0.026	0.090	0.307	0.759
<i>Detected infection status ~ Roost repertoire</i>	0.020	0.041	0.492	0.623

446

447 Table 2: Reliability of fit statistics of the MRQAP models. Roost sharing in these models was set to a timespan

448 within 4 days. The AIC,  $\Delta$ AIC, and Pseudo-R<sup>2</sup> as "(null deviance – residual deviance) / (null deviance – residual

449 deviance + numerator degrees of freedom)" are given. The best model is marked in bold.

Model	Covariate	AIC	$\Delta$ AIC	Pseudo-R <sup>2</sup>
A	Association strength	711.901	17.091	0.044
B	Subsequent roost sharing	696.862	2.052	0.069
<b>C</b>	<b>Association strength</b> <b>Subsequent roost sharing</b>	<b>694.810</b>	<b>0.000</b>	<b>0.076</b>

450

451

452 Table 3: Results of the MRQAP models. Roost sharing in these models was set to a timespan within 4 days. The

453 log odds estimate with SE, the odds ratio estimate, and the p-value are given. The results of the best fitted model

454 are marked in bold.

Model	Covariate	Estimate log odds	SE	Estimate odds ratios	P-value
A	Association strength	3.843	0.806	46.657	0.079
B	Subsequent roost sharing	0.115	0.019	1.122	0.024
C	<b>Association strength</b>	<b>1.856</b>	<b>0.926</b>	<b>6.400</b>	<b>0.319</b>
	<b>Subsequent roost sharing</b>	<b>0.093</b>	<b>0.022</b>	<b>1.098</b>	<b>0.030</b>

455

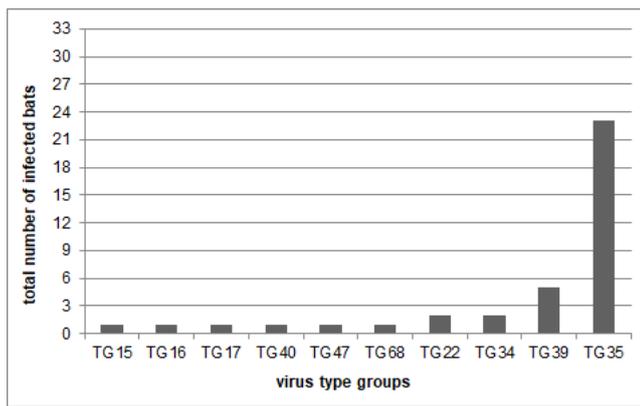


Figure 1

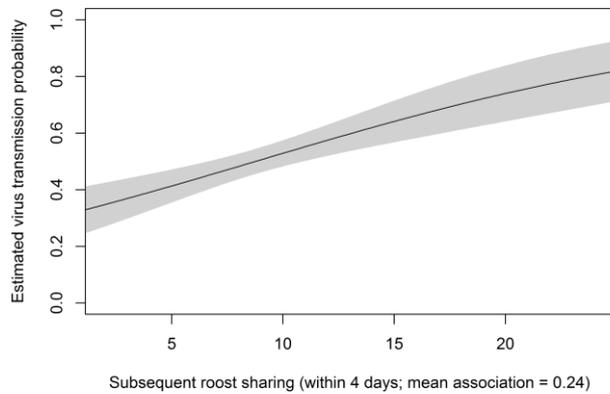


Figure 2



### **4.3 Roosting dynamics between co-occurring con- and heterospecific bat colonies**

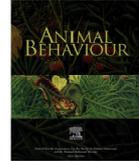
#### **Manuscript 3**

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## Conspecific and heterospecific social groups affect each other's resource use: a study on roost sharing among bat colonies



Veronika M. Zeus\*, Sébastien J. Puechmaille, Gerald Kerth

Zoological Institute and Museum, Greifswald University, Greifswald, Germany

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Sharing resources with conspecifics or heterospecifics can involve costs like increased competition or higher pathogen infection risks as well as benefits such as information on the location, quality and availability of resources. Depending on the consequences of sharing resources, the responses of individuals towards resources used by conspecifics and heterospecifics can range from ignoring them through avoidance to attraction. Within bats it is well known that colony members share information about day roosts and roost switching is often coordinated within the colony. However, little is known about roosting interactions between distinct colonies of conspecifics or heterospecifics. In this study, we investigated roosting interactions between five co-occurring bat colonies that belong to three forest-living species (*Myotis bechsteinii*, *Myotis nattereri*, *Plecotus auritus*). Occupied roosts were continuously monitored with an automatic RFID system over three maternity seasons. Furthermore, we used simulations to test whether colonies preferentially occupied recently used roosts of other colonies. We found no evidence that the roosting behaviour of the *M. bechsteinii* colony was influenced by the co-occurring heterospecific colonies. In contrast, *P. auritus* and *M. nattereri* frequently explored roosts of conspecific and heterospecific colonies, respectively. Nevertheless, with largely separated roosting ranges, the three *P. auritus* colonies avoided occupying roosts that had been inhabited by conspecific colonies. In contrast, *M. nattereri* specifically occupied recent roosts of all three *P. auritus* colonies. Our results give evidence that co-occurring colonies of conspecific and heterospecific bats can influence each other's roost usage. Our findings have implications for both our understanding of inter- and intraspecific resource sharing among distinct social groups and the management of forest-living bats that are of conservation concern.

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Individuals that co-occur in the same habitat typically compete for local resources (Boyce & McDonald, 1999; Manly, McDonald, Thomas, McDonald, & Erickson, 2010). This either leads to the exclusive use of a particular resource by one individual, e.g. through interference competition (Amarasekare, 2002), or to the sharing of the resource among many. Sharing of resources can have both costs and benefits. The actual cost–benefit ratio will often depend on the identity of the interacting individuals. For example, competition is generally higher within than between species (Connell, 1983). On the other hand, in many animals, individuals benefit from grouping with conspecifics (Krause & Ruxton, 2002). The cost–benefit ratio can also depend on the type of resources, e.g. whether they become depleted if shared. For instance, while a food patch may become quickly consumed when many individuals aggregate there, the

quality of a shelter may be less affected by the number of individuals using it.

However, even if resources can be shared without being depleted and in the absence of aggression towards other individuals through interference competition, aggregations of individuals may entail costs such as increased transmission risk for pathogens and parasites (e.g. Durrer & Schmid-Hempel, 1994; Leu, Kappeler, & Bull, 2010). As pathogens and parasites often remain infectious for some time after the host has left (e.g. Reckardt & Kerth, 2006) such costs can occur even if individuals use the same resource at different times. Moreover, if parasites are species specific, the transmission risk at shared resources will differ for conspecifics and heterospecifics. Benefits of resource sharing include the multiple grouping benefits that arise from animals gathering with each other (Krause & Ruxton, 2002). For example, by aggregating at resources, predation risks can be reduced per capita due to a dilution effect (Cresswell, 1994; Foster & Treherne, 1981; Schmidt & Ostfeld, 2001). Additionally, shared interest in

\* Correspondence: V. M. Zeus, Zoological Institute and Museum, Greifswald University, Johann Sebastian Bach Street 11/12, D-17489, Greifswald, Germany.  
E-mail address: [veronika.zeus@gmail.com](mailto:veronika.zeus@gmail.com) (V. M. Zeus).

the same resources may provide animals with the opportunity to gain information from conspecifics or even from heterospecifics on the locality, availability and quality of such resources (Dawson & Chittka, 2012; Poysa, 2006; Valone & Templeton, 2002).

The response of individuals to co-occurring conspecifics and heterospecifics is strongly dependent on the cost–benefit ratio of sharing resources with each other. Consequently, a wide variety of behavioural responses to the co-occurrence of conspecifics and heterospecifics has been reported, ranging from ignoring them through avoidance/aggression to attraction (Ferretti, Sforzi, & Lovari, 2008; Hoogland & Brown, 2016; Latham, Latham, Mccutchen, & Boutin, 2011; Losin, Drury, Peiman, Storch, & Grether, 2016; Mönkkönen, Forsman, Helle, & Monkkonen, 1996; Nazeri, Madani, Kumar, Salman Mahiny, & Kiabi, 2015). Thus, by analysing the respective behavioural response one can infer the likely costs and benefits of sharing resources. However, relatively little is known about the extent to which co-occurring conspecifics or heterospecifics of different social groups influence each other in their respective use of resources. The roosting behaviour of temperate zone forest-dwelling bats is particularly well suited for the investigation of this issue. In most of these species, females are philopatric to their natal maternity colony where they give birth and raise their young during the maternity season in summer (Burland & Wilmer, 2001; Kerth, 2008). Adult males are typically solitary but sometimes join the colonies, depending on the species and the respective population (McGuire & Boyle, 2013; Nardone et al., 2015; Safi, 2008). In late summer the colonies disband and the individuals leave the area to mate at swarming sites and subsequently hibernate until spring before returning to their maternity sites (Dietz, Helversen, & Nill, 2007; Parsons, Jones, & Greenaway, 2003).

The maternity colonies of forest-living bats frequently switch their day roosts (Kerth, Perony, & Schweitzer, 2011; Park, Masters, & Altringham, 1998), which are typically located in tree cavities or artificial bat boxes (Dietz et al., 2007). The colonies also often split temporarily into a variable number of subgroups that use separate roosts ('fission–fusion' dynamics, e.g. Garroway & Broders, 2007; Kerth & König, 1999; Popa-Lisseanu, Bontadina, Mora, & Ibáñez, 2008). It is not clear why bat colonies show such a highly dynamic roosting behaviour on an often-daily basis. However, different studies have assumed that roost switching allows for the selection of optimal roosting conditions and the avoidance of predation and parasites (Kerth, Weissmann, & König, 2001; Lewis, 1995; Patterson, Dick, & Dittmar, 2007). Fission–fusion, on the other hand, might enhance transfer of information about available roosts within the colony and further allows colony members to balance individual needs and group preferences (Fleischmann & Kerth, 2014; Kerth, Ebert, & Schmidtke, 2006; Kerth & Reckardt, 2003; Popa-Lisseanu et al., 2008; Pretzlaff, Kerth, & Dausmann, 2010). A side effect of this frequent roost switching is close proximity of co-occurring conspecific and heterospecific bat colonies that typically do not mix (August, Nunn, Fensome, Linton, & Mathews, 2014; Kerth & Van Schaik, 2012). Moreover, as a further consequence, colonies of many forest-living bat species need to regularly find new, suitable roosts. To do so, bats typically visit potential roosts briefly during several nights (hereafter referred to as 'prospecting'; Kerth & Reckardt, 2003; Reed, Boulinier, Danchin, & Oring, 1999) before later using them as day roosts. The information on potential roosts gathered in this way is shared between members of the colony (Kerth & Reckardt, 2003; Wilkinson, 1992), allowing for a coordinated roost switching of the colony members (Fleischmann et al., 2013; Kerth et al., 2006).

In the European temperate zone, 13 bat species have been described as forest dwelling, regularly using roosts in tree cavities and bat boxes for rearing their offspring, and often several of these

co-occur in a single forest patch (Dietz et al., 2007). Even within the same habitat, the degree of fission–fusion and roost-switching behaviour can vary between colonies and species, as does the ability to find new roosts (Baigger et al., 2013; Fleischmann & Kerth, 2014; Kerth et al., 2011; Ruczyński, Kalko, & Siemers, 2009). Thus, by following the roost choices of other conspecific or heterospecific colonies, bats could improve their efficiency when locating new roosts (Ruczynski, Kalko, & Siemers, 2007; Schöner, Schöner, & Kerth, 2010). A drawback to this would be that, by using roosts that have been previously occupied by other colonies, bats would risk exposure to infectious pathogens (Plowright et al., 2015) and parasites (Reckardt & Kerth, 2006) that remain infectious in the roosts for some time after the previous occupants left. Owing to the high species specificity of many pathogenic agents and parasites, roosts of conspecifics are expected to pose a higher infection risk than roosts of heterospecifics (Davies & Pedersen, 2008; Dick, 2007; Fischer et al., 2016; Streicker et al., 2010). Currently, however, almost nothing is known about the extent to which distinct bat colonies of conspecifics or heterospecifics influence each other's roosting behaviour, e.g. whether they follow each other in their roost choice or avoid the roosts of other colonies.

Using an extensive set of field data in combination with a simulation approach, this study assessed whether five different co-occurring bat colonies belonging to three European bat species influenced each other's roosting behaviour. A previous study on two of our three study species (Fleischmann & Kerth, 2014) demonstrated differences in their abilities to find roosts and in roost-switching behaviour. We therefore expected interest in the roost choice of other colonies to vary between our study species, e.g. depending on their respective need and ability to find new roosts. A previous survey on bat ectoparasites (Rupp, Zahn, & Ludwig, 2004) showed that, although some species of parasites that are typically transmitted via roosts (bat bugs, bat fleas and bat flies) can infest several host species, none of these parasites are shared between our three study species. The same is true for viruses that were detected in the faeces of our colonies by a study that accompanied our research (Fischer et al., 2016). Thus, if different colonies explore each other's roosts and subsequently occupy them, we expected these colonies to discriminate between roosts of conspecifics and heterospecifics.

## METHODS

### *Study Site and Subjects*

We collected roosting data in 2013 (133 days), 2014 (153 days) and 2015 (154 days) between April and September, for a total of 440 days in a forest near the city of Würzburg, Germany. The area comprises the home ranges of one *Myotis nattereri* sensu stricto colony ('Mnat', Puechmaille et al., 2012), one *Myotis bechsteinii* colony ('Mbec') and three *Plecotus auritus* colonies ('Paur1', 'Paur2' and 'Paur3'). All three species are medium-sized vespertilionid bats with a similar roosting ecology (Andreas, Reiter, & Benda, 2012; Dietz et al., 2007). Members of the same colony are defined as individuals that roost together at least intermittently while members of different colonies never roost together (Kerth et al., 2011; Kerth, Safi, & König, 2002).

We worked with a radio frequency identification system (RFID) that is well established for the automatic monitoring of bats (Baigger et al., 2013; Fleischmann & Kerth, 2014; Kerth & König, 1999; Kerth et al., 2011). RFID systems consist of a transponder, an antenna and a data logger (RFID reader). The antenna emits weak electromagnetic waves and if a transponder (RFID tag) is nearby, the antenna can read the identification number of the transponder without direct contact. This number is then stored

together with the respective date and time of the reading on the RFID reader attached to the antenna. These data can then be downloaded on a laptop for further analyses. Our adult bats are marked with individual RFID tags allowing the identification of all marked individuals within each colony. Every year before 20 May when the females became visibly pregnant and after the fledging of the offspring in late July and August, unmarked bats were captured from their day roosts by hand (about two to three times per year) and marked with RFID tags that were inserted via subcutaneous injection. Afterwards, tagged individuals were immediately returned to their colony.

In total, 134 bat boxes (type 2FN, diameter: 16 cm; height: 36 cm; Schwegler, Germany) have been installed in the study area in previous years. During the maternity season, from spring to late summer, all colonies use these boxes regularly as day roosts (hereinafter simply referred to as 'roosts') in addition to natural tree cavities. We checked all boxes each day without opening them using a standard protocol (Kerth et al., 2011; Kerth & Reckardt, 2003). At occupied boxes, we installed automatic RFID tag readers to monitor the activity of bats from dusk until dawn. Date, time and RFID tag number of bats passing the logger antenna placed at the box entrance were recorded. Bats that were recorded during the emergence from the bat box in the evening were assigned as having spent the day in the respective box (Kerth et al., 2011; Kerth & Reckardt, 2003). The rate of logger malfunctions due to technical problems such as battery failure or antennae damage was very low (3% of all monitored boxes).

#### Ethical Note

Handling and tagging of the bats were conducted under permits for species protection (55.1-8642.01-2/00) and animal welfare (55.2-2531.01-47/11 and 55.2-2532-2-20) that had been issued by the government of Lower Franconia.

#### Data Analysis

First, we analysed the respective roosting behaviours of all colonies to compare their demand for finding new roosts. To do so we quantified various roosting parameters linked to this need, e.g. roost fidelity or the number of occupied boxes/day, for each colony per year. As juvenile bats (young of the year) had only been marked after they had become volant in August, they were excluded from this analysis to avoid any bias due to their absence in the data before August of the respective year. As roosting behaviour in each of the 3 years showed comparable trends, years were pooled to improve overall sample size. The results of the individual years can be found in the [Supplementary material](#).

Second, to investigate the potential for roost sharing between colonies, we analysed the spatiotemporal occurrence of the colonies in the study area. The usage of the boxes was visualized using QGIS (version 2.10.1-Paris, <http://qgis.org>). In addition, we examined to what extent the roosting ranges of different colonies overlapped (i.e. how many day roosts were used by more than one colony during each maternity season). For each colony, the relative overlap was calculated by determining how many of the occupied boxes were also used by other colonies. We refrained from calculating the overlaps across the whole study period as this would not account for possible shifts of roosting ranges between years. Such pooling would result in an artificial inflation of overlaps in roosting areas even though within a given year there may be much less overlap between colonies. Therefore, we calculated the overlap between each pair of colonies separately per year and averaged these yearly overlaps across the whole study (see [Supplementary material](#) for single years).

Third, we assessed whether colonies displayed interest in roosts occupied by heterospecific colonies, or in the case of *P. auritus* additionally by other colonies of the same species. We used the nightly RFID recordings at each monitored day roost to evaluate whether and how often members of colonies briefly entered boxes at night (i.e. 'prospected') that had been used as day roosts by other bat colonies earlier the same day. Thereby, the recorded prospecting behaviour was evaluated independent of whether the colony whose roost had been visited had switched to a new roost the following morning. Moreover, we tested whether prospecting colonies preferred roosts of specific foreign colonies over others. For analysis of the behaviour of the colonies Mnat and Mbec towards roosts of heterospecifics, all roosts of the *P. auritus* colonies were pooled. Comparison within species for each Paur colony was performed against the pooled roosts of the other two conspecific colonies. For simplicity, roosts of both heterospecific and conspecific foreign colonies are referred to as 'foreign roosts'. For each prospecting colony, we counted over all nights during which the specific colony prospected: (1) the total number of foreign roosts of each species that had been occupied during the day but were not prospected by the focal colony and (2) the total number of the respective roosts that had been prospected by the focal colony. Using a two-tailed Fisher's exact test (95% confidence interval) we tested whether colonies prospected foreign roosts of the different species with equal frequencies.

#### Simulation

Subsequently, we evaluated whether colonies specifically choose foreign roosts for occupation. Therefore, we built a model using R (version 3.2.3; <https://r-project.org>) to generate random data sets and tested the hypothesis that colonies followed each other in the sense that a box had a higher chance of being occupied by colony A if it was currently or recently occupied (hereinafter referred to as 'previously occupied') by colony B. The roosting behaviour of the colonies and box usage in the study site were taken into account. The metric was calculated via a sliding window approach (width = 1, 2, 3 or 4 days; step = 1 day). We decided on a time frame of maximally 4 days to account for potential avoidance of the transmission of pathogens via roosts, as the persistence of astroviruses, the most frequent virus type in our three study species, is likely to strongly decline after this time span (Abad et al., 2001; Fischer et al., 2016). Colony A was defined as following colony B if it occupied the box after colony B within the window duration. The metric used to quantify and test the behaviour of colony A following colony B was the sum of the following events across boxes and sliding windows. If the value of the metric calculated from the real data set was larger than 95% of the values from simulated data sets, the test was declared significant at the 5% level. The following null model parameters were directly taken from the observed data sets. (1) Probability of occupancy: as we could not control for potentially important roosting parameters in the field such as temperature, humidity or parasitic load, we controlled for the colony-specific preferences for certain boxes by including a probability of occupancy. The probability of occupancy of a specific box by a specific colony on any given day (i.e. the probability of the box being occupied on one specific day) was estimated by counting the total number of times (i.e. days) that the box had been used by the colony across the study period divided by the sum of days of all boxes that were occupied by the colony. This probability of occupancy was estimated for each box and each colony. For example, if colony A occupied boxes 250 times and box 'X' was occupied by colony A 10 times, the probability of box X being occupied by colony A on any day is 0.04 (10 divided by 250). (2) Fission–fusion dynamics: given that members of some colonies

can occupy more than one box on any given day (colony fission–fusion), the occurrence of such events was estimated from the real data set and included in the null model. (3) Length of consecutive roost occupation: the number of consecutive days for which colonies/subgroups stayed in the same box was randomly drawn from the vector of observed values. The simulations, carried out at the colony level, were forward in time. The source code in R will be provided upon request by S.J.P.

## RESULTS

### Colony Roosting Behaviour

Over the 440 days of the study, we obtained 20 146 individual roosting observations in occupied bat boxes: 12 618 for Mnat, 2974 for Mbec, 1295 for Paur1, 2011 for Paur2 and 1248 for Paur3. In all colonies, the number of consecutive days a box was occupied differed greatly between boxes, ranging from 1 to 25 days (Table 1). Only a few bat boxes were used over a longer period while on average the colonies stayed just 1.3–2.8 consecutive days per box before switching. After a colony had switched roosts, on average between 54% (Mnat) and 21% (Paur2) of the used roosts were re-occupied by the same colony in the same year (Table 1). In comparison to all other colonies, the Mnat colony had the highest number of members and was most frequently present in the boxes (Table 1). The Mbec colony was distinctly smaller but could also be found most of the days in boxes (Table 1). In contrast, the Paur colonies were present in the boxes less frequently during the study (Table 1). They were occasionally absent from boxes during the maternity season and stopped using boxes as roosts shortly after weaning the young, while the colonies Mbec and Mnat were regularly found in boxes several weeks after the nursing period. Although the Mnat colony was about six to eight times larger than the other four colonies, the mean group size per box was very similar for all five colonies. Indeed, the Mnat colony split into subgroups much more often than the other four bat colonies (Table 1). Thus, it occupied the highest number of boxes both per day and over the course of the study (Table 1).

### Roosting Range

Members of the colony Mnat used a large number of boxes that were widely distributed over the study site (Fig. 1) in contrast to the rather restricted roosting areas of the other four colonies. The Mbec colony and two of the Paur colonies (Paur1 and Paur2) only occupied boxes in areas of the study site that had the highest box density. The Paur3 colony exclusively roosted near the southern edge of the site. On any given day, only members of the same colony shared the same box at the same time (thus when referring to our study, ‘sharing’ between colonies/species always refers to using the same boxes but not simultaneously). However, there was considerable overlap between the roosting ranges of the different

colonies (see Table 2). On average, all colonies shared between 44% and 86% of their boxes with at least one other colony in the same year, with the rather isolated Paur3 colony having the lowest overlap with the other colonies. Colonies of both Mnat and Mbec overlapped to some degree with all other colonies within their distribution. While all Paur colonies inhabited boxes that were also used by the other bat species (see Table 2), they showed strong spatial separation from each other. Overall, only one box was used by more than one Paur colony in the same year (Table 2). This box was used by Paur2 before Paur3 arrived at the study site in spring with a time lag of 7 weeks between occupations. Moreover, the Paur colonies did not occupy the box in immediate succession, as it was used by the Mnat colony in the interim. Overall, the large overlap in the roosting areas of the different colonies and the nonexclusive use of many boxes indicates a high potential for roost sharing among the study colonies, except for colony Paur3.

### Prospecting of Roosts

Individuals of all colonies prospected boxes that had been occupied by members of the other colonies on the same day. However, the frequency of prospecting differed strongly between colonies, ranging from two nights for colony Paur3 to 97 nights for the Mnat colony (see Fig. 2).

The colony Mbec showed no significant preferences for a particular species in their prospecting behaviour (two-tailed Fisher’s exact test:  $P_{\text{Mnat-Paur}} = 0.078$ ). In contrast, colonies Paur1 and Paur2 prospected boxes occupied by the respective conspecific colonies significantly more often than those used by the hetero-specific colonies Mbec or Mnat (for Paur1:  $P_{\text{Mnat-Paur}} < 0.001$ ,  $P_{\text{Mbec-Paur}} = 0.001$ ,  $P_{\text{Mbec-Mnat}} = 0.574$ ; for Paur2:  $P_{\text{Mnat-Paur}} < 0.001$ ,  $P_{\text{Mbec-Paur}} < 0.001$ ,  $P_{\text{Mbec-Mnat}} = 1.000$ ). As the Paur3 colony prospected only twice, no statement on preferences can be provided for this colony. Like two of the Paur colonies, members of Mnat discriminated between roosts of different species as they prospected boxes occupied by Paur colonies significantly more often than boxes occupied by the Mbec colony ( $P_{\text{Mbec-Paur}} < 0.001$ ). Interestingly, most of the nightly prospectings did not result in a subsequent use of the respective boxes as day roosts. Besides one occurrence in the colony Paur1, only members of the Mnat colony occupied boxes immediately after prospecting (Fig. 2).

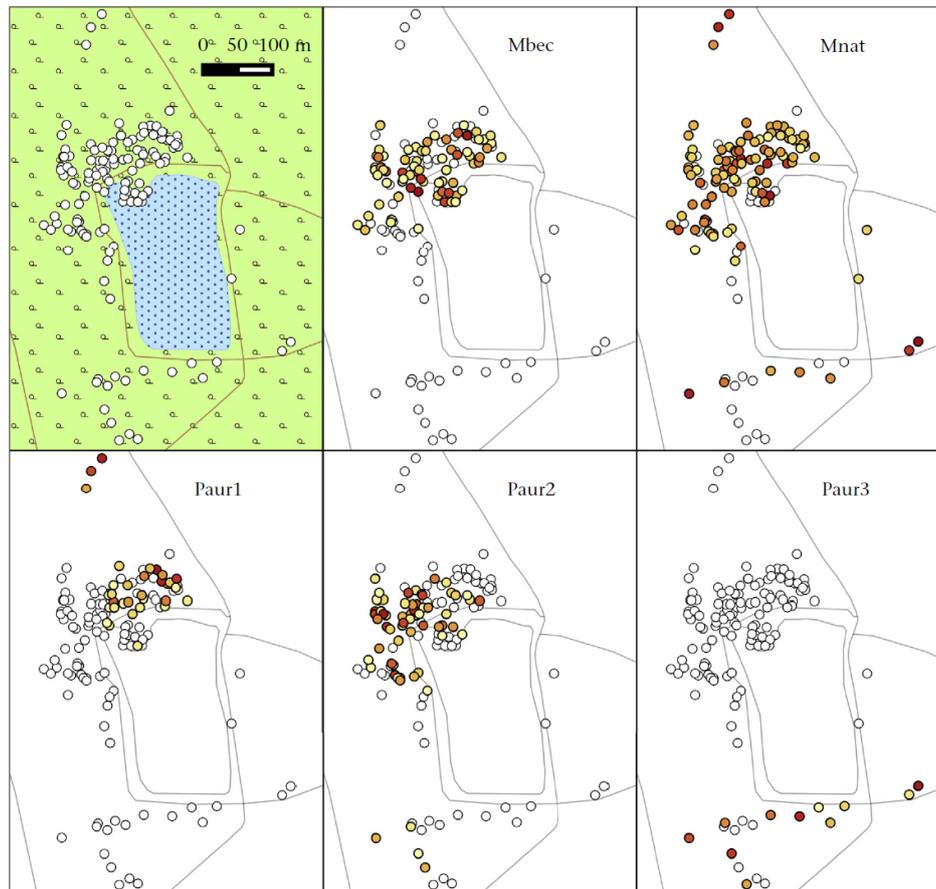
### Simulation

Considering the whole study period (three pooled maternity seasons), the Mnat colony occupied boxes previously occupied by colonies Paur1 ( $P_{\text{day1}} < 0.001$ ) and Paur2 ( $P_{\text{day1}} < 0.001$ ) within 1 day significantly more often than expected by chance. Although not significant, a similar trend was suggested for Mnat towards boxes previously occupied by Paur3 within 1 day ( $P_{\text{day1}} = 0.087$ ). Within longer time frames (2, 3, and 4 days), this roost-following behaviour of Mnat towards the previously occupied roosts of the Paur

**Table 1**  
Mean values for maximal colony sizes and roosting behaviour

	Mnat	Mbec	Paur1	Paur2	Paur3
Mean of maximal colony size/year	82.3	11.0	10.0	11.7	12.7
Mean % of days present	92.6	88.5	39.0	55.0	30.0
Mean no. of different boxes occupied/year	75.3	37.3	15.7	27.3	6.7
Mean % of days present in more than 1 box	76.5	16.8	9.2	3.7	1.7
Average median no. of used boxes/day	2.0 (1–5)	1.0 (1–3)	1.0 (1–2)	1.0 (1–2)	1.0 (1–2)
Average median of group size/box	12.3 (1–70)	8.0 (1–13)	8.0 (1–14)	9.0 (1–14)	10.5 (1–14)
Average median of consecutive days per box	1.3 (1–16)	1.3 (1–25)	1.8 (1–19)	1.8 (1–13)	2.8 (1–18)
Mean % of boxes that were reoccupied/year	53.7	44.1	29.4	20.9	31.0

For each colony, it is stated how often the bats were found in the area and how they used the boxes over the course of the study. The mean values across the 3 years are given. For the average of median values the whole range is given in parentheses. The respective values for each year can be found in the Supplementary Material, Table S1.



**Figure 1.** Roosting ranges of the colonies in the study area. An overview of the area with the swamp (dotted area) and surrounding woodland and existing walking paths is shown at the top left. Open circles represent bat boxes in the area. The darker coloured the boxes the higher the box usage over all years (2013–2015). Note that each of the five plots shows the entire study site with the roost use of the respective colony. Colony names are explained in the text.

**Table 2**  
Mean overlap of roosting ranges (%)

	Mnat boxes	Mbec boxes	Paur1 boxes	Paur2 boxes	Paur3 boxes
Mnat		54.0	75.9	70.1	35.6
Mbec	26.6		28.7	23.7	0.0
Paur1	15.2	12.9		0.0	0.0
Paur2	25.4	16.9	0.0		3.7
Paur3	3.4	0.0	0.0	1.1	
Total	62.6	67.3	85.9	81.6	43.5

The ratio of the number of boxes shared with other colonies per year to the total number of boxes used by the respective colony per year is given as the mean across all 3 years. The first five rows show the overlap of a colony's boxes with every other colony and the last row reflects the total overlap for the respective boxes (note that the latter number does not correspond to the sum of the ratios for each colony as some boxes had been used by more than two colonies).

colonies was significant for the boxes of all three Paur colonies (Paur1:  $P_{\text{day}2} < 0.001$ ,  $P_{\text{day}3} < 0.001$ ,  $P_{\text{day}4} < 0.001$ ; Paur2:  $P_{\text{day}2} < 0.001$ ,  $P_{\text{day}3} < 0.001$ ,  $P_{\text{day}4} < 0.001$ ; Paur3:  $P_{\text{day}2} = 0.027$ ,  $P_{\text{day}3} = 0.023$ ,  $P_{\text{day}4} = 0.020$ ). No such roost-following behaviour could be observed in any other combination of colonies when data were simulated over the entire study period (see Table S2 in the [Supplementary material](#)). Simulated results and the respective

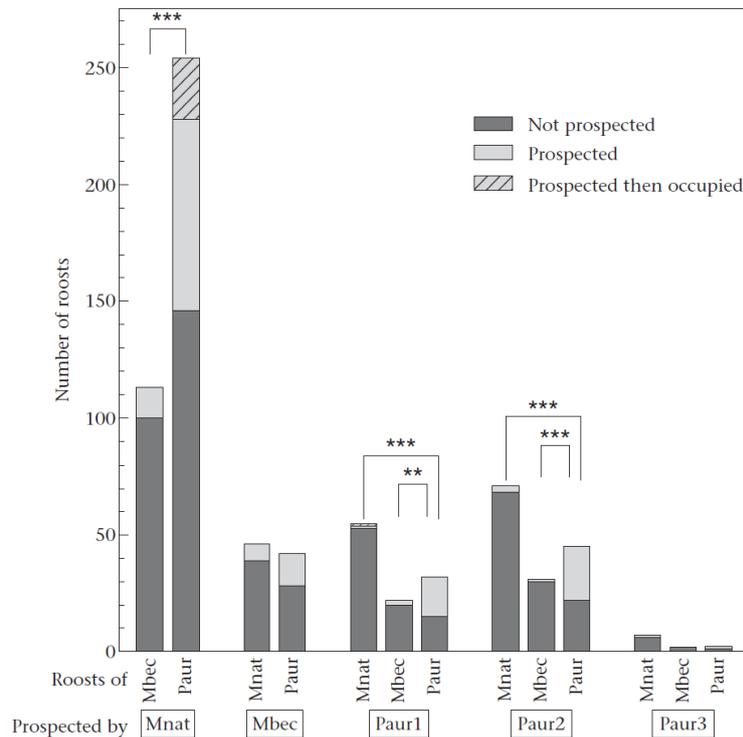
number of observed following behaviour of Mnat can be found in [Fig. 3](#). The  $P$  values and figures for all other colonies across the whole study period can be found in the [Supplementary material](#).

When simulated for each year separately, the Mnat colony occupied boxes previously used by at least two of the three Paur colonies significantly more often within all analysed time frames (1, 2, 3 and 4 days). Our RFID logger data showed that on several occasions individuals of the Mnat colony entered a box after the Paur colony had left for the night and occupied this box despite individuals of the Paur colony attempting to return in the morning. The spatially more isolated colony of Paur3 was only followed by the Mnat colony during 2014. Then, however, all four analysed time frames were significant (1, 2, 3 and 4 days). In 2014, the Mnat colony followed the Mbec colony within the longest analysed time frame of 4 days ( $P_{\text{day}4} = 0.024$ ). All  $P$  values and figures for the separate years can be found in the [Supplementary material](#).

## DISCUSSION

Sharing resources can imply costs as well as benefits and the affected animals may respond with a variety of different behaviours to optimize the outcome. Here, we examined whether co-occurring

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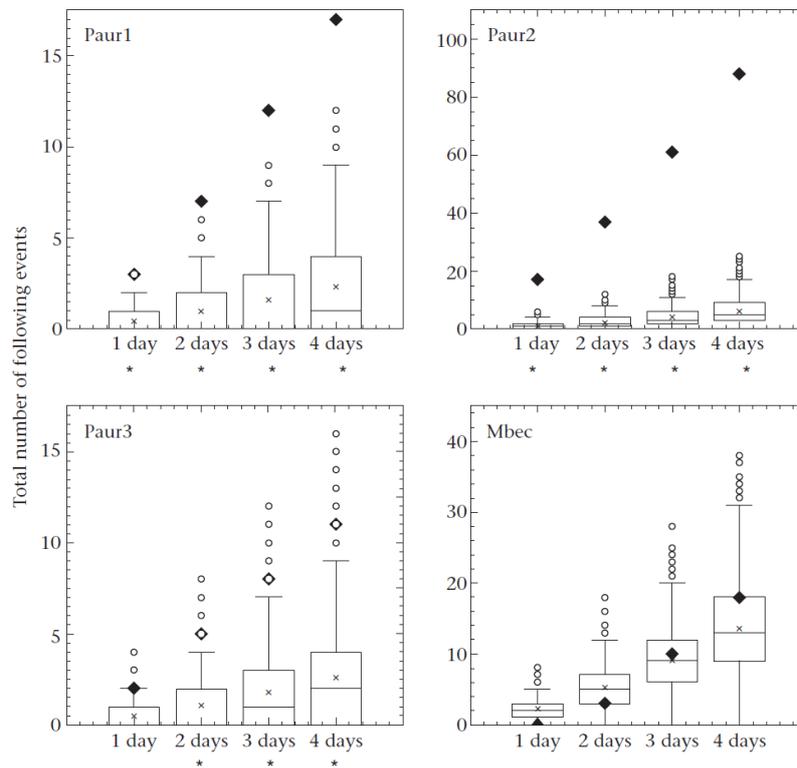
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**Figure 2.** Prospecting behaviour and preferred roosts. The ratios between the number of not prospected and prospected roosts were tested by two-tailed Fisher's exact test for each colony. \*\* $P < 0.01$ ; \*\*\* $P < 0.001$ . All  $P$  values can be found in the Results. Number of nights across the whole study period where the colony prospected:  $N_{Mnat} = 97$ ,  $N_{Mnbc} = 20$ ,  $N_{Paur1} = 20$ ,  $N_{Paur2} = 26$ ,  $N_{Paur3} = 2$ .

bat colonies of the same and of different species influence each other in their roosting behaviour. Among the five colonies studied, we found evidence for both avoidance and attraction towards roosts of other colonies, depending on the identity of the different colonies.

In general, shelters, such as burrows, caves, hollow trees or artificial bird and bat boxes are often attractive to many different animal species. Consequently, shelters are regularly used by several species simultaneously (Mori, Menchetti, & Balestrieri, 2015; Waterman & Roth, 2007). This has also been shown for bats, e.g. in cave-dwelling species (Kunz & Lumsden, 2003). In contrast, in our study different conspecific and heterospecific colonies never shared roosts at the same time despite large overlaps in their respective roosting ranges. This suggests that competition for roosts occurs between the co-occurring colonies (Schoener, 1983). Our findings also imply that colonies must often wait an unpredictable number of days while a roost is occupied by a foreign colony, which means previously accumulated knowledge on available roosts might become unreliable. However, even if currently occupied roosts are not used by different colonies at the same time, roost occupancy by foreign colonies might still be of interest to other colonies. For comparison, birds assess nest site quality by the breeding success of conspecifics or heterospecifics (Doligez, Danchin, & Clobert, 2002; Parejo, White, Clobert, Dreiss, & Danchin, 2007; Thomson, Forsman, & Mönkkönen, 2003). Such information on the resource use of other individuals or other social groups can later be used to decide which site to occupy when it becomes available again.

Playback experiments conducted by Schöner et al. (2010) demonstrated that the bat species we studied are responsive to cues signalling the presence of heterospecifics during roost selection. In their study, newly placed (and hence unfamiliar) bat boxes which were accompanied by playback recordings of heterospecific social calls were approached by all three species significantly more often than those without any social call recordings. Despite this documented interest towards social calls of heterospecifics, in our study the same species differed in their occupation of foreign roosts. While neither *M. bechsteinii* nor *P. auritus* showed interest in the previously used roosts of heterospecifics, *M. nattereri* specifically selected bat boxes that had been recently occupied by other bat colonies, with a strong preference for *P. auritus*. The *M. nattereri* colony had by far the largest number of occupied boxes each season and per day, as it showed the highest fission–fusion behaviour. Thus, in comparison to the other four bat colonies in our study area they most likely faced the highest pressure to gather information about potential roosts. All roost boxes had the same design with a general suitability for bats and thus could have been used as potential roosts by all five colonies. However, while the roosts were identical in their structural characteristics, roost availability and quality may be highly dynamic, e.g. due to weather-dependent differences in preferences for a certain roost temperature (Kerth et al., 2001). Thus the bats need to frequently update their information on potential roosts (Kerth et al., 2006). Regular exploration of potential roosts should be especially costly during the maternity season when time and energy reserves for females are constrained (Henry, Thomas, Vaudry,



**Figure 3.** Simulated and observed following behaviour of the Mnat colony across the whole study period. The simulated and observed occupation of foreign roosts (following events) by the Mnat colony within 1, 2, 3 or 4 days after the roost became vacant are shown. Box plots represent the simulated data. The median is indicated by a horizontal line and the mean by a small 'x'. Whiskers denote the lowest and highest values within  $1.5 \times$  IQR from the first and third quartiles. White circles depict outliers. The observed values are shown as black diamonds. Overlapping values of observed data and outliers are indicated by black diamonds with white circles. Asterisks below the number of days indicate significant differences ( $P < 0.05$ ) between the observed and simulated data for the respective time window.

& Carrier, 2002; Kunz, 1982). Consequently, unlike *M. bechsteinii*, which are very quick to explore available resources (Fleischmann & Kerth, 2014; Kerth & Reckardt, 2003), some colonies may not be able to cover their roost demand by exploring and information sharing within the colony alone. Considering current roost choices of other co-occurring colonies thus might increase the efficiency of finding suitable roosts.

Given the aforementioned dynamics in quality and availability of roosts, the time between assessment and occupation should be as short as possible to ensure topicality of the information gained (Doligez, Cadet, Danchin, & Boulinier, 2003). The observed capability of the *M. nattereri* colony to take over currently used roosts of the *P. auritus* colonies shortens the period before a box becomes available again and thus may prevent a degradation of information. Boxes occupied by *M. bechsteinii* may be a less attractive target for roost take-overs as *M. bechsteinii* is the largest of the three studied species and known to react aggressively towards foreign (conspecific) bats entering the roosts (Kerth et al., 2002). An influence of different roost or site preferences on the specific choice of previously occupied roosts of *P. auritus* can be largely ruled out, as the *M. nattereri* colony overlapped in its roost use with all other colonies in the area with the highest box density to a similar extent and because our simulation took preferences for certain boxes into account.

The favoured occupation of heterospecific roosts might also be linked to parasite and pathogen avoidance. The avoidance of

parasites such as bat flies and fleas that accumulate in shelters has been shown to influence the selection and switching of roosts in bats (Bartonička & Gaisler, 2007; Reckardt & Kerth, 2006, 2007) and similar findings exist for other animals such as birds and primates (e.g. Christe, Oppliger, & Richner, 1994; Hausfater & Meade, 1982). While sharing shelters carries the risk of indirect transmission of infections and parasites (Kallio, 2006; Leu et al., 2010), in our bat species, pathogens and ectoparasites are largely species specific (Fischer et al., 2016; Rupp et al., 2004). This implies that a colony is exposed to a lower infection risk when using roosts of heterospecifics, as in the case of the *M. nattereri* colony, instead of re-occupying their own roosts or using those of other conspecific colonies if available. In fact, our colonies reoccupied a considerably smaller proportion of their own boxes within 1 year (Table 1) compared to the overlap of roosts with heterospecifics (Table 2). Interestingly, few boxes within the roosting ranges of all colonies had never been occupied by any colony during the study (Fig. 2). Despite the identical box design, these unused boxes were seemingly not considered suitable or at least less so than previously occupied roosts; possibly due to local factors e.g. solar exposure or density of surrounding vegetation (Barclay & Kurta, 2007).

Disease and parasite avoidance could also explain the separated roosting ranges of the three *P. auritus* colonies. Members of *P. auritus* colonies harbour bat fleas (Rupp et al., 2004; personal observation) that are transmitted via roosts (Marshall, 1982) and they may also face a risk of cross-infection with astroviruses when

using recently used roosts of conspecific colonies (Fischer et al., 2016). In addition, the separated roosting ranges of conspecific colonies might be a strategy to avoid competition costs as spatial separation appears to be more cost efficient than direct confrontations (interference competition) at contested resources (Amarasekare, 2002; Ambrosio & Baeza, 2016; Goss-Custard, Durell, McGroarty, & Reading, 1982). Despite the observed separation of their roosting ranges, members of the *P. auritus* colonies showed strong interest in current roosts of the other conspecific colonies, displaying frequent prospecting behaviour. As this prospecting never led to the occupation of such roosts or communal roosting of members from the different colonies elsewhere in our study area, the reason for this behaviour remains unknown. Possibly, it could provide the *P. auritus* colonies with regular updates on their respective roosting ranges and thus an assessment of anticipated intraspecific competition (Doligez, Danchin, Clobert, & Gustafsson, 1999; Valone & Templeton, 2002). Reducing costs of intraspecific conflict is likely to be particularly important for the *P. auritus* colonies as they frequently lost some of their currently used roosts to the *M. nattereri* colony. The apparent low ability to compete with the *M. nattereri* colony might also have played a role in the relatively frequent absence of *P. auritus* colonies from the boxes and their overall shorter presence in the study area. Such competitive displacement has previously been shown in other mammals (Stewart, Bowyer, Kie, Cimon, & Johnson, 2002; Thulin, 2003).

In conclusion, using bat colonies as a model, our study demonstrates that co-occurring social groups can influence each other's use of resources that cannot be depleted. We have shown that this can happen through both avoiding and preferring resources that had been previously used by other groups of conspecifics or heterospecifics. The insights obtained allow for a better understanding of the dynamics and interdependencies between social groups in respect to resource use. Such data are important for understanding community structures (Louthan, Doak, & Angert, 2015) and provide relevant information for the planning of conservation measures such as provisioning of roosts for bats. For example, a sole focus on artificial shelters such as bat boxes with general suitability for a broad range of species (Alcalde et al., 2013; Dodds & Bilston, 2013; Fleischmann & Kerth, 2014) can lead to outcompeting of one target species by co-occurring species as indicated here between *P. auritus* and *M. nattereri*. This could be prevented by aiming for higher diversity in artificial shelters while also preserving a natural variety of shelter types (such as a tree cavities: Dodds & Bilston, 2013; Mering & Chambers, 2014; Rueeggger, 2016).

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#### Supplementary Material

Supplementary material associated with this article is available, in the online version, at <http://dx.doi.org/10.1016/j.anbehav.2016.11.015>.

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## SUPPLEMENT

Table S1: Colony size and roosting behaviour per year.

Parameter	Year	Colony				
		Mnat	Mbec	Paur1	Paur2	Paur3
Maximal colony size	2013	73	11	11	15	13
	2014	84	9	11	11	14
	2015	90	13	8	9	11
% days present	2013	94.7	90.2	47.4	50.4	16.5
	2014	95.4	92.2	35.9	59.5	39.2
	2015	87.7	83.1	33.8	55.2	34.4
N of different boxes	2013	69	38	22	26	4
	2014	82	32	17	31	9
	2015	75	42	8	25	7
% present in more than one box	2013	89.7	11.7	12.7	4.5	0
	2014	67.8	12.1	9.1	4.4	3.3
	2015	71.9	26.6	5.8	2.4	1.9
Median N of used boxes/day	2013	2	1	1	1	1
		(1-5)	(1-3)	(1-2)	(1-2)	(1-1)
		2	1	1	1	1
	2014	(1-5)	(1-3)	(1-2)	(1-2)	(1-2)
		2	1	1	1	1
		(1-4)	(1-3)	(1-2)	(1-2)	(1-2)
Median of group size/box	2013	10	8	10	13	9.5
		(1-42)	(1-11)	(1-11)	(1-14)	(2-13)
		13	7	7	8	11
	2014	(1-54)	(1-9)	(1-10)	(1-10)	(1-14)
		14	9	7	6	11
		(1-70)	(1-13)	(2-8)	(1-9)	(1-11)
Median of consecutive days per box	2013	2	1	2	2	2.5
		(1-14)	(1-11)	(1-8)	(1-10)	(1-16)
		1	1	1	1.5	2
	2014	(1-9)	(1-25)	(1-19)	(1-8)	(1-15)
		1	2	2.5	2	4
		(1-16)	(1-8)	(1-10)	(1-13)	(1-18)
% of boxes that were re-occupied	2013	57.4	45.9	27.3	19.2	0
	2014	53.1	40	23.5	23.3	50
	2015	50.7	46.3	37.5	20	42.9

Data are shown as median values with corresponding range in brackets. Per year the colonies were observed for the following number of consecutive days:  $N_{2013} = 133$ ,  $N_{2014} = 153$ ,  $N_{2015} = 154$ .

Table S2: Additional p-values of the simulation across the whole study period.

Time frame	Former box user	Following colony				
		Mnat	Mbec	Paur1	Paur2	Paur3
Day 1	Mnat	NA	1.000	0.518	1.000	1.000
	Mbec	1.000	NA	1.000	1.000	1.000
	Paur1	0.000	1.000	NA	1.000	1.000
	Paur2	0.000	1.000	1.000	NA	1.000
	Paur3	0.087	1.000	1.000	1.000	NA
Day 2	Mnat	NA	1.000	0.527	0.753	1.000
	Mbec	0.799	NA	1.000	1.000	1.000
	Paur1	0.000	1.000	NA	1.000	1.000
	Paur2	0.000	1.000	1.000	NA	1.000
	Paur3	0.027	1.000	1.000	1.000	NA
Day 3	Mnat	NA	1.000	0.548	0.772	1.000
	Mbec	0.421	NA	1.000	1.000	1.000
	Paur1	0.000	1.000	NA	1.000	1.000
	Paur2	0.000	0.395	1.000	NA	1.000
	Paur3	0.023	1.000	1.000	1.000	NA
Day 4	Mnat	NA	1.000	0.566	0.797	1.000
	Mbec	0.254	NA	1.000	1.000	1.000
	Paur1	0.000	1.000	NA	1.000	1.000
	Paur2	0.000	0.344	1.000	NA	1.000
	Paur3	0.020	1.000	1.000	1.000	NA

Table S3: P-values of the simulation for 2013.

Time frame	Former box user	Following colony				
		Mnat	Mbec	Paur1	Paur2	Paur3
Day 1	Mnat	NA	1.000	0.381	1.000	1.000
	Mbec	1.000	NA	1.000	1.000	1.000
	Paur1	0.005	1.000	NA	1.000	1.000
	Paur2	0.000	1.000	1.000	NA	1.000
	Paur3	1.000	1.000	1.000	1.000	NA
Day 2	Mnat	NA	1.000	0.381	0.368	1.000
	Mbec	0.337	NA	1.000	1.000	1.000
	Paur1	0.000	1.000	NA	1.000	1.000
	Paur2	0.000	1.000	1.000	NA	1.000
	Paur3	1.000	1.000	1.000	1.000	NA
Day 3	Mnat	NA	1.000	0.386	0.368	1.000
	Mbec	0.334	NA	1.000	1.000	1.000
	Paur1	0.000	1.000	NA	1.000	1.000
	Paur2	0.000	0.078	1.000	NA	1.000
	Paur3	1.000	1.000	1.000	1.000	NA
Day 4	Mnat	NA	1.000	0.394	0.370	1.000
	Mbec	0.276	NA	1.000	1.000	1.000
	Paur1	0.000	1.000	NA	1.000	1.000
	Paur2	0.000	0.078	1.000	NA	1.000
	Paur3	1.000	1.000	1.000	1.000	NA

Table S4: P-values of the simulation for 2014.

Time frame	Former box user	Following colony				
		Mnat	Mbec	Paur1	Paur2	Paur3
Day 1	Mnat	NA	1.000	1.000	1.000	1.000
	Mbec	1.000	NA	1.000	1.000	1.000
	Paur1	0.000	1.000	NA	1.000	1.000
	Paur2	0.064	1.000	1.000	NA	1.000
	Paur3	0.025	1.000	1.000	1.000	NA
Day 2	Mnat	NA	1.000	1.000	1.000	1.000
	Mbec	0.337	NA	1.000	1.000	1.000
	Paur1	0.000	1.000	NA	1.000	1.000
	Paur2	0.006	1.000	1.000	NA	1.000
	Paur3	0.004	1.000	1.000	1.000	NA
Day 3	Mnat	NA	1.000	1.000	1.000	1.000
	Mbec	0.087	NA	1.000	1.000	1.000
	Paur1	0.000	1.000	NA	1.000	1.000
	Paur2	0.001	1.000	1.000	NA	1.000
	Paur3	0.005	1.000	1.000	1.000	NA
Day 4	Mnat	NA	1.000	1.000	1.000	1.000
	Mbec	0.024	NA	1.000	1.000	1.000
	Paur1	0.000	1.000	NA	1.000	1.000
	Paur2	0.000	1.000	1.000	NA	1.000
	Paur3	0.005	1.000	1.000	1.000	NA

Table S5: P-values of the simulation for 2015.

Time frame	Former box user	Following colony				
		Mnat	Mbec	Paur1	Paur2	Paur3
Day 1	Mnat	NA	1.000	0.381	1.000	1.000
	Mbec	1.000	NA	1.000	1.000	1.000
	Paur1	0.006	1.000	NA	1.000	1.000
	Paur2	0.000	1.000	1.000	NA	1.000
	Paur3	1.000	1.000	1.000	1.000	NA
Day 2	Mnat	NA	1.000	0.381	1.000	1.000
	Mbec	1.000	NA	1.000	1.000	1.000
	Paur1	0.007	1.000	NA	1.000	1.000
	Paur2	0.000	1.000	1.000	NA	1.000
	Paur3	1.000	1.000	1.000	1.000	NA
Day 3	Mnat	NA	1.000	0.386	1.000	1.000
	Mbec	0.389	NA	1.000	1.000	1.000
	Paur1	0.006	1.000	NA	1.000	1.000
	Paur2	0.000	1.000	1.000	NA	1.000
	Paur3	1.000	1.000	1.000	1.000	NA
Day 4	Mnat	NA	1.000	0.394	1.000	1.000
	Mbec	0.184	NA	1.000	1.000	1.000
	Paur1	0.006	1.000	NA	1.000	1.000
	Paur2	0.000	0.156	1.000	NA	1.000
	Paur3	1.000	1.000	1.000	1.000	NA

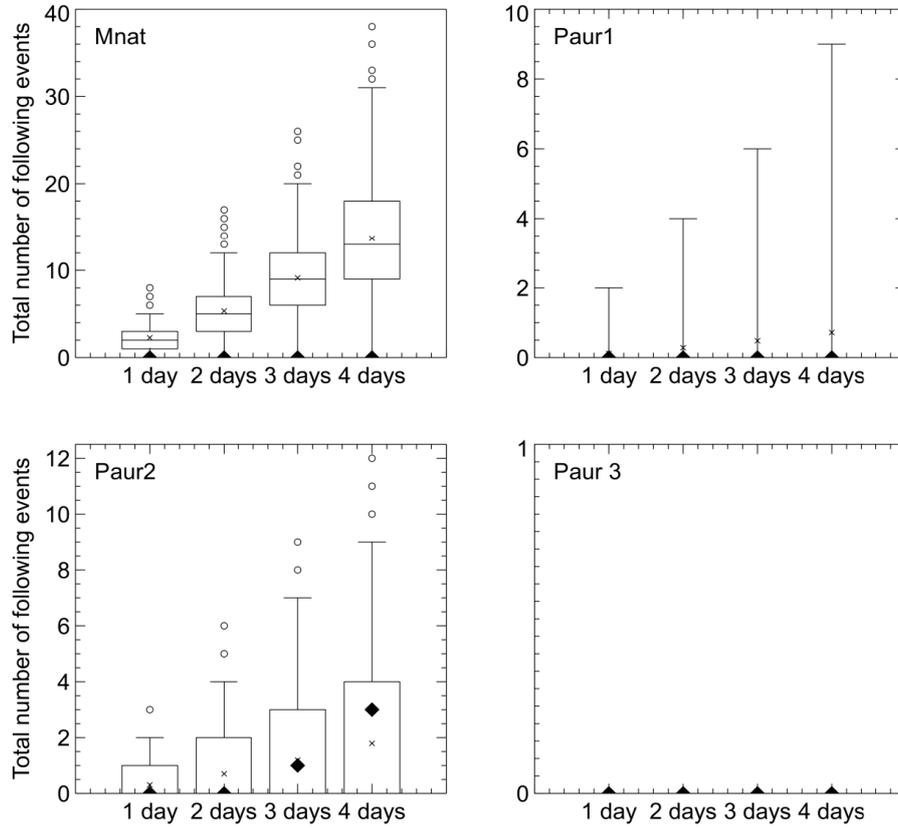


Figure S1: Simulated and observed following behaviour of the Mbec colony across the whole study period. The simulated and observed occupation of foreign roosts (following events) by the Mbec colony within one, two, three or four days after the roost became vacant are shown. Box plots represent the simulated data: Whiskers denote the lowest and highest values within  $1.5 \times$  IQR from the first and third quartiles; white circles depict outliers. The observed values are shown as black diamonds. Overlapping values of observed data and outliers are indicated by black diamonds with white circles. Asterisks below the number of days indicate significant differences between the observed and simulated data for the respective time window.

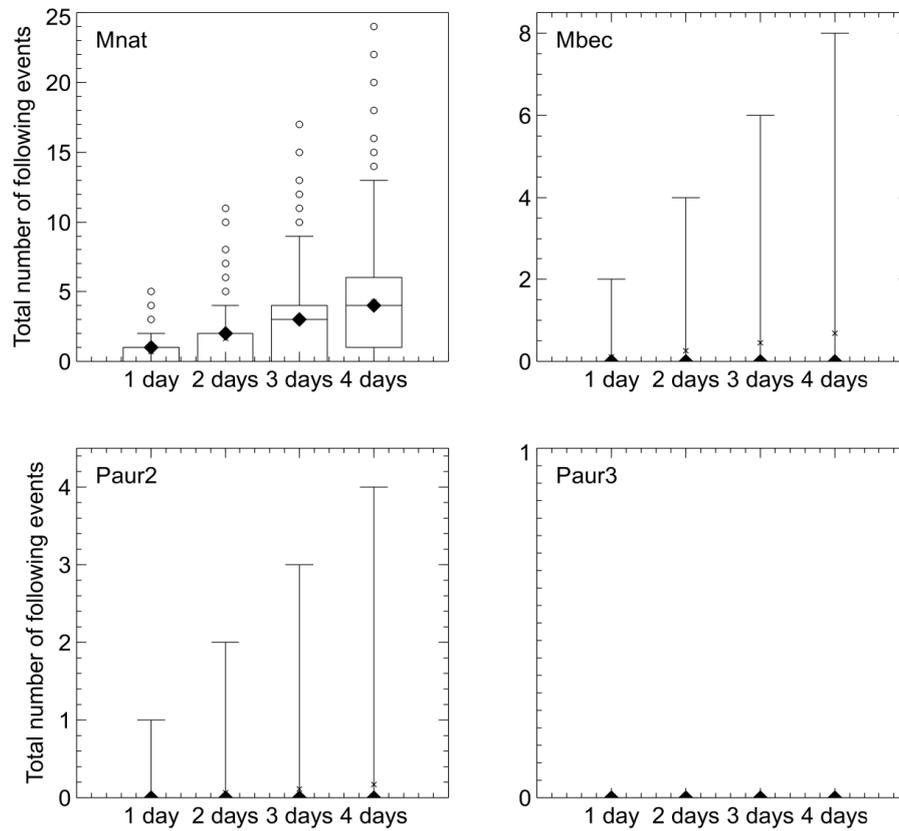


Figure S2: Simulated and observed following behaviour of the Paur1 colony across the whole study period. The simulated and observed occupation of foreign roosts (following events) by the Paur1 colony within one, two, three or four days after the roost became vacant are shown. Box plots represent the simulated data: Whiskers denote the lowest and highest values within  $1.5 \times \text{IQR}$  from the first and third quartiles; white circles depict outliers. The observed values are shown as black diamonds. Overlapping values of observed data and outliers are indicated by black diamonds with white circles. Asterisks below the number of days indicate significant differences between the observed and simulated data for the respective time window.

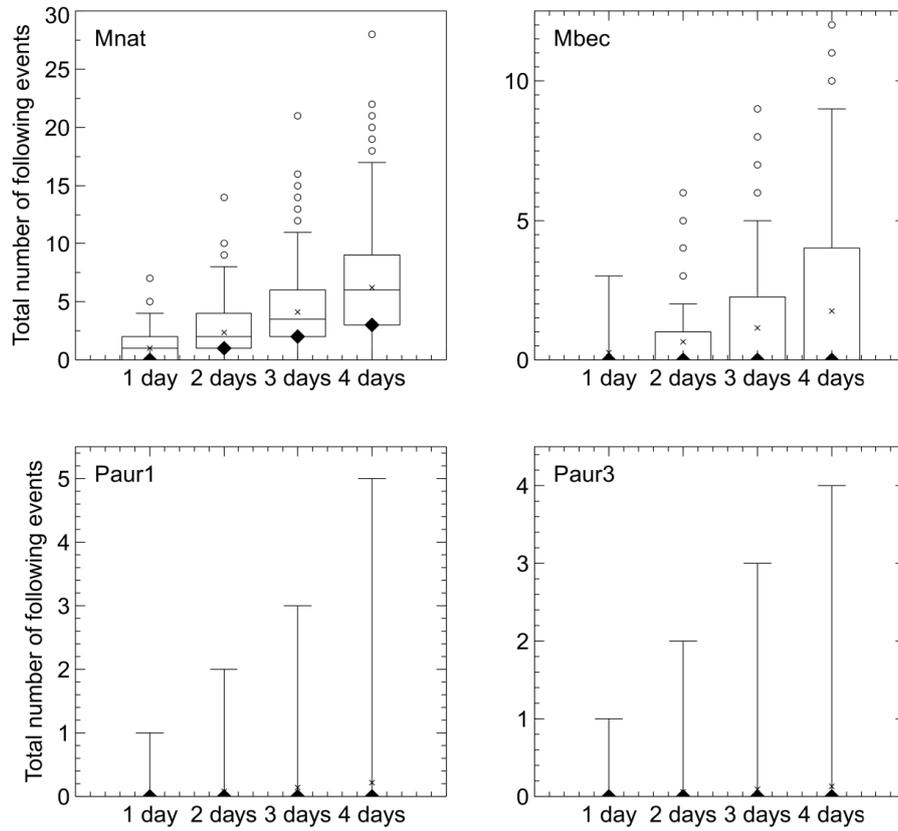


Figure S3: Simulated and observed following behaviour of the Paur2 colony across the whole study period. The simulated and observed occupation of foreign roosts (following events) by the Paur2 colony within one, two, three or four days after the roost became vacant are shown. Box plots represent the simulated data: Whiskers denote the lowest and highest values within  $1.5 \times$  IQR from the first and third quartiles; white circles depict outliers. The observed values are shown as black diamonds. Overlapping values of observed data and outliers are indicated by black diamonds with white circles. Asterisks below the number of days indicate significant differences between the observed and simulated data for the respective time window.

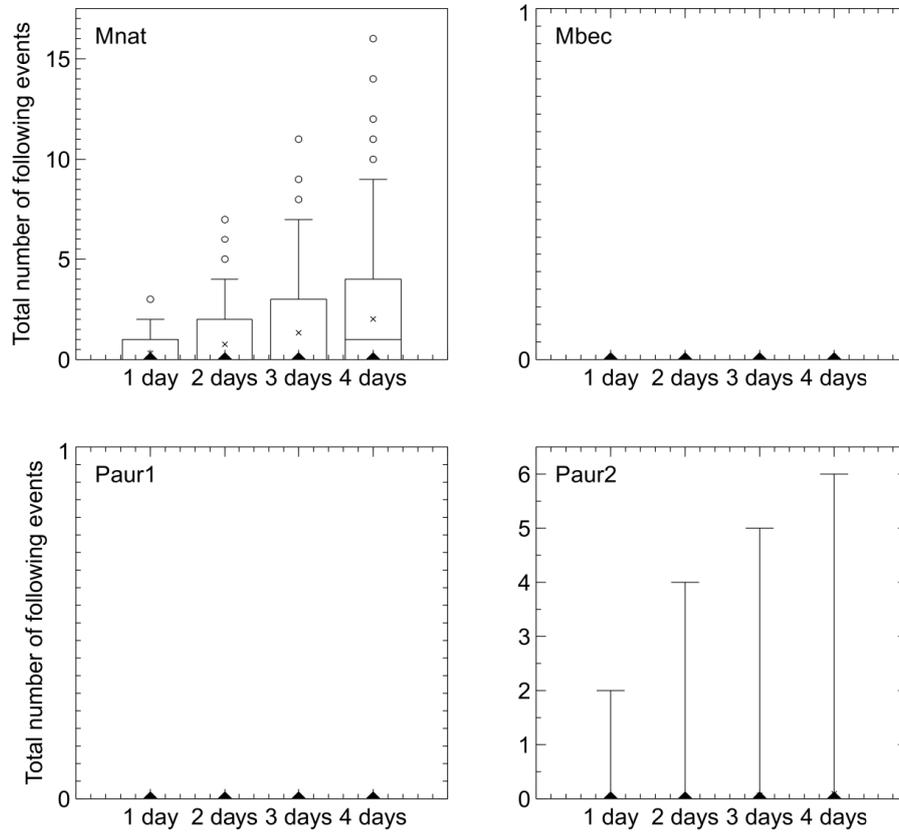


Figure S4: Simulated and observed following behaviour of the Paur3 colony across the whole study period. The simulated and observed occupation of foreign roosts (following events) by the Paur3 colony within one, two, three or four days after the roost became vacant are shown. Box plots represent the simulated data: Whiskers denote the lowest and highest values within  $1.5 \times$  IQR from the first and third quartiles; white circles depict outliers. The observed values are shown as black diamonds. Overlapping values of observed data and outliers are indicated by black diamonds with white circles. Asterisks below the number of days indicate significant differences between the observed and simulated data for the respective time window.

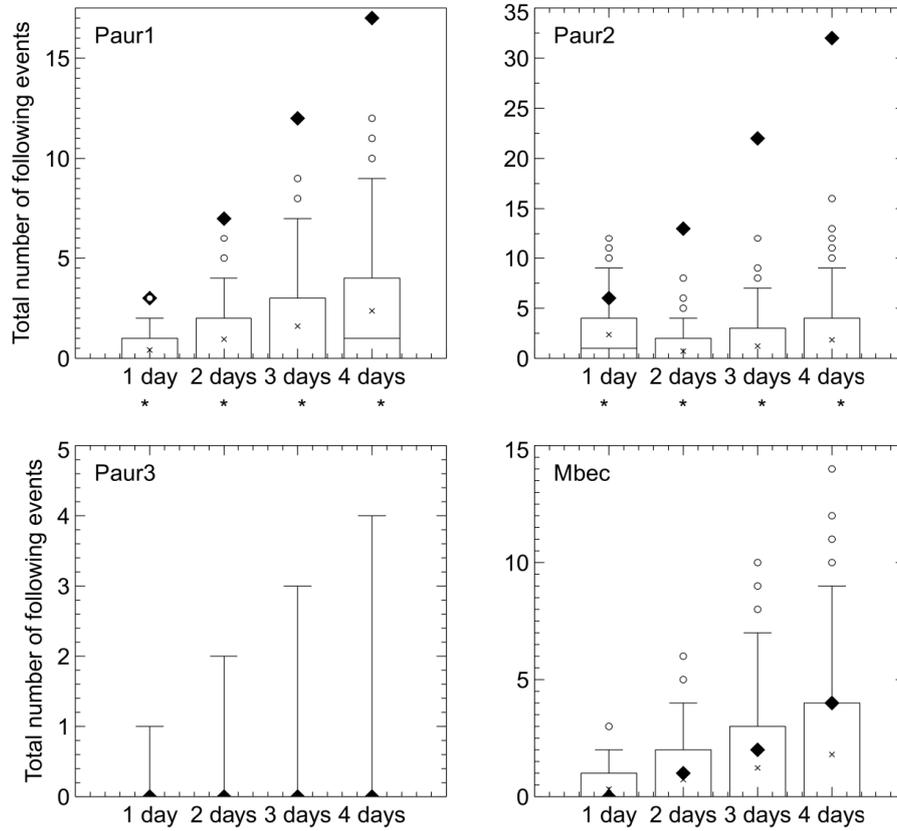


Figure S5: Simulated and observed following behaviour of the Mnat colony in 2013. The simulated and observed occupation of foreign roosts (following events) by the Mnat colony within one, two, three or four days after the roost became vacant are shown. Box plots represent the simulated data: Whiskers denote the lowest and highest values within  $1.5 \times$  IQR from the first and third quartiles; white circles depict outliers. The observed values are shown as black diamonds. Overlapping values of observed data and outliers are indicated by black diamonds with white circles. Asterisks below the number of days indicate significant differences between the observed and simulated data for the respective time window.

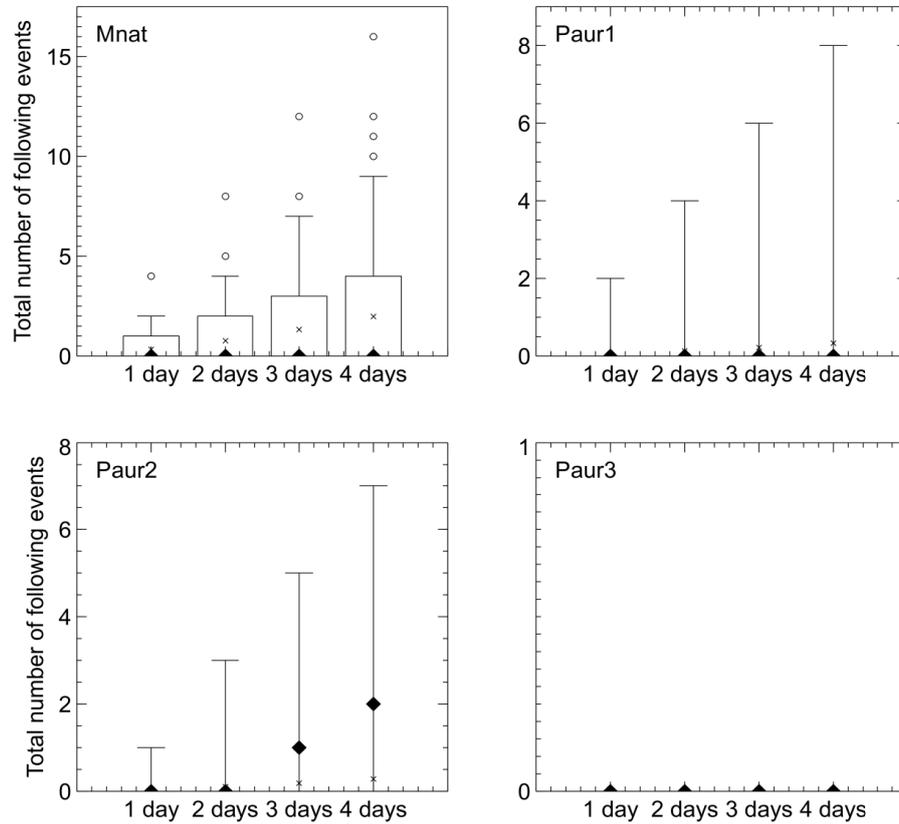


Figure S6: Simulated and observed following behaviour of the Mbec colony in 2013. The simulated and observed occupation of foreign roosts (following events) by the Mbec colony within one, two, three or four days after the roost became vacant are shown. Box plots represent the simulated data: Whiskers denote the lowest and highest values within  $1.5 \times \text{IQR}$  from the first and third quartiles; white circles depict outliers. The observed values are shown as black diamonds. Overlapping values of observed data and outliers are indicated by black diamonds with white circles. Asterisks below the number of days indicate significant differences between the observed and simulated data for the respective time window.

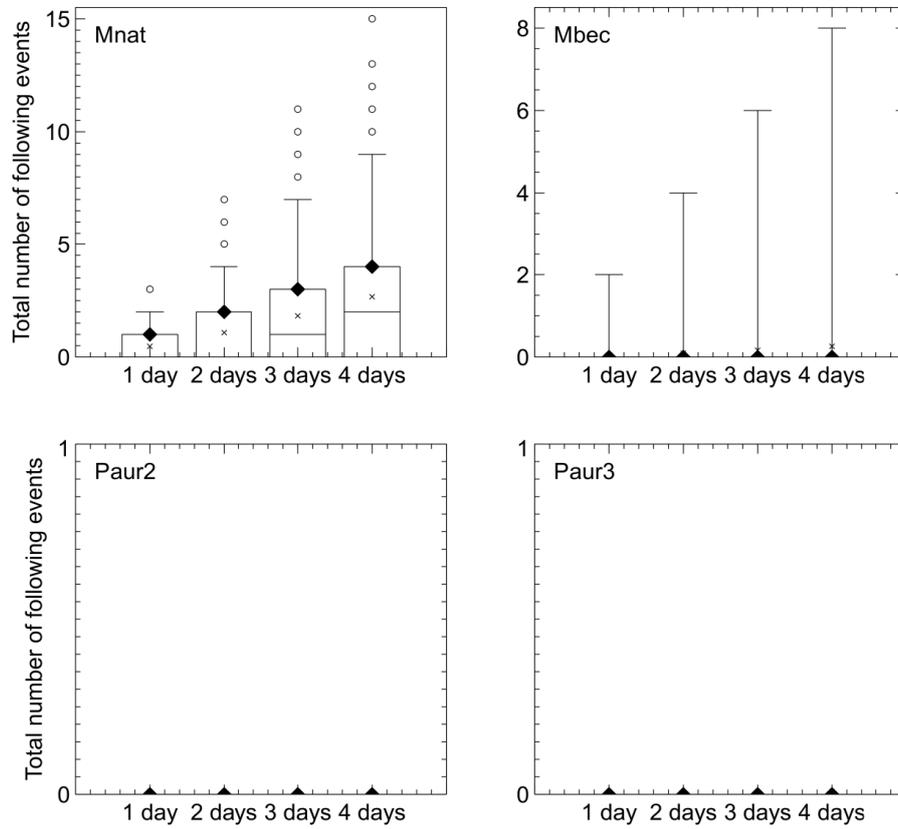


Figure S7: Simulated and observed following behaviour of the Paur1 colony in 2013. The simulated and observed occupation of foreign roosts (following events) by the Paur1 colony within one, two, three or four days after the roost became vacant are shown. Box plots represent the simulated data: Whiskers denote the lowest and highest values within  $1.5 \times$  IQR from the first and third quartiles; white circles depict outliers. The observed values are shown as black diamonds. Overlapping values of observed data and outliers are indicated by black diamonds with white circles. Asterisks below the number of days indicate significant differences between the observed and simulated data for the respective time window.

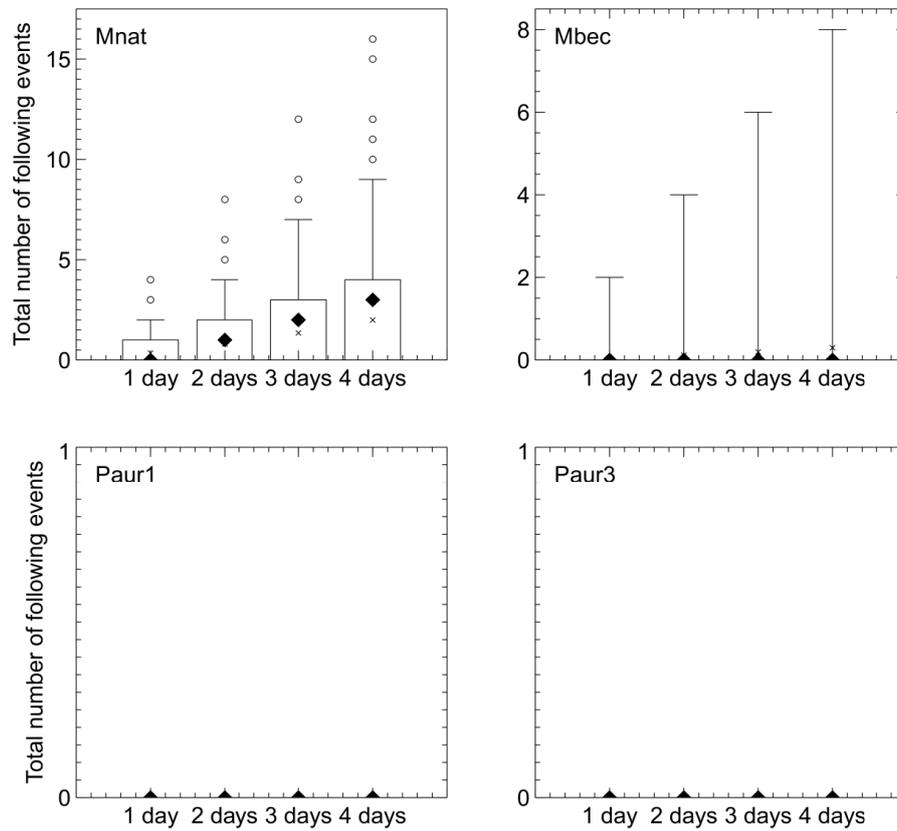


Figure S8: Simulated and observed following behaviour of the Paur2 colony across in 2013. The simulated and observed occupation of foreign roosts (following events) by the Paur2 colony within one, two, three or four days after the roost became vacant are shown. Box plots represent the simulated data; Whiskers denote the lowest and highest values within  $1.5 \times \text{IQR}$  from the first and third quartiles; white circles depict outliers. The observed values are shown as black diamonds. Overlapping values of observed data and outliers are indicated by black diamonds with white circles. Asterisks below the number of days indicate significant differences between the observed and simulated data for the respective time window.

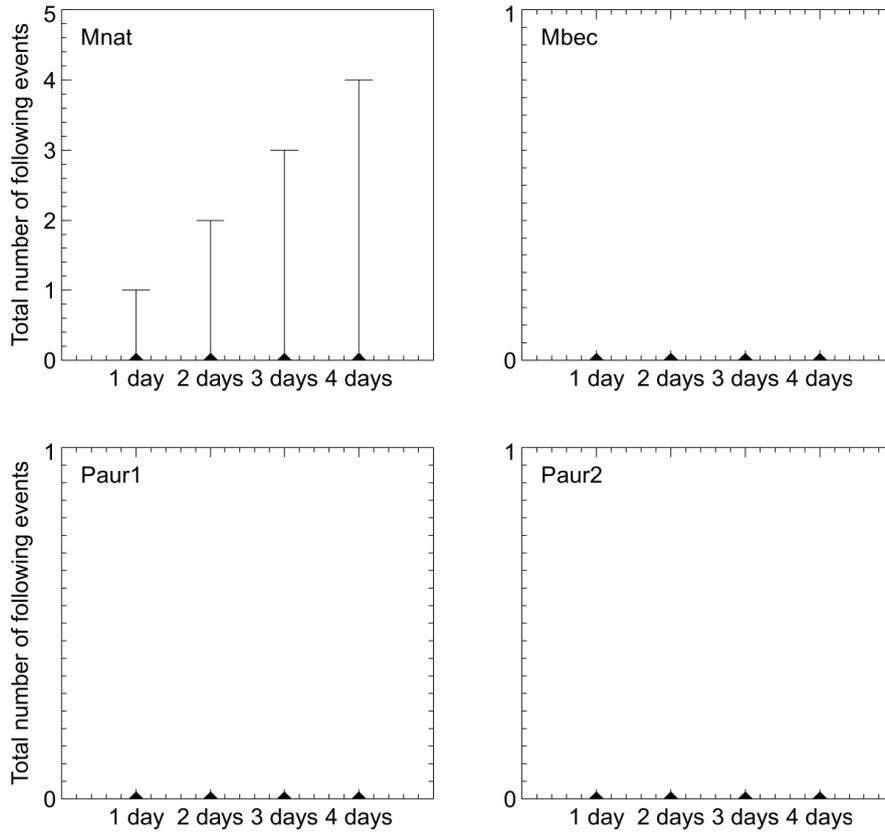


Figure S9: Simulated and observed following behaviour of the Paur3 in 2013. The simulated and observed occupation of foreign roosts (following events) by the Paur3 colony within one, two, three or four days after the roost became vacant are shown. Box plots represent the simulated data: Whiskers denote the lowest and highest values within  $1.5 \times$  IQR from the first and third quartiles; white circles depict outliers. The observed values are shown as black diamonds. Overlapping values of observed data and outliers are indicated by black diamonds with white circles. Asterisks below the number of days indicate significant differences between the observed and simulated data for the respective time window.

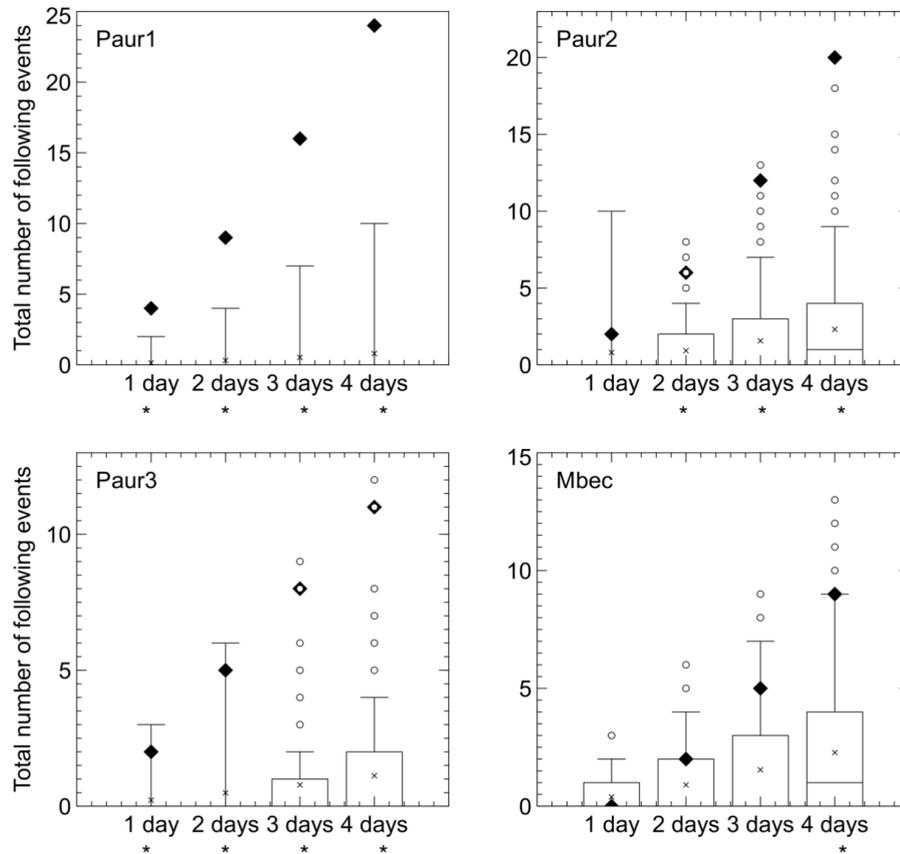


Figure S10: Simulated and observed following behaviour of the Mnat colony in 2014. The simulated and observed occupation of foreign roosts (following events) by the Mnat colony within one, two, three or four days after the roost became vacant are shown. Box plots represent the simulated data: Whiskers denote the lowest and highest values within  $1.5 \times \text{IQR}$  from the first and third quartiles; white circles depict outliers. The observed values are shown as black diamonds. Overlapping values of observed data and outliers are indicated by black diamonds with white circles. Asterisks below the number of days indicate significant differences between the observed and simulated data for the respective time window.

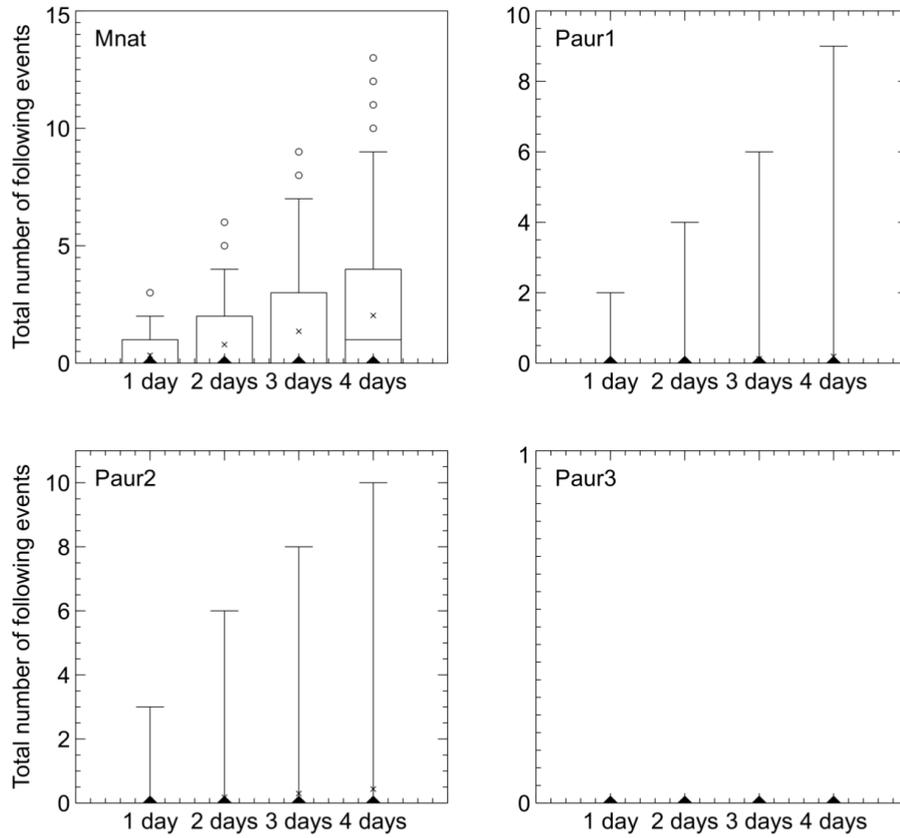


Figure S11: Simulated and observed following behaviour of the Mbec colony in 2014. The simulated and observed occupation of foreign roosts (following events) by the Mbec colony within one, two, three or four days after the roost became vacant are shown. Box plots represent the simulated data: Whiskers denote the lowest and highest values within  $1.5 \times \text{IQR}$  from the first and third quartiles; white circles depict outliers. The observed values are shown as black diamonds. Overlapping values of observed data and outliers are indicated by black diamonds with white circles. Asterisks below the number of days indicate significant differences between the observed and simulated data for the respective time window.

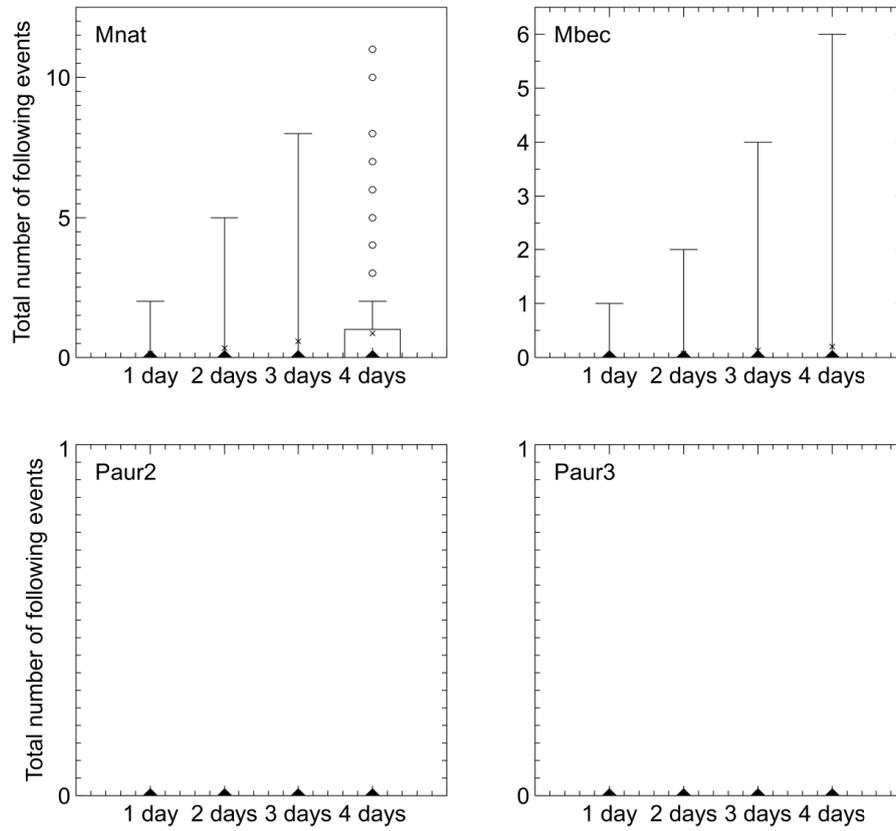


Figure S12: Simulated and observed following behaviour of the Paur1 colony in 2014. The simulated and observed occupation of foreign roosts (following events) by the Paur1 colony within one, two, three or four days after the roost became vacant are shown. Box plots represent the simulated data: Whiskers denote the lowest and highest values within  $1.5 \times \text{IQR}$  from the first and third quartiles; white circles depict outliers. The observed values are shown as black diamonds. Overlapping values of observed data and outliers are indicated by black diamonds with white circles. Asterisks below the number of days indicate significant differences between the observed and simulated data for the respective time window.

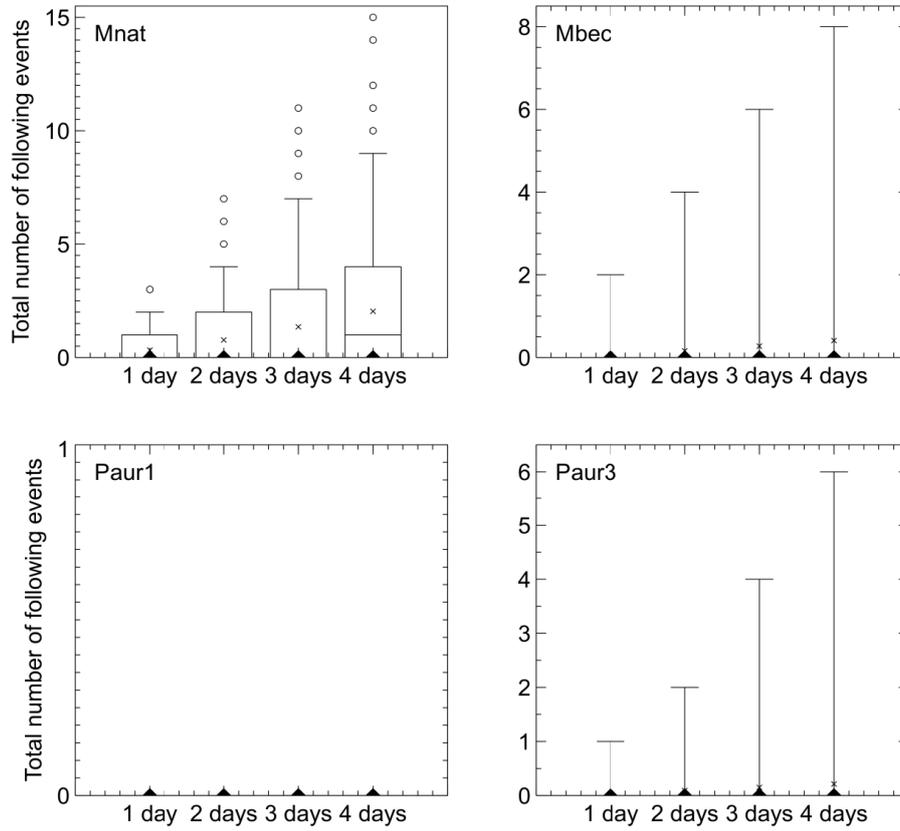


Figure S13: Simulated and observed following behaviour of the Paur2 colony in 2014. The simulated and observed occupation of foreign roosts (following events) by the Paur2 colony within one, two, three or four days after the roost became vacant are shown. Box plots represent the simulated data: Whiskers denote the lowest and highest values within  $1.5 \times$  IQR from the first and third quartiles; white circles depict outliers. The observed values are shown as black diamonds. Overlapping values of observed data and outliers are indicated by black diamonds with white circles. Asterisks below the number of days indicate significant differences between the observed and simulated data for the respective time window.

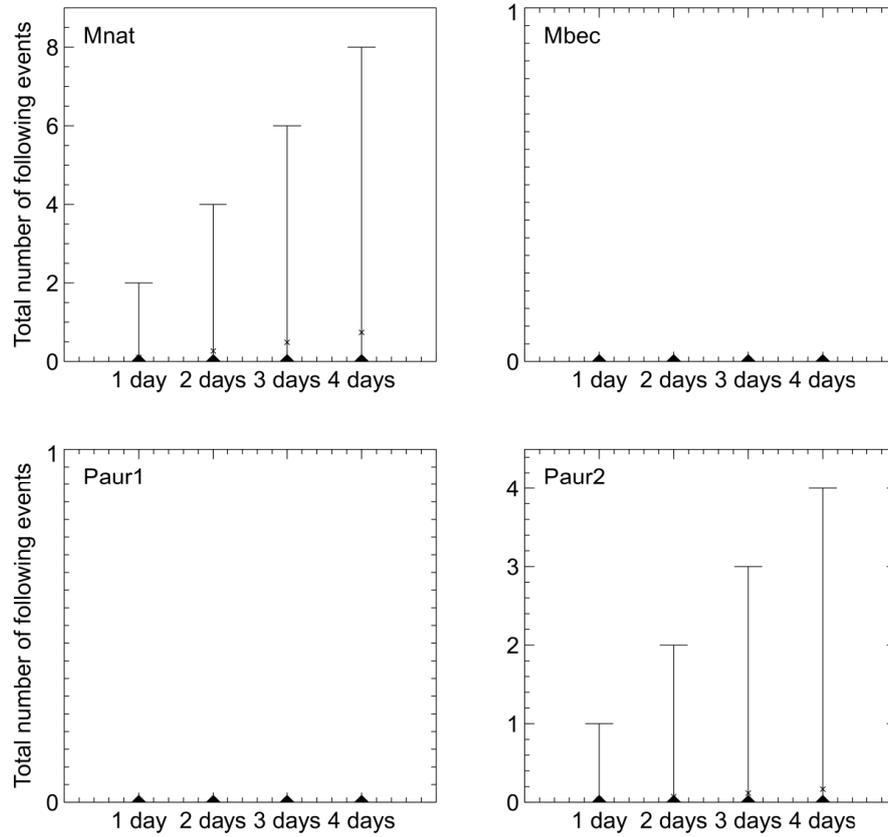


Figure S14: Simulated and observed following behaviour of the Paur3 colony in 2014. The simulated and observed occupation of foreign roosts (following events) by the Paur3 colony within one, two, three or four days after the roost became vacant are shown. Box plots represent the simulated data: Whiskers denote the lowest and highest values within  $1.5 \times \text{IQR}$  from the first and third quartiles; white circles depict outliers. The observed values are shown as black diamonds. Overlapping values of observed data and outliers are indicated by black diamonds with white circles. Asterisks below the number of days indicate significant differences between the observed and simulated data for the respective time window.

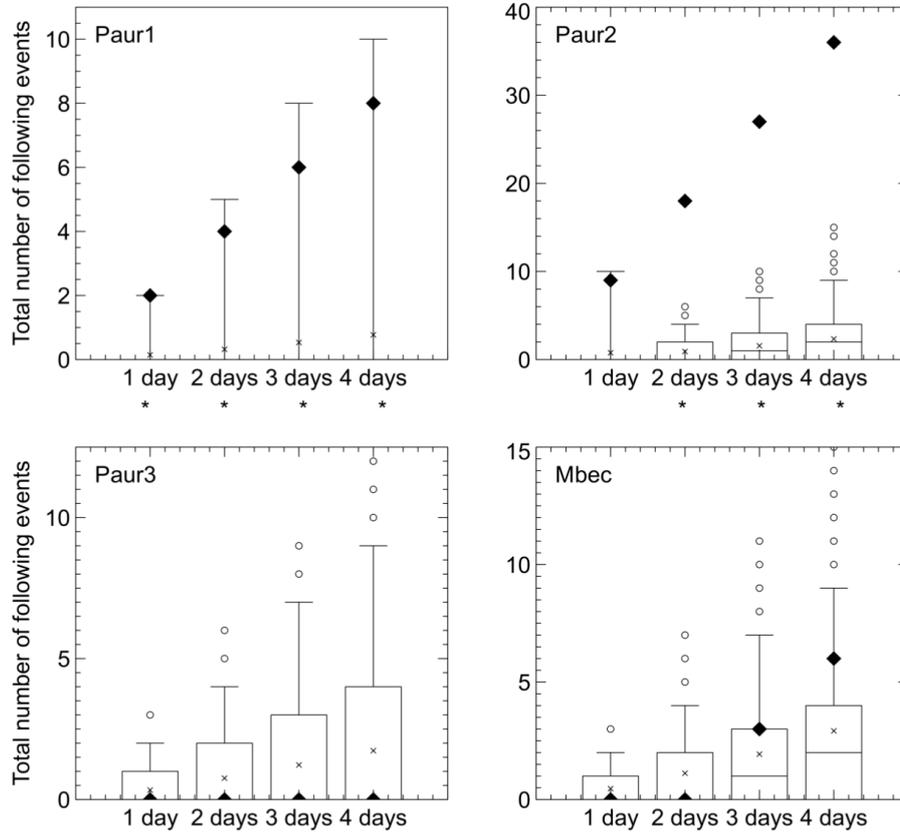


Figure 15: Simulated and observed following behaviour of the Mnat colony in 2015. The simulated and observed occupation of foreign roosts (following events) by the Mnat colony within one, two, three or four days after the roost became vacant are shown. Box plots represent the simulated data: Whiskers denote the lowest and highest values within  $1.5 \times$  IQR from the first and third quartiles; white circles depict outliers. The observed values are shown as black diamonds. Overlapping values of observed data and outliers are indicated by black diamonds with white circles. Asterisks below the number of days indicate significant differences between the observed and simulated data for the respective time window.

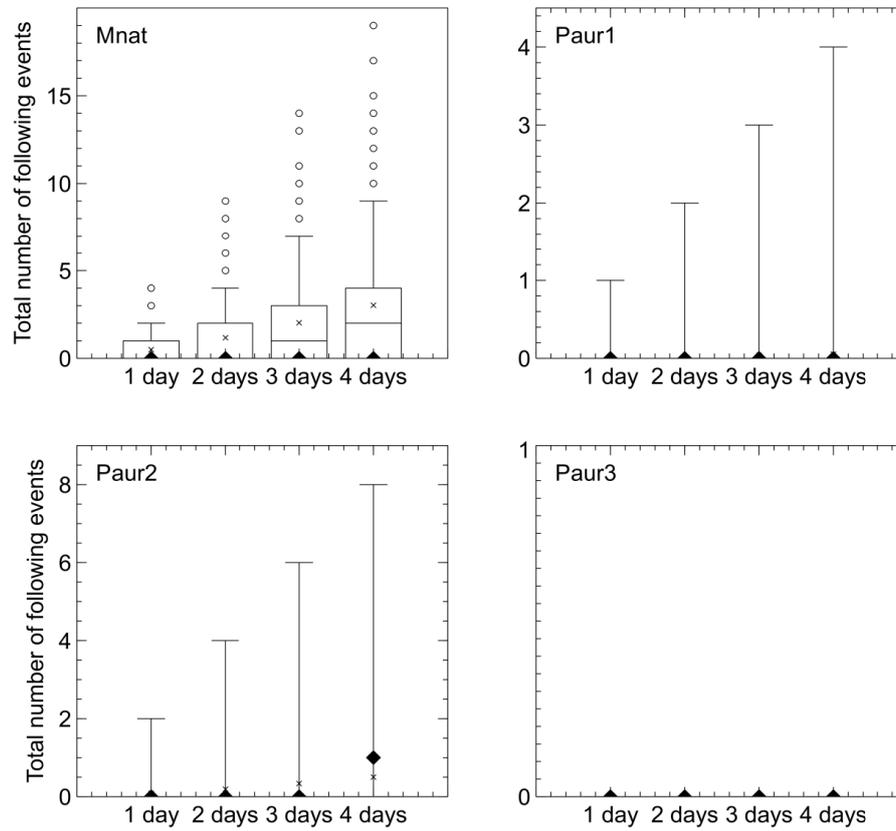


Figure S16: Simulated and observed following behaviour of the Mbec colony in 2015. The simulated and observed occupation of foreign roosts (following events) by the Mbec colony within one, two, three or four days after the roost became vacant are shown. Box plots represent the simulated data: Whiskers denote the lowest and highest values within  $1.5 \times \text{IQR}$  from the first and third quartiles; white circles depict outliers. The observed values are shown as black diamonds. Overlapping values of observed data and outliers are indicated by black diamonds with white circles. Asterisks below the number of days indicate significant differences between the observed and simulated data for the respective time window.

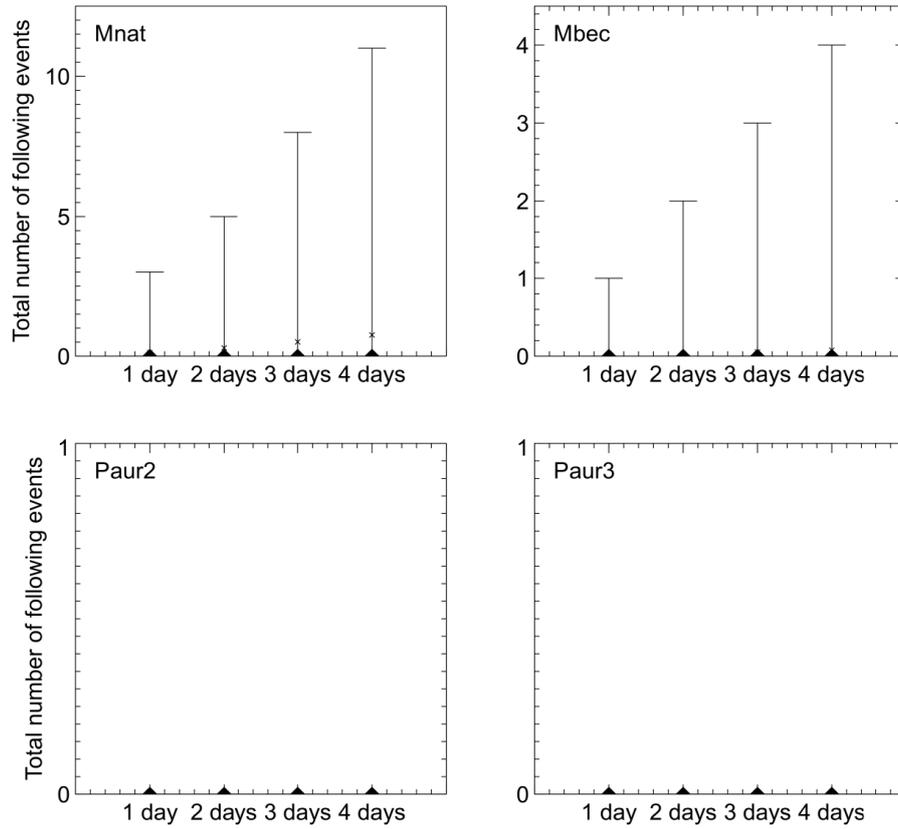


Figure S17: Simulated and observed following behaviour of the Paur1 colony in 2015. The simulated and observed occupation of foreign roosts (following events) by the Paur1 colony within one, two, three or four days after the roost became vacant are shown. Box plots represent the simulated data: Whiskers denote the lowest and highest values within  $1.5 \times \text{IQR}$  from the first and third quartiles; white circles depict outliers. The observed values are shown as black diamonds. Overlapping values of observed data and outliers are indicated by black diamonds with white circles. Asterisks below the number of days indicate significant differences between the observed and simulated data for the respective time window.

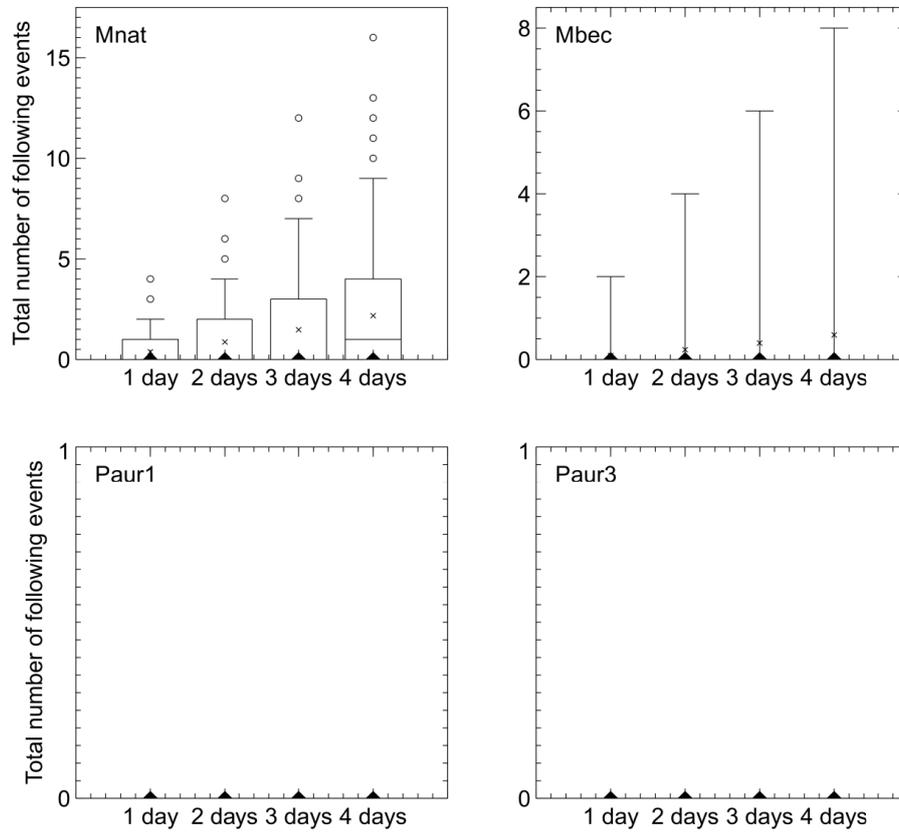


Figure S18: Simulated and observed following behaviour of the Paur2 colony in 2015. The simulated and observed occupation of foreign roosts (following events) by the Paur2 colony within one, two, three or four days after the roost became vacant are shown. Box plots represent the simulated data: Whiskers denote the lowest and highest values within  $1.5 \times \text{IQR}$  from the first and third quartiles; white circles depict outliers. The observed values are shown as black diamonds. Overlapping values of observed data and outliers are indicated by black diamonds with white circles. Asterisks below the number of days indicate significant differences between the observed and simulated data for the respective time window.

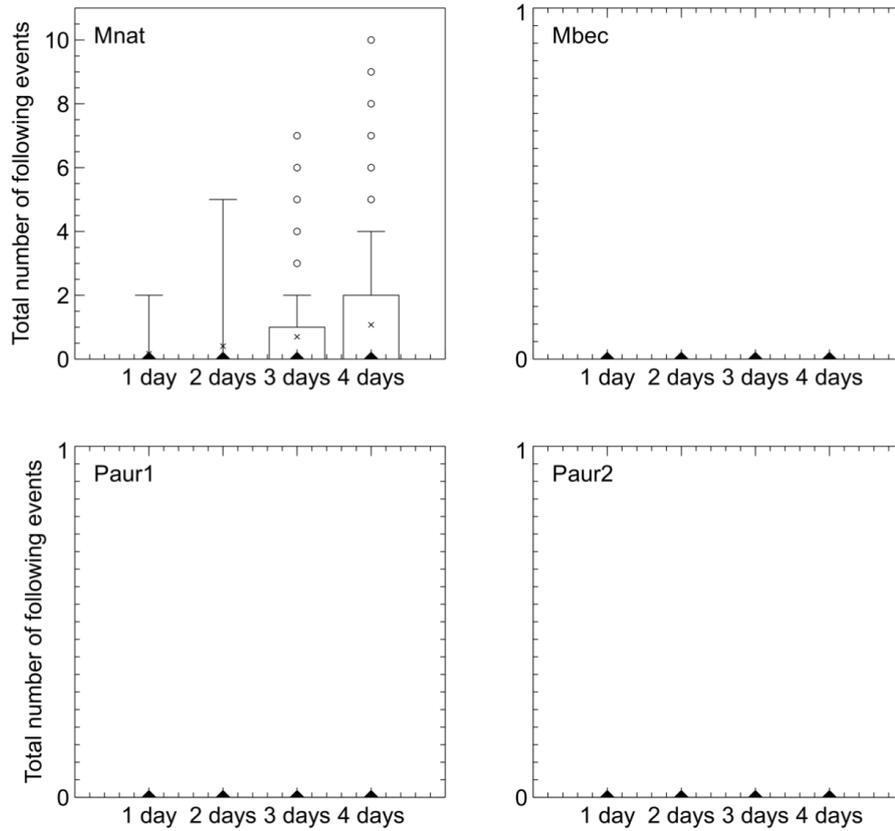


Figure S19: Simulated and observed following behaviour of the Paur3 colony in 2015. The simulated and observed occupation of foreign roosts (following events) by the Paur3 colony within one, two, three or four days after the roost became vacant are shown. Box plots represent the simulated data: Whiskers denote the lowest and highest values within  $1.5 \times$  IQR from the first and third quartiles; white circles depict outliers. The observed values are shown as black diamonds. Overlapping values of observed data and outliers are indicated by black diamonds with white circles. Asterisks below the number of days indicate significant differences between the observed and simulated data for the respective time window.

#### 4.4 Contribution to publications

##### Manuscript 1

**Zeus, V. M.**, Reusch, C., & Kerth, G. (2018). Long-term roosting data reveal a unimodular social network in large fission-fusion society of the colony-living Natterer's bat (*Myotis nattereri*). *Behavioral Ecology and Sociobiology*, 72(6), 99. <https://doi.org/10.1007/s00265-018-2516-4>

**VMZ** carried out the collection of the field data, conducted the analysis, wrote the R script together with CR, and wrote the first draft of the manuscript. All authors were involved in the design of the study, wrote on the manuscript and gave final approval for publication.

##### Manuscript 2

**Zeus, V. M.**, Köhler, A., Reusch, C., Fischer, K., Balkema-Buschmann, A. & Kerth, G. (submitted). Analysis of astrovirus transmission pathways in a free-ranging fission-fusion colony of Natterer's bats (*Myotis nattereri*).

**VMZ** carried out the collection and analysis of the field data, participated in writing the R script, and wrote the first draft of the manuscript. AK and KF analyzed the virus samples. CR wrote the R script. All authors were involved in the design of the study, wrote on the manuscript and gave final approval for publication

##### Manuscript 3

**Zeus, V. M.**, Puechmaille, S. J., & Kerth, G. (2017). Conspecific and heterospecific social groups affect each other's resource use: a study on roost sharing among bat colonies. *Animal Behaviour*, 123, 329–338. <https://doi.org/10.1016/j.anbehav.2016.11.015>

**VMZ** carried out the collection of the field data, conducted the analysis together with SJP who wrote the R Script, and wrote the first draft of the manuscript. All authors were involved in the design of the study, wrote on the manuscript and gave final approval for publication.

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## ACKNOWLEDGEMENT

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