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Social and genetic drivers of behaviour and life history in horseshoe bats

Social and genetic drivers of behaviour and life history in horseshoe bats

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Abstract

Organisms seldom exist in isolation. The transmission of an individual's genes often depends on its social environment. From conception, an individual's growth and survival are defined by the resources allocated by its mother. Similarly, in forming groups, the actions of conspecifics can mediate access to resources through competition or cooperation. Thus, phenotypic variation can be shaped by the dynamics of interactions with conspecifics, in addition to genetic effects. In this thesis, I explore questions related to the evolution of behavioural and life history strategies, using two species of long-lived horseshoe bat (Chiroptera: Rhinolophidae) as model systems. Chapter 2 examines whether bi-parental or maternal kinship influences spatial assortment in underground hibernacula among individual greater horseshoe bats (*Rhinolophus ferrumequinum*), driving population sociogenetic structure. Neither measure of kinship was an important predictor of assortment; rather, associations within roosts were age-structured. Chapter 3 investigates the potential benefits of group-living for hibernating lesser horseshoe bats (*R. hipposideros*), specifically the "social alarm-clock" hypothesis which proposes that torpid individuals may use the activity of nearby normothermic conspecifics as an inadvertent social cue indicating favourable conditions to arouse. Temperature cues proved most important for triggering arousals, but evidence for social transmission of arousals was found, particularly in Autumn. Chapter 4 applies quantitative genetic analysis to a long-term study of *R. ferrumequinum* to estimate the heritability and evolvability of morphological and life-history traits, and to investigate the role of maternal effects in shaping phenotypic variation. The results show high heritability but low evolvability for morphological traits and low heritability but high evolvability for life-history traits. Maternal effects were weak, implying limited maternal influence on offspring phenotypic variation for the traits studied. Overall, this thesis provides insight into the social and genetic factors driving phenotypic variation in bats.

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Author’s declaration

I declare that the work in this dissertation was carried out in accordance with the requirements of the University's *Regulations and Code of Practice for Research Degree Programmes* and that it has not been submitted for any other academic award. Except where indicated by specific reference in the text, the work is the candidate's own work. Work done in collaboration with, or with the assistance of, others, is indicated as such. Any views expressed in the dissertation are those of the author.

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Author contributions

My contributions to each chapter are specified by the 'LR' initial. Significant contributions from each chapter from Dr Roger Ransome (RR), Professor Gareth Jones (GJ), Professor Stephen Rossiter (SR), Professor Darren Croft (DC), Dr Michael Weiss (MW), Dr Helen Ward (HW), and Dr Megan Power (MP) are specified below:

Chapter 1: Original draft: LR; Review: LR and GJ

Chapter 2: Conceptualisation: LR, GJ, RR; Sample collection: Lead by RR with assistance from LR, GJ, SR, HW, MP and other volunteers as part of long-term field study; Lab work: LR, SR and HW; Data analysis: LR, with some code and assistance from MW in social network analysis. SP and HW constructed the pedigree ≤2011. LR constructed the pedigree 2012-2018; Original draft: LR; Review: LR, GJ, and SR.

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Greater horseshoe bats (*Rhinolophus ferrumequinum*) were caught and sampled under licences from the Home Office (held by LR) and Natural England (held by GJ and RR). Lesser horseshoe bats (*R. hipposideros*) were observed under licence from Natural England (held by GJ and RR).

Contents

List of Tables

List of Figures

List of Equations

List of Supplementary Tables and Figures

Chapter 1

General Introduction

The Cotswolds in a photo. Cattle-grazed pastures, deciduous woodlands, and appropriately managed hedgerows are vital for contemporary horseshoe bat populations. View from the Cotswolds Way towards the city of Bath.

Photo credit: L Romaine

1.1 Overview

The “struggle for existence” forms a major pillar of Charles Darwin’s theory of evolution by natural selection [1]. Here, the “struggle” is to reproduce one’s genetic material in the next generation when the ability to do so for all individuals is restricted. Succeeding in this endeavour – through natural selection – requires a phenotype where the limited resources acquired by an individual are allocated sufficiently according to the environment, and possessing genes that allow it to do so. A fundamental aim in evolutionary biology is to understand what makes phenotypes successful via their adaptions. Yet, no phenotype is identical. What is the genetic and environmental basis behind phenotypic variation? In this thesis, I focus on the social group as the agent of environmental variation, and explore how an individual’s social environment might shape how it allocates limited resources during its life history. I use two species of long-lived horseshoe bat (Chiroptera: Rhinolophidae) as model systems.

In this introduction, I outline the key concepts and current theory behind the evolution of sociality and highlight methodological approaches to studying the impact of social relationships on individuals and how they shape phenotypic variation in wild populations. Next, I introduce bats (Chiroptera), an extremely speciose and social order of mammals. I draw upon the current knowledge of sociality in bats and review how social network analysis has been applied to these organisms. Lastly, I introduce horseshoe bats as a study system to investigate questions relating to the social and genetic drivers of phenotypic variation.

1.2 The evolution of sociality

All animals live in some form of social system. Some exist mainly in solitude, seldom interacting with conspecifics until mating. Alternatively, some live in groups that represent, to many, some of the greatest phenomena of the natural world. It is hard to imagine a planet without the elegant courting of a mating pair of great-crested grebes (*Podiceps cristatus*), the several dozen bumblebees (*Bombus* sp.) building an underground hive, or the several million-strong herds of ungulates migrating across the plains of southern Africa. Indeed, sociality* is a remarkable trait. However, at first glance, it presents a Darwinian puzzle. There can be severe costs to being social. Large groups of prey animals are highly conspicuous to predators [2–4]. Additionally, sociality is associated with an increased probability of disease transmission [5,6]. For example, solitary male gorillas (*Gorilla gorilla*), are less vulnerable to the Ebola virus than their social counterparts [7]. Contacts among Tasmanian devils (*Sarcophilus harrisii*) predict susceptibility to facial cancer, transmitted via bites from conspecifics [8]. Furthermore, animals that forage in social groups face the cost of intraspecific food competition and the risk of kleptoparasitism [9]. Most unexpectedly, group living can bring direct reproductive costs. In meerkat (*Suricata suricatta*) society, a single female is responsible for the reproductive output of the group. The 'alpha' female will suppress the reproduction of her subordinates, through high levels of aggression, leading to miscarriage, or via infanticide. Subordinate females spend considerable amounts of their own resources foraging and protecting the group from rival groups and predators, and suckling the alpha females' pups [10,11].

Why do any animals live in groups instead of living alone? Wynne-Edwards (1962)[12] and Lorenz (1963)[13] believed that natural selection operated at the level of the social group, whereby an individual engages in costly behaviour for the "good of the species". A central tenet of Darwinian theory is ignored here – the importance of the individual, the gene it carries, and the potential for cheating. Evolution by natural selection relies on heredity of adaptive traits, and the unit of heredity is the gene. Apart from clones and monozygotic twins, every individual's genome is unique. Genetic mistakes, mutations, created during the

* In this thesis, I define 'sociality' according to Tinbergen (1951)[14]: "*An animal is called social when it strives to be in the neighbourhood of fellow members of its species when performing some, or all, of its instinctive activities*". A social system, therefore, broadly describes the set of social individuals, and the "*nature, quality, and patterning*" of their relationships [15].

formation of gametes, generate variation among individuals. With variation there is conflict. A gene variant (allele) more capable of replicating over another will eventually outcompete another and increase in frequency. In other words, an allele improving the fitness of its host (its relative reproductive success) will be more likely passed to the next generation than one that does not. This is the basis of evolution by selection, according to "selfish gene theory" [16–18].

As was realised by Trivers (1974)[19], a gene-centric view has surprising implications for the evolution of sociality. From this perspective, social relationships are not as harmonious as they appear superficially. For example, the most fundamental social bond – that between a mother and infant – is, in fact, the outcome of an evolutionary tug-of-war. After all, following fertilisation, the developing embryo shares only 50% of its genetic material with the mother (and future siblings), yet 100% with itself. Therefore, selection should favour alleles leading to increased energetic investment from mother to embryo, more so than would benefit the mother's own fitness i.e., the prospect of future offspring [19–21]. It is remarkable a mother cares for her infant in the first place. In truth, both parties can benefit. Taking a gene-centred view of evolution provides an explanation as to why this can be, despite the genetic conflict [17,19,22]. The higher the coefficient of relatedness (*r*) between two individuals, the greater the probability an allele is shared via inheritance from a common ancestor. An allele that promotes maternal investment in its host can increase in frequency provided it results in more shared alleles being transmitted to the next generation than if the behaviour were not performed. In the matter of parent-offspring conflict, where there is a 50% probability of a shared allele between mother and foetus (i.e. the coefficient of relatedness $r = 0.5$), no alleles can be transmitted if the pregnancy does not reach full-term. Despite the costs incurred, maternal investment (in its most fundamental form of pregnancy and parturition) pays off as it promotes the fitness of the offspring to which the mother is genetically related, and therefore promotes her own fitness.

In some species, parental care is directed entirely towards a relative's offspring, forgoing an one's own reproduction. This puzzling, altruistic behaviour, where direct fitness is sacrificed for another's, is exemplified in the communal breeders (see above) e.g. meerkats (*S. suricatta*). W.D. Hamilton's kin selection theory [17] explains how altruism, in this context, can evolve. This theory introduces the concept of "inclusive" fitness whereby an individual can

increase the transmission of alleles it possesses by promoting the direct fitness of close genetic relatives (kin) that share the same alleles. From a gene-centric view, altruistic behaviour is not so puzzling. As much as an individual is related to its own offspring, so it is too with full siblings (r = 0.5). Indeed, aiding the reproduction of three nieces (r = 0.25 x 3 = 0.75), yields an additional 25% genetic units transmitted indirectly to the next generation. The geneticist J.B.S Haldane famously quipped that he would be willing to sacrifice himself for two brothers or eight cousins [23].

Ultimately, the emergence of sociality depends on the fragile balance of costs and benefits to individuals [24]. As with any behaviour, the "decision" to be social is a trade-off [25], where the balance may tip the other way under alternative ecological, genetic, social, and individual contexts [26]. Hamilton's rule [17] predicts natural selection – will only favour altruism if the cost (C) does not exceed the benefit (B) to the recipient multiplied by the probability of shared alleles with the actor:

$$r \times B > C$$

Equation 1.1 *Hamilton's Rule*

Kinship, conferring indirect fitness benefits, is only one of many possible mechanisms driving social affiliation [27,28]. It is not a prerequisite [28]. Many mammals live in groups with non-relatives.

Outside of kin selection, observations of costly behaviour are usually explained by reciprocity or "reciprocal altruism" [29]. Here, behaviours expressed at a cost to the individual are stabilised by the expected future repayment – reciprocation.

Reciprocity is well-documented in primates, particularly in the context of grooming [30,31]. Grooming, involving the removal of ectoparasites from the fur of the recipient, can incur a temporary fitness cost to the actor through reduced vigilance and increased predation risk [32,33]. Even when costs are minimal, the groomer does not share the immediate benefit afforded by their own services. Particularly between non-kin, altruistic behaviour is unlikely to remain a stable strategy if payment is never returned. In some primate societies, notably vervet monkeys (*Chlorocebus pygerythrus*), grooming is exchanged for support by higher ranking individuals in potentially risky conflicts [34–36]. Grooming is often reciprocated with more grooming. A meta-analysis by Schino & Aureli (2007)[37] found female primates prefer

to groom those that groom them the most. Immediate reciprocation, where grooming is returned in short-term sessions, avoids the problem of "cheaters" building up debt [28,38,39]. Avoiding exploitation by cheaters can also be avoided individual recognition, necessitating a substantial social memory of helpful individuals [40].

Social affiliations are not always explained by altruism, rather, "selfishness". The fitness benefits to all involved individuals can be immediate. Hamilton's (1971)[41] "selfish herd" theory predicts why aggregations of prey species might evolve despite the costs of conspicuousness. Isolated brown fur seals (*Arctocephalus pusillus)* find themselves in an expanded "domain of danger", the area within which, when a predator enters it, they will be closer than neighbouring conspecifics. By aggregating amongst neighbours, the "domain" is much reduced, alongside the probability of this same individual being predated. Rather than for the intended benefit of others, by grouping, every individual receives an immediate benefit of diluting their own predation risk amongst others in their group [42,43]. A "selfish herd" is formed. Such formations tend to be more common for diurnal species, where attacks by visual predators are more likely, especially those living in open habitats [44].

Competition for resources among conspecifics remains a selective pressure against sociality and can explain why species relying on scarce resources tend to be in smaller groups, or live solitarily [45]. For example, patch size and density were identified as factors limiting foraging group size in Peruvian spider monkeys (*Ateles chamek*) [46]. However, it is doubtful these food patches could be identified without the help of others. When resources are unpredictable over space and time (i.e. patchy) [47], and thus costly for the individual to locate through trial-and-error, access to knowledge from already informed individuals can become beneficial [48,49]. For example, bottlenose dolphins (*Tursiops truncatus*) hunting ephemeral, mobile prey, maintain stronger group ties compared to their solitary counterparts targeting shellfish farms where resources are predictable [50]. In addition, rather than hunting cooperatively, many seabirds will forage in aggregations, relying on passive social cues (such as foraging behaviour) to locate food patches [51–53].

The spatio-temporal variation of social interactions has consequences for individuals in how they allocate resources to growth and reproduction and make decisions to improve survival [54]. In this next section I explore how we can capture this variation as a network and understand its impacts to evolution through quantitative genetics.

1.3 Studying sociality

1.3.1 Social network analysis

Identifying the structure and drivers behind social group formation is necessary to appreciate how animal social systems evolve. Although the methods used to tackle this most fundamental aim today have changed dramatically since the advent of behavioural ecology in the mid-20th century.[54,55]

Box 1. Social Network Theory Terminology. Definitions from Wey et al., (2008)[53] and Kurvers et al., (2014)[54].

Basic Terms

***Network or Graph*:** a visualisation of interrelationships between objects, represented as nodes connected by ties.

***Node or Vertex*:** a component of the network representing an individual or group.

Edge or Tie: a connection between two nodes in a network, representing interactions.

***Path Length*:** the shortest number of edges between two nodes.

Individual Measures

***Centrality*:** an individual's importance in the network, based on its position.

***Degree Centrality*:** the number of immediate interactions with a node.

***Closeness Centrality*:** the sum of the shortest number of dyadic interactions, or shortest path length, between one specific node and all other nodes.

***Betweenness Centrality*:** the most conservative number of vertices that intersect a node between other nodes.

***Eigenvector Centrality*:** the influence of a node on network structure.

Homophily or Assortativity: the tendency of individuals to associate with similar individuals.

***Bridge*:** an individual who connects two, otherwise unconnected, subgroups.

Intermediate Measures

***Cohesion*:** the degree of interconnectivity between groups of nodes.

***Cluster Coefficient*:** the connectedness of a node's immediate neighbours.

Group Measures

***Small-world network*:** a network where average path length increases logarithmically with the total number of nodes - characterised by a high clustering coefficient with a short average path length.

***Well-mixed population*:** all pairs of nodes have equal opportunity of interacting with each other.

Social network analysis (SNA) allows behavioural ecologists to examine sociality in a new light – multiple dyadic associations between individuals can be captured quantitatively in a standardised manner. Here, the consequences of social relationships to individuals, through to groups, and even whole populations can be examined [54–57].

SNA can trace its origins to graph theory in the 18th century [58], which interprets pair-wise relationships (edges) between objects (nodes) within mathematical structures. From the 1930s, research in the social sciences led to significant conceptual development and creation of meaningful, quantitative descriptors of networks (see Box 1). Yet, after an established history of human research, it is only relatively recently that the properties of non-human social structure have been studied quantitively [59,60].

Generally, social groups are not random assemblages. A key application of SNA is to examine the existence of non-random social preferences and the factors driving their formation. Studies of non-human primates pioneered the use of SNA in this way [60]. One of the earliest studies, by Simonds (1974) [61], observed groups of bonneted macaques (*Macaca radiata*). Building directed networks or "sociograms", weighted on grooming interactions between individuals, Simonds could readily visualise contrasting structures in alternate macaque troops and gave insight into the processes underpinning them. Connections amongst individuals developed with age, and distinct sex roles were apparent. The strength of interactions for males in one troop could be predicted by their dominance rank. Alpha males appeared to be particularly important for group cohesion. Indeed, the presence of preferred partners was clear. Female infants built early connections among close kin, whereas males were less selective. During the birthing season, the network developed clusters of unrelated females tending to new-borns.

Statistical tools have since become more sophisticated and powerful, greatly improving the scope and robustness of SNA studies. In contrast to Simonds' (1974)[61] more qualitative descriptions of social affiliation, researchers can now quantify the tendency to assort (*assortativity* - Box 1) between individuals of similar phenotypes. A recent study on killer whales (*Orcinus orca*) found that age and sex play a key role in driving interactions, such as physical contact, among individuals [62]. Males became less social (lower centrality) with age, spending more time foraging away from the central group. However, neither age nor sex predicted spatio-temporal associations. Instead, maternal kinship was a significant predictor of associations. By associating with close kin, individuals may obtain inclusive fitness benefits from targeted kin interactions – such as food sharing [63].

The properties of networks give insight into the evolution of life history strategies [54]. An individual's position within a network can impact their survival and reproductive success. An earlier study within the same population of killer whales found the centrality of males predicted mortality risk. Males with lower closeness centrality were less likely to survive in years of low salmon (*Oncorhynchus nerka*) abundance [64]. Closeness centrality (Box 1) predicts an individual's access to information from others in the network. For socially peripheral males, when resources are poor, their access to ecological knowledge is weak, reducing their survival chances. In this case, additional network analysis revealed older females demonstrate leadership during these periods of low salmon numbers. Female killer whales are exceptionally long-lived. By leading kin (particularly sons) to food patches, they promote the fitness of their kin, and their own inclusive fitness -favouring the selection of longevity [65].

Such studies exemplify how the properties of networks can have implications for selection on individuals. In other words, an individual's social environment can determine its fitness [66]. Populations are rarely homogeneous and well-mixed (Box 1). More likely, individuals occupy space and time in a non-random way (e.g., through habitat selection[26,67,68] or social preferences[69–71]) that leads to non-random differences in phenotypes between neighbours. Consequently, the selection environment becomes structured. However, fitness can be actively engineered by individuals, either by entering or creating [72,73] environments so selection proceeds in their favour. For example, in a study of house finches (*Haemorhous mexicanus*) [74], males with "unattractive" sexual ornamentation held more peripheral network positions and received fewer mates relative to males with more elaborated ornaments. However, they could enhance their own attractiveness by switching groups [75]. They improved their paternity success more so than those, of similar phenotype, that were more faithful to their original flock.

One of the most exciting advancements provided by the SNA framework is the ability to capture indirect connections [59,76]. This application has been put to great use in the field of social learning. An individual's actions rarely end with their neighbours - because their neighbours, too, have neighbours. The flow of contagions [77], be it disease or information, is a process that emerges from the connection of individuals through to the creation of a complex network. Network-based diffusion analysis (NBDA) was developed to enable

questions on contagion transmission to be answered [78]. In an experiment of wild tits (Paridae), association networks were constructed of individuals foraging together at feeders containing sunflower seeds. New feeders (i.e. artificial food patches) were introduced at random locations. In addition to random discovery, Aplin et al., (2012) [79] suggest social information was used to locate these novel food patches. Upon identification of a new feeder, the sequence of individuals to arrive corresponded to the structure of the network. In the wild, tits rely on ephemeral resources. While naturally selected traits can achieve limited success, passive acquisition of information through social associations results in mutual benefits for individuals in flocks - greatly increasing the efficiency at which food can be found. The use of NBDA has been pivotal in the understanding of complex cultural transmission in a range of taxa, including insects [80], birds [79,81,82], and mammals [83–85].

1.3.2 Quantitative genetics

The field of quantitative genetics (QG) is fundamentally concerned with identifying the additive genetic sources of phenotypic variation among individuals. Additive genetic effects arise from the inheritance of alleles from parent to offspring, and thus underpin the resemblance among relatives [86]. Consequently, they are primarily responsible for cross-generational changes in the distribution of phenotypes after selection has occurred i.e. evolution. The contribution of additive genetic effects to the observed phenotypic variation is defined under the concept of "heritability". Thus, the proportion of phenotypic variation that can persist to the next generation depends on the degree of which is "heritable" [87,88].

QG utilises pedigrees, or controlled breeding studies, to provide direct information on genetic relatedness, allowing the sources of variation (genetic and environmental) to be distinguished [87,89]. Under constant environmental conditions (as many animal breeders seek to achieve) heritability can, theoretically, achieve 100%. Thus, the magnitude of the genetic components of variation are best understood in the context of non-genetic components. Statistical approaches in QG, such as the animal model, are intended to assess the relative significance of these various factors that might influence the expression of any measurable phenotype that varies among individuals [90,91].

Since Darwin, we have recognised that while selection primarily operates on individuals (as discussed in section 1.2), rarely are phenotypic traits expressed in isolation. Fitness, after all, is a relative concept that relies on variation among individuals [92]. All individuals compete for

resources, be they for sustenance (food resources) or reproduction (mates), influencing the development of traits [66]. Therefore, if we are to understand trait heritability, and consequently evolutionary responses, it is imperative we understand the sources of social variation acting on individuals, that drive selection. West-Eberhard (1983)[93] defines "social selection" as "*differential success in social competition, whatever the resource at stake*". Sexual selection, as first defined by Darwin[94], where reproductive success varies according to mate competition and kin selection, where an individual's fitness can be determined by its relatives [17] are both forms of social selection.

One source of social variation that has seen increasing attention over recent decades are parental effects [95,96]. Firstly, in species with parental care (as in mammals), the shared parental environment among offspring can generate non-genetic, but heritable, variation [97]. Such confounding effects need to be accounted for to provide accurate estimates of additive genetic effects (heritability). Secondly, as the parental effects often have a genetic component, they themselves can be heritable, providing an additional, hidden source of genetic variance that can respond to selection [98].

Maternal effects have received the greatest level of focus, given paternal care (from the father) is rare in the majority of mammal species [96]. A recent meta-analysis by Moore et al., (2019)[99] revealed maternal effects often equated the contribution of additive genetic effects. This was most noticeable in juvenile traits, unsurprisingly, as the juvenile phase is when a mother exerts the most influence. Maternal effects may constitute the effect of direct investment, such as the sharing of ecological knowledge or milk provision in infancy, and as such can depend on environmental fluctuations [83,100,101]. Intriguingly, the mothers' genes may exert an influence on the offspring's phenotype beyond their direct inheritance. That is, an offspring's phenotype can depend on the expression of the parent's genotype post-fertilisation. For mothers, these 'indirect' genetic effects (IGEs) might comprise the effect of lactation gene expression on offspring growth [102]. On the other hand, relatively little is known about the impact of the father's genotype. This may be because male mammals typically disperse to breed in and contribute little to parental care. Nevertheless, recent studies have revealed the importance of paternal effects mediated through the ejaculate [103], warranting further exploration in wild mammal populations.

1.4 Sociality in bats

1.4.1 Why are bats social?

The Chiroptera (bats) are the second-most speciose order of mammals, with over 1400 species discovered as of 2023 [104], constituting almost two thirds of all wild mammal individuals [105]. Based on morphological features, the order was split traditionally into the suborders - Microchiroptera and Megachiroptera. Microchiroptera consisted largely of smaller, insectivorous bats, whereas the Megachiroptera encompassed the remaining large, frugivorous bats of Africa, Asia, and Oceania [106]. Springer et al., (2001)[107] proposed a new phylogeny based on molecular evidence. Chiroptera was re-divided into two new suborders: Yinpterochiroptera and Yangochiroptera. The Yinpterochiroptera consist of the traditional megabats (Pteropodidae) as well as the "microbat" families: Rhinolophidae, Rhinonycteridae, Craesonycteridae, Hipposideridae, Megadermatidae, and Rhinopomatidae. The Yangochiroptera hold the remaining 14 families, including the Vespertilionidae and Molossidae.

Across the diverse array of bat species is an exceptional diversity of social systems. Group sizes vary from a handful of individuals to several million. Very few are solitary (e.g. *Lasiurus cinereus* [108]). Group-living is the rule, rather than the exception, in bats [109]. Why might this be?

One hypothesis for sociality is roost limitation. Many species roost gregariously in caves, a habitat with limited distribution. However, group-living species exist that construct their own roosts [110] e.g. the tent-making bats [111,112], and switch between them regularly [113,114]– suggesting roosts are not always a limited resource. Thus, while roost limitation may provide an ecological constraint promoting sociality, it is certainly not the sole contributor [109].

Another explanation for sociality in bats is thermoregulation. Maternity colonies in temperate regions are common, where breeding females gather to give birth communally. Mothers cluster together tightly, potentially to limit their exposed surface area and reduce heat loss. Together, the colony achieves mutual warming benefits, offsetting the extensive energetic costs of parental care [115–118]. Similarly, mixed-sex groups of bats are often observed huddling to minimise heat and water loss during winter hibernation [119–123]. Social thermoregulation is not necessarily restricted to the temperate zones, and can be found in

the Neotropics, where temperatures too can fluctuate. For example, captive vampire bats (*Desmodus rotundus*), living in the Neotropics, huddle for warmth during cold spells – perhaps as they cannot endure multi-day bouts of torpor (hibernation) [124].

Group-living bats may benefit further from reduced predation risk. Many species roost colonially, and will emerge to feed in vast swarms, rather than individually [125]. Despite tremendous agility, bats can still fall prey to predation by raptors[126] and ground-dwelling carnivores [127–129]. As noted by Hamilton (1971) [41], collective emergence may serve to dilute predation risk [130,131] (see section 1.2). However, evidence supporting this hypothesis is mixed [125]. For example, serotine (*Eptesicus serotinus*) [132] and greater horseshoe bats (*Rhinolophus ferrumequinum*) emerged collectively upon the arrival of a trained barn owl (*Tyto alba*) and domestic cat (*Felis catus*) respectively [133]. In contrast, responses to owl models were weak in little brown bats (*Myotis lucifugus*) [134] and common pipistrelles (*Pipistrellus pipistrellus*) [130]. That being said, common pipistrelles, as do many bat species, emit low-frequency distress calls directed at conspecifics [135]. These potentially serve as a warning system, as observed in many mammal and bird species [136], so anti-predation strategies probably do contribute to their sociality. Interestingly, there is evidence that anti-predator evasion may serve to disrupt group formation in Neotropical bats [137].

Social groups of bats are not only reliant on such density dependent benefits. In many bat societies, bonds between individuals can persist over several years – an ability dependent largely on their extended lifespans. The evolution of flight, torpor, and relatively low predation rates has afforded bats life histories akin to mammals several times their size. Individuals of some species can exceed 30 year life spans [138]. One notable individual *M. brandtii* lived to at least 41 years old [139]. Longevity, combined with philopatry, leads to the sustained aggregation of individuals, and stable group structures [109,140]– a necessary pre-requisite for co-operative strategies to persist [141,142]. Herein a positive feedback loop is created, where the social environment, enabled by stable groups, facilitates the evolution of prosocial behaviours that, in turn, reinforce the benefits of group-living [56,143].

The significance of kinship for group-living in bats has attracted considerable research in recent years [144,145]. As discussed in section 1.2, individuals can achieve indirect fitness benefits by co-operating with, thus promoting the fitness of, close genetic relatives ('kin') [17] . In bats, kinship appears to be an important predictor of group-living in some species, but

not universally [145]. For example, female greater spear-nosed bats (*Phyllostomus hastatus*) exist in highly stable, but un-related social units [145,146]. On the other hand, both male and female Spix's disk-winged bats (*Thyroptera tricolor*) associate among relatives. A key determinant of these patterns appears to be which sex (if any) disperses. In *P. hastatus*, offspring are not recruited to their natal colony, precluding any opportunity for kin to associate. In contrast, *T. tricolor* offspring remain faithful to their natal group.

Even where kinship predicts associations, the same cannot always be said for interactions among individuals. The text-book case is the common vampire bat (*D. rotundus*). Females form stable communities, more likely among relatives. Two of the three species of sanguivorous (blood-feeding) vampire bats, *D. rotundus* and *Diphylla ecaudata,* are known to share blood meals with conspecifics [147,148]. The majority of donations are from mother to offspring, but a large proportion are shared among adults. While some nepotistic sharing occurs, favoured by kin selection, kinship itself is not the only predictor of donation rates. Instead, receiving a blood meal is contingent on the recipient having previously groomed or shared a meal with the donator [144,149]– an example of reciprocal co-operation, similar to that observed in primates (see section 1.2). An individual, by increasing its roster of donations beyond close relatives, can expand the rate of donations it receives [150].

Beyond food sharing, complex social behaviours can be observed across the Chiroptera. Kinship, again, appears to not be a necessary pre-requisite. For example, while allogrooming (grooming conspecifics) in female Bechstein's bats *M. bechsteinii* was correlated with kinship [151], non-relatives were frequently recruited to vacant roosts, potentially to exchange mutual benefits of social thermoregulation during the parturition season. Additionally, evening bats (*Nycticeius humeralis*) were recorded following roost mates (likely unrelated) that had had prior foraging success to new food patches [152]. Reciprocal relationships within pairs were formed, where leaders become followers, and *vice versa*, on subsequent foraging trips.

Females of some bat species have been reported nursing unrelated pups. While this may constitute parasitism on the part of the non-offspring, there are potential adaptive benefits for the individual female if the risk of mastitis is reduced and her decreased weight improves foraging success [153].

1.4.2 Network analysis in bats

Given high levels of gregariousness, social complexity, and ecological diversity as previously discussed, bats would appear to be natural candidates for social network studies. Webber & Wal (2019)[154] conducted a systematic literature review of animal social network studies published up until 2016. Mammals constituted the majority (55 %) of species studied. Over half of these were either primates (32 %) or even-toed ungulates (20 %). Overall, 13 % of species were bats. A taxonomic bias towards larger, more charismatic mammals is evident. Yet, the use and application of SNA in bats has seen growth in recent years (**Figure 1.1**), owing to technological innovations, and has allowed researchers to ask increasingly complex questions. I extended the literature database provided by the authors to deepen my understanding of the key areas SNA has been applied in bats.

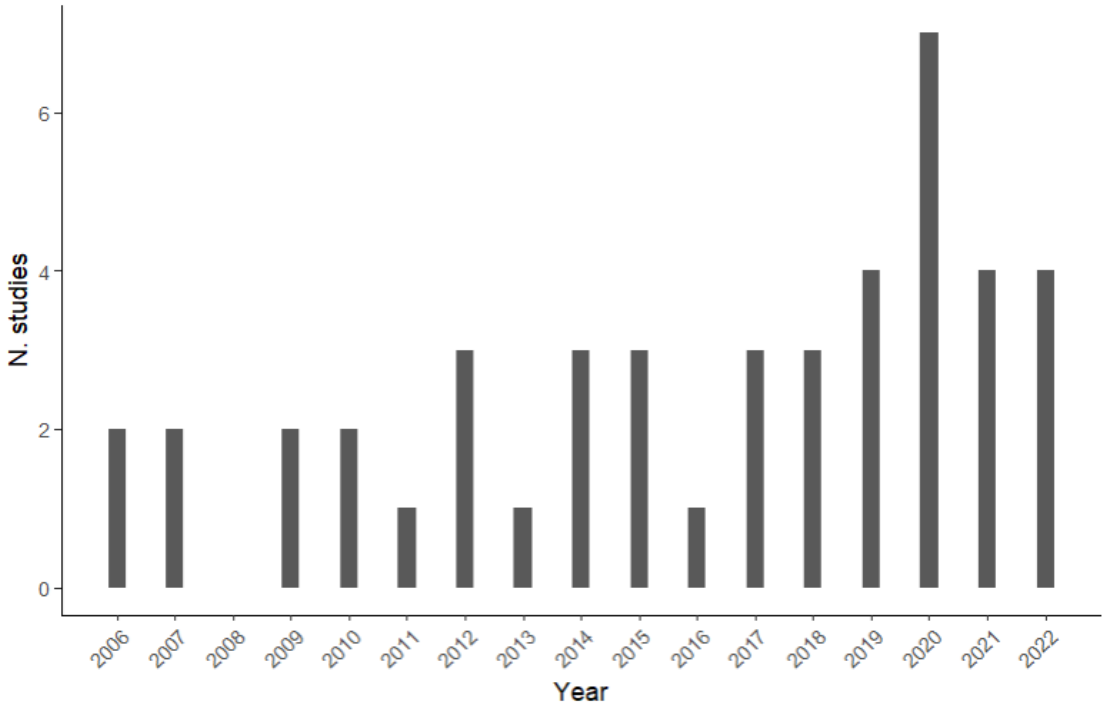

Figure 1.1 *The number of social network studies on bats has grown gradually over recent years.*

I collated a comprehensive database of 46 social network studies involving bats published as of February 2023 (**Table 1.1**). The vast majority of studies (77 %) constituted basic research, testing specific hypotheses related to the formation of social groups and the non-random structure of associations. For example, a long-term study of Bechstein's bats (*M. bechsteinii*) revealed that social contacts could be remarkably resilient, with individuals maintaining social bonds over several years – despite the regular fission-fusion of groups and

demographic fluctuations. Long-term social bonds may provide opportunity for individuals to reciprocate co-operative behaviours, such as allogrooming [155,156].

A promising area of applied social network analysis in bats focuses on patterns of disease transmission (11 % studies). Bats play a critical role in the ecology of viral pathogens [157] – owing to their high species diversity [158], and unique anti-viral immunity [159]. Recent studies have revealed social network structure, and an individual's position within it, can have tractable consequences for the spread of disease [160]. Zeus et al., (2020)[161] constructed networks based on shared viral sequences, roost association and contact frequency in Natterer's bats (*M. nattereri*). Frequent contacts were associated with sharing of the same viral sequence, eluding to direct transmission. Indirect transmission through contamination of the shared roost also appeared to play an important role. Bechstein's bats (*M. bechsteinii*) appear to use regular roost switching and avoidance of recently contaminated sites as a pathogen avoidance mechanism – though this may in fact aid in further pathogen transmission across roosts [162].

Experiments in *D. rotundus* revealed how individuals can shape their social environment upon infection, and how the transmission of pathogens can be context dependent. Bats injected with an immune-challenging substance isolated themselves from the group – reducing their network centrality (see Box 1). So-called "sick" bats produced fewer prosocial contact calls [163], groomed neighbours less frequently [164], and physically distanced themselves from most individuals [165]. This response presumably slows the transmission of pathogens to neighbours, though the advantage to the already infected individual is less certain. One possibility is that this potentially altruistic behaviour could be re-paid upon novel infection to the group at a later date. Kin selection may provide an alternate explanation. However, grooming rates reduced markedly among non-kin, but remained unaffected between mother and offspring, suggesting kin selection may instead favour social interactions over social isolation [166].

There is unlikely to be a single explanation, rather multiple contributing factors that interact, and ultimately favour the selection of group-living in the majority of bat species. The application of SNA to bat research has allowed increasingly complex hypotheses related to the causes and consequences of bat sociality to be tested, with promising results. However, there is still much progress to be made if the diversity of bats is to be reflected in

the number of publications. One overlooked family of bats is the Rhinolophidae – the horseshoe bats – a species rich group with a global distribution. They will be the subject of the next section where I outline their ecology, and highlight the need for further study into their sociality and life history.

1.5 Study species: the British horseshoe bats

Named after their striking and ornate nose leaves, the horseshoe bats (Rhinolophidae), are a family of 114 species [104] all within the genus *Rhinolophus*. The horseshoe bats exist across an expansive range from the Palearctic to the Australasian regions – in tropical and temperate forests, deserts, and mountainous habitats. Five species reside within Europe, and two – *R. ferrumequinum* (the greater horseshoe; **Figure 1.5**) and *R. hipposideros* (the lesser horseshoe; **Figure 1.6**) can be found in the British Isles [167]. *R. ferrumequinum* is considerably larger than its lesser counterpart. Within Great Britain, the distributions of both species are largely confined to southwest England and mid-Wales (**Figure 1.2**), though historical records exist as far east as London and Kent [168].

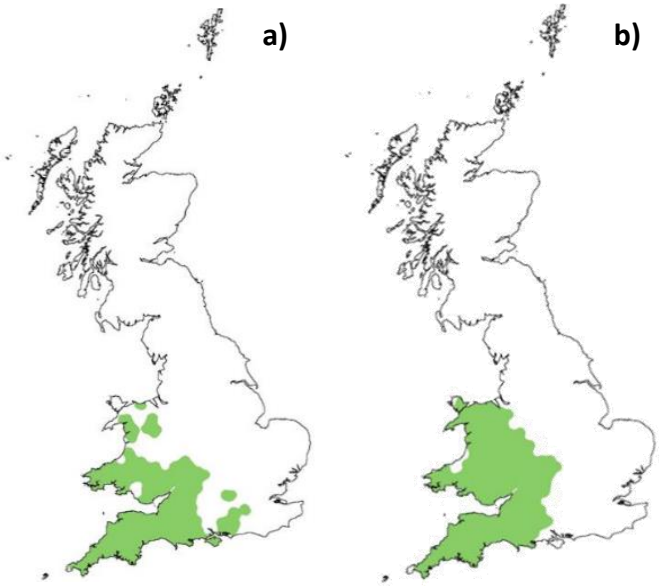

Figure 1.2 *Distribution of* ***a)*** R. ferrumequinum *and* ***b)*** R. hipposideros *in the British Isles (excluding the Isle of Man) based on occurrence data 1995-2016* [169].

The horseshoe bats possess a highly sophisticated echolocation system, emitting ultrasonic calls through their noses, allowing interception of prey on the wing or gleaning off vegetation. All species are insectivorous [167]. The greater horseshoe bat's relatively larger size is reflected in its diet, where larger prey items, such as dung beetles (*Aphodius* sp. and

Geotrupes sp.), cockchafers (*Melolontha melolontha*), and large Lepidoptera (e.g. Noctuidae) are generally favoured [170–174] over the small Diptera (e.g. Tipulidae) and Trichoptera seen largely in the diet of *R. hipposideros* [175,176].

Caves and dis-used mines, fragments of deciduous woodland, unmanaged hedgerows, and cattle pastures in lowland landscapes are characteristic of these species' distribution in Britain – permitting connectivity, foraging and hibernating opportunities [177]. Both species, as all bats, in Britain are thought to have undergone a dramatic decline over the 20th century, owing to intensifying agricultural practices and loss of roosts [178]. Mediated by strong legislative protection, and possibly climate change [179], a modest recovery has been seen over the past 25 years, from an estimated 5,000 and 14,000 [180] to 13,000 and 50,400 [169] in *R. ferrumequinum* and *R*. *hipposideros* respectively. *R. ferrumequinum* is the second rarest species of bat in Britain (behind the grey long-eared bat *Plecotus austriacus*) for which reliable population estimates can be made [169]. Great Britain now represents a stronghold for these species within Europe, despite being at the northernmost edge of their range, providing a potential climate refugium amidst ongoing environmental change [181]. Unsustainable development, through light pollution and habitat degradation (roost disturbance and habitat loss), alongside extreme weather events, could threaten further recovery [177,182].

Both species provide great utility as model subjects in the study of social behaviour and life history in bats. Their tendency to roost in large cavities i.e. loft spaces, and avoidance of tight crevices (unlike most vespertilionids), alongside high natal philopatry in females, enables high re-capture rates. These are critical to be able to understand patterns of dispersal between social groups, and to accurately define key events in an individual's life history, namely reproduction and mortality [183].

1.5.1 The horseshoe bat year

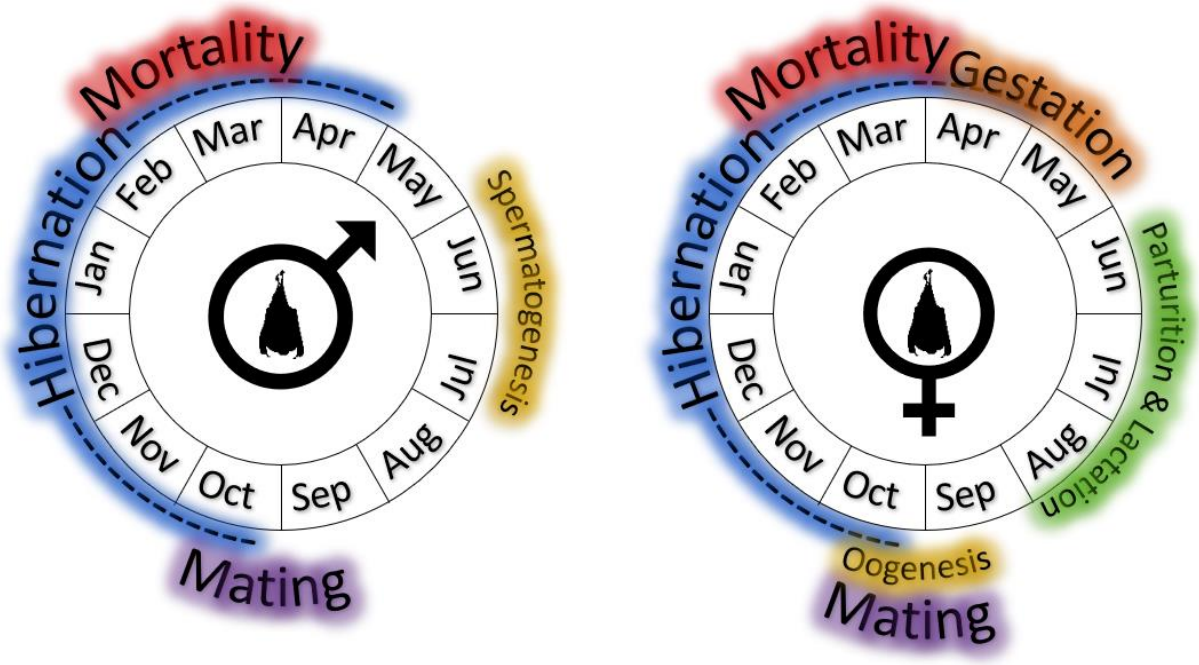

Figure 1.3 *The British horseshoe bat year. The annual life cycle of horseshoe bats within Great Britain is alike that of most temperate bats, where patterns of reproduction and dispersal following seasonal changes in resource availability. The coloured phases shown represent the approximate time period within which life history events are most likely to occur.*

As with most temperate species of mammal, the lives of both species of British horseshoe bat follow the seasons (**Figure 1.3**), and show fission and fusion of social groups likely in response to changes in the distribution of resources [109,184–186]. Within these seasons, particularly during winter, relatively little is known of the structure of these groups and the drivers leading to their formation.

Females gather in maternity colonies to give birth and wean young from late spring through to late summer [187]. This maternal investment imposes significant physiological stress [188]. Yet, few studies in bats have explored its role in the development of offspring phenotype, particularly if there are lasting effects on offspring fitness. Concurrently, spermatogenesis occurs in males [189–191], with territories beginning to be established, approaching the mating season [170]. In the Autumn, the females leave to join the males in mating sites, before the core hibernation period in winter [170]. Consequently, the dispersal of males is thought to be key to maintaining genetic connectivity between populations [192–194]. After mating, a vaginal plug is formed which may serve to prevent further insemination from other males [195]. However, females may have the ability to eject these plugs to allow for additional mating

[117]. Sperm are stored in the uterus until fertilisation in early spring [196]. Weather conditions over the hibernation period are a key factor limiting population growth [197]. Poor weather over the hibernation period, particularly in early spring, can restrict feeding opportunities, imposing physiological stress [198], potentially leading to starvation [197,199]. Consequently, milder winters and springs due to climate warming may have aided the recent modest increase in horseshoe bat populations [179] .

1.6 Study system: Woodchester Mansion

Long-term studies, where individual life histories can be tracked and kinship is known, are a rich source of data for researchers seeking to understand the structure of animal societies and its consequences for lifetime reproductive success and survival [200]. In this regard, bats - long-lived, species-rich, and highly social, are an undervalued study system [201]. Long-term studies exist now across a range of bat species [201], including the Bechstein's bats (*M. bechsteinii*) in southern Germany, the greater sac-winged bats (*Saccopteryx bilineata*) in Costa Rica [202], and the greater mouse-eared bats (*M. myotis*) in Brittany, France [203]. The study of greater horseshoe bats (*R. ferrumequinum*) at Woodchester Mansion, southwest England is the longest running of these with continuous data collection commencing in the late 1950s.

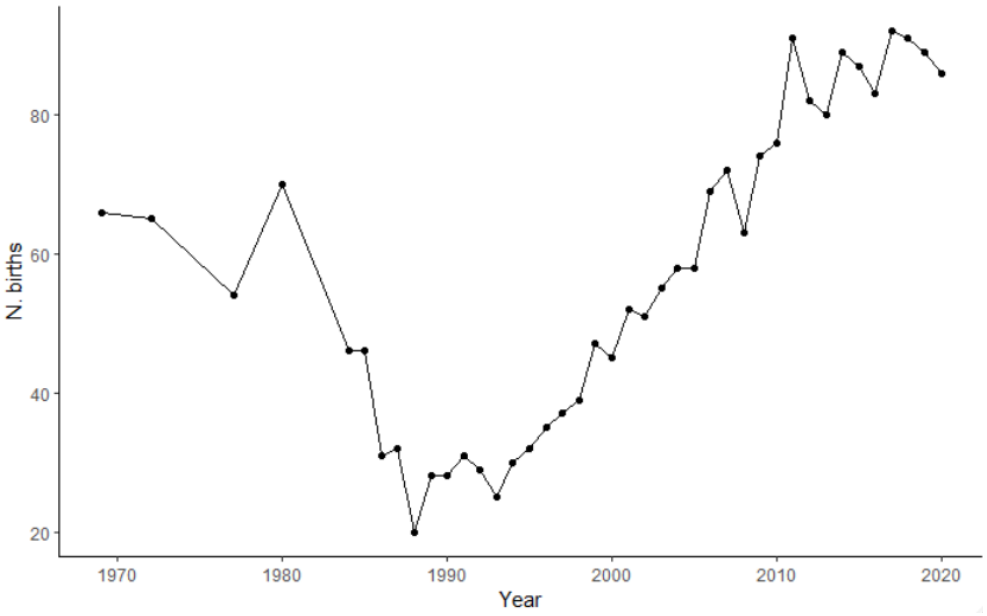

Figure 1.4 *Number of births at the Woodchester Mansion colony 1969-2020. As females typically produce one pup a year, births provide an index of effective population size. Following a sharp decline in the mid-1980s, the population has since stabilised at around 100 breeding females. Data provided by Roger Ransome.*

Travel to the Gloucestershire Cotswolds, amongst its woodland valleys, honey-coloured cottages, and rolling hills, you will find Woodchester Mansion. An uninhabited, neo-gothic house – its primary residents are two large colonies of lesser (*R. hipposideros*) and greater horseshoe bats (*R. ferrumequinum*).

Dr Roger Ransome has been studying the population of greater horseshoe bats continuously since 1956. Since 1993, wing biopsies have been taken of all young born plus the majority of adult individuals captured within local hibernacula. DNA are extracted and genotyped at microsatellite loci to determine offspring parentage. At each capture, morphometric measurements (the length of the forearm, fifth finger digit, and body mass) are recorded, as well as reproductive status. Consequently, the project has generated a wealth of data, constituting the life histories of over 2000 individuals. Using these data, studies have identified the role of weather in driving population dynamics [197,204], determinants of reproductive success [205,206], the sharing of foraging areas [207], unusual mating patterns [208], and life history variation in relation to telomere length [188].

Today, the core colony consists of around 100 breeding females (**Figure 1.4**). They assemble in the summer, where mothers give birth and raise their young together. By the Autumn, the colony has dissolved. Individuals disperse and seek refuge in underground hibernacula in the surrounding landscape – to enter torpor and to mate.

My thesis utilises this unique system to explore key questions on social and genetic drivers of behaviour and life history in greater detail.

1.7 Thesis Outline

In this thesis, I investigated how behaviour and life history phenotype are influenced by genotype and the social environment in horseshoe bats. Each chapter of this thesis represents a specific question or hypothesis (or series of) that I examined, integrating a range of methodological approaches.

Does genetic relatedness drive spatial assortment in hibernating bats? In **Chapter 2**, I applied a social network approach to elucidate the socio-genetic structure of the population of *R. ferrumequinum* hibernating within 25 km of Woodchester Mansion. Kinship is known to underpin spatial assortment in several mammal species, including some bats. Using field survey data collected over 12 winters, I assessed the influence of kinship in relation to roost sharing and spatio-temporal associations in loose clusters.

Why do hibernating rhinolophid bats form groups in winter? Rhinolophid bats commonly form loose clusters during the hibernation period. In the absence of social thermoregulation, explanations for this behaviour are unclear. There are multiple potential (non-mutually exclusive) hypotheses, including the dilution of predation risk and roost limitation. In **Chapter 3**, *R. hipposideros* were observed in a loose cluster in the hibernaculum at Woodchester Mansion. I tested the "social alarm clock" hypothesis that proposes individuals collectively arouse to forage by either actively or passively disturbing their roost mates. A thermal imaging camera was used to capture arousals and social interactions.

In **Chapter 4**, I used the long-established pedigree of *R. ferrumequinum* at Woodchester Mansion, and applied evolutionary quantitative genetic techniques to isolate genetic and environmental components of phenotypic variation in morphological and life history traits, including reproductive success. Does a mother's behaviour influence the phenotype of her offspring? I aimed to understand the effects of maternal investment (genetic and environmental) on offspring phenotype. Primarily, this chapter aimed to estimate this population's adaptive potential amid ongoing environmental change.

Finally, in **Chapter 5**, I synthesise my findings and suggest future avenues of investigation.

Table 1.1 *All known studies of bats involving social network analysis. Rows given to each study are according to each species investigated.*

Author	Year	Title	Source	doi	Family	Species
Rhodes et al.,	2006	Applying network analysis to the conservation of habitat trees in urban environments: a case study from Brisbane, Australia	Conservation Biology	10.1111/j.1523-1739.2006.00415.x	Molossidae	*Tadarida australis*
Campbell et al.,	2006	Resource distribution and social structure in harem-forming Old World fruit bats: variations on a polygynous theme	Animal Behaviour	10.1016/j.anbehav.2006.03.002	Pteropodidae	*Cynopterus brachyotis*
Garroway & Broders	2007	Non-random association patterns at northern long-eared bat maternity roosts	Canadian Journal of Zoology	10.1139/Z07-079	Vespertilionidae	*Myotis septentrionalis*
Rhodes	2007	Roost fidelity and fission–fusion dynamics of white-striped free-tailed bats (*Tadarida australis*)	Journal of Mammalogy	10.1644/06-MAMM-A-374R1.1	Molossidae	*Tadarida australis*
Dechmann et al.,	2009	Experimental evidence for group hunting via eavesdropping in echolocating bats	Proceedings of the Royal Society B: Biological Sciences	10.1098/rspb.2009.0473	Noctilionidae	*Noctilio albiventris*
Fortuna et al.,	2009	The roosting spatial network of a bird-predator bat	Ecology	10.1890/08-0174.1	Vespertilionidae	*Nyctalus lasiopterus*
Chaverri	2010	Comparative social network analysis in a leaf-roosting bat	Behavioral Ecology and Sociobiology	10.1007/s00265-010-0975-3	Thyropteridae	*Thyroptera tricolor*
Patriquin et al.,	2010	Do social networks of female northern long-eared bats vary with reproductive period and age?	Behavioral Ecology and Sociobiology	10.1007/s00265-010-0905-4	Vespertilionidae	*Myotis septentrionalis*
Kerth et al.,	2011	Bats are able to maintain long-term social relationships despite the high fission–fusion dynamics of their groups	Proceedings of the Royal Society B: Biological Sciences	10.1098/rspb.2010.2718	Vespertilionidae	*Myotis bechsteinii*
Johnson et al.,	2012	Roost networks of northern myotis (*Myotis septentrionalis*) in a managed landscape	Forest Ecology and Management	10.1016/j.foreco.2011.11.032	Vespertilionidae	*Myotis septentrionalis*
Johnson et al.,	2012	Social networks of Rafinesque's big-eared bats (*Corynorhinus rafinesquii)* in bottomland hardwood forests	Journal of Mammalogy	10.1644/12-MAMM-A-097.1	Vespertilionidae	*Corynorhinus rafinesquii*
Ancilloto et al.,	2012	Spatial proximity between new-borns influences the development of social relationships in bats	Ethology	10.1111/j.1439-0310.2011.02016.x	Vespertilionidae	*Pipistrellus kuhlii*
Baigger et al.,	2013	Bechstein's bats maintain individual social links despite a complete reorganisation of their colony structure	Naturwissenschaften	10.1007/s00114-013-1090-x	Vespertilionidae	*Myotis bechsteinii*

Silvis et al.,	2014	Association, roost use and simulated disruption of *Myotis septentrionalis* maternity colonies.	Behavioural processes	10.1016/j.beproc.2014.01.016	Vespertilionidae	*Myotis septentrionalis*
Silvis et al.,	2014	Roosting and foraging social structure of the endangered Indiana bat (*Myotis sodalis*)	PLoS ONE	10.1371/journal.pone.0096937	Vespertilionidae	*Myotis sodalis*
August et al.,	2014	Sympatric woodland myotis bats form tight-knit social groups with exclusive roost home ranges	PLoS ONE	10.1371/journal.pone.0112225	Vespertilionidae	*Myotis daubentonii*
August et al.,	2014	Sympatric woodland myotis bats form tight-knit social groups with exclusive roost home ranges	PLoS ONE	10.1371/journal.pone.0112225	Vespertilionidae	*Myotis nattereri*
Silvis et al.,	2015	Effects of hierarchical roost removal on northern long-eared bat (*Myotis septentrionalis*) maternity colonies	PLoS ONE	10.1371/journal.pone.0116356	Vespertilionidae	*Myotis septentrionalis*
Godinho et al.,	2015	Network analysis reveals cryptic seasonal patterns of association in Gould's wattled bats (*Chalinolobus gouldii*) roosting in bat-boxes	Behaviour	10.1163/1568539X-00003315	Vespertilionidae	*Chalinolobus gouldii*
Ancilloto et al.,	2015	Sociality across species: spatial proximity of new-born bats promotes heterospecific social bonding	Behavioural Ecology	10.1093/beheco/aru193	Vespertilionidae	*Hypsugo savii*
Ancilloto et al.,	2015	Sociality across species: spatial proximity of new-born bats promotes heterospecific social bonding	Behavioural Ecology	10.1093/beheco/aru193	Vespertilionidae	*Pipistrellus kuhlii*
Webber et al.,	2016	Social network characteristics and predicted pathogen transmission in summer colonies of female big brown bats (*Eptesicus fuscus*)	Behavioral Ecology and Sociobiology	10.1007/s00265-016-2093-3	Vespertilionidae	*Eptesicus fuscus*
Rathinakumar	2017	Social grooming among Indian short-nosed fruit bats	Behaviour	10.1163/1568539X-00003410	Pteropodidae	*Cynopterus sphinx*
Monks & Donnell	2017	Social implications of a colony collapse in a highly structured vertebrate species (long-tailed bat, *Chalinolobus tuberculatus*)	Animal Conservation	10.1111/acv.12324	Vespertilionidae	*Chalinolobus tuberculatus*
Nad'o et al.,	2017	Structural, temporal and genetic properties of social groups in the short-lived migratory bat *Nyctalus leisleri*	Behaviour	10.1163/1568539X-00003444	Vespertilionidae	*Nyctalus leisleri*
Zeus et al.,	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	10.1007/s00265-018-2516-4	Vespertilionidae	*Myotis nattereri*
Harten et al.,	2018	Persistent producer-scrounger relationships in bats	Science Advances	10.1126/sciadv.1603293	Pteropodidae	*Rousettus aegyptiacus*
Garg et al.,	2018	Social structure of the harem-forming promiscuous fruit bat, *Cynopterus sphinx*, is the harem truly important?	Royal Society Open Science	10.1098/rsos.172024	Pteropodidae	*Cynopterus sphinx*

Stockmaier et al.,	2018	An immune challenge reduces social grooming in vampire bats	Animal Behaviour	10.1016/j.anbehav.2018.04.021	Phyllostomidae	*Desmodus rotundus*
Harten et al.,	2019	Food for sex in bats revealed as producer males reproduce with scrounging females	Current Biology	10.1016/j.cub.2019.04.066	Pteropodidae	*Rousettus aegyptiacus*
Wilkinson et al.,	2019	Kinship, association, and social complexity in bats	Behavioral Ecology and Sociobiology	10.1007/s00265-018-2608-1	Phyllostomidae	*Artibeus jamaicensis*
Wilkinson et al.,	2019	Kinship, association, and social complexity in bats	Behavioral Ecology and Sociobiology	10.1007/s00265-018-2608-1	Phyllostomidae	*Desmodus rotundus*
Wilkinson et al.,	2019	Kinship, association, and social complexity in bats	Behavioral Ecology and Sociobiology	10.1007/s00265-018-2608-1	Vespertilionidae	*Myotis bechsteinii*
Wilkinson et al.,	2019	Kinship, association, and social complexity in bats	Behavioral Ecology and Sociobiology	10.1007/s00265-018-2608-1	Vespertilionidae	*Myotis septentrionalis*
Wilkinson et al.,	2019	Kinship, association, and social complexity in bats	Behavioral Ecology and Sociobiology	10.1007/s00265-018-2608-1	Vespertilionidae	*Nycticeius humeralis*
Wilkinson et al.,	2019	Kinship, association, and social complexity in bats	Behavioral Ecology and Sociobiology	10.1007/s00265-018-2608-1	Phyllostomidae	*Phyllostomus hastatus*
Wilkinson et al.,	2019	Kinship, association, and social complexity in bats	Behavioral Ecology and Sociobiology	10.1007/s00265-018-2608-1	Emballonuridae	*Rhynchonycteris naso*
Wilkinson et al.,	2019	Kinship, association, and social complexity in bats	Behavioral Ecology and Sociobiology	10.1007/s00265-018-2608-1	Emballonuridae	*Saccopteryx bilineata*
Wilkinson et al.,	2019	Kinship, association, and social complexity in bats	Behavioral Ecology and Sociobiology	10.1007/s00265-018-2608-1	Thyropteridae	*Thyroptera tricolor*
Ripperger et al.,	2019	Proximity sensors on common noctule bats reveal evidence that mothers guide juveniles to roosts but not food	Biology Letters	10.1098/rsbl.2018.0884	Vespertilionidae	*Nyctalus noctula*
Ripperger et al.,	2019	Vampire bats that cooperate in the lab maintain their social networks in the wild	Current Biology	10.1016/j.cub.2019.10.024	Phyllostomidae	*Desmodus rotundus*
Zeus et al.,	2020	Analysis of astrovirus transmission pathways in a free-ranging fission-fusion colony of natterer's bats (*Myotis nattereri*)	Behavioral Ecology and Sociobiology	10.1007/s00265-020-02932-y	Vespertilionidae	*Myotis nattereri*
Bachorec et al.,	2020	Egyptian fruit bats do not preferentially roost with their relatives	Journal of Zoology	10.1111/jzo.12816	Pteropodidae	*Rousettus aegyptiacus*

Stockmaier et al.,	2020	Sickness effects on social interactions depend on the type of behaviour and relationship	Journal of Animal Ecology	10.1111/1365-2656.13193	Phyllostomidae	*Desmodus rotundus*
Welch et al.,	2020	Social experience of captive Livingstone's fruit bats (*Pteropus livingstonii*)	Animals	10.3390/ani10081321	Pteropodidae	*Pteropus livingstonii*
Flores et al.,	2020	Social structure and relatedness in the fringe-lipped bat (*Trachops cirrhosus*)	Royal Society Open Science	10.1098/rsos.192256	Phyllostomidae	*Trachops cirrhosus*
Bachorec et al.,	2020	Spatial networks differ when food supply changes: foraging strategy of Egyptian fruit bats	Spatial networks differ when food supply changes: Foraging strategy of Egyptian fruit bats	10.1371/journal.pone.0229110	Pteropodidae	*Rousettus aegyptiacus*
Ripperger et al.,	2020	Tracking sickness effects on social encounters via continuous proximity sensing in wild vampire bats	Behavioral Ecology	10.1093/beheco/araa111	Phyllostomidae	*Desmodus rotundus*
Teague O'Mara & Dechmann	2021	Greater spear nosed bats in Panamá do not use social proximity to improve foraging efficiency	bioRxiv	10.1101/2021.09.30.462631	Phyllostomidae	*Phyllostomus hastatus*
Yarlagadda et al.,	2021	Social convergence of gut microbiomes in vampire bats	Biology Letters	10.1098/rsbl.2021.0389	Phyllostomidae	*Desmodus rotundus*
Ripperger & Carter	2021	Social foraging in vampire bats is predicted by long-term cooperative relationships	PLoS Biology	10.1371/journal.pbio.3001366	Phyllostomidae	*Desmodus rotundus*
Waag et al.,	2021	Social networks based on frequency of roost cohabitation do not reflect association rates of *Myotis lucifugus* within their roosts	Ecology and Evolution	10.1002/ece3.7244	Vespertilionidae	*Myotis lucifugus*
Rensel et al.,	2022	Maternity colony social structure of myotis in British Columbia, Canada	Maternity colony social structure of myotis in British Columbia, Canada	10.1007/s00265-022-03265-8	Vespertilionidae	*Myotis lucifugus*
Sunga et al.,	2022	Roost fidelity partially explains maternity roosting association patterns in *Myotis lucifugus*	Animal Behaviour	10.1016/j.anbehav.2022.09.008	Vespertilionidae	*Myotis lucifugus*
Finch et al.,	2022	Social networks of the greater horseshoe bat during the hibernation season: a landscape-scale case study	Animal Behaviour	10.1016/j.anbehav.2022.03.019	Rhinolophidae	*Rhinolophus ferrumequinum*
Edwards et al.,	2022	Social roles influence cortisol levels in captive Livingstone's fruit bats (*Pteropus livingstonii*)	Hormones and Behavior	10.1016/j.yhbeh.2022.105228	Pteropodidae	*Pteropus livingstonii*

Figure 1.5 *A greater horseshoe bat* R. ferrumequinum *ringed at Woodchester Mansion. Photo credit: G Jones*

Figure 1.6 *A lesser horseshoe bat* R. hipposideros *photographed at Woodchester Mansion. Photo credit: G Jones*

Figure 1.7 *A lone lesser horseshoe bat* R. hipposideros *in torpor. Photo credit: L Romaine*

Figure 1.8 *A small cluster of lesser horseshoe bats* R. hipposideros*. Photo credit: L Romaine*

Figure 1.9 *A ringed greater horseshoe bat* R. ferrumequinum *in torpor. Photo credit: L Romaine*

Chapter 2

Sociogenetic structure of a greater horseshoe bat (*Rhinolophus ferrumequinum*) population during the hibernation period

An active juvenile greater horseshoe bat (*Rhinolophus ferrumequinum*) in a hibernaculum near Nailsworth, Gloucestershire. Photo credit: L Romaine

2.1 Abstract

Some mammal species inhabiting temperate zones hibernate when conditions deteriorate in the winter. In bats, hibernation typically involves the selection of hibernacula with diverse thermal properties, often leading to spatio-temporal overlap of associations. The sociogenetic structure of such associations may have important consequences for individuals, such as the potential for inbreeding, but has remained largely unexplored in temperate systems. A long-studied population of greater horseshoe bats (*Rhinolophus ferrumequinum*) was recorded at hibernation sites around Nailsworth, Gloucestershire for 12 winters. Combining field data on hibernaculum-use with a multigenerational pedigree, I ask whether kinship influences the sharing of space in underground hibernacula. Assessing bi-parental and maternal relatedness, I investigate social structure and assortment at the roost level and among loose clusters of individuals within sites. Kinship was not an important predictor of assortment among bats, regardless of spatial scale. Intriguingly, bats born in the same cohort were more likely to share a roost than expected by chance – indicating a degree of age structure within the population. These results give insight into the factors structuring populations of temperate bats during the hibernation period, with important implications for their conservation.

2.2 Introduction

The predictable arrival of winter's harsh and frigid weather conditions, bringing a rapid depletion of resources, has shaped the evolution of species living in temperate ecosystems [209,210]. Rather than avoidance (migration to warmer climes), many mammals adopt a strategy of hibernation – bouts of extended torpor where body temperature reaches near-ambient levels and metabolic functions are dramatically reduced [211,212]. For cavernicolous bats, space use forms a critical component of the hibernation strategy [213]. Depending on physiological condition, individuals can select specific microclimates in thermally diverse, underground hibernacula (hibernation sites) that either promote energy conservation in torpor or arousal to normothermia [214–218], providing the opportunity to perform beneficial behaviours such as mating and foraging [213].

Variation in space use generates fine-scale population structure, influencing the exposure of individuals to selection over environmental gradients [219,220]. An often-overlooked source of selection is the social environment – the arrangement of conspecifics around an individual [74,92,93,221–223]. In many animal social systems, social assortment is based on genetic relatedness [27]. This can potentially expose populations to risk of inbreeding, and to competition for limited resources [224–227]. However, there can also be tangible benefits for an individual's fitness. Hamilton's (1964)[228] kin selection theory predicts directing affiliative behaviour towards close genetic relatives can improve the chances of propagating shared genes. Whether by actively promoting social cohesion, or occurring passively through limited dispersal, kin-biased associations lead to population sub-structuring [229–231], shaping the selection environment an individual experiences. However, little is known of whether kinship shapes space use during the hibernation period in cavernicolous bats.

Temperate cavernicolous bats provide an interesting system through which to explore spatial associations during the hibernation period. Extensive intraspecific variation exists regarding roost choice [122,232]. For example, individuals with low energy reserves may choose sites with higher average temperatures as to trigger arousals during warm weather [233]. Furthermore, hibernation appears to have played a key role in the evolution of extended lifespans in these species, where prolonged torpor bouts facilitate somatic maintenance [234,235], and delay telomere shortening [188]. The sociogenetic environment during hibernation

provides context for the expression of this unusual life history. Yet studies of kinship in bat societies outside of the pup-rearing season are still rare [207,236–243], giving only a snapshot of the full breadth of social structures possible in seasonal environments.

The population of greater horseshoe bats *Rhinolophus ferrumequinum* living in and around Nailsworth, Gloucestershire has been studied continuously for over 60 years, providing a unique opportunity for studying the sociogenetic structure of a wild temperate bat population during the hibernation period. Social organisation in the summer is typical of temperate bats [140,244] – with males dispersing, while females are philopatric to their natal roost, forming maternity colonies [170]. Genetic differentiation between such colonies is typically weak within regions due to male-biased dispersal [192,194,245]. While average relatedness within the maternity colony approximates zero, there is substantial genetic sub-structure among matrilines [207], similar to other temperate bat societies [238,243]. As autumn approaches, the maternity colony dissolves. Most bats hibernate in local hibernacula within 25 km of the maternity roost [170]. The patchy distribution of suitable sites leads to the spatio-temporal aggregation of individuals – the social unit of focus in this study. While many individuals may share a roost, seldom are they in bodily contact. Within this population, *R. ferrumequinum* typically roosts alone, sparsely separated from conspecifics, though there are occasional instances where loosely arranged clusters are found [199,246].

Maternal kin structure might be expected in the associations of *R. ferrumequinum* co-habiting roosts in winter. Maternal relatedness is a common predictor of association patterns in species where females are philopatric [145,247–252]. However, given the low mean relatedness within the maternity colony adjacent to hibernacula [207], active assortment of kin is required for genetic structure to emerge [253]. Thus, one potential mechanism for kin structure is maternal site inheritance. Previous work in this colony detected significant range overlap among matrilineal relatives during foraging [207]. Individuals travel independently; thus, offspring may vertically inherit foraging sites through maternal relatives. Indeed, information sharing might constitute maternal investment, as juveniles orphaned post-weaning may experience higher mortality rates [254]. A juvenile learning the location of suitable hibernaculum from its mother, or other maternal relatives is plausible. There is evidence from a several long-lived mammals, including bats, that individuals may share ecological knowledge to improve survival of conspecifics [64,237,255–257]. Nonetheless, the

role of kinship in the sharing of information on roost location and suitability is not always straightforward, with some species exhibiting maternal guidance [258,259] and others relying on experienced, non-related individuals [152,162,257,260].

A second potential mechanism that may lead to associations among kin is shared mate preference within matrilines. Rossiter et al., (2005)[208] observed that related females mated with the same male over consecutive years. Mating occurs throughout the winter, (as demonstrated by the rise in vaginal plugs [261,262]; **Figure S 2.1**), when males probably hold territories and are visited by females [170,208]. Therefore, I expect most associations among breeding-age individuals to reflect mate choice – with related females roosting alongside a territorial male.

Here I report on the sociogenetic structure of a *R. ferrumequinum* population during the hibernation period. Specifically, this study investigates the influence of bi-parental and maternal kinship in shaping space-sharing among *R. ferrumequinum* in hibernacula. Pedigree-based estimates of kinship are combined with field data on the co-roosting of hibernacula surrounding Nailsworth, Gloucestershire. A network approach is applied to quantify spatio-temporal associations. Social network analysis is commonly employed in studies of kin structure in wild populations, given its ability to uncover cryptic social structure among fission-fusion societies (e.g.[156,263,264]). I address three questions: 1) is there non-random structure of associations and are they persistent over the long-term? 2) are roost aggregations composed primarily of close kin? 3) does kinship drive non-random associations? I examined spatio-temporal associations at two spatial scales: among bats sharing roosts, and those in loose clusters within roosts. Active assortment of maternal kin is hypothesised to explain associations, potentially due to mechanisms such as maternal site inheritance or mate sharing within matrilines. Since philopatric females have greater opportunities for long-term social interactions, including with relatives, in the maternity roost, it is predicted that the most stable bonds over winters will be formed by females.

2.3 Materials and Methods

2.3.1 Study sites, surveys, and handling

To locate co-roosting *R. ferrumequinum,* surveys were carried out each winter between 2007/08 and 2018/19, in all known hibernacula within ~25 km of the maternity roost at Woodchester Mansion, Gloucestershire, England (51°43'N, 2°18'W) (**Table S 2.1**). Each hibernaculum was visited three times annually, corresponding to 'early', 'mid', and 'late' hibernation stages - Late-October/November, January/February, and March/April respectively. Searches of hibernacula were conducted by torchlight by a minimum of two people. Located entirely within the Cotswolds Area of Outstanding Natural Beauty, all sites (n=23) are abandoned stone mines, except for the natural cave at site T (**Figure 2.1**). Linear distances between roosts range from ~5 metres (e.g. at sites R1, R2, and R3; **Table S 2.1**) to ~20 km. The surrounding landscape is characterised by small settlements, fragments of beech (*Fagus sylvatica*) woodland, and limestone grassland. Thorough searches of all roosts are infeasible within a single day. Instead, surveys were split over two days between large core and smaller outlier sites (**Figure 2.1**).

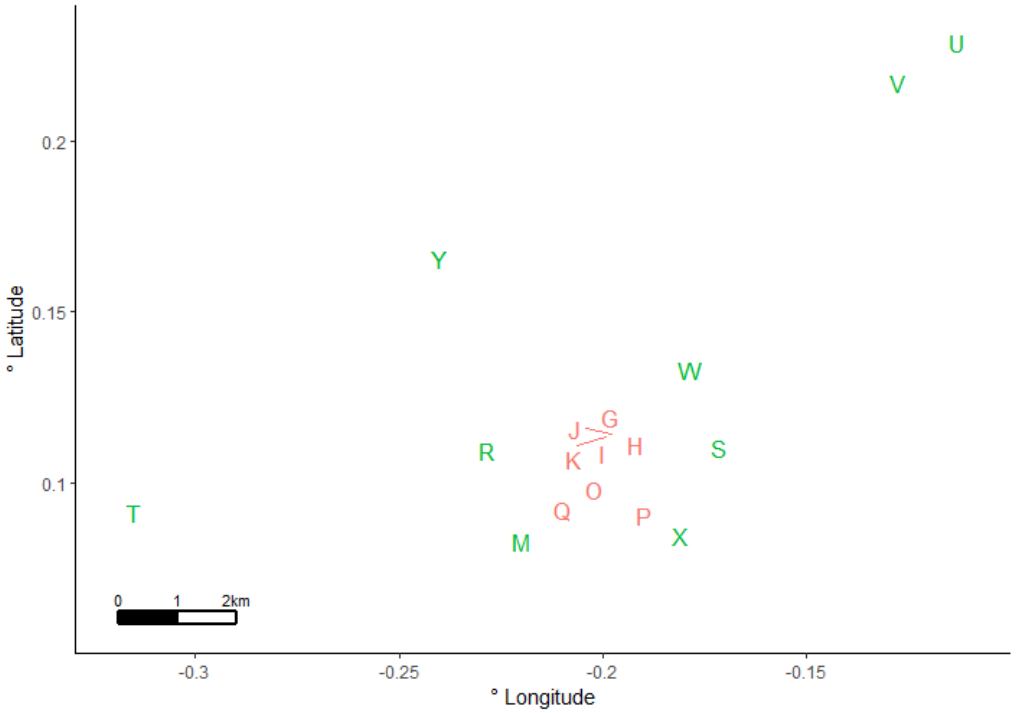

Figure 2.1 *Spatial relationships between hibernacula surrounding Nailsworth, Gloucestershire, England. A geographic map is not shown to protect locations of sites. Letters represent ID of hibernacula visited during the study period according to Ransome et al., (1968) (**Table S 2.1**). Red letters indicate core sites and green indicate outliers. Core sites contain the highest numbers of bats and are surveyed prior to the smaller, outlier sites. Some letters represent several small sites clustered in close proximity to each other (see **Table S 2.1**).*

All torpid *R. ferrumequinum* individuals identified were captured in hand nets, or by a gloved hand if reachable. Bats were placed in separate cloth bags before processing. Collection of morphometric and life-history data proceeded as detailed in Ward et al., (2014)[205] and Power et al., (*in press*). In brief, all individuals born at Woodchester Mansion are ringed within four days of birth, and so can be aged with high precision [197]. Age (before weaning) can also be calculated from growth curves [265]. Individuals from other colonies (hereon referred to as "immigrants") are ringed upon capture. Measurements are then taken of body mass (g), sex, radius and fifth digit length (mm). Juveniles can be identified according to their grey pelage and smoothly tapered epiphyseal-diaphyseal joints in the finger bones. Older bats (brown pelage and knobbly finger joints) are assigned a minimum age of two if un-ringed, relating to the minimum age of sexual maturity [170].

Each bat has a tissue sample taken from the uropatagium (tail-membrane) using a sterile 3 mm skin biopsy punch (Medisave UK, Weymouth, Dorset, UK). Using sterilised forceps, samples are placed in a labelled tube containing 90 % ethanol and stored in a -20 °C freezer until DNA extraction.

All handling was conducted under licence from Natural England, and biopsy sampling under licence from Natural England and the Home Office.

2.3.2 Quantifying kinship

I took a pedigree-based approach to quantifying kinship among individuals. I drew upon parentage data from a long-term genetic database, started in 1993 and completed in 2011. Here I report on the methods I used to assign parentage for individuals born from 2012 until 2018.

2.3.3 DNA extraction

For each tissue sample, DNA was extracted using the DNeasy Blood and Tissue Kit (QIAGEN), following manufacturer's instructions.

2.3.4 PCR optimisation

All individuals captured (in the maternity roost or in hibernacula) until 2011 were genotyped at up to 40 microsatellite loci, using methodology detailed in Ward et al., (2014)[205]. I revised this methodology for individuals captured from 2012. Between 2003 and 2011, individuals were genotyped at a reduced set of 33 loci, after seven were discarded (see [205] and [266]). Polymerase chain reaction (PCR) primers matching these loci were arranged across ten multiplex PCR panels (**Table S 2.2**), consisting of between three and four primer pairs each [266]. To increase throughput of genotyping for the remaining cohorts, minimising the use of reagents and sample DNA, the number of multiplex primer panels was halved to five, whilst maintaining, initially, the full set of 33 loci.

The value of multiplex PCR is the ability to amplify DNA at multiple loci within the same reaction [267–269]. Primers can be bonded with fluorescent tags (fluorochromes) that fluoresce when processed through capillary electrophoresis [270]. Using a combination of different fluorochromes allows for multiple loci to be amplified together, even if the ranges of possible allele sizes overlap [271]. Secondly, it is important to consider the optimal annealing temperatures of the PCR primers [271,272]. 'Annealing' is a key component of the PCR cycle, where the primers bond to the DNA template so that the DNA polymerase enzyme can amplify the relevant sequence [272]. To limit heterogenous amplification of loci, annealing temperatures of primers within the same panel should be similar [271]. The QIAGEN Type-It® Multiplex PCR Kit uses a synthetic factor to stabilise primers and increase their concentration local to the DNA template, thus mitigating against small differences in optimal annealing temperature between primers [269]. Ward (2013)[266] tested a range of annealing temperatures for each primer that led to successful amplification of target loci.

In creating a streamlined set of multiplex panels, a primary aim was to minimise the number of panels whilst also minimising the variance in annealing temperatures between primers and maximising the distance (in base pairs) between primers tagged with the same fluorochrome. The annealing temperatures, allele ranges, and multiplex panels are shown in **Table S 2.2**. To optimise the concentration, annealing temperature, and membership of primers within the new set panel set, DNA from a set of six candidate individuals, selected *ad libitum*, were amplified in duplicates per panel set, using a thermal cycler (MasterCycler Nexus gradient, Eppendorf, Germany) and PCR thermocycling profiles outlined by Ward et al., (2014)[205].

Prior to PCR, DNA concentrations were diluted to ≤ 50 ng ul^{-1}. To confirm successful amplification, PCR fragments were resolved using gel electrophoresis, on a 1.5% agarose gel, and viewed under UV light. The PCR fragments are then diluted by a factor of 1:150 using MilliQ® water. Fragment lengths were then analysed with an Applied Biosystems™ 3730 DNA Analyser® by a third party (DBS Genomics, Durham, UK).

Levels of fluorescence were measured using GENEIOUS® v. 10 [273] and assigned a categorical level of intensity (**Table S 2.3**) used by Ward (2013)[266]. Primers in which peaks were present, but low, were tested in separate PCR reactions with double their previous concentration. If allele size ranges were compatible, primers which failed to amplify at all were assigned to a new panel with an annealing temperature closer to their optimum. If not compatible, the entire panel containing the problem primer was tested at a new annealing temperature closer to that primer's optimum.

The revised set of panels were then tested again using the same procedure. Only SN91 and RHA118 failed to show quality peaks and were discarded. This left a set of 31 microsatellite loci, well above the typical 10-20 loci used in similar parentage analysis studies e.g. [274–277].

2.3.5 Microsatellite genotyping

For the cohorts born between 2012 and 2018, up to 31 microsatellite loci were amplified, and processed using the aforementioned protocol. Alongside all PCRs, negative and positive controls were used to, respectively, screen for the presence of potential contaminating DNA, and confirm successful DNA amplification, as well as enable calibration of genotypes with previous cohorts (see below).

An important stage in genotyping is correctly identifying fragment lengths from fluorescence peaks [278]. However, it is here where microsatellite genotyping has some disadvantages. The results risk being non-reproducible if not appropriately calibrated [279–281]. Laboratory conditions (the technician, the type of dyes used, and status of the machine) can vary between fragment capillary electrophoresis runs. Calibration with these data can be achieved with a suitable number of controls – samples in which the allele sizes are already known. A correction factor, reflecting the change in laboratory conditions, can then be applied to the assigned fragment length [271]. To ensure consistency and to calibrate fragment lengths between each fragment analysis run I used a set of eight control samples from the 2012 cohort. In addition, to ensure consistency to the pre-existing cohorts (1993-2011), I included a pre-2011 individual (the positive control) whose genotypes were known, alongside each PCR run.

Applying the relevant correction factor, a new calibrated set of allele sizes within the newly created primer panels was used as a reference to score fragment lengths (genotypes) in GENEIOUS®[273]. There are multiple, common sources of error that reduce confidence in parentage assignment. Scoring of microsatellite allele sizes can be challenging largely due to the presence of stutter peaks, where the *Taq* polymerase enzyme slips during its replication of the target fragment, resulting in deletion or expansion in the number of short-tandem repeats [278]. This can become a problem if alleles differ by a few base pairs (known as adjacent allele heterozygotes), and stutter artefacts can be mis-interpreted as a true allele, or vice versa. Following Hoffman & Amos (2005)[282], true alleles were distinguished from stutter peaks by comparison with known homozygotes of both putative alleles. Additional human error can arise through clumsy data entry and clerical mistakes. These, and the presence of potential adjacent allele heterozygotes mis-reads, were accounted for by a second investigator (M. Power) verifying fragment lengths in GENEIOUS® [273] and comparing with the final allele scores.

Another significant source of error is allelic drop out and null alleles, which can arise through the non-amplification of an allele in a true heterozygote, leading to inaccurate genotyping as a homozygote. I calculated the incidence of these errors through two ways. Of the total 1935 individuals genotyped to date, 144 (7%) were genotyped at least twice. I used the PEDANT[283] software to estimate the probability of allelic drop out and additional

mistyping per loci, using these duplicated genotypes. Next, I used CERVUS v 3.0.7 [284] to estimate allele frequencies and overall error rate per loci, using Mendelian discrepancies between known mother-offspring pairs (439 pairs) captured together in the summer maternity colony. I also used CERVUS to detect particularly erroneous alleles through significant deviations from the Hardy-Weinberg equilibrium. Five loci were removed (**Table S 2.2**), leaving the final average error rate from mother-offspring mismatches in CERVUS as 0.0294 and repeated genotypes in Pedant as 0.0261 for the remaining 26 loci. I opted for the most conservative option (0.0294) in downstream analyses. Error rates per locus are reported in **Table S 2.2**.

2.3.6 Parentage analysis and pedigree construction

Assignment of parentage and pedigree construction for individuals born between 1993 and 2011 were conducted according to Ward et al., (2014)[205], using a suite of up to 40 microsatellite loci. Cohorts from 2012 onwards used identical methodology with some minor modifications. The results of this analysis are reported here. In brief, parentage analyses were implemented by cohort, primarily in CERVUS, using an error rate of 0.0294. *R. ferrumequinum* give birth to a maximum of one pup a year. Previous work [274] has established all pups found attached to a female are that female's offspring. Excluding those mothers who were found with a pup attached, a set of candidate mothers was created by inspecting traits indicative of reproduction over the parturition season, i.e. pregnancy, lactation, baldness surrounding teats [170]. Candidate fathers included all males genotyped at more than 10 loci, at least 2 years old, thought to be alive during the mating period preceding the parturition season in question. Simulations for calculating confidence values of maternity assignments were performed with a sampling parameter depending on the ratio of known and candidate mothers to offspring. For example, in 2013, 60 pups were found attached, and 19 unattached. For those 19 pups, 15 candidate mothers were identified, giving a sampling rate of 95%. The same simulations for paternity assignments used a conservative sampling rate of 70% [274]. Maternity was assigned first, with a minimum pair confidence of 95%. If a mother was assigned, paternity was assigned at a minimum trio confidence of 95%. For pups born from 2012, the combined exclusion probability of a candidate father when an offspring's mother was known was 0.999 for the 26 loci.

The program COLONY v 2.0.6.9[285] was used to determine parentage of any remaining pups that could not be assigned through CERVUS. COLONY takes an alternative approach to parentage assignment, using pedigree information beyond parent and offspring. I included the five loci, previously discarded in CERVUS, as COLONY is more robust to significant deviations from HWE and higher error rates [286]. Analyses were performed per cohort, with the male mating system classified as polygamous and female as monogamous (as they can only mother a single pup per year). Error rates were inputted per locus (**Table S 2.2**). Putative parentage or sibships assignments were accepted with a confidence estimate of ≥ 80%, following Ward et al., (2014)[205].

An additive genetic matrix, calculating the pair-wise relatedness coefficient (*r*), was calculated using the *pedantics* R package [287] from the full pedigree covering 2154 individuals. In total, 664 were caught at least once during the study period (November 2007-April 2019). Individuals included within the pedigree, but not captured during the study period were removed from the kinship matrix. Relatedness to captured individuals not included within the pedigree (n=110) e.g. immigrants that were not identified as parents of pups born at Woodchester, was assumed to be zero.

2.3.7 Social network analysis

2.3.7.1 Social network construction

Group memberships were constructed by assembling co-roosting individuals into a "group-by-individual" matrix using the *asnipe* R package [288]. To explore the possibility of social structure at differing spatial scales, I defined associations in two ways: 1) among individuals sharing hibernacula and 2) among individuals found together in a loose 'cluster'. Here, a cluster is defined as an aggregated group of individuals within at least twenty body-lengths (~1 metre). Clusters were typically sparsely distributed within sites and were easily distinguishable.

Group-membership data were then used to construct association indices – the probability of association for a pair of individuals. The most common index is the "simple ratio" (SRI), defined as the number of sampling periods individuals A and B are found together, divided by the number of sampling periods where at least one is seen [289,290] (**Equation 2.1**).

$$\frac{x}{x + y_{AB} + y_A + y_B}$$

Equation 2.1 *The simple ratio index (SRI) where x is the number of sampling periods individuals A and B are found together; yA and yB, the number of sampling periods where only individual A or B was observed respectively; yAB, the number of sampling periods where both A and B were observed, but not associating.*

The SRI comes with the important assumption that all individuals and their associates can be identified accurately within a sampling period. While roosting bats tend to be conspicuous, locating all individuals within a site is not guaranteed, especially if sections are inaccessible to observers. Therefore, I opted for the more conservative "both-identified index" (BII), a modified form of the SRI, which calculates only the probability of association given both individuals in a dyad are observed on a given date [289,291] (**Equation 2.2**).

$$\frac{x}{x + y_{AB}}$$

Equation 2.2 *The both-identified index (BII), where x is the number of sampling periods individuals A and B are found together, and y_{AB} the number of sampling periods where both A and B were observed, but not associating.*

Furthermore, estimated association probabilities between individuals only captured once or twice are less likely to reflect true social preferences [289]. Therefore, to reduce uncertainty in my association indices, further analysis was restricted to only those bats observed at least three times (the median capture rate; **Figure S 2.2**).

2.3.7.2 Question 1 – Is there non-random structure of associations and are they persistent over the long-term?

I examine social structure by estimating social differentiation (*S*)– calculated as the coefficient of variation of the association probabilities in the "true" network (CV_{true}), that which would be obtained if 100% of dyads were observed. The coefficient of variation describes the extent of variation in dyadic association rates. In the extreme case where all individuals associate with one another, there is no variation of association indices – giving a homogenous network with no social preferences. An *S* of 0 indicates no social preferences. An *S* of at least 1 suggests strong social preferences in a highly segregated network [289].

Dividing the estimate of *S* by the coefficient of variation of the observed network (CV_{obs}) provides a means to quantify how well the observed network matches the underlying, true network, and so gives a measure of statistical power of further analyses [292]. Explanatory power of the observed network is given as a correlation coefficient – *r* (**Equation 2.3**).

According to Whitehead (2008)[292], an *r > 0.4* represents a good approximation of the true social structure.

$$r = \frac{S}{CV_{obs}}$$

Equation 2.3 *The correlation coefficient (r) of the true association indices (S) and the observed (CV_{obs})*

Social differentiation (*S*), the observed CV (CV_{obs}), and correlation between the observed and true networks (*r*) were calculated under the *social_differentiation* function in the *aninet* package in R [293]. Standard error and 95% confidence intervals were obtained through the default 100,000 parametric bootstraps of the raw data. In addition, I calculated the modularity (*Q*) of the observed network using the *igraph* package [294]. Values of *Q* range between 0 and 1, with those above 0.5 indicating the presence of distinct communities within the network, where individuals share greater associations among others in their community than those outside it [295].

2.3.7.2.1 Temporal stability of associations

A key element of social structure is the temporal stability of associations [15,296]. Using all individuals in the dataset, I estimated standardised time-lagged association rates (LAR) for the full network and among same-sex dyads within SOCPROG v 2.9 [297]. The LAR gives the probability two individuals will be found re-associating after a given time period. I opted for the standardised form as I could not be certain all true associates of a captured individual could be identified on a given survey. The LAR was plotted against a continuous time lag using a moving average of 20,000 associations to smoothen spurious noise. Included in the output plot (**Figure 2.4**) is the standardised null association rate, which gives the probability of two individuals randomly associating after a given time lag. An LAR curve falling below the null association rate indicates no preferred associations beyond that time point. The uncertainty around the lagged association rates was estimated with a jack-knifing procedure over 30-day periods (as surveys within the same month are not assumed to be independent).

2.3.7.3 Question 2 – Are roost aggregations composed primarily of close kin?

Next, I test for the presence of kin structure within the hibernating *R. ferrumequinum* population. Specifically, I test the hypothesis that groups of closely related individuals are formed more than expected by chance.

This analysis was implemented using the *gbi_MCMC* function in the *aninet* package v 0.1. The function adopts the method suggested by Bejder et al., (1998)[298], where the Markov Chain Monte Carlo (MCMC) algorithm is used to create an appropriate null distribution of group relatedness against which to compare the original observed pattern – while maintaining group size.

Taking the group-by-individual matrix, group permutations were restricted within year to ensure individuals not present in the population at a given time were not included in the randomised groups. The function was run using the MCMC routine for 500,000 permutations, over four chains, with a thinning interval of 50,000 and a burn-in of 500. Output plots were checked to ensure chain convergence and close approximation of the desired target null distribution.

2.3.7.4 Question 3 - Does kinship drive non-random associations?

Here I perform a complementary analysis, and test whether biparental and/or matrilineal kin are more likely to form non-random associations. 'Matriline' was defined as the oldest female founder in a maternal lineage (including at least two individuals descended directly from the founder through the female line), and extracted via the *founderLine* function in the *nadiv* R package [299]. This pedigree-based calculation of matriline is an imperfect proxy of maternal kinship, and assumes 'matriline = mitochondrial genotype', though this may not always be the case. In addition, matriline was not known for all bats. The use of mitochondrial DNA (mtDNA) may not always be appropriate for populations that have undergone founder effects and subsequent expansions, with the potential for unfamiliar individuals from different colonies to have identical mtDNA genotypes [300–302].

Those individuals above the capture threshold, but without a known matriline (n=62) were excluded, giving a final sample size of 345 bats for the roost network and 250 for the cluster network. A dyadic regression model was applied in the form of a general linear model quadratic assignment procedure (GLMQAP), with the association index (edge strength) as the response variable, and matrices of phenotypic similarity as predictors. The GLMQAP (as

described in [303] and [304]) was implemented using the *glmqap* function in the *aninet* R package [293], with a binomial error family (appropriate for association indices). The denominator (**Equation 2.2**) specified the sampling effort per dyad. The double-semi-partialling permutation procedure [305] was chosen as it allows for the testing of predictors whilst accounting for the effects of others. Similarity matrices of phenotypic variables were built using the *attribute_similarity* function, again in *aninet*. A recent study showed age and sex were important in driving assortment in *R. ferrumequinum* networks [306], so these factors were controlled for in the analysis. Sex and matriline were set to binary variables (same or not). Age (year of birth) was set as a continuous variable, with similarity defined by pair-wise negative absolute difference between birth year. Energy reserves may also play role in assortment, because individuals with different body mass were found to differ in their choice of hibernacula [199]. As a proxy for energy reserves [307], a body condition index at the time of capture was calculated according to Ransome (1995)[308] - (body mass (g) / radius length (mm)) x population mean radius length (mm). The mean body condition across all captures was used to create an average body condition index per individual. A similarity matrix was constructed based on the pair-wise negative absolute difference between average body condition indices.

Factors driving space-sharing may depend on sex and life history, for example, females may be more likely to associate with maternal relatives in hibernacula given the possibility of maternal transmission of roost information within the maternity colony. Mature males may be more tolerant of kin in neighbouring territories [27]. Therefore, GLMQAP analyses were repeated for sex (male-male and female-female dyads) and age segregated networks. The latter were separated according to whether an individual was younger (immature) or older (mature) than the typical age of sexual maturity (2 years old). To control for multiple testing, *p*-values were adjusted using the false discovery rate (FDR) correction method [309,310]. The FDR threshold was set to 0.05.

2.3.7.4.1 Post-hoc analysis of age structure

The analysis revealed that age significantly influenced roost sharing and cluster associations among immature and mature bats respectively (see 'Results' section). Assortativity could reflect similar roost preferences leading to co-roosting and/or cluster membership [199]. However, social attraction cannot be ruled out. Assortativity among bats (irrespective of

relatedness) has been reported where individuals gain familiarity through prolonged spatial proximity earlier in life [311–313]. For example, Kuhl's pipistrelle (*Pipistrellus kuhlii*) pups that spent more time together in early life developed stronger social ties in adulthood. Pre-weaning, *R. ferrumequinum* pups are reliant on their mothers leaving the roost to forage, subsequently aggregating in tight clusters [265,314]. These "crèches" may facilitate the formation of social bonds among pups that could persist until they reach hibernacula, or perhaps adulthood. Positive assortment among immature, and adult bats of the same cohort would be consistent with this hypothesis. Therefore, I conducted a post-hoc analysis [315] on immature and mature dyads, repeating the dyadic regression analysis previously mentioned, but setting age to a binary variable (same cohort or not).

2.4 Results

2.4.1 Data summary

Over 12 winters, 23 hibernacula, and 81 surveys, a total of 664 bats (287 males, 377 females) were identified, leading to over 2833 individual encounters. The largest portion of these (41%) took place in 'mid-hibernation', or January/February (**Figure S 2.3**). A mean intermediate rate of identification [289] (the proportion of the study population in a given year identified per sampling period) of 25% ± 16% SD was obtained. The mode and mean age of individuals at capture was 1 and 3.431 ± 3.586 SD respectively. 529 individuals (80%) were born in the Woodchester Mansion maternity colony, and 135 from elsewhere. These individuals were captured 1-26 times (median = 3, SD = 3.389; **Figure S 2.2**), resulting in 479 roost-level groups with a mean of 5.921 bats per group (SD=7.016, range=1-59). 386 (58%) individuals were found at least once in a cluster, resulting in 202 cluster-level groups with a mean of 3.620 and median of 2 bats per cluster (SD=2.950, range=2-20). Membership of a cluster was infrequent, with a mean of 21% of individuals found in a cluster on a given survey (SD=19%, range=0-72%). Individuals were rarely found in the same roost they were previously captured in within the same winter (65% of re-captures), indicating frequent roost switching and low fidelity to single sites. Group sizes i.e. roost counts, were not uniformly distributed among roosts. **Figure S 2.4** shows variation in the numbers of bats recorded at each core and outlier site over the study period. Over 50% of captures occurred within three sites (H, I and O1).

2.4.2 Parentage and kinship

During the study period, 622 bats (355 females, 267 males) captured in hibernacula were genotyped at between 10 and 40 microsatellite loci to obtain measures of kinship. For 85% of these individuals, at least one parent was assigned. Parentage was fully assigned for 62% of individuals, with only maternity assigned for 22% and only paternity for 0.6%. No parentage was assigned for 110 bats, of which 109 were immigrants.

Mating pairs, defined by the pedigree, were rarely found co-roosting. On 40 occasions, a female was found with the male she reproduced with the following summer. However, on 359 occasions, a female was present with a male but did not reproduce with him.

A matrix representing pair-wise additive genetic relatedness was generated from the pedigree, representing the opportunity for individuals to passively associate with kin. Kin availability was low, as average pair-wise kinship approximated to zero (0.017 ± 0.060 (SD)). Among the potential pairings, 1% were among first-order relatives ($r > 0.25$) (**Figure 2.2**). Matrilineal data were associated with 345 individuals (211 females, 134 males) who were caught at least three times. This restricted set was used in downstream social network analysis.

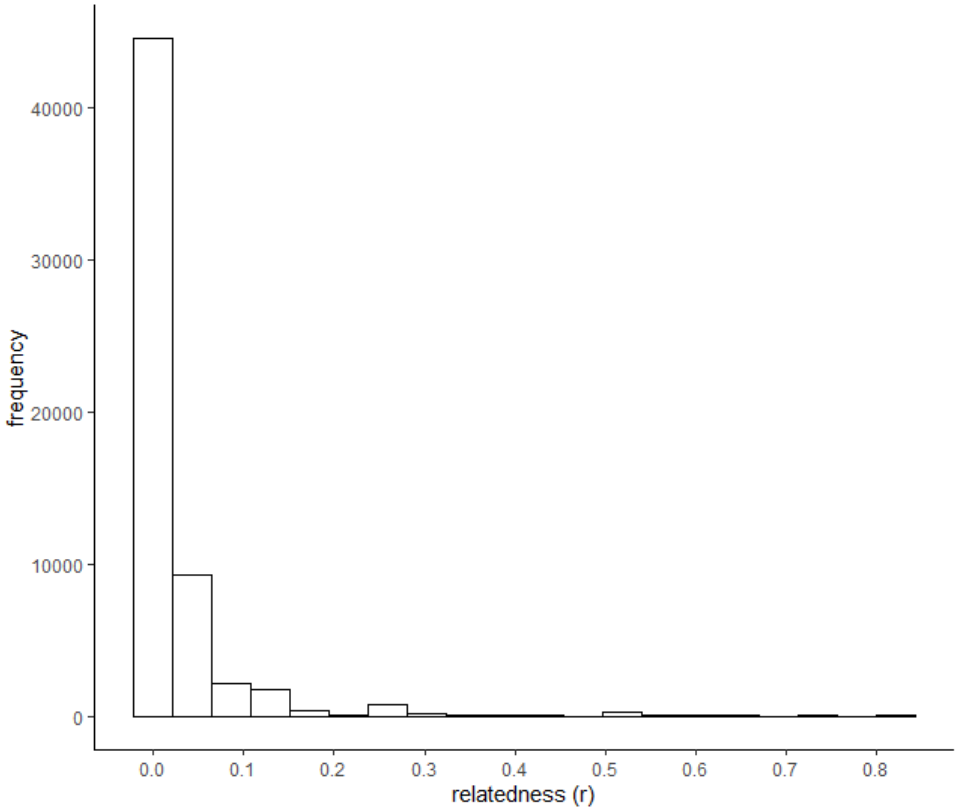

Figure 2.2 *Distribution of pairwise relatedness coefficients - calculated via the pedigree-based additive genetic relatedness matrix.*

2.4.3 Question 1 - Is there non-random structure of associations and are they persistent over the long-term?

Despite random structure regarding relatedness (see section 'Parentage and Kinship' above), the social differentiation in the roost-sharing network was moderate (*S* ± SE = 0.738 ± 0.013). Social differentiation in the cluster network was high (1.306 ± 0.099). Among co-roosting bats, social differentiation was highest among mature female dyads (0.866 ± 0.038, **Figure 2.3**). The correlation between the observed and underlying network, and therefore power of this analysis, ranged from 0.319 ± 0.023 (*r* ± SE) in the cluster network to 0.694 ± 0.050 in the immature male network. The correlation coefficient again between the observed restricted (individuals caught at least three times) and underlying network was 0.516 ± 0.009 – indicating a fair representation of underlying social patterns. Restricting candidates to at least three captures had little effect on the social differentiation (0.734 ± 0.114), nor statistical power (0.491 ± 0.008) of the roost-sharing network. The same applied for the cluster network (**Figure 2.3**). The modularity (*Q*) of the restricted roost-sharing network was 0.330, indicating a lack of distinct social communities, and a well-mixed population with limited spatial segregation of associations. In contrast, *Q* for the cluster network was 0.618, indicating distinct separation of social communities.

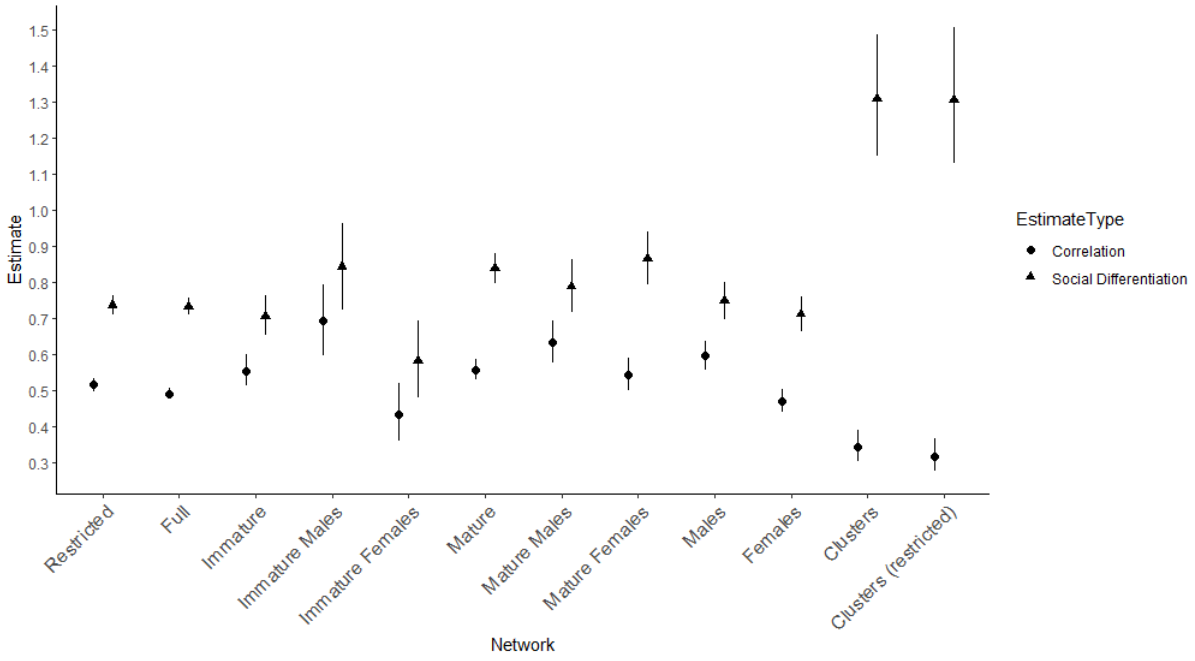

Figure 2.3 *Estimated social differentiation (blue) and correlation between the "true" and observed association indices (red) for each network. Error bars indicate upper and low 95% confidence intervals. Social differentiation is defined according to* [289]*, as the coefficient of variation of the true association indices. Values close to zero indicate little, or no, social complexity. Values closer to 1, or higher, indicate higher levels of social complexity in the study population. The correlation between the true and observed association indices gives an estimate of the accuracy, or power, of observed social patterns. According to Whitehead (2008)*[292]*, a correlation above 0.4 gives a reasonable representation of the underlying social structure.*

2.4.3.1.1 Temporal stability of associations

To investigate the temporal stability of associations between individuals I calculated standardised lagged associated rates (LARs) to investigate the probability of two individuals re-associating after a given time period. Among all dyads, standardised LARs remained above the null association rate for up to 2000 days (~5.5 years). Associations among males appear to be maintained for longer periods than females. Standardised LARs among female dyads fell below the null expectation by 2000 days, whilst they always remained above null expectations for males (**Figure 2.4**).

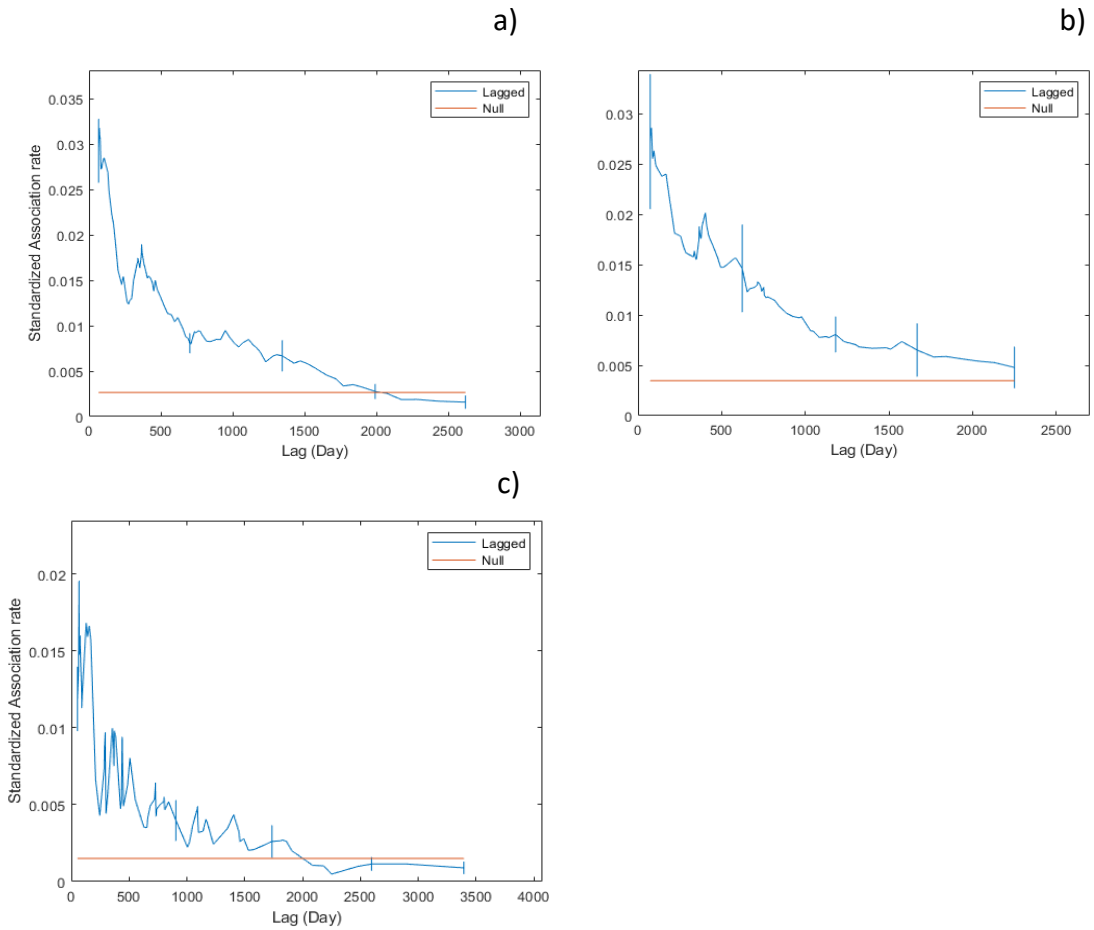

Figure 2.4 a) *Standardised lagged association rates (LARs) among female-female dyads,* ***b)*** *male-male dyads* ***c)*** *and all dyads plotted against time in days. Standard errors were estimated through jack-knifing. The red horizontal line indicates the null standardised association rate. Estimates and plots were generated in SOCPROG v 2.9.*

2.4.4 Question 2 - Are roost aggregations composed primarily of close kin?

Kin structure was not detected among co-roosting bats. That is, most individuals found together in a roost were unrelated to each other. The MCMC algorithm converged at 500,000 iterations (**Figure S 2.5a,c**), generating a null distribution (mean ± SD = 0.0198 ± 0.002, **Table S 2.4**, **Figure S 2.5b**) that did not significantly differ from the observed value of low average group kinship (mean ± SD = 0.0181 ± 0.0438).

2.4.5 Question 3 - Does kinship drive non-random associations?

Neither bi-parental (**Figure S 2.6**) nor maternal kinship (**Figure S 2.7**) were important predictors of roost sharing, regardless of the network tested (all *p > 0.05;* **Table S 2.5**). However, individuals of similar age were more likely to be found together in a roost than expected by chance in all (*p* < 0.01*;* **Table S 2.5***,* **Figure 2.5**), except for the immature

networks (all $p > 0.05$*;* **Table S 2.5**). Average body condition was not significantly related to roost sharing, except among immature bats (β = 0.080 ± 0.019, $p<0.05$; **Table S 2.5**). Significant positive assortment by sex was also detected among mature bats (β= 0.144 ± 0.034, $p < 0.001$*;* **Table S 2.5**).

With bats roosting in clusters (**Figure 2.6**), similarly, age predicted positive assortment (β =0.245 ± 0.0018, p<0.001). Neither bi-parental, maternal kinship, sex, nor body condition were important predictors of association rates (all $p > 0.05$*;* **Table S 2.5**).

2.4.6 Post-hoc analysis of age structure

Cohort effects for both immature and mature dyads were positive and significant at the roost (immature: β = 0.201, $p<0.05$; mature: β= 0.258, $p<0.001$; **Table S 2.6**) and cluster level (β= 1.293, $p<0.001$; **Table S 2.6**), consistent with the hypothesis that individuals have the ability to form bonds among cohort members in early life that persist until adulthood.

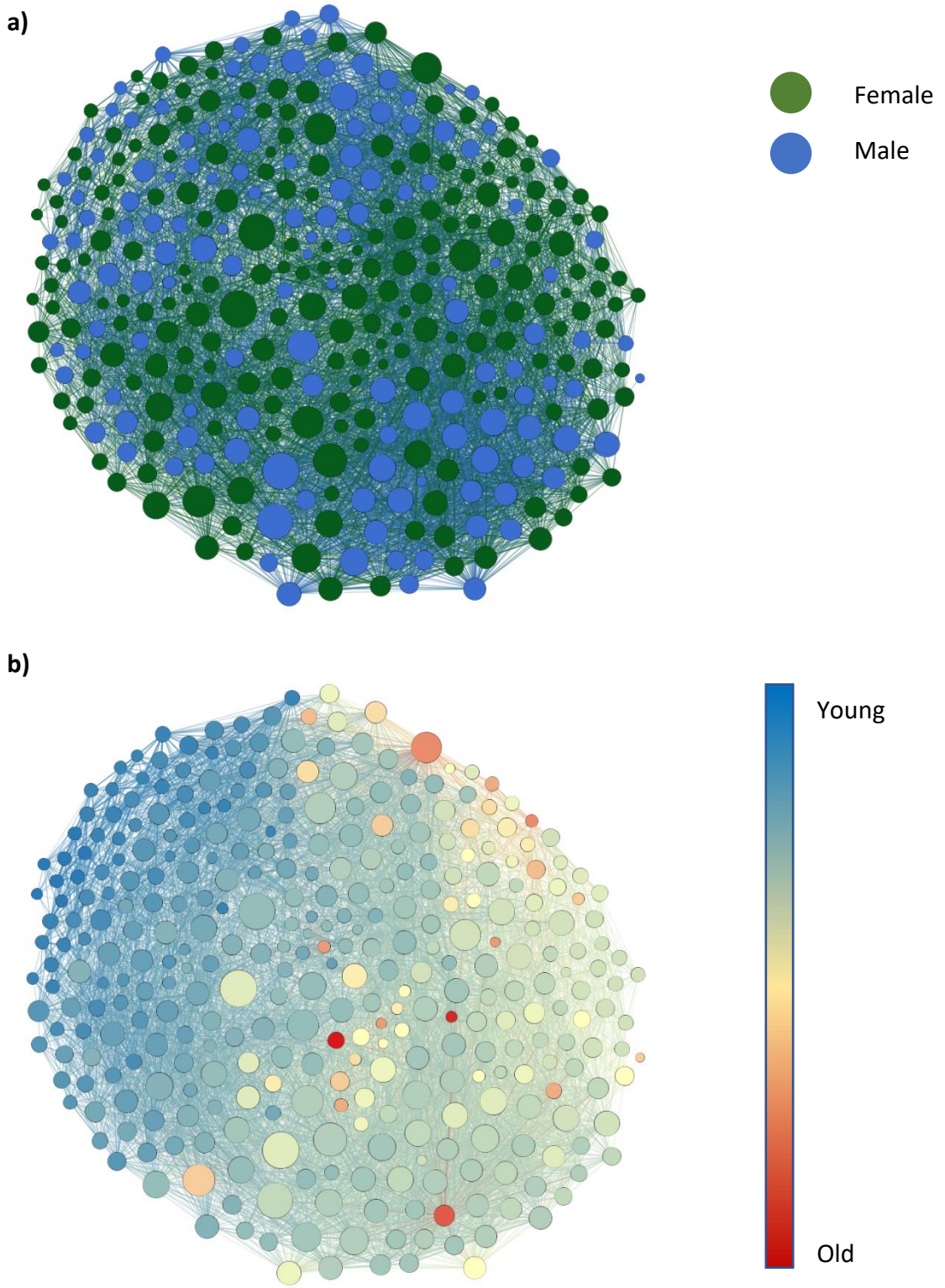

Figure 2.5 *Roost sharing network (n=345). Edges represent the 'both seen index' (see 'Social Network Construction' section). Node size varies according to degree (number of connections).* ***a)*** *Blue nodes=males, green nodes=females.* ***b)*** *Node colour varies along a gradient according to age, from the oldest (red) to youngest (blue).*

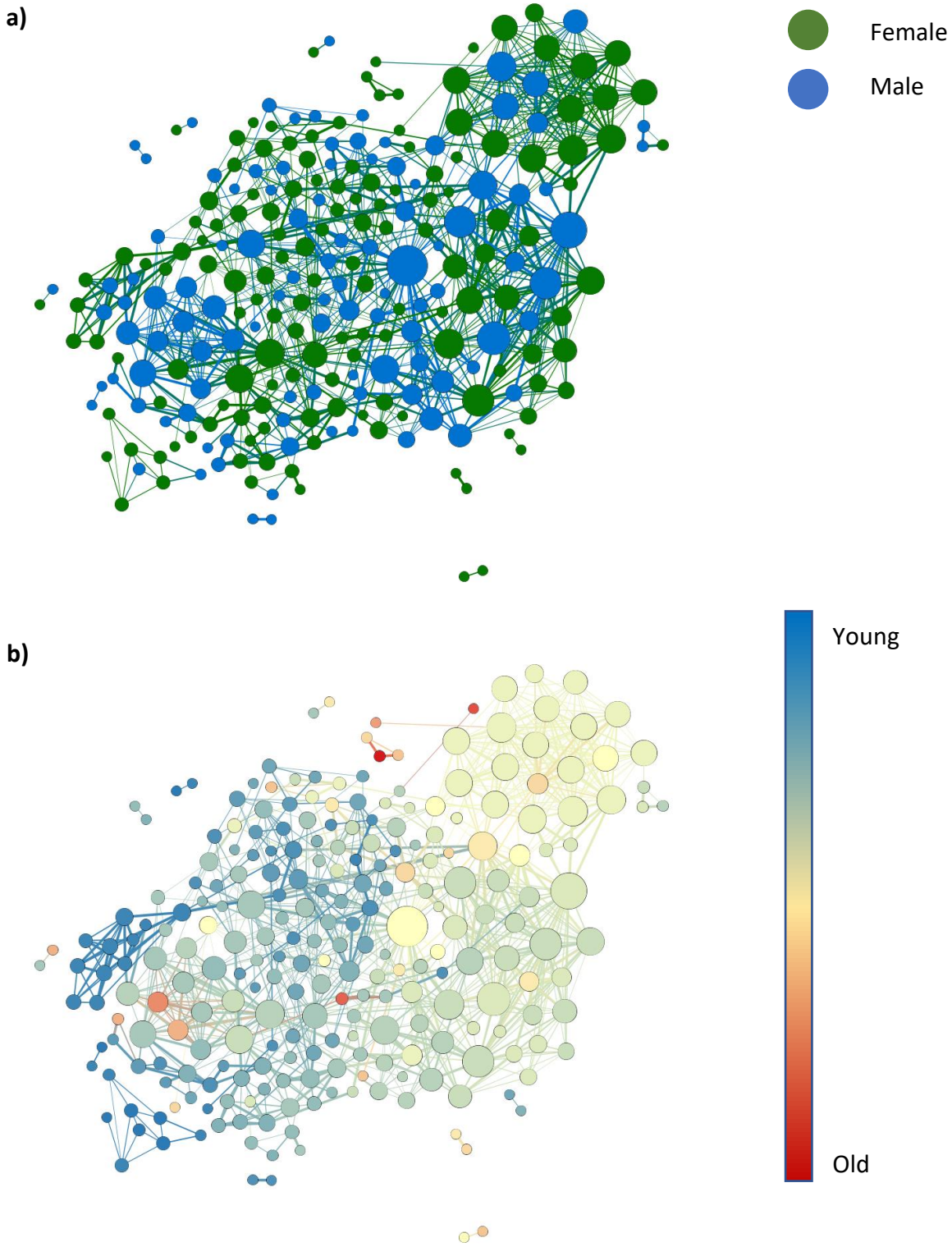

Figure 2.6 *Cluster network (n=267). A 'cluster' constitutes a group of bats roosting within an approximate 1 metre radius. Edges represent the 'both seen index' (see 'Social Network Construction' section). Node size varies according to degree (number of connections).* ***a)*** *Blue nodes=males, green nodes=females.* ***b)*** *Node colour varies along a gradient according to age, from the oldest (red) to youngest (blue).*

2.5 Discussion

Seasonality in local ecological conditions can generate predictable intraspecific social variation [316]. As such, the presence of kin structure should not be an assumed stable feature within all species. The supply of resources can fluctuate over space and time, leading to dynamic changes in the spatial distribution of individuals, local demographics (reproduction and mortality), and consequently the fitness pay-offs of remaining with kin [317]. Temperate bat societies exhibit an annual fission-fusion where female colonies disband, and individuals disperse to hibernacula in the winter [185]. While kin structure in this summer maternity colonies has been well-studied[207,236–238,242,243,318–320], very little is known about genetic relationships among individuals in the hibernation period. Here I tested predictions on bi-parental and maternal kinship and space use in *R. ferrumequinum* over 12 winters. Contrary to my expectations, I did not find significant kin-directed assortment. Regardless of the spatial scale tested, co-roosting *R. ferrumequinum* found in hibernacula were no more related than expected by chance. Neither did maternally related bats preferentially roost together. However, I detected significant social structure regarding age over all networks, except those of immature bats. Additionally, positive assortment was detected among mature individuals of the same sex.

2.5.1 Question 1 - Is there non-random structure of associations and are they persistent over the long-term?

Network analyses detected the presence of fission-fusion dynamics, driven by the formation and breaking of associations within and between winters. *R. ferrumequinum* formed moderately structured associations in the hibernation period, probably as a consequence of differential roost selection among individuals. However, modularity (Q) was low, indicating a well-mixed population, and weak spatial segregation in dyadic associations over time. Nevertheless, any population with spatial structure will exhibit social network structure [321], as associations between individuals with differential site fidelity are less likely. Studies by Ransome (1968; 1971)[199,233] revealed *R. ferrumequinum* may choose hibernacula according to the ambient temperature variability and air flow. All hibernating bats undergo periodic arousals during which they may re-hydrate [322], feed to recuperate spent fat reserves [323–325], and/or move between roosts [246,326]. The temperature and air flow regime of the hibernaculum are key determinants of arousal frequency; thus, a bat must choose the site carefully as to avoid arousing during unfavourable periods [233,327–330]. Favourable foraging

habitat close to the roost may be advantageous to limit energy costs spent during dispersal [324]. Optimal roost selection is critical for bats with poor body condition, as they may need to better detect rises in ambient temperature, and arouse to capitalise on feeding opportunities [233,323,325,327,331,332]. Immature *R. ferrumequinum* tend to have lower body reserves, regardless of sex [199]. Positive assortment among immature bats according to average body condition may reflect sharing of such sites that promote well-timed arousals.

The sexual segregation of associations in mature bats was surprising, given that mating is thought to take place over winter. Captures during January constituted the majority of associations (**Figure S 2.3**), as did a previous study of a neighbouring population [306] that reported the same finding. Thus, the observed association patterns may, primarily, reflect contrasting reproductive, energy conservation, and consequently space-use strategies during mid-winter. Adult males use arousals as a means to not only recover energy reserves lost during mating, but to capitalise on mating opportunities lost while in torpor [182]. According to the "thrifty female hypothesis", females, in contrast, may prefer prolonged torpor and energy conservation, given survival to the following summer is essential for their reproduction [333,334]. Conflicting priorities in the selection of sites according to their microclimates may therefore drive a separation of co-roost associations.

Hibernacula form a predictable resource, promoting site fidelity and repeated associations [335]. Lagged association rates for both sexes were consistently above null expectations for approximately five years. I expected a slower decay in females, as they have a greater capacity to maintain social bonds while in the maternity colony. Rather, males maintained a longer period of re-associations, possibly aided by fidelity to shared sites where they achieved paternities. For example, songbirds are known to return to sites where they bred successfully [336].

Bats of a similar age were more likely to be found together (**Table S 2.5**, **Figure 2.5**, **Figure 2.6**). This result is consistent with the findings of Finch et al., (2022)[306], and could be due to similar microclimate preferences among similarly aged individuals [199], and/or population turnover [337]. The latter could also explain the decay of repeat association probabilities seen over five years. Nevertheless, these age-related association patterns contrast with those previously reported for temperate bats during the summer, where maternity colonies

typically contain several generations. For example, no significant correlation between age and association was identified in a female Bechstein's bat (*M. bechsteinii*) maternity colony [156]. In northern long-eared bats (*M. septentrionalis*), older females were frequently found alongside younger adults [338]. The relatively lower capture rate reported for older bats in this study warrants additional investigation. High mortality rates are unlikely, given frequent re-captures in the summer (*pers. obs*). Rather, one possible explanation is that less accessible parts of hibernacula, or those outside the study area, are preferred.

Further analyses determined age-structured roost and cluster sharing included bats of the same cohort (**Table S 2.6**). Cohort effects were consistent among both young, immature and older, mature bats. Considering repeat non-random associations among pairs can be maintained for five years at most, these results indicate a capacity for individuals born together to maintain social bonds through to adulthood. Yet, under the fission-fusion system, maintenance of these individually-specific long-term bonds presents a significant cognitive challenge, requiring repeat partner reunification and recognition [186]. Belonging to a cluster, rather than roosting alone may facilitate the locating of specific individuals. Such findings open the door to further study to deduce whether cohort mates actively associate (as observed in *P. kuhlii*[312] and *Phyllostomus hastatus*[146]) and if there is benefit to doing so, or whether the observed patterns are explained by population turnover and passive roost selection alone.

2.5.2 Question 2 & 3 - Are roost aggregations composed primarily of close kin & does kinship drive non-random associations?

The social environment experienced by hibernating *R. ferrumequinum* is not structured by kinship. In contrast to the many social mammal populations where individuals live among kin[27] (e.g. in ungulates[339,340], cetaceans[341,342], and some bats [151,242,243,343]), I found no evidence of either biparental or maternal kin structure within the co-roosting associations of this species – regardless of maturity status and sex. Average pair-wise relatedness approximated zero, with approximately 1% of relationships exceeding 0.25 (**Figure 2.2**), indicating a negligible opportunity for kin to associate passively, as do individuals in highly kin-structured societies [344]. Thus, any kin-biased associations would require active recognition [153,253] of kin within the roost, or co-ordinated dispersal of related individuals from the maternity roost [243,259]. The apparent random associations regarding kinship

suggest neither of these mechanisms occur, or that kin-biased associations are likely to confer sufficient fitness benefits.

The findings differ from those in temperate bat species such as *Myotis septentrionalis* and *M. bechsteinii*, where closely related females maintained strong associations despite low average colony relatedness [238,242,243]. In *R. ferrumequinum*, the selection of summer foraging sites was kin-biased. However, the absence of kin-biased associations during winter raises the question of what might explain this. Bachorec et al. (2020) [345] found seasonal variation in patterns of genetic relatedness among groups of foraging Egyptian fruit bats (*Rousettus aegyptiacus*), where relatives foraged together when resources were plentiful, but a drop in food supply in spring led to greater sharing of foraging sites and overall dilution of the previous kin structure. This may explain the contrasting results of this study and Rossiter et al. (2002) [207], where differences in the abundance and spatial distribution of resources could influence kin structure. The localised and small number of hibernacula available to Woodchester Mansion's population limits the opportunity for potential kin groups to spatially segregate. Naïve individuals, presumably, find little difficulty in locating the full selection of sites. Foraging sites are widely dispersed and likely diverse in quality, potentially making it beneficial for kin to exchange information on their location [207,242]. However, caution is necessary in generalising these results and interpretations, as roost availability and distribution will depend on the landscape under investigation.

There may be fitness benefits to associating with non-kin. Notably, outbreeding positively influences offspring survival, particularly in males. Inbreeding may increase mortality risk in females [193]. Assuming that mating can occur in hibernacula, individuals may choose to avoid kin to improve access to unrelated mates. Furthermore, as sneak copulations by males have been observed in horseshoe bats (see *Chapter 3*), associations with non-kin may be necessary to avoid inadvertent mating with relatives.

Surprisingly, I did not detect maternal kin-biased associations among mature females. An intralineage polygynous mating strategy (i.e. matrilineal relatives sharing breeding partners) was previously reported in this species [208]. Therefore, it may be the case that females of the same matriline do not form stable groups, rather, that visits by females to male territories are fleeting. Alternatively, mating patterns may have changed. The previous study [208] focused on the population when it was at low numbers, with a limited number of males

available for females to choose from, possibly inflating the opportunity for matrilineal kin to choose the same male. That being said, co-roosting data from the three annual surveys were a poor approximation of mating patterns according to the pedigree. It is possible that mating groups of maternal kin form, most likely only in autumn and perhaps only transiently. These groups may form infrequently enough to not be detected during hibernation surveys.

The lack of kin-biased associations reported in this study does not mean kin-biased interactions do not occur. Association-based network studies assume interaction between individuals as associations provide opportunity for finer-scale interaction [62,346]. Yet interactions may not occur with equal probability among all associates. A recent study of sulphur-crested cockatoos (*Cacatua galerita*)[347] revealed negligible kin structuring among roosting groups, yet individuals showed strong preferences for interactions with kin. The proximity-based relationships represented in the present study may not be accurate representations of social interactions. *R. ferrumequinum* is known to arouse from torpor and move within roosts [348]. Social calls have also been recorded [349], thus kin-biased interactions within hibernacula are plausible, though they probably do not occur at equal frequency among all co-roost associates.

2.5.3 Conservation implications

Understanding the sociogenetic structure of the wintering *R. ferrumequinum* population is important for assessing how gene flow may operate in populations of slow life history species in human-modified landscapes. Habitat fragmentation is of particular concern for this species due to its apparent reliance on linear habitat features, such as hedgerows, that are vulnerable to disturbance and removal throughout its range [350–352]. Fragmentation is typically evident in the sociogenetic structure of a population as associations, and therefore geneflow, are segregated within isolated habitat patches [353,354], often leading to higher levels of inbreeding and significant kin structure [355–357]. The lack of kin structure among hibernating *R. ferrumequinum* sharing roosts indicates the opportunity for inbreeding in this population is limited. The capture of 135 bats from other maternity colonies (some >25 km away) demonstrates the possibility for gene flow among populations.

In addition to facilitating gene flow, movement between hibernacula may be necessary for individuals seeking new microclimates that are not presently available [199,326]. The low levels

of roost fidelity observed, as indicated by low re-capture rates at single sites within winters, combined with the low modularity score, suggest regular inter-hibernacula movements occur. However, quantification of connectivity between all sites at a landscape scale would be worthwhile. Nevertheless, roost counts were not evenly distributed (**Figure S 2.4**), with most bats found at just three sites - emphasising the role only a few sites can play in facilitating social connections and population processes, such as gene flow.

2.6 Conclusion

Taken together, the data suggest kinship is not an important force driving co-roosting associations in *R. ferrumequinum* during the hibernation period, given little opportunity for encounters with first-order relatives. Future studies could explore the influence of kinship on interactions, such as acoustic communication. The social environment for hibernating *R. ferrumequinum* appears to be largely driven by age-structured associations. Further study is warranted into associations among individuals in early life and the potential benefits conferred to individuals.

In this chapter, I examined the sociogenetic structure of associations in *R. ferrumequinum* during the hibernation period. These associations include loose clusters that are commonly observed in hibernacula. In the following chapter, I explore the functional significance of these clusters in greater detail using a closely-related species, *R. hipposideros*.

2.7 Supplementary Tables & Figures:

Table S 2.1 *IDs, total number of* R. ferrumequinum *recorded, number of males and females, median count per survey, median age, size, and typical temperatures experienced (thermal regime) at each core and outlier site according to qualitative assessment by Ransome (1968)*[199]*. Most sites contain a range of microclimates according to their variable structure. 'WM' refers to the hibernaculum and attic at Woodchester Mansion. Sites in very close proximity are given a common letter ID.*

ID	Site Type	Total number recorded	N. males	N. females	Median count	Median age	Thermal Regime	Size
G	Core	46	15	31	2	5	Stable	Medium
H	Core	429	217	212	13	1	Regions with all regimes	Large
I	Core	535	231	304	15	1	Variable cool and variable	Medium
J	Core	102	32	70	3	1	Variable	Very small
K	Core	180	64	116	6	1	Variable cool	Small
M	Outlier	119	60	59	4	1	Stable & variable	Medium
O1	Core	471	231	240	13	1	Stable and variable cool	Very large
O2	Core	11	9	2	1	8	Stable	Small
O3	Core	19	10	9	2	1	Stable and variable cool	Medium
P	Core	64	27	37	3	3	Stable and variable	Large
Q	Core	60	29	31	1	1.5	Stable and variable	Large
R1	Outlier	76	29	47	2	2	Stable	Medium
R2	Outlier	25	8	17	1	1	Variable and cool	Small
R3	Outlier	10	8	2	1	2	Variable	Small
S	Outlier	17	8	9	2	4	Stable and variable	Medium
T	Outlier	273	57	216	9	2	Variable	Medium
U	Outlier	20	11	9	2	1.5	Variable	Small
V	Outlier	111	22	89	4	4	Variable	Medium
W	Outlier	86	45	41	3	1	Variable cool	Small
WM1	Outlier	34	17	17	7	<1	Variable	Small
WM2	Outlier	19	10	9	3	<1	Regions with all regimes	Small
X	Outlier	45	14	31	2	5	Stable and variable cool	Small
Y	Outlier	81	14	67	4	4	Stable	Small

Table S 2.2 *33 microsatellite loci used to genotype* R. ferrumequinum *in this study. Multiplex panels constructed by Ward (2013) and the author are represented as "HW" and "LR" respectively. Thereafter "HW" refers to whether a particular marker was found to deviate significantly from Hardy-Weinberg equilibrium. "Tm (°C) range" gives the optimised annealing temperatures tested in Ward (2013). "Tm (°C)" is the annealing temperature from the original publication (see Reference column). The fluorochrome tag allocated to each PCR primer is provided. Error rates calculated from known mother-pup mismatches, observed (Hobs), and expected heterozygosity (Hexp) were calculated in CERVUS. CERVUS error rates were compared to those derived from COLONY (mother-pup mismatches) and PEDANT software (duplicated genotypes). Microsatellite motifs can be found via the accession number in the NCBI database.*

Microsatellite	HW Panel	LR Panel	Fluorochrome	N. alleles	Allele size range (bp)	H_{obs}	H_{exp}	HW	CERVUS error	PEDANT error	T_m range	T_m	Accession N.	Reference
RHA8	P2HW	LR1	6FAM	9	146-177	0.709	0.709	NS	0.0037	0.014	55-57	56	JF750631	-
E95	P6HW	LR1	HEX	5	111-129	0.779	0.773	NS	0.0186	0.019	55-59	55	EU737094	Mao et al. (2009)[358]
RHA101	P3HW	LR1	HEX	5	143-151	0.504	0.512	NS	0.0179	0.012	56-57	56	JF750632	-
B63	P1HW	LR1	HEX	7	191-210	0.707	0.72	NS	0.0192	0.015	55-59	55	EU737086	Mao et al. (2009)[358]
RHA102	P3HW	LR1	HEX	4	286-299	0.555	0.561	NS	0.0076	0.008	55-61	56	-	-
Rhpu-PH30	P5HW	LR1	NED	4	175-186	0.354	0.361	NS	0	0.007	57-65	56	EF423561	Hua et al. (2009)[359]
C09/Rferr01	P3HW	LR2	6FAM	5	122-130	0.578	0.593	NS	0.065	0.037	56-59	55	AF160200	Rossiter et al. (1999)[360]
SHEF5/Rferr16	P10HW	LR2	6FAM	7	192-205	0.477	0.482	NS	0.108	0.010	59-65	58	AJ560696	Dawson et al. (2004)[361]
RHA104	P2HW	LR2	6FAM	6	272-299	0.371	0.381	NS	0	0.109	55.5-57	56	JF750633	-
SHEF3/Rferr13	P5HW	LR2	HEX	7	89-104	0.333	0.409	***	0.240	-	-	56	AJ560694	Dawson et al. (2004)[361]
A26	P2HW	LR2	HEX	6	187-212	0.656	0.656	NS	0.040	0.031	55-57	58	EU737082	Mao et al. (2009)[358]
Rhsi-SN91	P4HW	LR2	HEX	4	222-229	0.487	0.508	NS	0.103	0.038	57-63	63	EU780431	Liu et al. (2009)[362]
Rhpu-PH69A	P5HW	LR3	6FAM	4	107-119	0.576	0.625	**	0.025	0.035	57	56	EU559249	Hua et al. (2009)[359]
Rhsi-SN80	P1HW	LR3	6FAM	9	156-184	0.69	0.713	NS	0.029	0.037	57-64.5	61	EU780430	Liu et al. (2008)[362]
Rhpu-PD3	P5HW	LR3	6FAM	5	212-221	0.672	0.692	NS	0.014	0.027	57	57	EF423565	Hua et al. (2009)[359]
Rhpu-H3	P4HW	LR3	6FAM	11	280-320	0.82	0.829	NS	0.047	0.021	55-63	57	EF423570	Hua et al. (2009)[359]

Rhpu-A4	P5HW	LR3	HEX	4	190-202	0.421	0.411	NS	0.015	0.010	57	56	EF423560	Hua et al. (2009)[359]
E7	P1HW	LR3	HEX	9	274-295	0.709	0.747	***	0.052	0.053	55-59	56	EU737089	Mao et al. (2009)[358]
h07/Rferr12	P10HW	LR3	NED	5	215-229	0.393	0.392	NS	0.035	0.015	63.5-65	55	AF160211	Rossiter et al. (1999)[360]
D6	P6HW	LR4	6FAM	5	207-217	0.609	0.684	***	0.149	0.082	55-61.5	60	EU737088	Mao et al. (2009)[358]
SHEF13/Rferr30	P8HW	LR4	6FAM	12	243-290	0.784	0.779	NS	0.003	0.012	-	59	AJ560713	Dawson et al. (2004)[361]
Rhpu-E11A	P4HW	LR4	HEX	8	99-141	0.429	0.427	NS	0.026	0.012	57	60	EU559248	Hua et al. (2009)[359]
SHEF14/Rferr27	P7HW	LR4	HEX	6	161-183	0.623	0.622	NS	0.005	0.023	56-61	58	AJ560710	Dawson et al. (2004)[361]
SHEF2/Rferr18	P10HW	LR4	HEX	2	196-202	0.303	0.301	NS	0.018	0.029	59-65	62	AJ560698	Dawson et al. (2004)[361]
D02/Rferr03	P6HW	LR4	HEX	10	213-240	0.734	0.741	NS	0.018	0.020	57-65	57	AF160202	Rossiter et al. (1999)[360]
SHEF12/Rferr29	P10HW	LR4	HEX	19	276-354	0.863	0.884	NS	0.008	0.016	64.5-65,	59	AJ560712	Dawson et al. (2004)[361]
SHEF9/Rferr24	P8HW	LR4	HEX	3	375-379	0.196	0.278	***	0.923	-	-	59	AJ560706	Dawson et al. (2004)[361]
SHEF10/Rferr28	P8HW	LR5	6FAM	3	146-155	0.611	0.6	NS	0.019	0.032	55-65	62	AJ560711	Dawson et al. (2004)[361]
RHA118	P7HW	LR5	6FAM	3	217-231	0.354	0.36	NS	0	0.072	55-65	59	JF750636	-
SHEF11/Rferr25	P9HW	LR5	6FAM	13	298-392	0.819	0.843	NS	0.049	0.042	-	62	AJ560708	Dawson et al. (2004)[361]
SHEF6/Rferr19	P9HW	LR5	HEX	3	199-205	0.268	0.262	NS	0.036	0.010	-	62	AJ560702	Dawson et al. (2004)[361]
RHA4	P7HW	LR5	HEX	5	270-280	0.508	0.511	NS	0.068	0.023	55-65	59	-	-
SHEF8/Rferr22	P9HW	LR5	NED	7	184-197	0.397	0.382	NS	0.050	0.020	61.5-64.5	63	AJ560704	Dawson et al. (2004)[361]

Table S 2.3 *Categorical levels of fluorescence intensity measured in relative fluorescence units (RFU) for fluorochrome tags bound to PCR primers, as used by Ward (2013)*[266].

Fluorescence level	**RFU**
very low	0-500
low	500-1000
medium	1000-1500
high	1500-2500
very high	> 2500

Table S 2.4 *Null Distribution and P-Values for a Group-by-Individual Matrix with Year-Restricted Permutations using MCMC. The function was run with 500,000 permutations, over four chains, with a thinning interval of 50,000 and a burn-in of 500.* ***a)*** *shows the mean, median, standard deviation, and 95% upper and lower confidence intervals of the target statistic (mean group relatedness) obtained from the permutations. "ESS" refers to effective sample size - a measure of how well the MCMC algorithm has converged.* ***b)*** *shows the P-values for observing a random value greater than the observed value, less than the observed value, and two-tailed p-value, along with their 95% upper and lower confidence intervals.*

a)

Statistic	**Value**	**95% Upper CI**	**95% Lower CI**
Mean	0.0198	0.0167	0.0238
Median	0.0197	NA	NA
SD	0.00182	NA	NA
ESS	1787.164	NA	NA

b)

Comparison	***p*-Value**	**95% Upper CI**	**95% Lower CI**
Random > Observed	0.266	0.246	0.287
Random < Observed	0.734	0.713	0.754
Two-Tailed	0.532	0.509	0.556

Table S 2.5 *Results from GLMQAP analyses relating maternal and bi-parental kinship, body condition and age (year of birth) homophily to dyadic association probabilities. Maternal kinship was defined by shared matriline (0 or 1). Bi-parental kinship (here referred to simply as 'kinship') was derived by a pedigree-based additive genetic matrix. Original and adjusted p-values (via false discovery rate correction) are given.*

All Dyads

Predictor	Estimate	STD. ERROR	Z	*P*	P_{adj}	Predictor	Estimate	STD. ERROR	Z	*P*	P_{adj}
(Intercept)	-0.734	0.024				(Intercept)	-0.746	0.025			
Matriline	-0.042	0.035	-1.204	0.346	0.586	Kinship	0.275	0.186	1.477	0.227	0.459
Age	0.070	0.004	19.999	<0.0001	0.001	Age	0.070	0.004	20.060	<0.0001	<0.001
Sex	0.034	0.023	1.487	0.227	0.459	Sex	0.034	0.023	1.492	0.223	0.459
Body Condition	0.755	0.512	1.475	0.403	0.615	Body Condition	0.746	0.512	1.457	0.406	0.615

Female-Female Dyads

Predictor	Estimate	STD. ERROR	Z	*P*	P_{adj}	Predictor	Estimate	STD. ERROR	Z	*P*	P_{adj}
(Intercept)	-0.796	0.034				(Intercept)	-0.801	0.034			
Matriline	-0.044	0.058	-0.757	0.570	0.822	Kinship	-0.012	0.308	-0.040	0.991	1.000
Age	0.050	0.005	9.844	<0.0001	0.001	Age	0.050	0.005	9.884	<0.0001	0.001
Body Condition	0.059	0.016	3.815	0.027	0.094	Body Condition	0.059	0.016	3.789	0.025	0.090

Male-Male Dyads

Predictor	Estimate	STD. ERROR	Z	*P*	P_{adj}	Predictor	Estimate	STD. ERROR	Z	*P*	P_{adj}
(Intercept)	-0.379	0.052				(Intercept)	-0.400	0.053			
Matriline	-0.123	0.086	-1.418	0.267	0.499	Kinship	0.284	0.471	0.603	0.626	0.891
Age	0.140	0.012	11.961	<0.0001	0.001	Age	0.140	0.012	11.942	<0.0001	0.001
Body Condition	-0.050	0.027	-1.837	0.196	0.430	Body Condition	-0.050	0.027	-1.828	0.200	0.430

Immature Dyads

Predictor	Estimate	STD. ERROR	Z	*P*	P_{adj}	Predictor	Estimate	STD. ERROR	Z	*P*	P_{adj}
(Intercept)	-0.329	0.051				(Intercept)	-0.341	0.052			
Matriline	-0.089	0.053	-1.672	0.146	0.371	Kinship	0.127	0.578	0.220	0.853	1.000
Age	0.201	0.062	3.254	0.020	0.078	Age	0.202	0.062	3.270	0.021	0.079
Sex	-0.088	0.086	-1.016	0.393	0.615	Sex	-0.089	0.053	-1.677	0.138	0.371
Body Condition	0.080	0.019	4.152	0.008	0.037	Body Condition	0.080	0.019	4.145	0.005	0.025

Mature Dyads

Predictor	Estimate	STD. ERROR	Z	*P*	P_{adj}	Predictor	Estimate	STD. ERROR	Z	*P*	P_{adj}
(Intercept)	-0.959	0.039				(Intercept)	-0.972	0.039			
Matriline	-0.047	0.051	-0.921	0.449	0.667	Kinship	0.270	0.260	1.037	0.374	0.609
Age	0.046	0.005	9.828	<0.0001	0.001	Age	0.047	0.005	9.889	<0.0001	<0.001
Sex	0.144	0.034	4.184	<0.0001	0.001	Sex	0.144	0.034	4.197	<0.0001	<0.001
Body Condition	-0.032	0.012	-2.736	0.063	0.199	Body Condition	-0.032	0.012	-2.758	0.063	0.199

Immature Female-Female Dyads

Predictor	Estimate	STD. ERROR	Z	*P*	P_{adj}	Predictor	Estimate	STD. ERROR	Z	*P*	P_{adj}
(Intercept)	-0.509	0.066				(Intercept)	-0.446	0.069			
Matriline	0.282	0.133	2.118	0.082	0.244	Kinship	-1.487	0.998	-1.490	0.197	0.430
Age	0.342	0.101	3.390	0.012	0.051	Age	0.328	0.101	3.252	0.015	0.061
Body Condition	0.070	0.031	2.298	0.144	0.371	Body Condition	0.071	0.031	2.317	0.146	0.371

Immature Male-Male Dyads

Predictor	Estimate	STD. ERROR	Z	*P*	P_{adj}	Predictor	Estimate	STD. ERROR	Z	*P*	P_{adj}
(Intercept)	-0.205	0.125				(Intercept)	-0.264	0.127			
Matriline	-0.519	0.237	-2.186	0.068	0.208	Kinship	-0.272	1.664	-0.164	0.900	1.000
Age	0.051	0.162	0.313	0.793	1.000	Age	0.054	0.161	0.334	0.783	1.000
Body Condition	0.098	0.056	1.766	0.158	0.371	Body Condition	0.092	0.055	1.659	0.176	0.404

Mature Female-Female Dyads

Predictor	Estimate	STD. ERROR	Z	*P*	P_{adj}
(Intercept)	-0.854	0.054			
Matriline	-0.122	0.087	-1.394	0.263	0.499
Age	0.033	0.007	4.795	0.004	0.021
Body Condition	0.038	0.020	1.874	0.232	0.459

Predictor	Estimate	STD. ERROR	Z	*P*	P_{adj}
(Intercept)	-0.875	0.054			
Kinship	0.223	0.417	0.536	0.640	0.898
Age	0.034	0.007	4.895	0.004	0.021
Body Condition	0.036	0.020	1.804	0.251	0.488

Mature Male-Male Dyads

Predictor	Estimate	STD. ERROR	Z	*P*	P_{adj}
(Intercept)	-0.400	0.076			
Matriline	-0.107	0.117	-0.908	0.466	0.682
Age	0.125	0.017	7.372	<0.0001	0.001
Body Condition	-0.066	0.034	-1.940	0.150	0.371

Predictor	Estimate	STD. ERROR	Z	*P*	P_{adj}
(Intercept)	-0.407	0.077			
Kinship	-0.199	0.671	-0.297	0.813	1.000
Age	0.125	0.017	7.365	<0.0001	0.001
Body Condition	-0.065	0.034	-1.913	0.152	0.371

Clusters

Predictor	Estimate	STD. ERROR	Z	*P*	P_{adj}
(Intercept)	-2.584	0.069			
Matriline	-0.135	0.107	-1.270	0.355	0.589
Age	0.245	0.018	13.677	<0.0001	0.001
Sex	0.083	0.064	1.295	0.294	0.529
Body Condition	4.543	1.621	2.802	0.158	0.371

Predictor	Estimate	STD. ERROR	Z	*P*	P_{adj}
(Intercept)	-2.612	0.069			
Kinship	0.654	0.571	1.146	0.348	0.586
Age	0.247	0.018	13.728	<0.0001	0.001
Sex	0.084	0.064	1.322	0.284	0.521
Body Condition	4.560	1.623	2.810	0.154	0.371

Table S 2.6 *Results from post-hoc GLMQAP analysis relating maternal and bi-parental kinship, body condition and year of birth homophily to dyadic association probabilities. Year of birth similarity was given a binary score (0= different year of birth, 1= same year of birth). Maternal kinship was defined by shared matriline (0 or 1). Bi-parental kinship (here referred to simply as 'kinship') was derived by a pedigree-based additive genetic matrix. Original and adjusted P-values (via false discovery rate correction) are given.*

Immature Dyads

Predictor	**Estimate**	**STD. ERROR**	**Z**	***p***	p_{adj}
(Intercept)	-0.530	0.073			
Year of Birth	0.201	0.062	3.254	0.001	0.040
Sex	-0.088	0.086	-1.016	0.408	0.615
Body Condition	0.080	0.019	4.152	0.005	0.025

Mature Dyads

Predictor	**Estimate**	**STD. ERROR**	**Z**	***p***	p_{adj}
(Intercept)	-1.134	0.039	NA	NA	
Year of Birth	0.258	0.043	5.965	0.000	0.001
Sex	0.131	0.034	3.822	0.001	0.007
Body Condition	-0.018	0.011	-1.603	0.298	0.529

Clusters

Predictor	**Estimate**	**STD. ERROR**	**Z**	***p***	p_{adj}
(Intercept)	-0.530	0.073			
Year of Birth	0.201	0.062	3.254	0.001	0.040
Sex	-0.088	0.086	-1.016	0.408	0.615
Body Condition	0.080	0.019	4.152	0.005	0.025

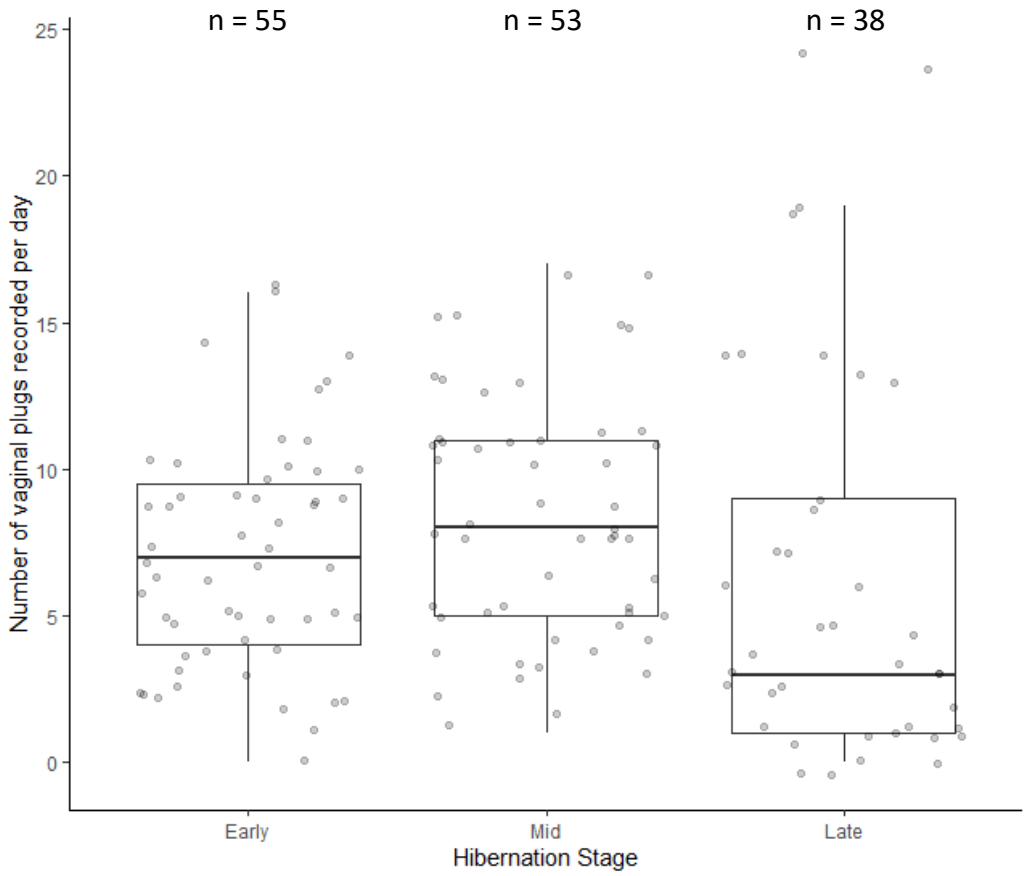

Figure S 2.1 *Counts of vaginal plugs increase in mid-hibernation and decline by late-hibernation, potentially following ejection by the breeding female. Data from each 'stage' are collected through two surveys conducted within a month-long period across core and outlier sites. 'Early', 'Mid', and 'Late', correspond to Late-October/November, January/February, and March/April respectively. Vaginal plugs form post-copulation from secretions emitted by both male and female. They can be detected in mature females via palpation of the lower abdomen.*

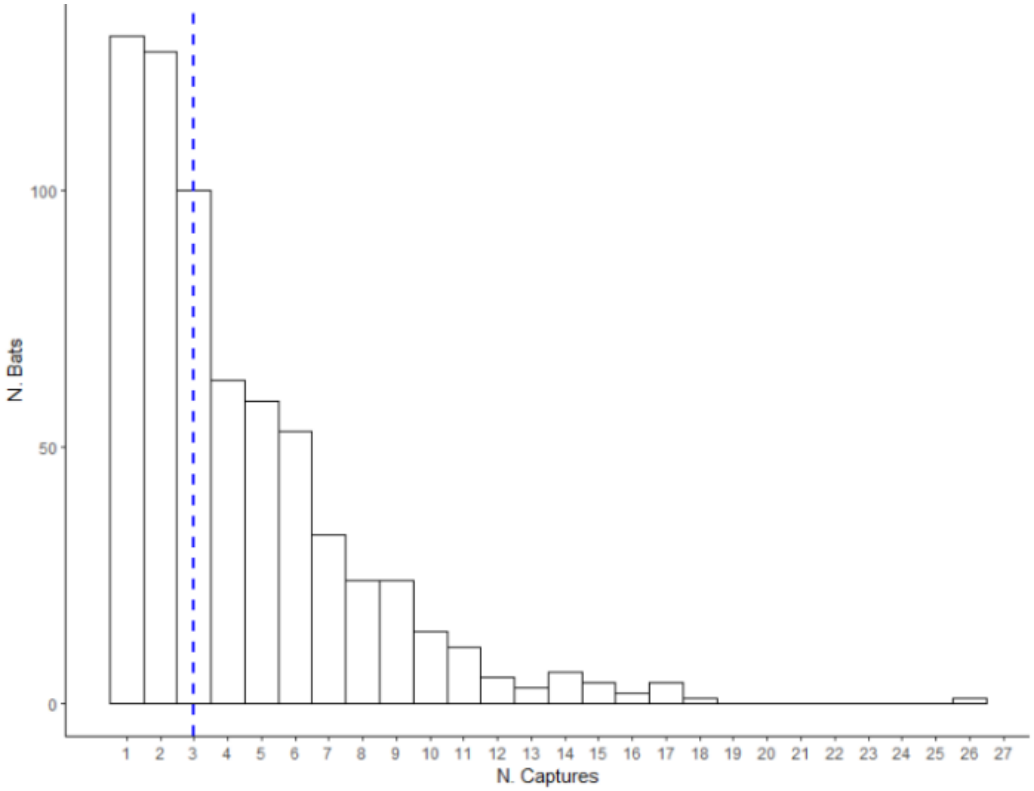

Figure S 2.2 *Number of captures per bat over the 12-year study period. I included only individuals caught at least the median number of times (dashed line) in network analyses to reduce uncertainty in association indices.*

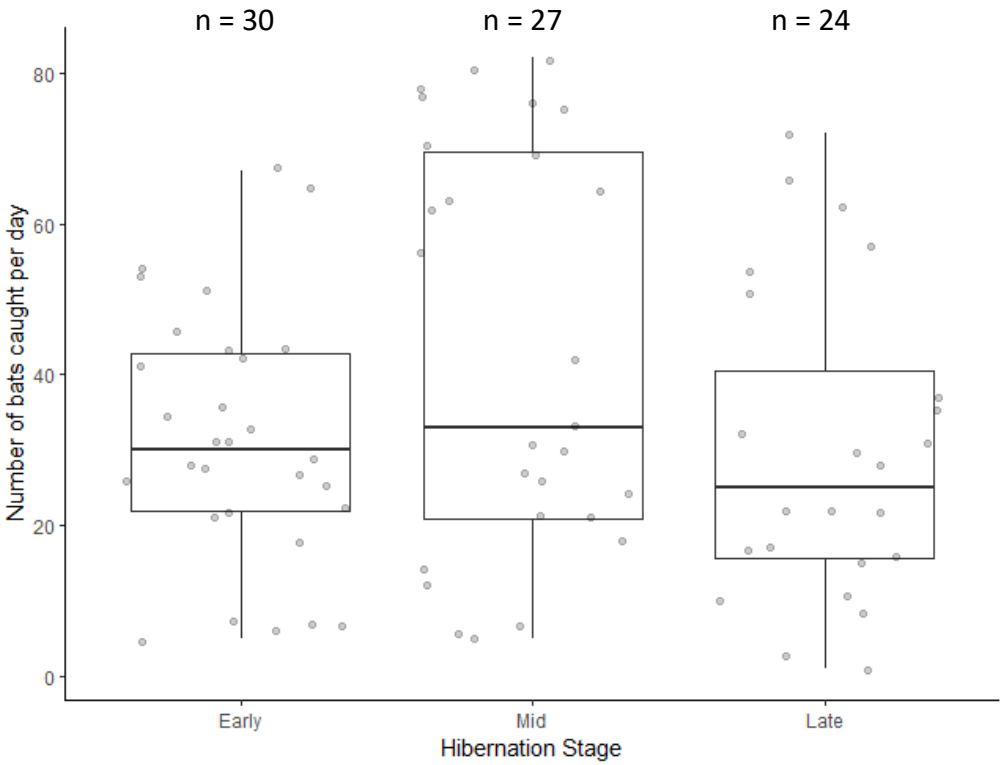

Figure S 2.3 *Variation in hibernation roost counts per hibernation stage. The plurality of counts occurs in mid-hibernation. Data from each 'stage' are collected through two surveys conducted within a month-long period across core and outlier sites. 'Early', 'Mid', and 'Late', correspond to Late-October/November, January/February, and March/April respectively.*

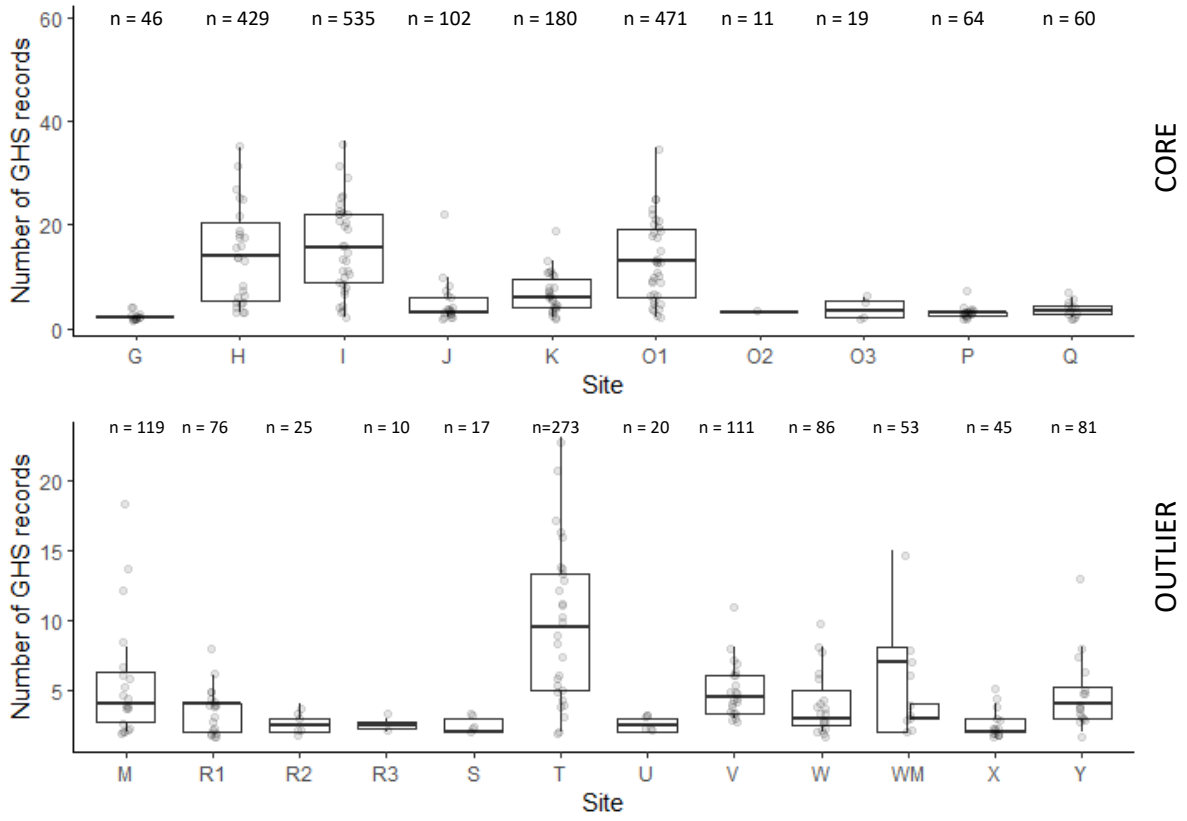

Figure S 2.4 *Boxplots showing variation in hibernation roost counts of R. ferrumequinum (GHS) at core and outlier sites. "WM" refers to the attic and the adjacent hibernaculum at Woodchester Mansion, Gloucestershire. See* ***Table S 2.1*** *for site IDs.*

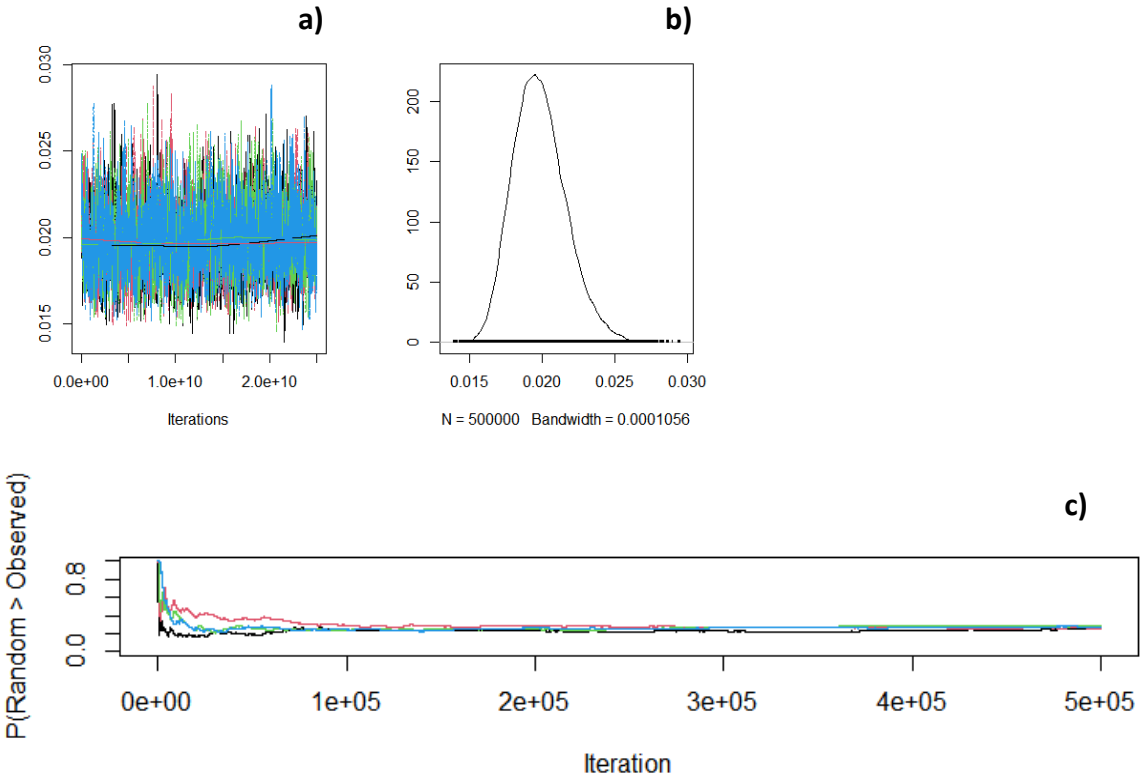

Figure S 2.5 *Diagnostic plots used to check convergence of the MCMC chains.* ***a)*** *Trace plot showing convergence of the MCMC chains. Y-axis shows sampling of the target statistic – mean group relatedness* ***b)*** *The null distribution of the target statistic – mean group relatedness (x-axis).* ***c)*** *shows changes in the estimated p-values for each chain over 500,000 iterations*

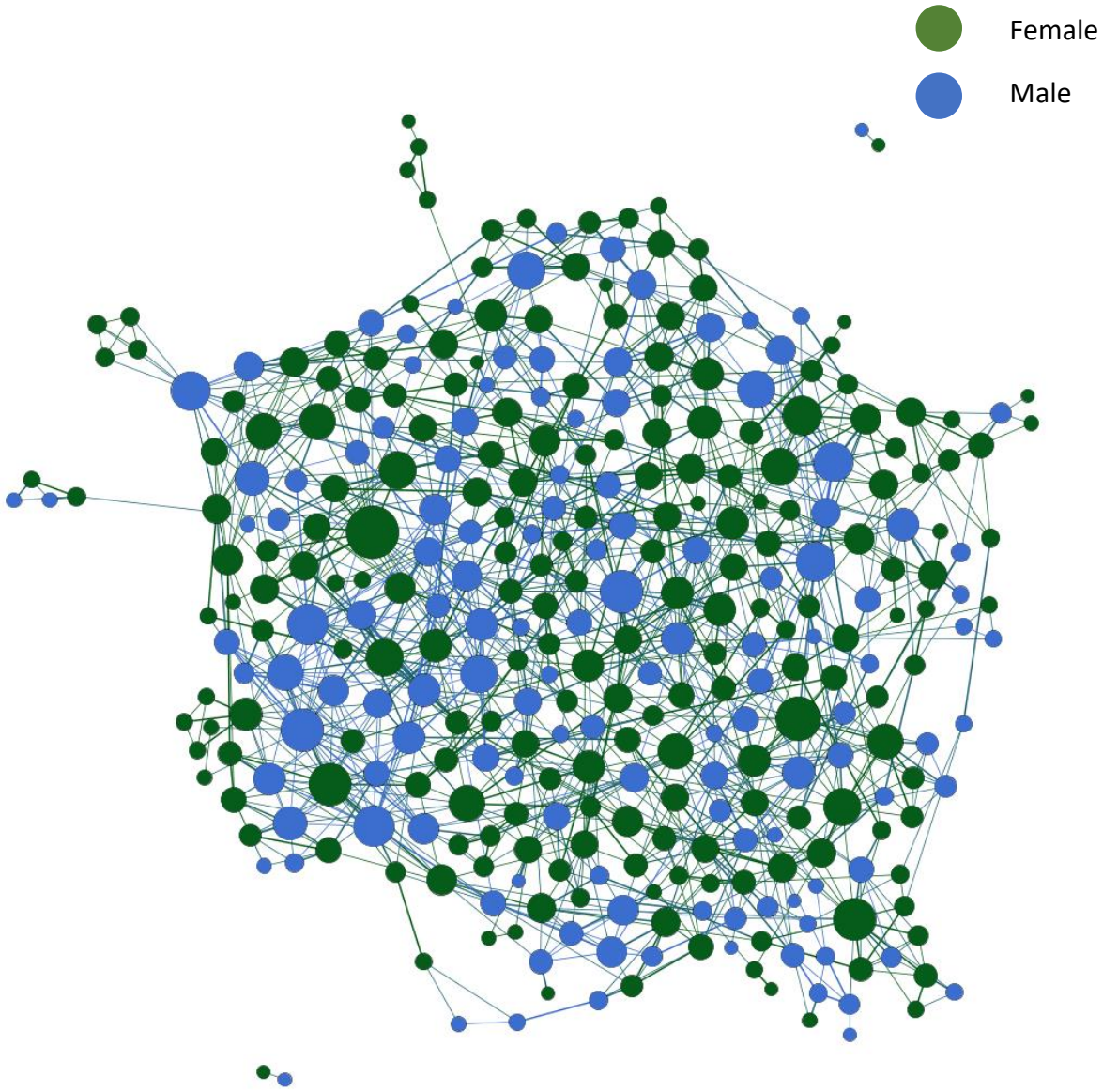

Figure S 2.6 *Bi-parental kinship network (n=345). Blue nodes=males, green nodes=females. Edges represent first-order relations (r > 0.24) among bats. Bats with no relations are not shown. Node size varies according to degree (number of connections).*

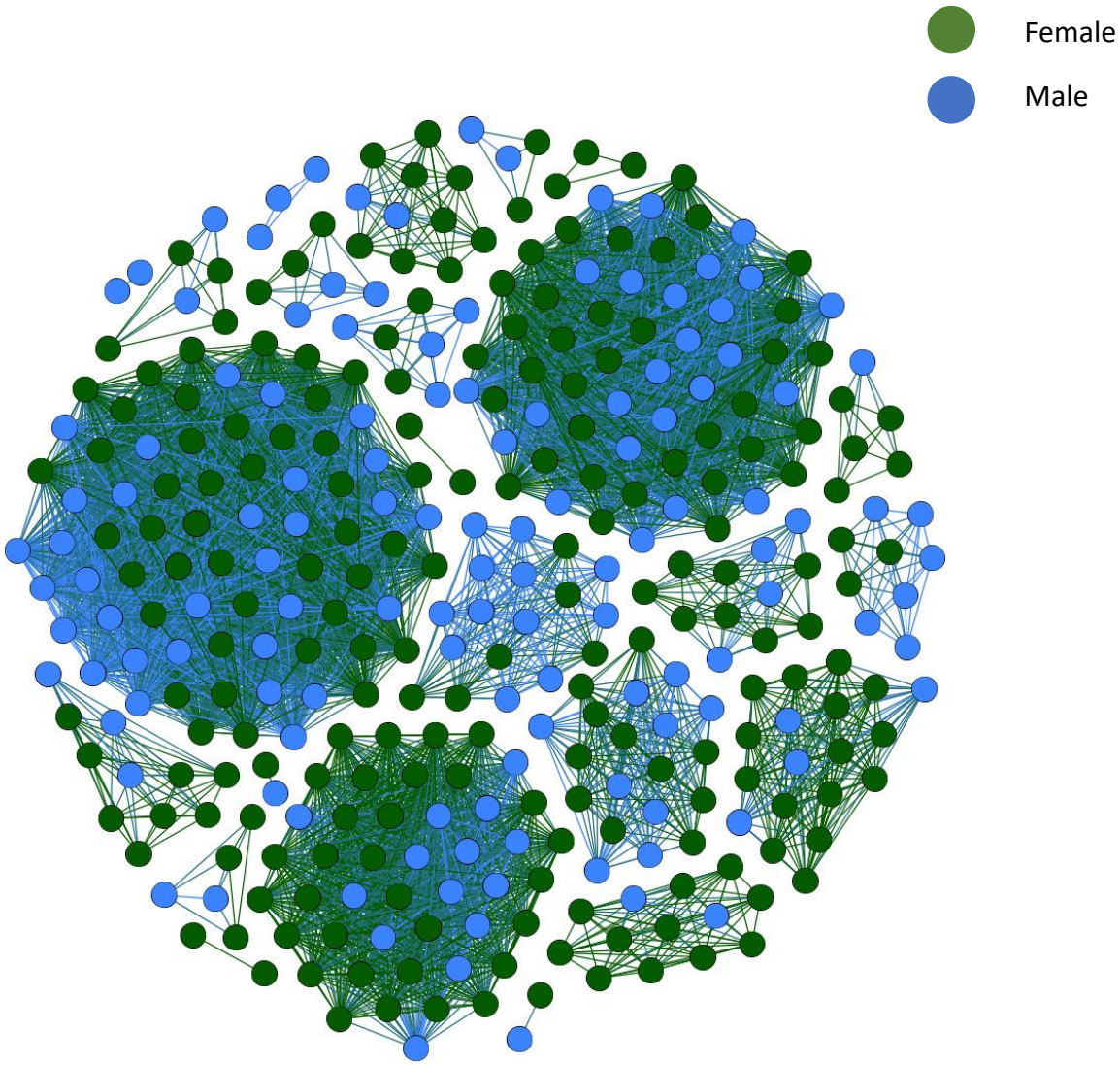

Figure S 2.7 *Matriline Network (n=345). Edges represent shared matriline. Blue nodes=males, green nodes=females. Matriline' was defined as the oldest female founder in a maternal lineage to which an individual belonged.*

Chapter 3

The social alarm-clock hypothesis: do lesser horseshoe bats (*Rhinolophus hipposideros*) arouse neighbours from hibernation?

The cluster of lesser horseshoe bats (*Rhinolophus hipposideros*) in the hibernaculum at Woodchester Mansion, Gloucestershire. Photo credit: L Romaine

3.1 Abstract

Hibernating bats need to make effective decisions regarding the timing of energetically costly arousals when fat reserves and prey availability are limited over winter. For some species, such decisions are made in a group context. Rhinolophid bats form loose clusters in which skin-contact is rare. Why they should do so is intriguing considering individuals receive little benefit of social thermoregulation alongside the potential cost of disturbance from conspecifics. One potential benefit is access to social information (provided it is accurate). Over winter, suitable foraging conditions are an ephemeral, but critical, resource in maritime climates. The "social alarm-clock" hypothesis proposes that torpid individuals may use the activity of nearby normothermic conspecifics as an inadvertent social cue indicating favourable conditions to arouse, typically during warm nights at sunset. Consequently, arousal is hypothesised to spread via contagion with individuals with the greatest access to social information (likelihood of disturbance) being the most likely to arouse. A thermal camera was used to record periodic arousals and social interactions within a cluster of lesser horseshoe bats (*Rhinolophus hipposideros*) over winter. Group structure, defined by spatial proximity, was estimated as a social network. Sequential arousal cascades were observed, meeting the prediction of social contagion. However, individuals largely used temperature cues to decide when to arouse from torpor on warm nights and were not solely reliant on the actions of conspecifics. Nevertheless, I find significant evidence for social transmission of arousals in October, but not in later months. Deep torpor in mid- to late-winter may preclude sensitivity to social cues. In addition, generalised estimating equations within a general linear model framework revealed that centrally positioned bats were more likely to arouse – supporting the social-alarm clock hypothesis. While not conclusive, my results provide insight into the proximate benefits of group-living, as well as the factors influencing periodic arousals in hibernating bats.

3.2 Introduction

Living in a seasonally cold environment can present significant challenges upon the arrival of inclement weather and restricted food availability. To overcome these conditions, many animals have evolved the ability to enter torpor, allowing metabolic function to sharply decline and body temperature (T_b) to fall within 0.5-2 °C of the ambient temperature (T_a) [183,363,364]. Extended bouts of torpor – hibernation – represent a critical energy-saving adaptation against severe environmental conditions [211], allowing for increased survival rates, and ultimately, enhanced life spans [234,235].

Contrary to popular belief, most animals that hibernate – hibernators - do not remain torpid continuously [121,233,325,365,366]. Doing so can reduce opportunities to reproduce and to restore limited fat reserves through foraging [175,189,213,324,325,333]. Prolonged torpor can result further in suppressed immunological function [367], the accumulation of metabolic waste [366], sleep deprivation [368], and predation [213,369]. Rather, bouts of torpor are periodically interrupted by arousals and a return to normothermic T_b [121,233,325,365,366]. Yet, re-warming and staying warm, too, comes at a significant energetic cost - representing 80-90 % of the energy expended during the hibernation period [370,371]. Small endotherms experience this problem acutely, suffering from a high thermal conductance with the external environment, due to their high surface-area-to-volume ratio and limited room to insulate with fat, fur, or feathers [372]. The optimal hibernation strategy, therefore, should involve decisions that carefully balance the torpor-normothermia trade-off, minimising energetic and ecological costs [213,373].

Phenology is a critical element of the hibernation strategy. A hibernator's energy balance is regulated according to when and for how long periods of torpor or normothermy are maintained. Optimal decisions is this respect are contingent on when opportunities to accumulate or conserve energy occur [209,233,374–376]. For example, a strong synchronisation of arousal time and sunset has been demonstrated in studies of hibernating insectivorous bats in temperature latitudes [323,325,377–379]. This is probably so that bats can capitalise on foraging opportunities during mild winter nights when insects are in higher abundance and predation risk is lower [233,325,380–382].

Mechanisms underlying the timing of arousal are not fully understood, but probably rely on a combination of endogenous and external cues [121,322,365,383]. An established body of

evidence exists regarding the influence of microclimate selection on arousal frequency, given the temperature dependence of torpor expression [213,214,233,384]. However, an individual's social environment may also play an important role [216]. Sensitivity to both tactile and non-tactile disturbance (e.g. temperature increase and noise), including from conspecifics, is retained by bats in torpor [385–390]. Even in the absence of photoperiodic stimuli, social cues from conspecifics can initiate "arousal cascades" as shown in studies of *Myotis* bats, whereby a normothermic individual disturbs and triggers the arousal of a neighbouring individual, initiating a domino effect of arousals across the group [387,391,392]. For species that huddle in groups, these collective arousals may be critical to sharing mutual thermal benefits [119–121,393] and deterring fungal infection [394], offsetting the energetic costs of disturbance. However, if disturbance is frequent, responding to these 'false alarms' may become maladaptive [121,387]. That said, individuals appear to be capable of deciding when to fully arouse through a mechanism known as "cold arousal", a brief rise and fairly rapid decline in T_b, during which the bat might classify the quality of the external stimuli (e.g. a predator vs inadvertent conspecific disturbance) [391,392,395].

For species that do not huddle, the benefits to group-living are less apparent, given equivalent exposure to conspecific disturbance without the benefits of social thermoregulation [109]. Rhinolophid bats rarely maintain bodily contact when roosting in small clusters over winter [122,199,246], despite being potentially vulnerable to high thermal conductance and water loss during periodic arousals [363,396–398].

The integration of social information to guide effective decision-making is a well-studied benefit of group-living in animals, including bats [399–401]. In groups, conspecific cues can provide access to information beyond an individual's own sensory inputs [402–404]. For instance, an individual engaged in foraging behaviour might indicate to others the presence of feeding resources that they would otherwise learn through costly trial-and-error [79,405]. I propose if personal information derived by interpreting abiotic cues e.g. T_a change, is either costly to acquire, or not sufficiently persuasive to trigger arousal alone, a nearby normothermic bat could represent useful, inadvertent, social information on the presence of favourable foraging conditions, a predator, or potential mate (assuming the individual is sensitive to such cues). I term this the "social-alarm clock" hypothesis. Interestingly, observations by Ransome (*pers. obs*.) of lesser horseshoe bats (*Rhinolophus hipposideros*)

actively nudging their roost mates suggests some individuals play an active role in arousing conspecifics from torpor. Alternatively, asocial learning may be preferred in cases where acting on social information is risky. For example, if conspecific cues are unreliable (i.e. if conspecific arousals are not frequently associated with useful information) and/or the interests of those transmitting information conflict significantly with the receiver (such as contrasting physiological requirements) [406,407].

I focused on a hibernating cluster of *R. hipposideros* to explore the possibility of a social-alarm clock i.e. social transmission of arousals, in rhinolophid bats during later autumn and winter. A thermal camera was positioned to record periodic arousals and social interactions within the cluster. I tested the "social-alarm clock" hypothesis, which proposes that individual *R. hipposideros* group together to receive socially transmitted cues on suitable foraging conditions. Such cues could be transmitted between individuals by tactile or non-tactile stimuli e.g. echolocation and radiant heat.

I evaluated support for social transmission of arousals in bats by using several methods. First, I examined whether the bats synchronised their arousals with sunset, and did so collectively in cascades, as might be expected if arousals were socially facilitated. Equally, such behaviour might be explained by synchrony of endogenous circadian rhythms. Next, I estimated the spatial arrangement and connectivity of groups as a social network. By combining data on individual arousal phenology and association patterns, I identified the sequence of arousal propagation and tested whether it spreads through pairwise interactions. I predicted individuals with weaker ties would be less likely to receive 'social information' (stimuli from a normothermic conspecific) and arouse. Lastly, I studied the effect of emergent group structure on collective arousal time. Ransome (1968)[199] observed that a tightly clustered group of *R. ferrumequinum* aroused fully in less time than one where individuals were sparsely distributed. Consequently, I predicted well-connected groups might generate more 'social information' and propagate it faster [402], leading to lower variance in arousal time. When variance in arousal timing is low, collective arousals become more beneficial for individuals due to diluted predation risk [125,134,380,408].

3.3 Materials and Methods

3.3.1 Study site

The study was carried out over the winter of 2020/2021 within a disused wood storage tunnel, adjacent to Woodchester Mansion (51°43'N, 2°18'W). The tunnel is sub-divided into chambers to achieve contrasting airflow regimes, intended to suit the thermal requirements of rhinolophid bats during the hibernation period. A single chamber (2.5 m x 2.5 m x 3 m), the focus of this study, is used by up to ~80 wild *R. hipposideros* each winter (**Table S 3.1**). During the study, the chamber experienced variable temperatures of between 5.9 °C and 16.8 °C, with a mean temperature of 9.6 °C (**Table S 3.1**, **Figure S 3.2**). The surrounding habitats are managed to provide foraging opportunities for rhinolophid bats, including the stocking of sheep and cattle over winter to promote the availability of dung-dwelling insects [324].

3.3.2 Equipment set-up

Thermogenesis and arousal from torpor in the bats was captured using infrared thermography (IRT). The use of IRT has been used successfully to capture arousals in hibernating mammals, providing a less invasive alternative to temperature-sensitive radio-transmitters [409–411]. IRT provides additional utility in that tactile social interactions leave a visible thermal trace [412]. However, the ability to capture and account for individual variation is compromised as individual identities, at least in this species, cannot be recognised.

I measured the relative skin surface temperature (T_{skin}) as a T_b proxy of the roosting bats with a thermal imaging camera (Optris PI640 thermal imaging camera, 640×480-pixel resolution, 33° x 25° lens (F=0,8), 0.1°C-temperature resolution; Optris GmbH, Berlin, Germany) attached to a tripod. The camera was positioned in the chamber and directed at approximately 50° towards, and 2.5 metres away from, the cluster of bats on the ceiling (**Figure 3.1**). The chosen position and angle of the camera was a compromise between maximising the number of bats in frame and minimising damage to the camera from falling debris, water, and excreta. However, an acute angle between the camera and subject can create perspective distortion, foreshortening objects that are equidistant, potentially reducing the accuracy of measurements of distance between bats. The distance of the IRT camera to the object may further impact the accuracy of temperature measurements, thus values of T_{skin} shown here should be viewed as an index rather than true measurements [409].

The camera was calibrated according to default reference data using Optris PI Connect Software (Optris). I set the camera's internal mechanical calibration device to flag every 12 seconds to account for thermal drift [409]. A USB extension lead connected the camera to a laptop computer (Dell XPS 15, Dell, Austin, TX, USA) in a separate chamber to minimise disturbance of the bats during the recording initialisation (**Figure 3.1**). Recordings were scheduled to start at least one hour before astronomical sunset – the time at which the sun disappears entirely below the horizon using Optris PI Connect Software v 120 (Optris), recording in *.ravi* video format at three frames s^{-1}. Video recording took place for 69 days over the months of October, December, February, and March. Recording start times were re-adjusted at the beginning of each period to reflect changes in day length and sunset time (**Table 3.1**). I recorded five hours per night (320 hours total). Five nights (10th October 2020, 8th, 12th, 14th, 16th March 2021) were lost due to technical malfunctions, leaving a final total of 64 nights of recordings. 24-hour activity data derived from echolocation call analysis (unpublished) showed a peak in activity around sunset. The five-hour recording window was chosen as a compromise between maximising the amount of activity recorded per night and minimising file size, thus allowing more nights to be captured sequentially without human disturbance to the cluster. Nevertheless, some arousal cascades outside of the recording window may have been missed.

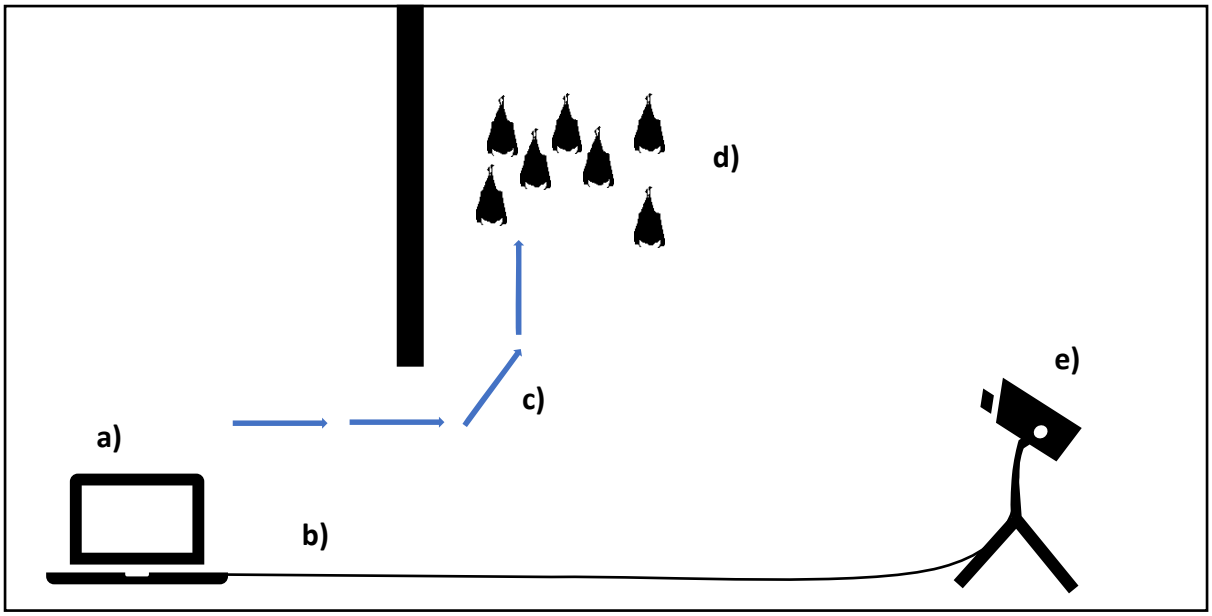

Figure 3.1 *Simplified schematic showing the equipment set-up (not to scale). In one chamber, a laptop* ***a)*** *was connected via a USB cable* ***b)*** *to a thermal imaging camera* ***e)*** *in a second chamber* ***c)****. The camera was angled towards a hibernating cluster of* R. hipposideros ***d)*** *roosting on the arched wall/ceiling. The blue arrows show the assumed direction of air flow from the first into the second chamber.*

Table 3.1 *Details of sampling blocks and recording schedule for each month over the study period (October 2020-March 2021)*

Sampling block	Month	Recording Days	Time of recording start (UTC+0)	Mean sunset time (UTC+0)
1	October	5th-13th	16:00	17:30
2	October	23rd-31st	16:00	16:52
3	December	16th-31st	14:00	16:04
4	February	7th-22nd	16:00	17:23
5	March	1st-19th	16:30	18:05

3.3.3 Determination of arousals

Each video recording was analysed using Optris PI Connect software. All torpid bats maintained a consistent ~0.3ºC T_{skin} above the T_a and were readily detectable against the background surface (**Figure 3.2**). Counts made through video recordings were compared to counts made by torchlight at the beginning of each sampling block to ensure accuracy. All bats in frame were assigned an ID number. Those that aroused during the video sequence were assigned a 5-pixel diameter circular measurement area to extract time series of maximum T_{skin}. These included 'cold' arousals – a moderate rise and decline in T_{skin} [391,392]. Another 5-pixel measurement area was assigned to the same section of wall to record a proxy of T_a.

The timing of arousal for each bat with time-series data was determined using non-parametric changepoint analysis, which does not assume a normal distribution of the underlying data [413,414]. Changepoint analysis seeks to identify the optimal segmentation of time series data, allocating a changepoint or "breakpoint" between segments when the statistical properties of the time series change significantly – thus providing a more objective and higher-throughput alternative to manual analysis of biological time series data [415,416].

I used the 'changepoint.np' function from the *changepoint.np* package [417] in R v 4.2.2 [418]. Multiple changepoints were detected using the pruned exact linear time (PELT) algorithm, which compares most favourably in terms of computational cost, whilst maintaining sufficient accuracy, against alternative algorithms e.g. the segment neighbourhood method [416,419]. I assigned a minimum segment length of 120 seconds (equivalent to two minutes of video) and the number of quantiles as 4(log)n, as recommended in Haynes et al., (2016) [414]. The PELT algorithm employs a cost function, whereby a penalty value (β) is employed to prevent overfitting. Higher values result in fewer changepoints, and *vice versa*. Given the irregularity of each timeseries dataset e.g. differences in length and number of artefacts, I was unable to find a consistently appropriate penalty value for every timeseries. A solution is to use the Changepoint for a Range of Penalties (CROPS) algorithm proposed by Haynes et al., (2017)[420]. CROPS scans systematically across a given range of β (βmin, βmax) and locates the optimal segmentations. I used a βmin of 8 and βmax of 800. The approach suggested by Lavelle (2005)[421] uses the output to give the number of changepoints as a function of β. The

"elbow" point represents the shift from under to over fitting, giving the most parsimonious choice of β [414]. To maintain a consistent definition of the "elbow" point, I used the ElbowFinder function within the *RclusTool* R package [422]. Here, a line is drawn from the first to the last point of the curve. The "elbow" point represents the point of maximum curvature.

The constraints of this model frequently resulted in extraneous changepoints that were not always of biological interest. For downstream analysis, I manually selected three points of interest according to the following strict definitions: arousal start (a consistent increase in T_{skin}), arousal end (plateau of T_{skin}), and exit (a sudden drop in T_{skin} when the bat leaves its roosting post). In cases where at least one changepoint of interest was not identified, an alternative segmentation solution given by CROPS was chosen (105 cases), or the missing changepoint was manually selected according to the above definitions (38 cases).

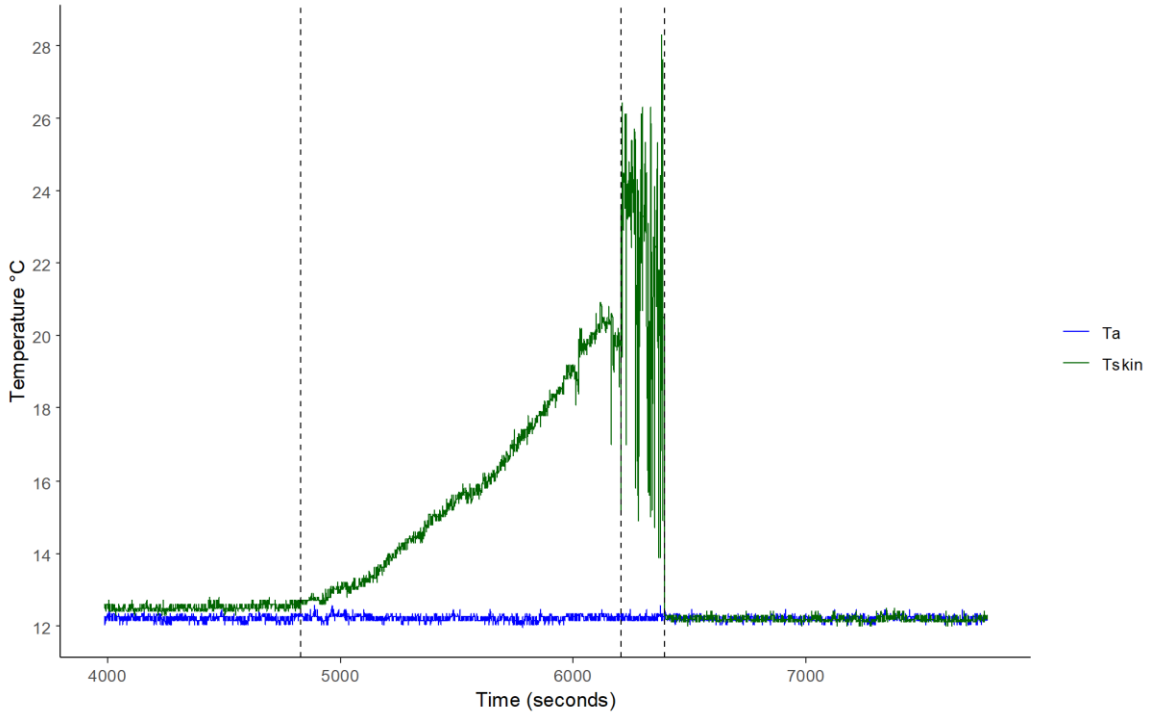

Figure 3.2 *Time series of skin surface temperature (T_{skin}) for an individual* R. hipposideros *(shown in dark green) compared to the ambient temperature (T_a), measured as the wall surface temperature (blue). Also shown are the assigned changepoints (dashed black line) for the start of arousal, end of arousal, and the bat exiting its roost position.*

3.3.4 Constructing spatial proximity networks

I assume the intensity of disturbance to torpid conspecifics (the opportunity for social transmission) is a function of distance from the nearest normothermic bat. Therefore, the spatial proximity should give the probability of an individual being disturbed (learning the target behaviour from social information) [423]. For each night, I produced undirected weighted networks based on the inverse Euclidean distances between all torpid bats in the cluster. XY coordinates of each bat were measured from screenshots (3840 x 2160 pixels) of video footage using ImageJ v 1.53 [424]. Euclidean distance matrices were calculated in R and inversed to give a measure of edge weight (**Figure 3.3a**). Individual centrality measures were then calculated using the *strength* function in the igraph R package [294], by summing edge weights adjacent to each individual (node) (**Figure S 3.3**).

3.3.5 Statistical analysis

3.3.5.1 Collective synchronisation with sunset

To test whether the bats collectively synchronised their arousals with sunset, I first converted the arousal start time of each bat to seconds before or after sunset in radians. Astronomical sunset times for the study site (51°43'N, 2°18'W) were extracted by the suncalc R package [425]. Within the circular R package [426], I performed a Rayleigh test of uniformity for each separate month, where the alternate hypothesis is a unimodal (random) distribution with an unknown mean direction (*mu*). The mean direction was set to 0 (sunset). Finally, time-series were inspected to identify whether arousal episodes occurred in isolation (not overlapping with another conspecific) or in sequences of overlapping arousals i.e. "cascades". Observation of arousal cascades would be consistent with the hypothesis of social transmission of arousals [427], or simply individual delay in response to an abiotic cue [423,428].

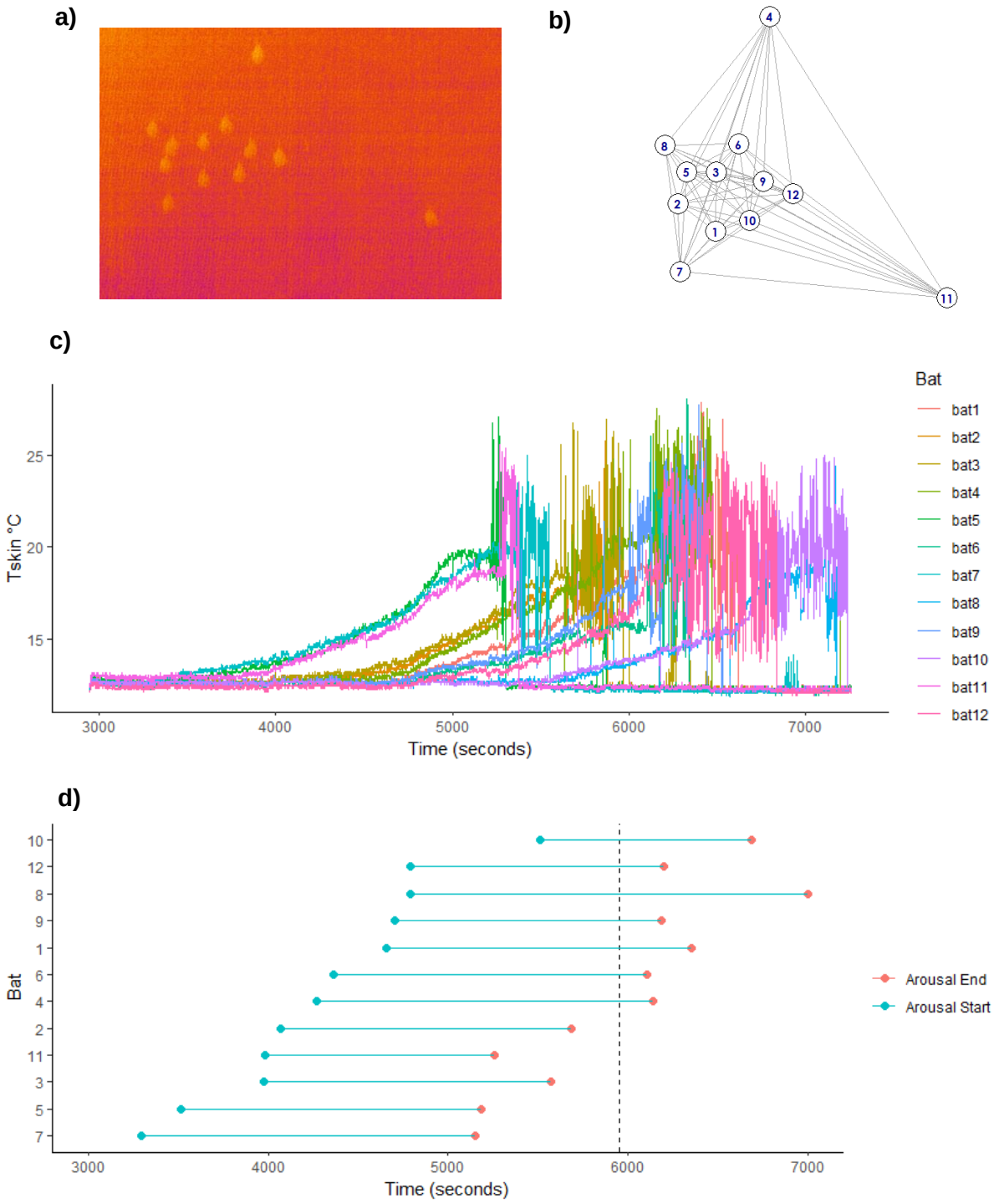

Figure 3.3 a) *A thermal image of the* R. hipposideros *cluster* ***b)*** *A representative spatial-proximity network of hibernating* R. hipposideros*. Node positions show roosting positions of individual bats.* ***c)*** *Raw time series of skin temperature (T_{skin}) in °C for each individual bat, represented by a line colour.* ***d)*** *A representative arousal cascade. Individual bats are ordered by arousal start time. Blue and red dots show the start and end of an arousal episode, respectively, as determined by changepoint analysis (see section 3.3.3). The vertical dotted line shows sunset on the night of observation (5th October 2020). Bat IDs on the y-axis correspond directly to T_{skin} traces in panel c and node labels in panel b.*

3.3.5.2 Evaluating support for social transmission

Second, following a similar approach to Aplin et al., (2012)[79], I used three methods to investigate whether or not arousal could be socially transmitted. If so, the pattern of associations should predict the observed pattern of arousals. Network-based diffusion analysis (NBDA) identifies whether or not the diffusion of behaviour through the group reflects network structure [78,429–431]. NBDA models a 'simple' contagion, where an individual acquires a behaviour ('learns') directly from its neighbours, making no judgement, and the likelihood of learning depends on the sum of one's connections [77,432]. If social transmission by simple contagion is identified, two measures are reported: 1) *s* the rate of social transmission per unit network connection compared to the baseline asocial learning rate. 2) *%ST* the percentage of events occurring by social transmission [429,430].

Next, I ask whether network position can predict whether a) an individual arouses on a given night ('arousal state') and b) the timing of arousal. Individuals with varying network position should also vary in their ability to receive information and express arousal.

Given my less invasive approach, I could not account for the likely repeated measures of the same individuals present over multiple nights. Hence, parameter estimates should be interpreted with caution, considering potential non-independence of residuals [433]. All continuous response variables in linear mixed models (LMMs) were transformed to normality using the bestNormalize R package [434], which identifies the optimal transformation to achieve the best fit to a normal distribution.

3.3.5.3 Network-based diffusion analysis (NBDA)

Following the protocol and code provided by Hasenjager et al., (2021)[430] within the NBDA package, I fitted an Order of Acquisition (OADA) model. OADA assumes the baseline asocial acquisition rate (the rate at which an individual "learns" a behaviour without social transmission) is the same for all individuals, but does not assume its shape over time, unlike less conservative alternatives such as Time of Acquisition Diffusion Analysis (TADA) [431]. Individuals were ordered by their arousal start time. I combined data from all nights where at least two individuals aroused, into a single model, to increase statistical power.

Akaike's information criterion corrected for small sample size (AICc) was used to compare support between the social and asocial models, ranking the probability that either model was the best Kullback-Leibler model, given the dataset [435–437]. Models within two ΔAICc are

assumed to have equal support [437]. In addition, a likelihood ratio test (LRT) was used to estimate the strength of evidence against the null hypothesis (asocial learning), and to receive a p-value. 95% confidence intervals (CI) around the values of *s* and *%ST* were then used to evaluate the strength of evidence for social transmission. 95% CIs were generated following the profile likelihood technique approach recommended by Morgan (2010)[438], which allows for asymmetry in the uncertainty of parameter estimates. Profile log-likelihood curves are then generated against the parameter estimate. Where the curve crosses the y-intercept, at 1.92 units above the minimum value of the negative log-likelihood of the fitted model, is the upper and lower 95% CI (**Figure S 3.4**).

3.3.5.4 Individual-level analysis

To investigate the influence of network measures on individual arousals I used (generalised) linear mixed models (G)LMM in R (*lme4* package [439]). First, a GLMM with a binomial (Bernoulli) error distribution and logit link function was fitted to test whether network measures affected the probability of an individual to arouse (1) or remain torpid (0) on a given night ('arousal state') (**Figure 3.4**). Second, a LMM was used to determine whether the same network measures affected arousal time. Arousal start time relative to sunset (in seconds) was used as the response variable and transformed via ordered quantile (ORQ) normalisation [434] to improve model fit. Continuous covariates were centred and scaled to improve model convergence. In addition, strength centrality was scaled within groups by dividing an individual's strength by the maximum strength of that group. An identical set of covariates were used in both global models (**Table 3.2**). Barometric pressure (BP), which may provide an important arousal cue [121,382,440], was not measured, nor included in models. Park et al., 1999[327] did not find a significant effect of BP on activity patterns in *R. ferrumequinum*, justifying its exclusion here.

The date corresponding to each data point was fitted as a random intercept to account for variability between nights. The effect of month on each response variable appeared to vary in a potentially non-linear fashion during exploratory data analysis. Therefore, month was included in the global model as an ordered factor with orthogonal polynomial contrasts (linear, quadratic, and cubic) to allow for a potentially non-linear relationship between month and the response variable while still treating month as a categorical variable.

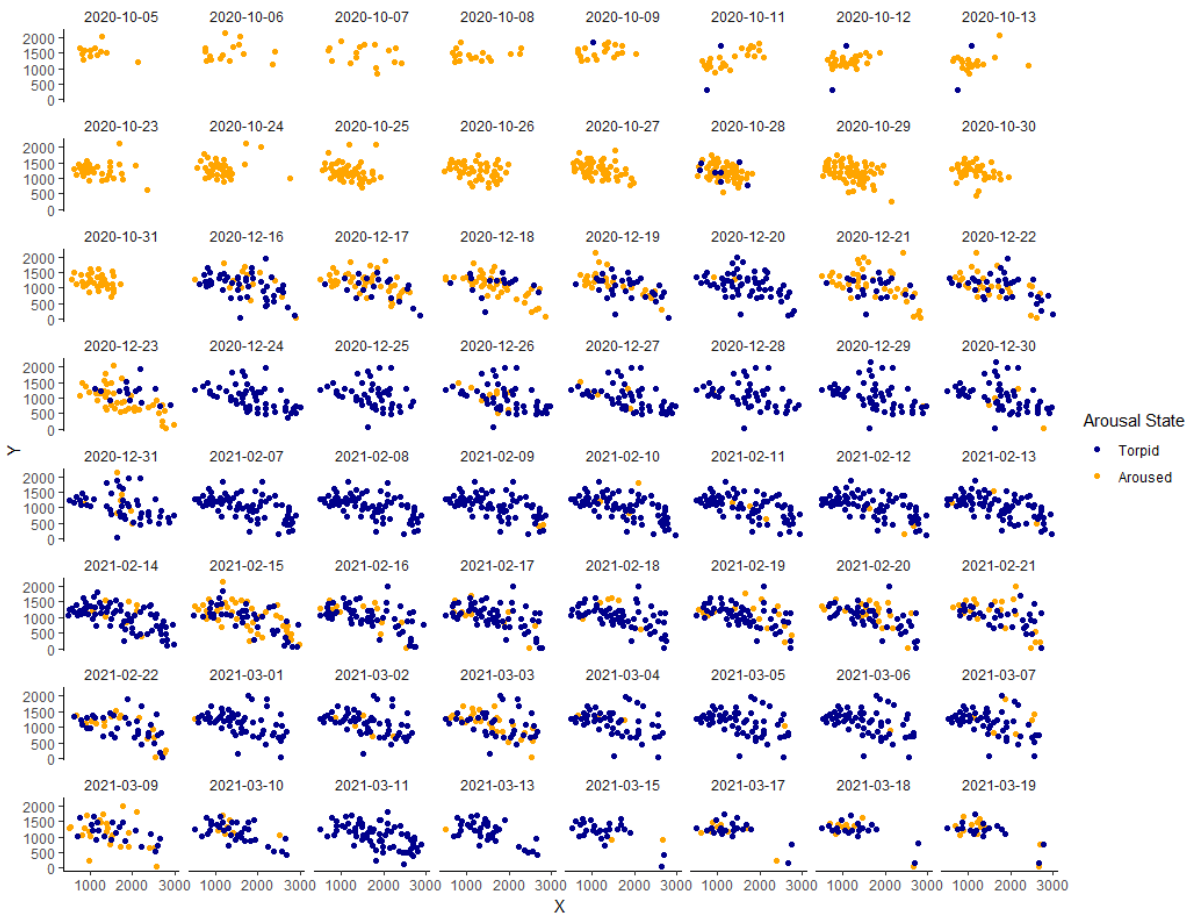

Figure 3.4 *Roost positions of individual* R. hipposideros *during the study period (October 2020 – March 2021). Dots indicate individual bats (n=3206). Dot colour indicates whether or not that individual aroused ('arousal state') during the 5 hour recording window (blue=torpid, orange=aroused).*

Table 3.2 *Explanatory variables/covariates used in (G)LMMs and justification for their inclusion*

covariate	**justification**
mean T_a (mean wall surface temperature during the video recording, °C).	Individuals may be more likely to arouse on warmer nights.
Month	Arousal responses are expected to change with month according to seasonal changes in abiotic conditions.
X coordinate (pixels) Y coordinate (pixels)	Subtle changes in temperature might be expected to be detected by individuals roosting further towards the chamber entrance (**Figure 3.1**).
Group size	More bats arousing may increase disturbance and the likelihood of social transmission.
Relative strength centrality (strength centrality relative to most central bat in the network on the given night).	Following the initial arousal, centrally positioned bats may be more likely to intercept arousal cascades spreading through the group. For example, individuals with high centrality have an increased likelihood of infection in disease-transmission networks.

From the global model with the maximum possible number of covariates, candidate models containing all possible subsets, including an intercept-only null model, were ranked using the *dredge* function (MuMIn R package[441]) based on AICc – applying an information-theoretic approach [442,443]. To account for model selection uncertainty, I assumed models with ΔAICc ≤2 of the best-supported model (lowest AICc) to have equivalent support [437]. With this final model set, I obtained model-averaged parameter estimates, unconditional 95% confidence intervals and standard errors. Significance was assessed if 95% confidence intervals did not cross zero.

Values of some explanatory covariates (e.g. group size and mean T_a) were clustered within month (**Table S 3.1**, **Figure S 3.1**, **Figure S 3.2**). However, mixed models typically require at least five levels of a random effect to robustly estimate variance [444,445]. Therefore, to examine whether fixed effects occurred independently of variance according to month, models were refitted using generalised estimating equations (GEE) in a GLM framework using the geepack R package [446]. GEEs are better suited to account for unmeasured dependence between observations, including temporal autocorrelation. Unlike (G)LMMs where effect estimates represent the change in the response variable for a unit change in a

predictor variable, GEEs give a population-averaged effect i.e. an estimate of the average effect of a predictor variable on the response over the entire group. In addition, an autoregressive correlation structure of order 1 ("ar1") was specified for the repeated measures within each cluster of observations (month). This correlation structure assumes that the correlation between observations within a month decreases exponentially as the time between observations increases.

I performed sequential Wald tests, comparing a full model that included all covariates to two nested null models. Each null model excluded one of the covariates predictive of social transmission: group size and relative strength centrality. In this case, the null hypothesis is that the addition of group size and/or relative strength centrality does not improve model fit. I used a likelihood ratio test (*anova* function in R) to compare the models and assess significance.

Residual diagnostics for all models were assessed using the *DHARMa* v 0.4.6 [447] and *performance* R packages [448], to check the overdispersion and normality of residuals, significant outliers, and correlation between the residuals and model predictions.

3.3.5.5 Group-level analysis

I conducted a group-level analysis, building a simple linear model, to investigate whether emergent group structure [402] might affect collective arousal. More cohesive groups were expected to have a lower variance in their arousal times. The variance of arousal times within each group was calculated using the var function in R and transformed via ORQ normalisation to improve fit within a linear model. Continuous individual-level covariates were averaged per night to produce a group-level metric. For example, mean relative strength centrality provides a measure of group social cohesion. Again, month was fit in the global model as a factor with orthogonal polynomial contrasts (linear, quadratic, and cubic).

As before, I ranked candidate models, including an intercept-only null model, by their AICc values using the dredge function (MuMIn Package[441]). I then calculated model-averaged parameter estimates, unconditional 95% confidence intervals, and standard errors using the top set of candidate models within 2 ΔAIC of the best-supported model (i.e., the model with the lowest AIC). I assessed significance by checking whether the 95% confidence intervals crossed zero.

3.3.6 Observations of active stimulation

I sought to understand that if arousals were socially transmitted, whether this was achieved through active or passive stimuli. I define an active stimulus as when a normothermic bat, not present previously, touches a torpid bat, and initiates an arousal episode. A passive stimulus is defined as when a normothermic bat inadvertently initiates an arousal episode in a nearby torpid bat e.g. due to radiant heat or echolocation.

Potential instances of active stimulation (disturbance) were identified by observing groups in a 50% subset of the video recordings. For each interaction I noted the contact type (**Table** 3.3) and the recipient – either the ID number or as "other" if an normothermic bat had returned to roost from off-screen. Mating was identified according to descriptions by Gaisler et al., (2011)[449]. Lastly, I noted whether interactions occurred between normothermic bats, a normothermic and torpid bat, or whether the recipient torpid bat later aroused.

Table 3.3 *Ethogram of physical contact behaviour of* R. hipposideros*. See* ***Figure S 3.5*** *for visual ethogram.*

Behaviour	**Definition**
Walking contact	Lands close by and walks into roosting bat.
Aerial contact	Flies towards roosting bat, flips over and nudges the roosting bat before flying away.
Nose contact	Perches adjacent to roosting bat and nudges bottom with nose. A heat signature often remains.
Roosting contact	Roosting bat is nudged either by grooming activity, wing stretching, or bat landing in close proximity.
Hanging	Lands on and hangs on roosting bat.
Mating	Mounting from back, wrapping wings around roosting bat for several minutes or seconds (attempted mating).
Wing spreading	Facing towards approaching bat, wings outstretched in response.

3.4 Results

3.4.1 Data summary

Across 64 nights and 320 hours of thermal video footage, I extracted 1033 T_{skin} traces from 3206 (pseudo-replicated) bats. In total, 2173 bats (67.8%) did not arouse. Of the 32.2% that aroused, 20 (2% arousals) swiftly returned to torpor (i.e. 'cold arousal') (e.g. **Figure S 3.6**). 'Cold arousals' were most frequent in December (9 counts), with 4, 4 and 3 cold arousals counted in October, February, and March respectively. In the remaining individuals, the mean arousal rate was 1895 seconds (31.6 minutes), SD = 551 (9.18 minutes). The median group size was 55 bats (SD = 17.4 , range = 12-79).

3.4.2 Collective synchronisation with sunset

The circular distribution of arousal times relative to sunset was not consistent with a unimodal distribution in October, December, and March, indicating non-random synchronisation of arousals with sunset for these months (**Table 3.4**). In February, I accepted the alternate hypothesis of a unimodal distribution, suggesting weaker synchronisation with sunset, if at all (**Table 3.4**).

Within the recording window, 98% of individuals aroused collectively with conspecifics in an apparent cascade (e.g. **Figure 3.3d**). Only a small number of arousal episodes (n = 24, or 2%) occurred in isolation, without overlapping with another conspecific. Isolated arousals were more frequent in December, February, and March, with 7, 7, and 8 arousals respectively. In contrast, there were only two isolated arousals in October.

Table 3.4 *Results of Rayleigh test of uniformity for arousal times for each month. Arousal time is defined relative to sunset (in seconds). The specified mean direction is 0 (sunset) and the alternate hypothesis is a unimodal (random) distribution. Means and standard deviations of arousal time are also provided in minutes to facilitate interpretation.*

Month	**Mean arousal time ± SD (sec)**	**Mean arousal time ± SD (min)**	**Z**	***p***
October	-684 ± 846	-11.4 ± 14.1	-0.050	0.943
December	384 ± 2111	6.4 ± 35.2	0.026	0.278
February	4883 ± 5535	81.4 ± 92.2	0.322	<0.0001
March	756 ± 4036	12.6 ± 67.3	0.047	0.250

3.4.3 Network-based diffusion analysis (NBDA)

The NBDA revealed significant evidence for social transmission in October. However, for the remaining months, the data were better fit by asocial models, with both the social transmission rate (s) and the proportion of arousal events due to social transmission (*%ST*) being 0 (**Table S 3.2, Figure 3.5**). Despite this, the upper 95% confidence intervals (CI) were well above 0, indicating that while evidence for social transmission is weak, its existence cannot be discounted.

In October, the social transmission model outperformed the asocial model with a better fit (ΔAICc = 5.609), receiving 16.522 more support and being 7.617 times more likely based on the likelihood ratio test ($p < 0.01$). This made it the best Kullback-Leibler model given the data. The rate of social transmission was moderate ($s = 38.821$), with a lower CI of 4.778 and an upper CI of 306.837 (**Table S 3.2**). The proportion of arousal events due to social transmission was 55.353%, with a lower CI of 16.463% and an upper CI of 84.484% (**Table S2, Figure 4**). However, the wide confidence intervals for both *s* and *%ST* (**Table S 3.2, Figure 3.5, Figure S 3.4**) indicate high levels of uncertainty regarding the strength of social transmission.

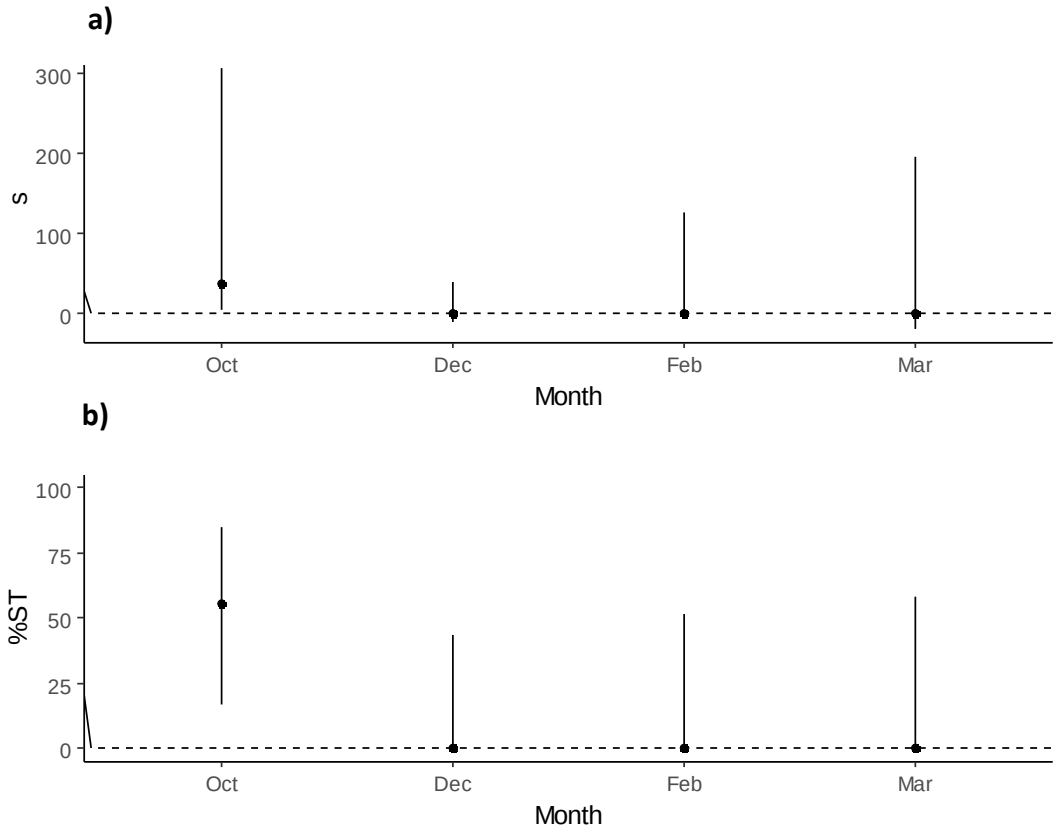

Figure 3.5 *Outputs of order of acquisition diffusion analysis (OADA) providing evidence for the relative strength of social transmission in each month.* ***a)*** *'s' is the rate of social transmission of arousal per unit network connection relative to the baseline asocial learning rate.* ***b)*** *%ST is the percentage of arousal events occurring by social transmission. 95% confidence intervals, calculated by the profile log-likelihood technique (****Figure S 3.4****), are provided for both parameters.*

3.4.4 Individual-level analysis

I used model averaging to account for model selection uncertainty when estimating the effect of covariates on each response variable. I considered a set of candidate models that included all possible combinations of covariates and selected them based on their AICc values if they fell within 2 ΔAIC. The model weights were calculated using Akaike weights (**Table S 3.3**, **Table S 3.5**). First, a GLMM with a binomial error family was run using final arousal state (0 = torpid, 1 = aroused) as a binary response variable. The unconditional model-averaged estimates for the effect of relative strength centrality and group size were 0.010 (SE = 0.035, 95% CI = [-0.059, 0.079]) and -0.025 (SE = 0.114, 95% CI = [-0.247, 0.198]) respectively, but were non-significant (**Table S 3.4**). The only significant effects were mean T_a (β = 2.241, SE = 0.590, 95% CI = [1.084, 3.397]; **Figure 3.6**) and month as a quadratic (β = 1.300, SE = 0.521, 95% CI = [0.274, 2.317]) and cubic (β = -1.270 , SE = 0.374, 95% CI = [-2.003, -0.537]; **Table S 3.4**, **Figure 3.6**) term.

A linear mixed model was fit with arousal time relative to sunset (transformed via ORQ normalisation) as the response variable. The unconditional model-averaged estimate for relative strength centrality was positive, but not significant (β = -0.032, SE = 0.033, 95% CI = [-0.097, 0.034]; **Table S 3.6**). However, a significant, positive effect of group size was detected, with individuals in larger groups tending to arouse after sunset compared to smaller groups (β = 0.471, SE = 0.076, 95% CI = [0.322, 0.619]; **Table S 3.6**, **Figure 3.7a**). Additionally, significant, positive effects of X and Y coordinates were identified (X coordinate: β = 0.085, SE = 0.035, 95% CI = [0.016,0.154]; Y coordinate: β = 0.117, SE = 0.031, 95% CI = [0.056,0.177]; **Table S 3.6**, **Figure 3.7c,d**), suggesting individuals roosting lower and leftward were more likely to arouse earlier than sunset, and vice versa. Similarly, warmer mean T_a was associated with earlier arousal relative to sunset (β = -0.510, SE = 0.185, 95% CI = [-0.872, -0.147]; **Table S 3.6**, **Figure 3.7e**). The effect of month fitted as a negative linear effect was significant (β = -1.077, SE = 0.361, 95% CI = [-1.786, -0.368]; **Table S 3.6**, **Figure 3.7b**), with individuals predicted to arouse earlier in later months.

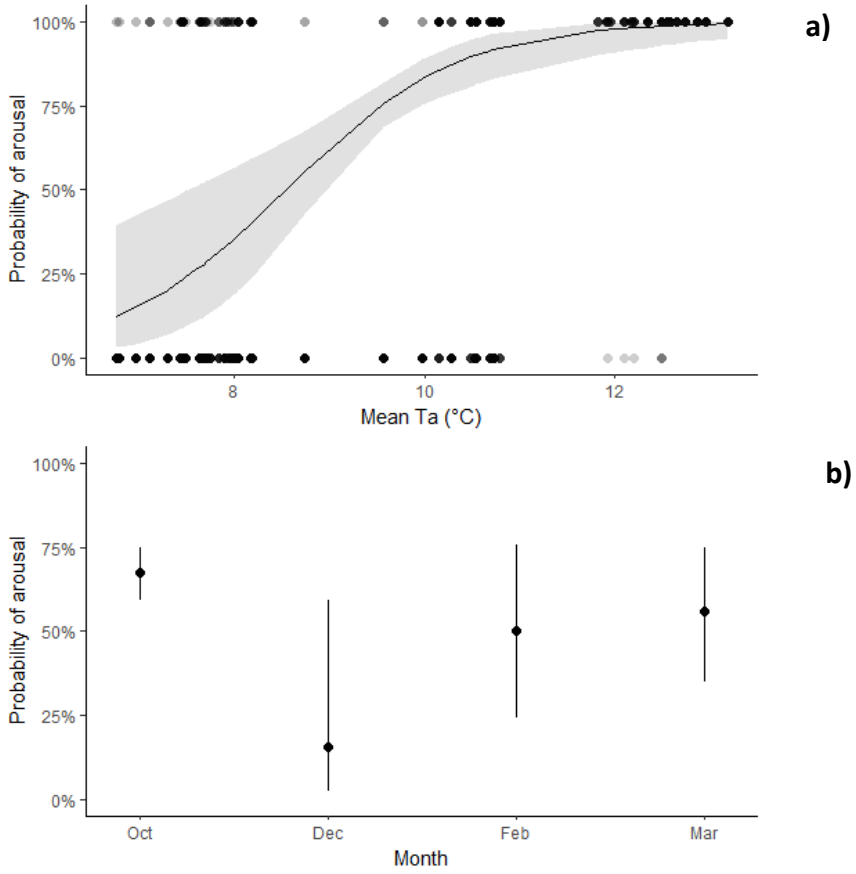

Figure 3.6 a) *The predicted effects of mean ambient temperature (T_a °C) and* ***b)*** *month on the probability of arousal in a given individual* R. hipposideros*.* ***b)*** *shows the effect of month and the probability of arousal as a cubic orthogonal polynomial.*

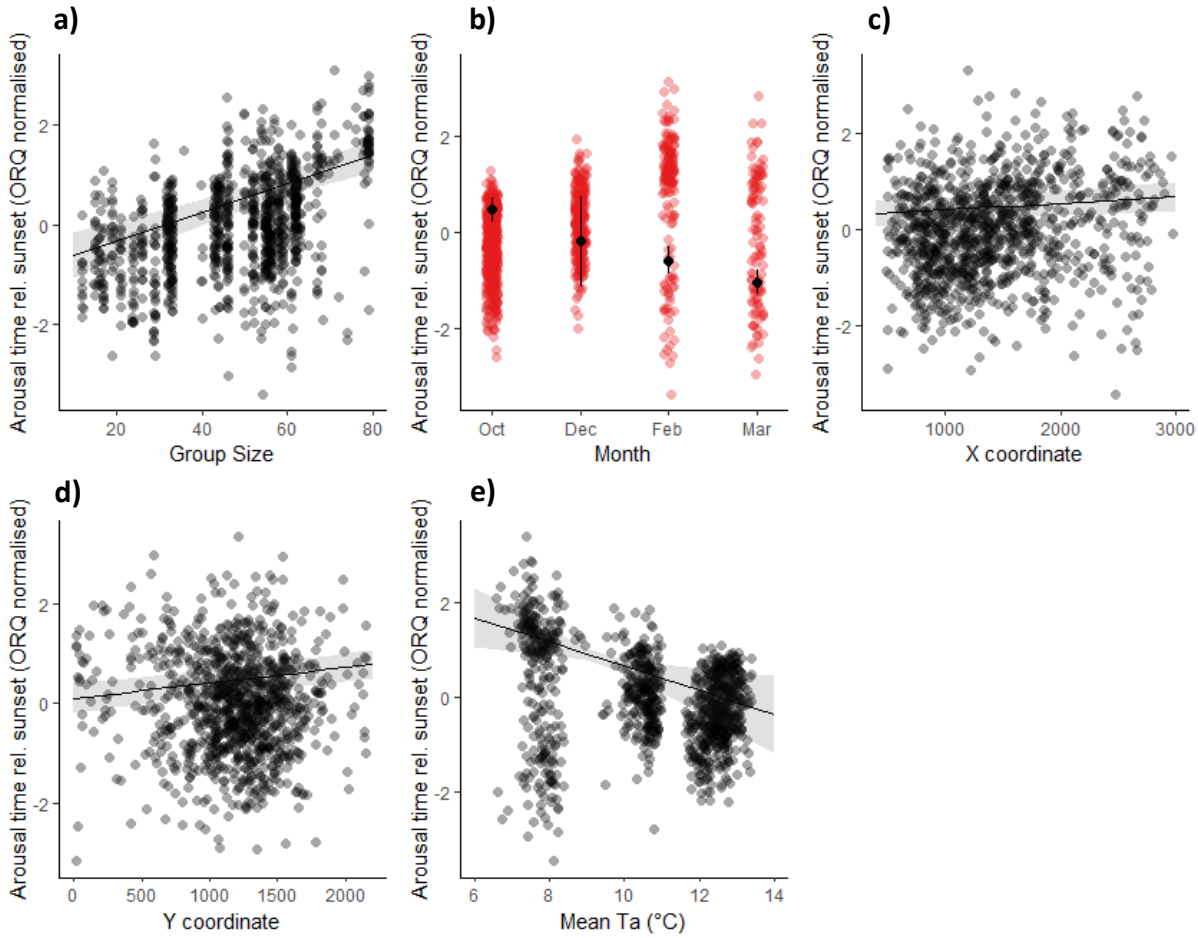

Figure 3.7 a) *The linear effects of group size,* ***b)*** *month,* ***c)*** *X coordinate (pixels),* ***d)*** *Y coordinate (pixels), and* ***e)*** *mean ambient temperature (T_a °C) on an individual's arousal time relative to sunset (0). The response variable was transformed via ordered quantile (ORQ) normalisation to satisfy assumptions of normality in a linear mixed model (LMM). Positive values indicate later arousals and vice versa.*

The same response variables and covariates were re-analysed using GEE in a GLM framework [446], assuming an ar1 correlation structure with month as the grouping factor, better accounting for unknown dependencies i.e. autocorrelation, between observations within the same month than in the (G)LMMs. GEE models generate a population-average response representing the average change in the response variable for a unit change in a predictor variable across all individuals in the population, whilst controlling for non-independence within the grouping factor i.e. month [433,450,451].

For the 'arousal state' binomial GEE-GLM models, a sequential Wald's test, comparing the global model (all covariates) to two nested null models (excluding group size and relative strength respectively) showed that the model containing relative strength centrality was significantly different to the null model (excluding relative strength centrality) (relative strength centrality: $x^2 = 40.4$, df = 1, $p < 0.001$; group size: $x^2 = 1.33$, df = 1, $p > 0.05$). In addition, a positive effect of relative strength centrality was statistically significant, controlling for the other covariates (β = 0.168, SE = 0.026, Wald = 40.400, $p < 0.001$; **Figure 3.8a**). As found previously in the LMM, a significant, strong positive effect was found for mean T_a (β = 1.089, SE = 0.478, Wald = 5.190, $p < 0.05$; **Figure 3.8b**).

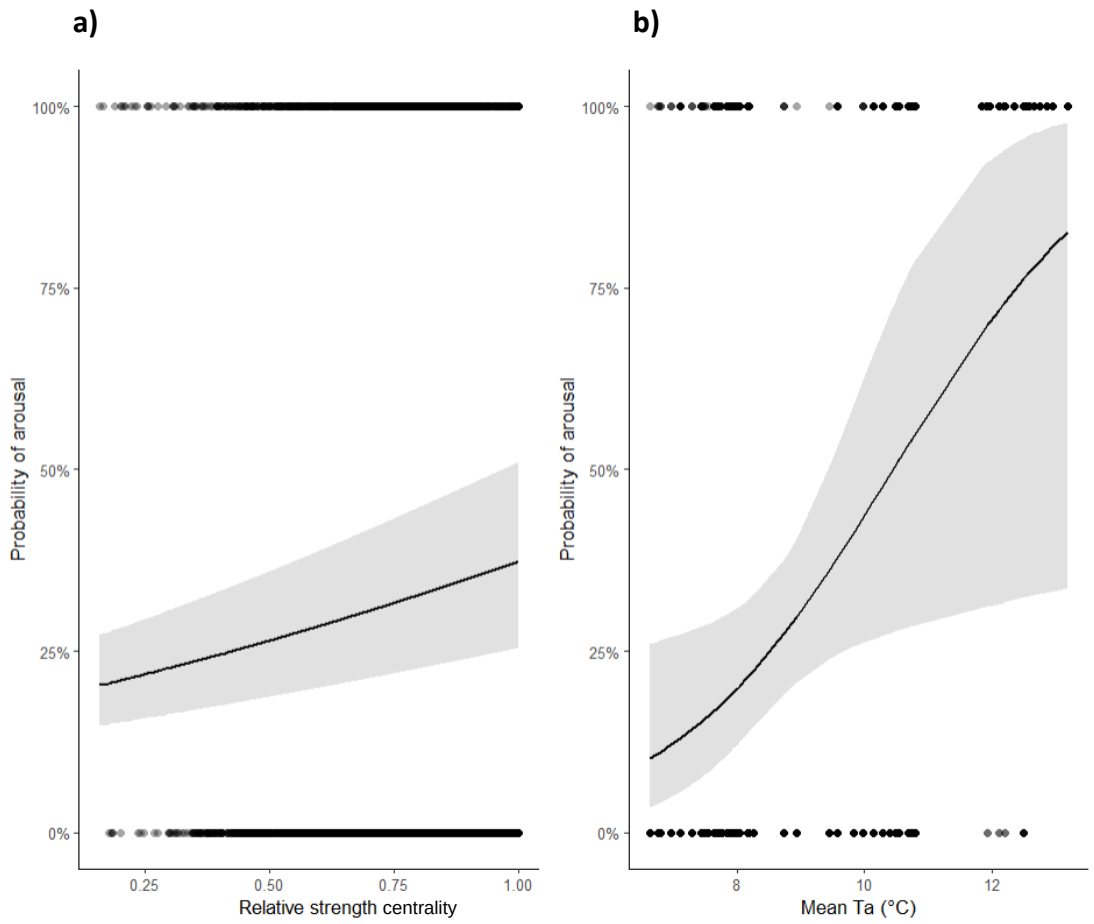

Figure 3.8 a) *Population-average effect of relative strength centrality and* ***b)*** *mean ambient temperature on the probability of arousal, as predicted in a generalised estimating equation (GEE) model, with an ar1 autoregressive correlation structure and month as the grouping factor. 'Arousal state' (0=torpid, 1=aroused) was the binary response variable.*

For the GEE-GLM models of arousal time, a sequential Wald's test showed the model containing group size was significantly different from the null model (excluding group size) (relative strength centrality; $x^2 = 2.35$, df = 1, $p > 0.05$; group size: $x^2 = 8.84$, df = 1, $p < 0.01$). In addition, significant positive effects were identified for group size (β = 0.520, SE = 0.175, Wald = 8.840, $p < 0.01$; **Figure 3.9a**), X and Y coordinates (X coordinate: β = 0.031, SE = 0.015, Wald = 4.000, $p < 0.05$; Y coordinate: β = 0.072, SE = 0.023, Wald = 9.490, $p < 0.01$; **Figure 3.9b,c**), but not mean T_a (β = -0.032, SE = 0.085, Wald = 0.140, $p > 0.05$), while controlling for other covariates.

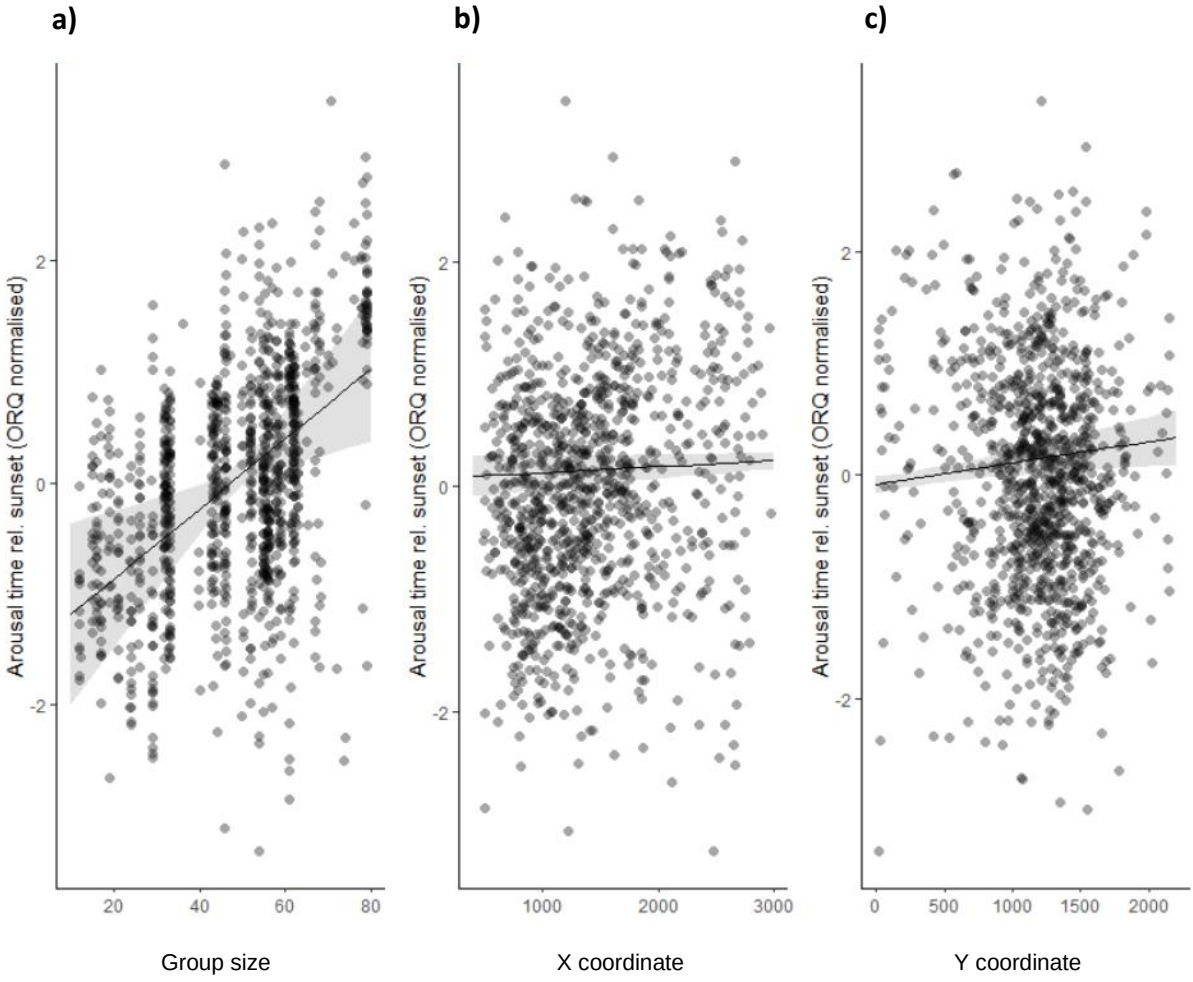

Figure 3.9 a) *Population-average effects of group size,* ***b)*** *X coordinate (pixels),* ***c)*** *Y coordinate (pixels) on arousal time relative to sunset (0), as predicted in a generalised estimating equation model, with an ar1 autoregressive correlation structure and month as the grouping factor. The response variable was transformed via ordered quantile (ORQ) normalisation to satisfy assumptions of normality in a Gaussian GEE-GLM. Positive values indicate later arousals and vice versa.*

3.4.5 Group-level analysis

A linear model was fit to test the effects of emergent group structure (e.g. group size and social cohesion) on the variance of arousal time on a given night. As before, model averaging was used to account for model selection uncertainty when estimating the effect of covariates on the response. Model weights were calculated using Akaike weights (**Table S 3.7**). The top selection of models (within 2 ΔAICc of the lowest AICc model) contained average relative strength centrality, group size, and mean ambient temperature (T_a) as covariates (**Table S 3.7**). The unconditional model averaged effect estimate of mean T_a on arousal time variance was negative and statistically significant (β = -0.574, SE = 0.121, 95% CI = [-0.816, -0.331]; **Table S 3.8**).

3.4.6 Observations of active disturbance

Active tactile disturbance from conspecifics was not primarily responsible for arousals over the 32 nights sampled. 379 arousal events (82%) were not associated with active physical contact with another bat, compared with 31 arousals events (6.7%) that occurred post-contact. Of these, the time between contact and arousal was considerable (mean = 65.063 minutes ± 79.078 SD, range = 1.36 - 301.75), making it difficult to establish cause and effect. The remaining 51 arousal events (11%) occurred before the bat was disturbed. An additional 164 instances of contact were between unlabelled normothermic bats (labelled as 'other' – see section 3.3.6). Overall, 324 instances of physical contact (49%) were not followed by an arousal, with the recipient bat remaining torpid.

Overall, I recorded 667 instances of physical contact of seven different types. Walking and roosting contacts were the most frequent physical encounters (146 and 176 respectively) between individuals (**Figure 3.10a**). Mating was the least common contact type (29 occurrences, **Figure 3.10a**). The frequency of contact types varied between months (**Figure 3.10b**). Wing spreading and aerial contacts were most frequently observed in October, becoming less frequent relative to roosting and walking contacts that both peaked in December. Mating was not observed in October.

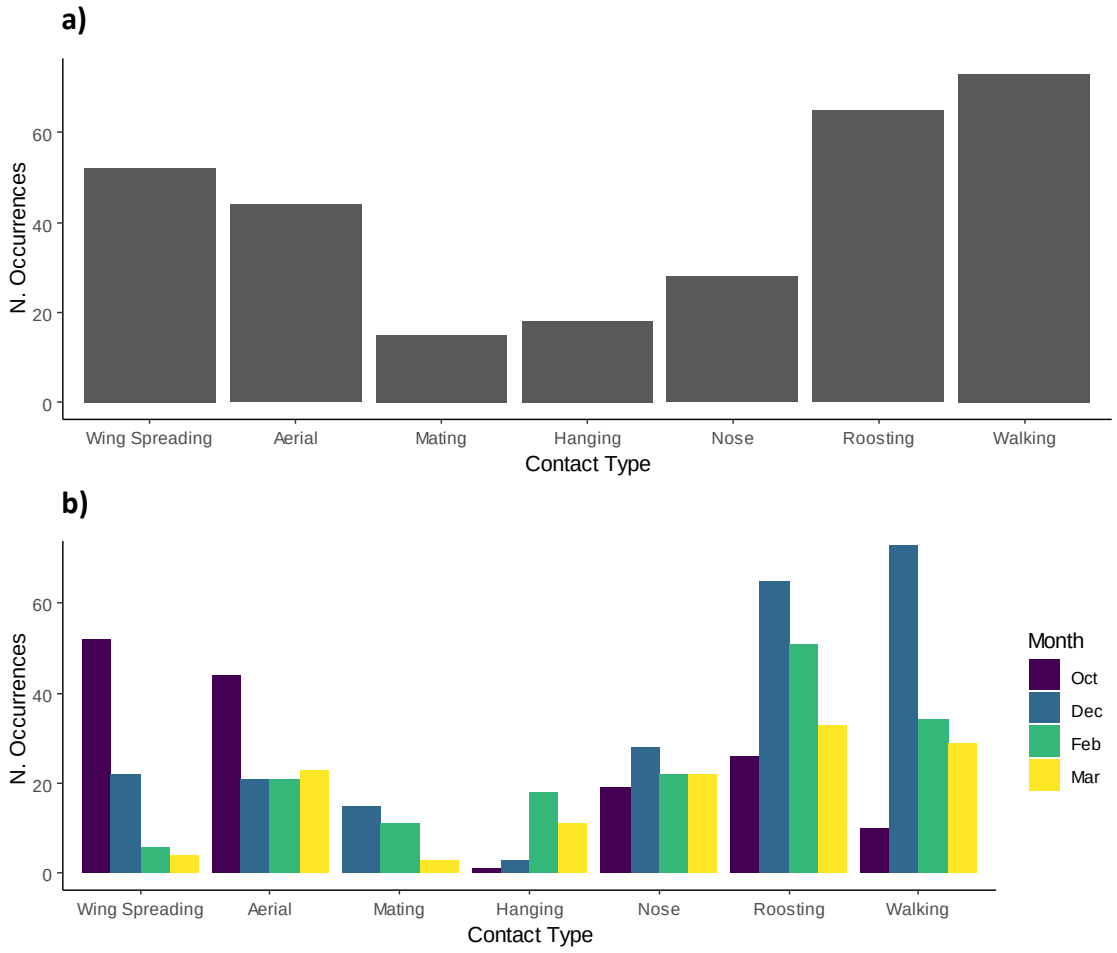

Figure 3.10 a) *Total occurrences of active stimuli (tactile disturbance) from conspecifics on roosting bats* ***b)*** *Occurrences of tactile disturbance from conspecifics on roosting bats per month*

3.5 Discussion

Access to social information can provide a selective benefit to individuals living in groups. Whether through direct interactions or indirect social cues, individuals can receive biologically relevant information more efficiently than through isolated experience [403,405]. For hibernating rhinolophid bats, making effective decisions about when to remain torpid or arouse is critical for maintaining energy supply and surviving the winter. Suitable foraging conditions over winter are an ephemeral, potentially unpredictable, resource that individuals require knowledge of if they are to maintain their energy budgets [213,373]. The "social alarm-clock" hypothesis proposes that the arrival of a normothermic individual in close proximity provides inadvertent information about the presence of suitable foraging conditions. Results from a group of *R. hipposideros* show that individuals largely use temperature cues to decide when to arouse from torpor on warm nights and are not solely reliant on the actions of conspecifics. However, social transmission also plays a role in initiating some arousals, supporting the social-alarm clock hypothesis, and potentially providing a selective advantage of group-living.

3.5.1 Asocial arousal cascades

Behavioural cascades can arise when individuals indiscriminately copy the behaviour of their conspecifics [427]. This may happen when the cost of differentiating one's own information from that of a conspecific becomes too high. For example, escape waves observed in shoals of fish[452] and flocks of birds [453] upon the arrival of a predator are model examples of cascades. In such cases, social information becomes valuable if not all individuals are aware of the stimulus [454]. In the cluster of *R. hipposideros,* individuals need information on the optimal time to arouse, which is typically during warm nights at sunset [233,325,380–382]. My results demonstrate a strong synchronisation of arousals with sunset, with individuals emerging from torpor sequentially in a cascade. However, this pattern is more likely an artefact driven by response latency to changes in air temperature and/or deviation in the endogenous circadian rhythms between individuals [325,377,455,456], than the spread of social information. Air temperature (T_a) as experienced by individuals through external air flowing into the hibernaculum, had the strongest effect on both the probability and timing of these arousals, including the rate at which the group collectively aroused.

A marked increase in arousal probability was observed in air temperatures rising above 10°C (**Figure 3.6** & **Figure 3.8**), aligning with the findings of Park et al., (2000)[325] in *R. ferrumequinum*. While temperature thresholds for flight may vary across and within species [457,458], ~10 °C is sufficient for flight in most temperate Diptera and Lepidoptera [175,187,458,459] important constituents of the winter diet in *R. hipposideros*. Where air flow into the chamber is present, temperature could provide a reliable cue on relative prey availability [199,233,381]. Consequently, individuals might choose hibernacula expressly if the T_a of the hibernacula tracks external temperatures accurately [199,233,330]. The decline in the probability of arousal in December and subsequent rise thereon, may not reflect the availability of warm nights as expected. All temperatures exceeding 10 °C were within October and the earlier part of the sampling block in December (**Figure S 3.2**). The increase in the probability of arousal, despite relatively lower temperatures, could indicate rising levels of energetic stress as energy supplies wane, prompting individuals to forage [408]. The negative trend in arousal time over successive months might reflect increasing energetic demands [408] or a weakening of circadian rhythms after extended bouts of torpor [120,121] . However, it is important to note the relative changes in mean values of arousal time (**Table 3.4**, **Figure S 3.7**) do not necessarily reflect the linear relationship predicted under the LMM (**Figure 3.7**).

Why might an individual favour T_a over social cues to initiate arousal? Conspecific activity is unlikely to be consistently reliable, given some bats may arouse for reasons other than foraging i.e. mating [216,333]. Thus, responding to all social cues indiscriminately may be maladaptive [121,387]. Observations of cold arousals within cascades (e.g. **Figure S 3.6**) – potentially in response to conspecific disturbance - indicate individuals are capable of controlling whether or not they arouse, regulating energy expenditure [391,392,395]. Furthermore, arousal following mating was rare, despite the presumed male maintaining contact with the presumed female for long periods. Arousal following mating is unlikely to be adaptive for females where energy budgets are limited and survival to spring is imperative for reproduction [333].

3.5.2 Social arousal cascades

A role for social transmission in explaining the observed arousal cascades cannot be overlooked. Relative strength centrality had a positive effect on the average probability of arousal for this colony of *R. hipposideros*. That is, on average, bats with better access to social information were more likely to arouse. In addition, the NBDA found significant support for the use of social information in initiating arousals in October. Approximately 50% of arousal episodes were predicted to have occurred via social transmission (**Figure 3.5**). This means that, following the first arousal, the observed order at which individuals aroused depended on a combination of individuals being disturbed by a neighbour and detecting T_a changes. After October, however, I can be much less certain over the use of social information in prompting arousal. The rate of social transmission and the percentage of arousal events initiated by social transmission were both zero. A potential explanation for this is the reduced probability of arousal during the mid-winter months, with individuals less responsive to conspecific disturbance in deep torpor [460,461].

3.5.3 Larger clusters aroused later than smaller groups.

Roost emergence following arousal has functional significance in bats. A probable trade-off exists between predation risk and foraging gains. Early emergence increases predation risk by visual predators, while later emergence reduces foraging success amidst greater competition for increasingly limited prey post-sunset [462]. The optimal emergence time for an individual or colony may depend on various factors including prey availability, sex, hunger [462], light conditions[463–465], weather conditions [466,467], and group size [468]. In some species, collective emergence can dilute an individual's predation risk [131,469], with larger groups potentially facilitating earlier emergence [468]. In this study, controlling for seasonal effects, larger clusters were found to arouse, and likely emerge, later than smaller clusters. However, this finding may be an artefact of sample size, as larger groups take longer to arouse entirely, positively skewing the mean group arousal time. This is consistent with Hristov et al., (2010)[470] who reported a similar correlation between colony size and emergence duration, and Swift (1980)[471] who found an increased emergence rate in proportion to colony size.

The observed trend in cluster size over the study period was comparable to that observed by [472] in central Italy, and may be explained by changes in T_a [473]. Further study is needed to

understand the functional significance of cluster size during hibernation. The observed increase in cluster size during December and February (**Figure S 3.1**), when few arousals occurred, is counter to the hypothesis that larger numbers of bats would increase disturbance levels, facilitating social transmission and collective arousal.

3.5.4 Roost position affected arousal time.

Alongside group size, an individual's roost position had a significant effect on arousal time. Individuals roosting in lower and leftward positions (closer to the chamber entrance) were more likely to arouse earlier relative to sunset (*vice versa*) (**Figure S 3.8**). When investigating the spatial spread of a behaviour there is often a well-founded concern of false positives. For example, delay in a direct, asocial, response to a localised stimulus according to distance can be difficult to distinguish from propagation of a behaviour via a social contagion [423]. Incidentally, the earliest bats to arouse in cascades were clustered around a leftward position in the chamber (**Figure S 3.9**). However, no consistent spatial thermal gradient on the wall surface was apparent enough to better explain potentially social arousal cascades in this study. Nevertheless, individual *R. hipposideros* might prefer specific roost positions if unmeasured microclimatic variables differ sufficiently within the chamber [122,474–476]. Bats possess sensory hairs enabling them to sense air flow, possibly enabling detection of subtle changes in temperature [477,478]. Thus, it is feasible individuals roosting closer to the chamber entrance (**Figure 3.1**) could detect suitable foraging conditions before bats elsewhere.

An additional explanation is that roost position co-varies with an individual's social connections (measured here as strength centrality). Inspection of spatial density plots reveals a strong clustering of roost positions on the lefthand side of the chamber (**Figure S 3.10**). Subsequently, most individuals with high strength centrality roosted within a limited area. Following my finding that strength centrality increases the average probability of arousal, roosting close to neighbours with high strength might prompt earlier arousal relative to sunset through conspecific disturbance. Indeed, individuals may adjust their roost position to mediate their predicted arousal time. Interestingly, further examination of IRT video recordings suggests the lefthand side of the chamber is a consistent nucleating point from which the cluster grows, even without prior bats in the roost, indicating potential roosting preferences. Ransome (1971)[233] observed similar roosting behaviour in *R. ferrumequinum*.

3.5.5 Active social stimuli had little effect on triggering arousals

I found little evidence to support active disturbance from conspecifics being the main trigger of arousals. In fact, 82% of the recorded arousal events occurred without any interaction with another bat. Thus, a passive component (e.g. noise production [388] and radiant heat [385–387,389,390]) to social transmission is more likely.

Most interactions occurred between bats that were either already awake or remained torpid, suggesting that they interact for reasons other than triggering arousals in torpid bats. However, since some interactions did occur prior to arousals in torpid bats, it remains possible that they played a role in triggering some arousals.

Adaptive explanations for the contact behaviours observed are, as of yet, unclear (except mating). One hypothesis, albeit speculative, is competition for roost position. Wing-spreading behaviour involved an individual being approached and responding by facing the pursuer with wings outstretched, causing the pursuer to leave – akin to territorial and threat displays seen in birds [479–481] and bats [482–484]. Inspection of video footage and T_{skin} traces suggests some individuals roosting in the same position a bat (potentially the same individual) had previously left (**Figure S 3.11**), suggesting some individuals may prefer a precise roosting position. Accordingly, an individual may engage in aerial, roosting, and/or walking contacts in attempting to reclaim its original roost spot after being displaced [485].

My observation of nose contacts resembles observations of biting behaviour in mating pairs of *R. hipposideros* by Gaisler et al., (2011)[449]. Here the male would bite the female's dorsal fur towards the posterior, during apparent mating. In this study, I did not always observe any prolonged mating behaviour following nose contacts. Rather, this behaviour could represent inspection of potential female mates by prospective males. Some bats (e.g. *Tylonycteris pachypus*[486]*, Eptesicus fuscus*[487]*, M. bechsteinii*[488]*, Mops condylurus*, *Chaerephon pumilus*[489], and *Pipistrellus pipistrellus*[490]) are known to use olfaction for sex discrimination and individual recognition.

The peak mating periods for *R. hipposideros* are thought to be in the Autumn and Spring [491,492]. Therefore, the higher frequency of mating behaviour recorded in December was surprising. In most cases, a normothermic bat (presumed male) approached a torpid bat (presumed female). This contrasts with the previous description by Gaisler et al., (2011)[449]

where both participants were normothermic. Forced copulations of torpid females have been observed previously in *M. lucifugus* [493,494]. The mating system of *R. hipposideros* is not well understood, but it may be similar to this species. For example, *M. lucifugus* has a promiscuous mating system with little to no male defence of females, given forced copulations by "sneaker" males would undermine a defending males' energetic investment [493,495]. Non-random reproductive success is achieved via sperm competition [140,496].

It is possible that mating behaviour occurred in October, even though it was not recorded. Bats were more likely to return to the roost within the 5-hour recording window in later months, possibly due to shorter foraging bouts. This allowed for more contact behaviour within the roost to be captured.

3.5.6 Comparing effects in (G)LMM and GEE-GLM models

The (G)LMM and GEE-GLM statistical models produced different results regarding the effect of relative strength centrality on arousal state and mean T_a on arousal time. The GEE-GLM model showed a moderate positive effect of relative strength centrality. In contrast, the GLMM model showed a weak and non-significant model-averaged effect. Mean T_a had a strong negative model-averaged effect on arousal time in the GLMM, but a weak and non-significant effect in the GEE-GLM model.

These differences may be due to the contrasting model selection approaches and/or the assumptions each type of model made about the dependence structure of the data. GEE-GLM models account for the correlation between observations within a group (i.e. month), while (G)LMMs account for both within-group (not accounted for here) and between-group variability (i.e. date), potentially leading to different conclusions [450,451]. For example, the GEE-GLM models accounted for the strong clustering of data within months. The distribution of T_a, in particular, was largely restricted to month (e.g. **Figure 3.7**, **Figure S 3.2**). Thus, the significant effect of T_a on arousal time in the LMM reflects month-dependent differences in arousal time, rather than a direct effect of T_a alone.

Additionally, GEE-GLM models focus on population-average effects, while (G)LMMs focus on individual-specific effects [450,451]. For example, the GEE-GLM model examined how relative strength centrality affects the average probability of arousal for all bats in a cluster, while the GLMM model examined how strength centrality affects a given individual bat's

probability of arousal. Unaccounted for individual-level effects such as sex and body condition may better explain the probability of arousal for individuals in the GLMM. These findings highlight the value in considering both population average and individual-specific effects. Further research is warranted to fully understand the role of network position in *R. hipposideros* arousals. One possible approach is to use RFID tags to account for individual identity (e.g. [497]).

3.5.7 Alternative models of social contagion

I took an information theory approach to model selection in the NBDA, using ΔAICc to compare support for models that included or excluded social transmission of arousal. However, no matter the information criterion chosen, they only provide an estimate of relative, not absolute, goodness-of-fit [435,436,443]. Therefore, there may be alternate models that better explain the social transmission of arousals than those examined in this study.

Firstly, there are several unmodelled individual-level variables (ILVs)[429–431] that might influence asocial or social propagation of arousal within a cluster. For example, individuals with poorer body condition may arouse closer to sunset [325] and be more responsive to temperature changes or conspecific disturbance, affecting the order they arouse within a cascade. The depth of torpor an individual is in prior to a cascade may also affect their sensitivity to external stimuli. For example, ground squirrels (Marmotini) become more sensitive to disturbance in the latter half of a torpor bout. Incorporating an individual's sensitivity to disturbance could reduce uncertainty around the estimated rate of social transmission [460,461]. For example, a non-random distribution of highly-receptive individuals in close proximity may generate a spurious effect of social transmission [431].

Secondly, the social propagation of arousals may not entirely follow a simple contagion model. A simple contagion assumes the likelihood of social learning is proportionate to an individual's total social connections to "informed" individuals [432,498,499]. Instead, the spread of a more complex contagion relies on the proportion of informed neighbours. In these circumstances, the likelihood of social learning can depend on multiple exposures to a behaviour, with the individual making a judgement before the behaviour is acquired [498–500].

3.5.8 Alternative hypotheses of group formation

My methodological approach did not allow me to test alternative hypotheses that might better explain group formation in this species. Firstly, as discussed, individuals may compete for the same roost space if the availability of hibernacula, or specific microclimates within them, is limited [109]. Second, individuals may wish to associate with kin or familiar individuals (see *Chapter 2*), potentially to exchange social information or other cooperative benefits while normothermic [17,27]. Third, individuals may aggregate to reduce their chance of predation. Owls (Strigiformes) [501,502], wood mice (*Apodemus sylvaticus*) [503], and even great tits (*Parus major*)[369] have been known to prey on defenceless hibernating bats. Thus, grouping together may dilute an individual's chance of predation (the selfish herd effect)[41], although, this is likely to be rare where light levels are extremely limited and bats hibernate at height. These hypotheses are potentially non-exclusive with the social-alarm clock hypothesis. For example, social transmission of arousals may aid predator avoidance. Further exploration may provide a better understanding of the factors driving group formation.

3.6 Conclusion

This chapter presents novel evidence supporting the hypothesis that arousal from torpor can spread via social contagion in hibernating *R. hipposideros*. In October at least, these bats likely use a combination of asocial and social environmental cues to facilitate optimal arousals when foraging conditions are favourable – potentially providing an advantage to group formation. Future research would benefit from an experimental approach that manipulates individual identities, group sizes, relative distances between individuals, and controls for environmental conditions. Nevertheless, the approach used in this study provides a less invasive route to observing torpor-arousal strategies in wild bats.

In spring, female rhinolophid bats leave their hibernacula and return to maternity roosts to raise their pups. The maternal bond is crucial for offspring development and reproductive success in many animal societies [504]. The next, and final, data chapter examines phenotypic variation within the Woodchester Mansion colony and quantifies the relative contributions of maternal and additive genetic effects using the pedigree developed in Chapter 2.

3.7 Supplementary Tables & Figures

Table S 3.1 *Ambient temperatures, group sizes, and mean relative strength centrality for each night over the study period*

Month	Date	Mean T_a	Group size	Proportion of group aroused	Mean Strength (± SD)
Oct	05/10/2020	11.907	12	1.000	0.754 ± 0.245
Oct	06/10/2020	11.955	15	1.000	0.686 ± 0.215
Oct	07/10/2020	11.828	16	1.000	0.764 ± 0.128
Oct	08/10/2020	12.172	17	1.000	0.778 ± 0.192
Oct	09/10/2020	10.725	19	0.947	0.816 ± 0.117
Oct	11/10/2020	12.195	24	0.917	0.803 ± 0.166
Oct	12/10/2020	12.098	26	0.923	0.72 ± 0.194
Oct	13/10/2020	11.925	21	0.905	0.66 ± 0.258
Oct	23/10/2020	13.187	32	1.000	0.632 ± 0.211
Oct	24/10/2020	12.740	33	1.000	0.728 ± 0.231
Oct	25/10/2020	12.860	44	1.000	0.717 ± 0.178
Oct	26/10/2020	12.953	43	1.000	0.757 ± 0.125
Oct	27/10/2020	12.553	46	1.000	0.769 ± 0.165
Oct	28/10/2020	12.495	52	0.865	0.754 ± 0.147
Oct	29/10/2020	12.338	56	1.000	0.745 ± 0.158
Oct	30/10/2020	12.584	33	1.000	0.718 ± 0.184
Oct	31/10/2020	12.654	32	1.000	0.719 ± 0.158
Dec	16/12/2020	10.288	63	0.222	0.748 ± 0.155
Dec	17/12/2020	10.145	62	0.677	0.733 ± 0.141
Dec	18/12/2020	10.482	55	0.709	0.714 ± 0.191
Dec	19/12/2020	10.692	55	0.455	0.724 ± 0.162
Dec	20/12/2020	10.527	59	0.034	0.749 ± 0.158
Dec	21/12/2020	10.547	62	0.629	0.747 ± 0.163
Dec	22/12/2020	10.740	59	0.441	0.714 ± 0.166
Dec	23/12/2020	10.795	58	0.741	0.662 ± 0.162
Dec	24/12/2020	10.400	53	0.000	0.686 ± 0.155
Dec	25/12/2020	9.827	46	0.000	0.74 ± 0.177
Dec	26/12/2020	9.577	56	0.161	0.698 ± 0.159
Dec	27/12/2020	9.980	56	0.089	0.719 ± 0.142
Dec	28/12/2020	9.447	47	0.021	0.764 ± 0.166
Dec	29/12/2020	8.935	54	0.019	0.699 ± 0.159
Dec	30/12/2020	8.742	56	0.071	0.696 ± 0.166
Dec	31/12/2020	7.982	52	0.135	0.74 ± 0.156
Feb	07/02/2021	8.268	67	0.000	0.758 ± 0.137
Feb	08/02/2021	7.640	69	0.000	0.662 ± 0.135
Feb	09/02/2021	7.502	70	0.043	0.749 ± 0.13
Feb	10/02/2021	7.302	71	0.042	0.693 ± 0.137
Feb	11/02/2021	6.973	72	0.042	0.706 ± 0.14

Feb	12/02/2021	6.767	74	0.041	0.715 ± 0.14
Feb	13/02/2021	6.800	76	0.039	0.717 ± 0.144
Feb	14/02/2021	7.118	78	0.115	0.67 ± 0.133
Feb	15/02/2021	7.462	79	0.494	0.756 ± 0.135
Feb	16/02/2021	7.895	67	0.179	0.696 ± 0.148
Feb	17/02/2021	7.640	67	0.149	0.734 ± 0.169
Feb	18/02/2021	7.718	65	0.092	0.733 ± 0.168
Feb	19/02/2021	7.695	68	0.221	0.732 ± 0.161
Feb	20/02/2021	8.047	61	0.311	0.706 ± 0.166
Feb	21/02/2021	8.193	54	0.389	0.638 ± 0.154
Feb	22/02/2021	8.172	50	0.340	0.773 ± 0.137
Mar	01/03/2021	6.633	55	0.018	0.705 ± 0.173
Mar	02/03/2021	7.955	59	0.085	0.764 ± 0.158
Mar	03/03/2021	7.657	61	0.410	0.741 ± 0.158
Mar	04/03/2021	7.997	52	0.058	0.675 ± 0.182
Mar	05/03/2021	7.750	58	0.017	0.699 ± 0.161
Mar	06/03/2021	7.545	57	0.018	0.694 ± 0.163
Mar	07/03/2021	7.442	57	0.105	0.705 ± 0.174
Mar	09/03/2021	7.445	46	0.543	0.705 ± 0.184
Mar	10/03/2021	7.463	40	0.175	0.701 ± 0.197
Mar	11/03/2021	7.760	67	0.000	0.598 ± 0.122
Mar	13/03/2021	7.750	36	0.028	0.738 ± 0.201
Mar	15/03/2021	7.755	29	0.069	0.597 ± 0.202
Mar	17/03/2021	8.045	29	0.276	0.707 ± 0.238
Mar	18/03/2021	7.840	29	0.310	0.715 ± 0.23
Mar	19/03/2021	7.935	29	0.414	0.685 ± 0.223

Table S 3.2 *Results of multiple diffusion order of acquisition analysis (OADA) for each month. 's' is the rate of social transmission of arousal per unit network connection relative to the baseline asocial learning rate. %ST is the percentage of arousal events occurring by social transmission. Lower (L) and upper (U) 95% confidence intervals (CI), calculated by the profile log-likelihood technique, are provided for both parameters. AICc shows relative fit between social and asocial models (ΔAICc), with lower values explaining the data better, giving the best Kullback-Leibler information. The exponential of half the difference between the AICc values ('e') gives the relative support for the social vs the asocial model. 'Δloglik' shows the relative difference in log-likelihood (a measure of model fit) between social and asocial models. The p-value was obtained via a likelihood ratio test and indicates whether there is a significant difference between social and asocial models.*

Month	**s**	**s LCI**	**s UCI**	***%ST***	***%ST* LCI**	***%ST* UCI**	**AICc (social)**	**AICc (asocial)**	**ΔAICc**	***e***	**Δ_{loglik}**	***p***
October	38.822	4.778	306.837	0.554	16.500	84.500	2605.509	2611.118	5.609	16.522	7.617	<0.01
December	0.000	-9.757	39.041	0	0	43.400	1256.037	1254.021	2.016	0.365	0.000	1
February	0.000	-5.000	127.094	0	0	51.300	634.400	632.375	2.025	0.363	0.000	1
March	0.000	-19.000	195.476	0	0	58.400	365.595	363.555	2.040	0.361	0.000	1

Table S 3.3 *Top set of candidate models selected from a model averaging procedure for a binomial generalised linear mixed model with 'arousal state' (0=torpid and 1=aroused) as the response variable. The covariates for each candidate model as well as the degrees of freedom (df) are included. Log-likelihoods (logLik), AICc, and Akaike weights (ω_i) were used to assess the relative support for each model.*

Covariates	**df**	**logLik**	**AICc**	**ΔAICc**	**ω_i**
Month + Mean Ta + X coordinate	7	-1021	2056	0.000	0.260
Month + Mean Ta + Y coordinate	7	-1021	2057	0.500	0.200
Month + Mean Ta + X coordinate + Y coordinate	8	-1020	2057	0.740	0.180
Month + Mean Ta + Group size + X coordinate	8	-1021	2058	1.470	0.130
Month + Mean Ta + Relative strength centrality + Y coordinate	8	-1021	2058	1.620	0.120
Month + Mean Ta + Relative strength centrality + X coordinate	8	-1021	2058	1.750	0.110

Table S 3.4 *Unconditional model averaged parameter estimates for a binomial generalised linear mixed model (GLMM) of 'arousal state' (0=torpid, 1=aroused). Significance was assessed if either of the 95% confidence intervals (CI) crossed zero. SE gives the standard error.*

	β	SE	Adjusted SE	*z*	Lower 95% CI	Upper 95% CI	*p*
(Intercept)	-0.197	0.187	0.187	1.050	-0.563	0.169	0.292
Month (Linear)	0.084	1.107	1.108	0.080	-2.087	2.255	0.940
Month (Quadratic)	1.296	0.521	0.521	2.490	0.274	2.317	0.013
Month (Cubic)	-1.270	0.374	0.374	3.400	-2.003	-0.537	0.001
Mean Ambient Temperature	2.241	0.590	0.590	3.800	1.084	3.397	<0.0001
X coordinate	-0.078	0.075	0.075	1.040	-0.226	0.069	0.298
Y coordinate	0.050	0.067	0.067	0.740	-0.081	0.180	0.457
Group size	-0.025	0.114	0.114	0.220	-0.247	0.198	0.829
Relative strength centrality	0.010	0.035	0.035	0.290	-0.059	0.079	0.774

Table S 3.5 *Top set of candidate models selected from a model averaging procedure for linear mixed model with arousal time relative to sunset (ORQ transformed) as the response variable. The covariates for each candidate model as well as the degrees of freedom (df) are included. Log-likelihoods (logLik), AICc, and Akaike weights (ω_i) were used to assess the relative support for each model.*

Covariates	**df**	**logLik**	**AICc**	**ΔAICc**	**ω_i**
Month + Mean Ta + Group size + Relative Strength Centrality + X coordinate + Y coordinate	11	-1291	2604	0	0.63
Month + Mean Ta + Group size + X coordinate + Y coordinate	10	-1293	2605	1.04	0.37

Table S 3.6 *Unconditional model averaged parameter estimates for a linear mixed model (LMM) arousal time relative to sunset (ORQ transformed). Significance was assessed if either of the 95% confidence intervals (95% CI) crossed zero. SE gives the standard error.*

	β	SE	Adjusted SE	*z*	Lower 95% CI	Upper 95% CI	*p*
(Intercept)	-0.325	0.126	0.126	2.580	-0.571	-0.078	0.010
Month (Linear)	-1.077	0.361	0.362	2.980	-1.786	-0.368	0.003
Month (Quadratic)	0.096	0.192	0.192	0.500	-0.281	0.472	0.619
Month (Cubic)	-0.074	0.121	0.122	0.610	-0.312	0.164	0.543
Mean Ambient Temperature	-0.510	0.185	0.185	2.760	-0.872	-0.147	0.006
X coordinate	0.085	0.035	0.035	2.420	0.016	0.154	0.015
Y coordinate	0.117	0.031	0.031	3.780	0.056	0.177	<0.001
Group size	0.471	0.076	0.076	6.220	0.322	0.619	<0.001
Relative strength centrality	-0.032	0.033	0.033	0.950	-0.097	0.034	0.343

Table S 3.7 *Top set of candidate models selected from a model averaging procedure for a linear model with group arousal time variance (ORQ transformed) as the response variable. The covariates for each candidate model as well as the degrees of freedom (df) are included. Log-likelihoods (logLik), AICc, and Akaike weights (ω_i) were used to assess the relative support for each model.*

Covariates	**df**	**logLik**	**AICc**	**ΔAICc**	**ω_i**
Average relative strength + Mean Ta	4	-59.3	127	0	0.55
Average relative strength + Mean Ta + Group size	5	-58.9	129	1.63	0.24
Mean Ta	3	-61.4	129	1.9	0.21

Table S 3.8 *Unconditional model averaged parameter estimates for a linear model with group arousal time variance (ORQ transformed) as the response variable. Significance was assessed if either of the 95% confidence intervals (95% CI) crossed zero. SE gives the standard error.*

	β	**SE**	**Adjusted SE**	***z***	**Lower 95% CI**	**Upper 95% CI**	***p***
(Intercept)	0.000	0.105	0.108	0.000	-0.212	0.212	1.000
Average relative strength centrality	-0.175	0.132	0.134	1.310	-0.437	0.087	0.190
Mean Ta	-0.574	0.121	0.124	4.640	-0.816	-0.331	<2e-16
Group size	0.028	0.081	0.082	0.340	-0.133	0.188	0.740

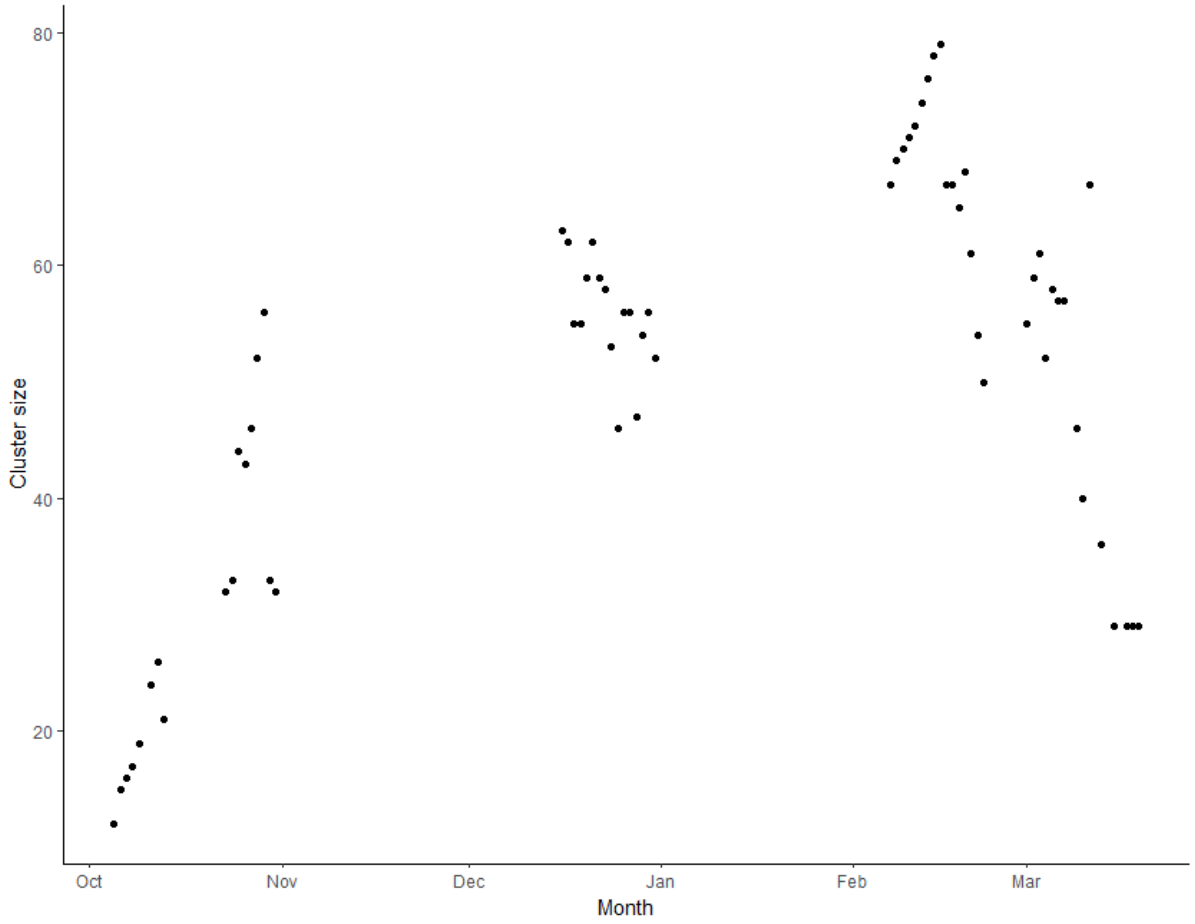

Figure S 3.1 *Distribution of cluster sizes in the hibernaculum over the study period (October 2020-March 2021).*

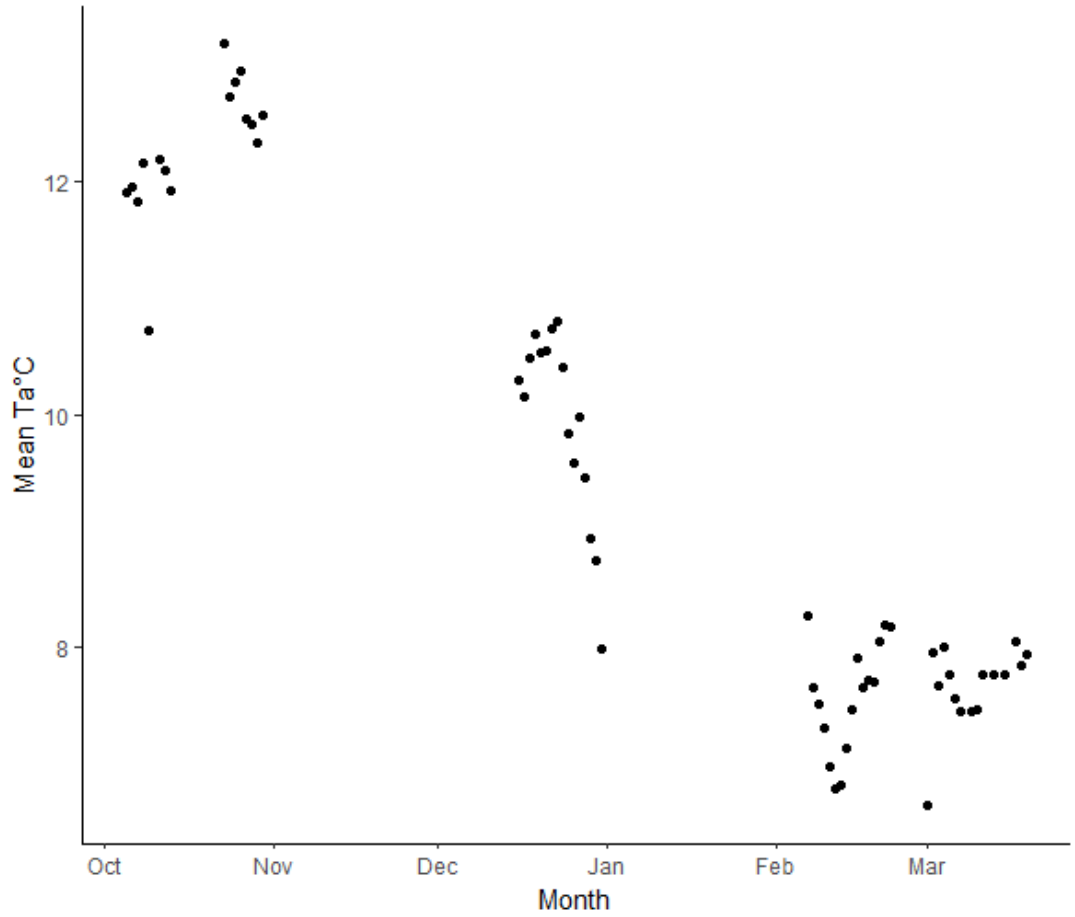

Figure S 3.2 *Distribution of mean ambient temperatures T_a (° C) in the hibernaculum over the study period (October 2020-March 2021).*

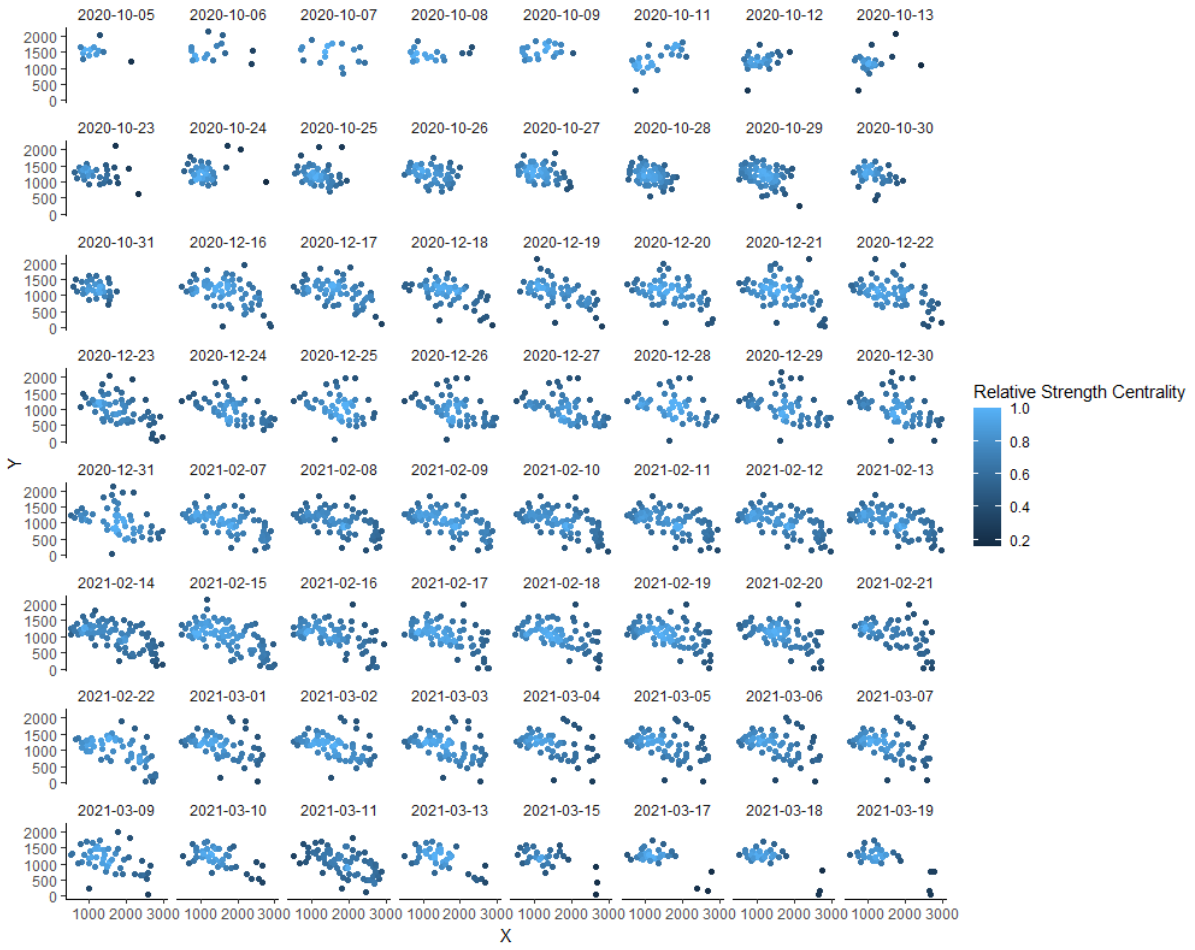

Figure S 3.3 *Roost positions of individual* R. hipposideros *(blue dots, n=3206) during the study period (October 2020 - March 2021). Shade of blue represents an individual's strength centrality relative to the maximum strength of the group on that day. Strength centrality was calculated as the sum of an individual's edge weights in a spatial proximity network. Note that a strength centrality of 0 is not possible as the network is fully connected.*

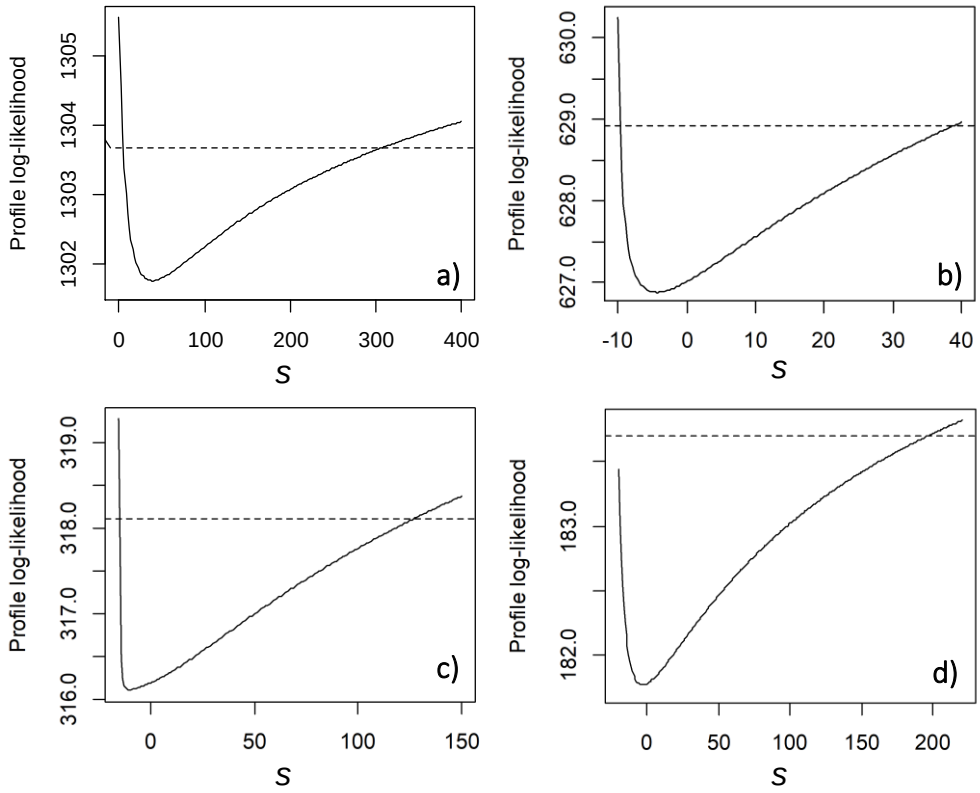

Figure S 3.4 *Profile log-likelihood plot of 95% confidence intervals (CI) for the rate of social transmission (s) for October (a), December (b), February (c), and March (d). The dashed line represents 1.92 units above the minimum negative log-likelihood (the estimate of s). The points at which the curve crosses the dashed line give the lower and upper confidence intervals. NB d) I was unable to plot the lower CI of -19 s. In addition, lower confidence intervals below zero are reported as zero in the above text, as negative values of s are not theoretically possible.*

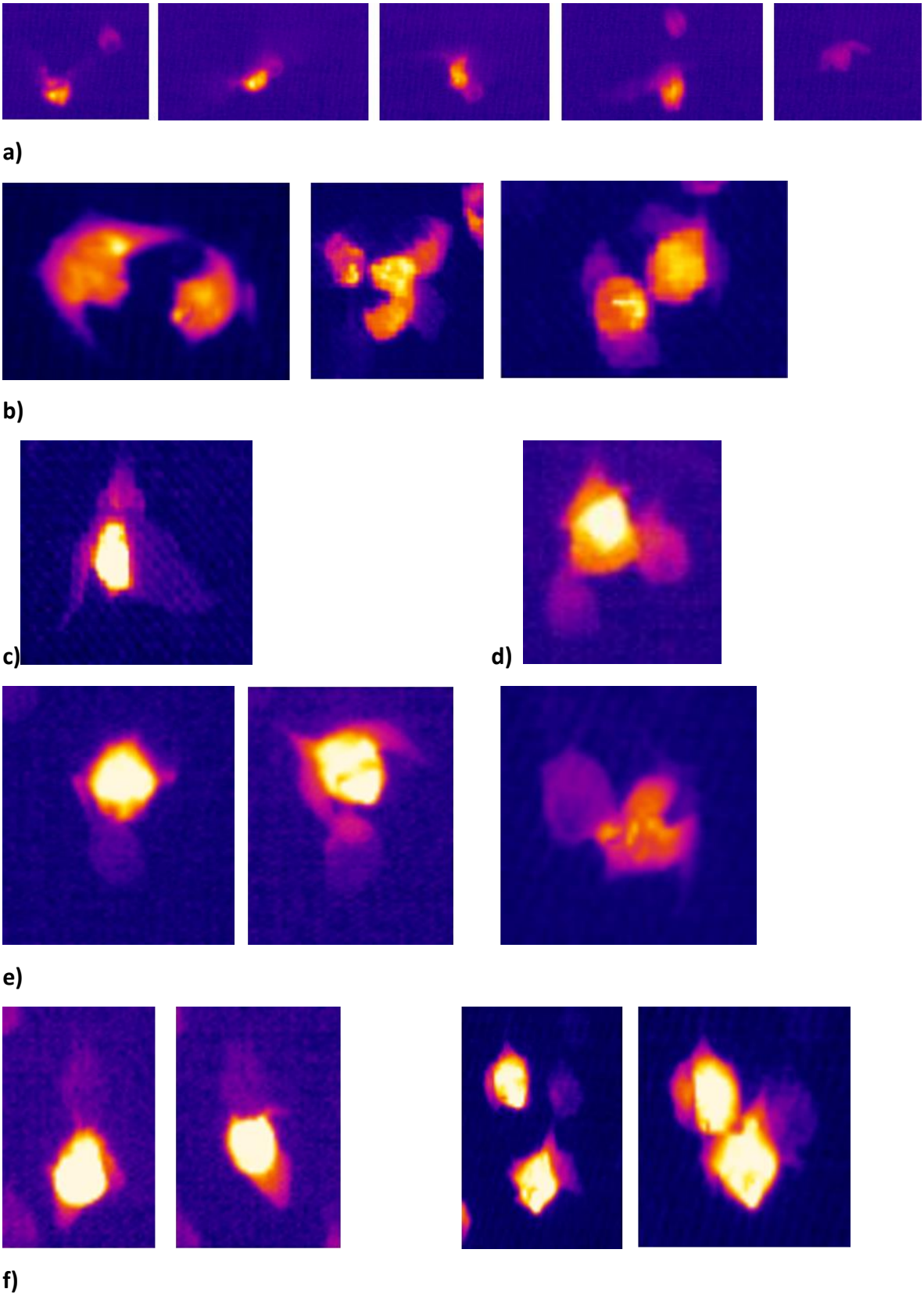

Figure S 3.5 *Visual ethogram giving representative examples of contact behaviours observed in a hibernating cluster of* R. hipposideros *using a thermal imaging camera.* ***a)*** *Aerial contact – sequence showing from left to right an individual flying up to a torpid conspecific, maintaining momentary contact, then leaving.* ***b)*** *Wing-spreading* ***c)*** *Hanging* ***d)*** *Roost contact* ***e)*** *Nose contact* ***f)*** *Two sequences of walking contact. An individual roosts in position before walking up and into another roosting bat.*

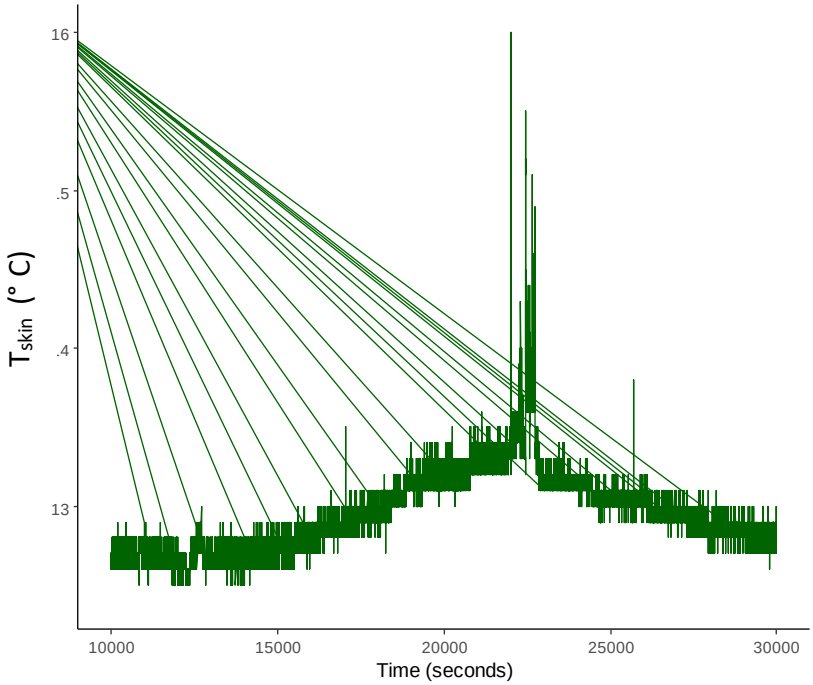

Figure S 3.6 *Example of a "cold" arousal. A T_{skin} (°C) trace shows a momentary rise in skin surface temperature before trending towards torpor.*

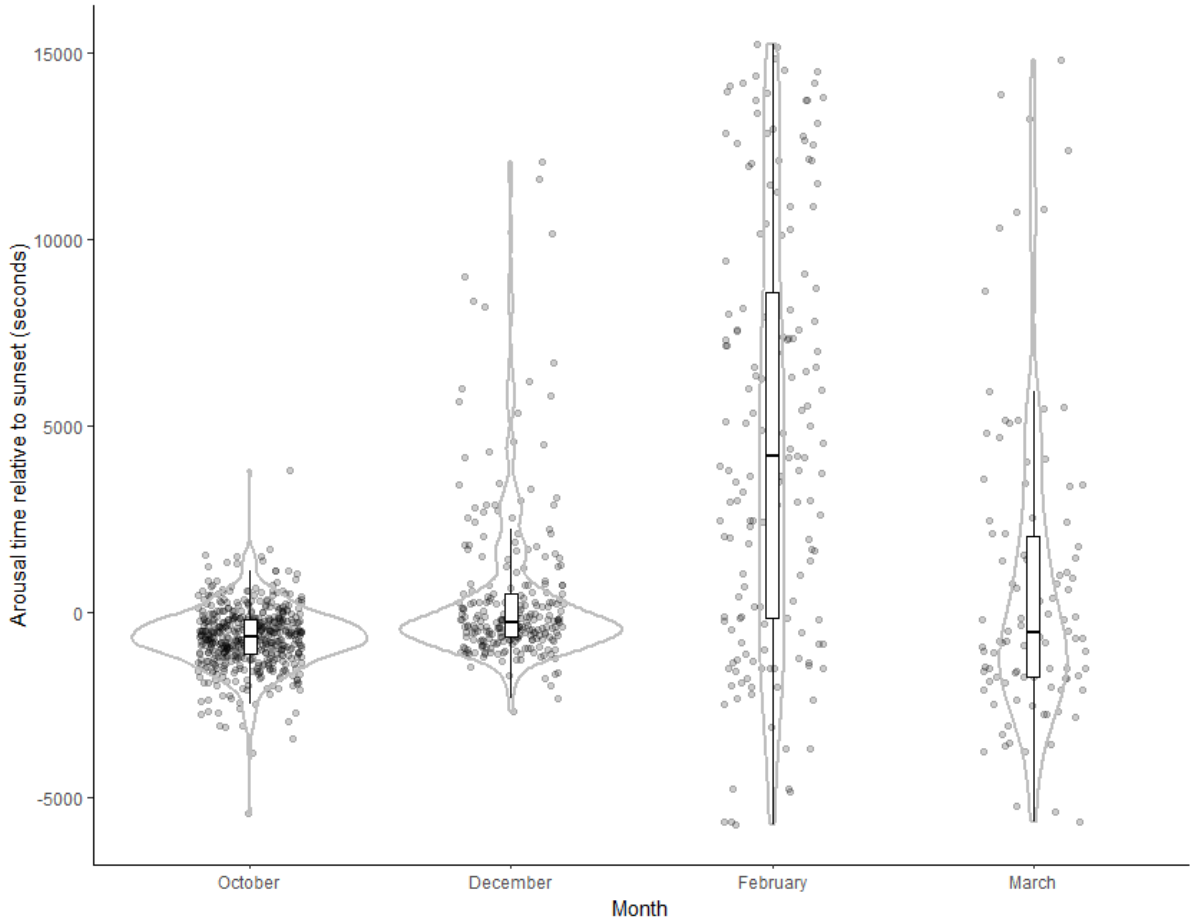

Figure S 3.7 *Boxplots showing variation in arousal time (seconds) relative to sunset (0) for each month in the study period. Violin plots show density of raw data (grey points).*

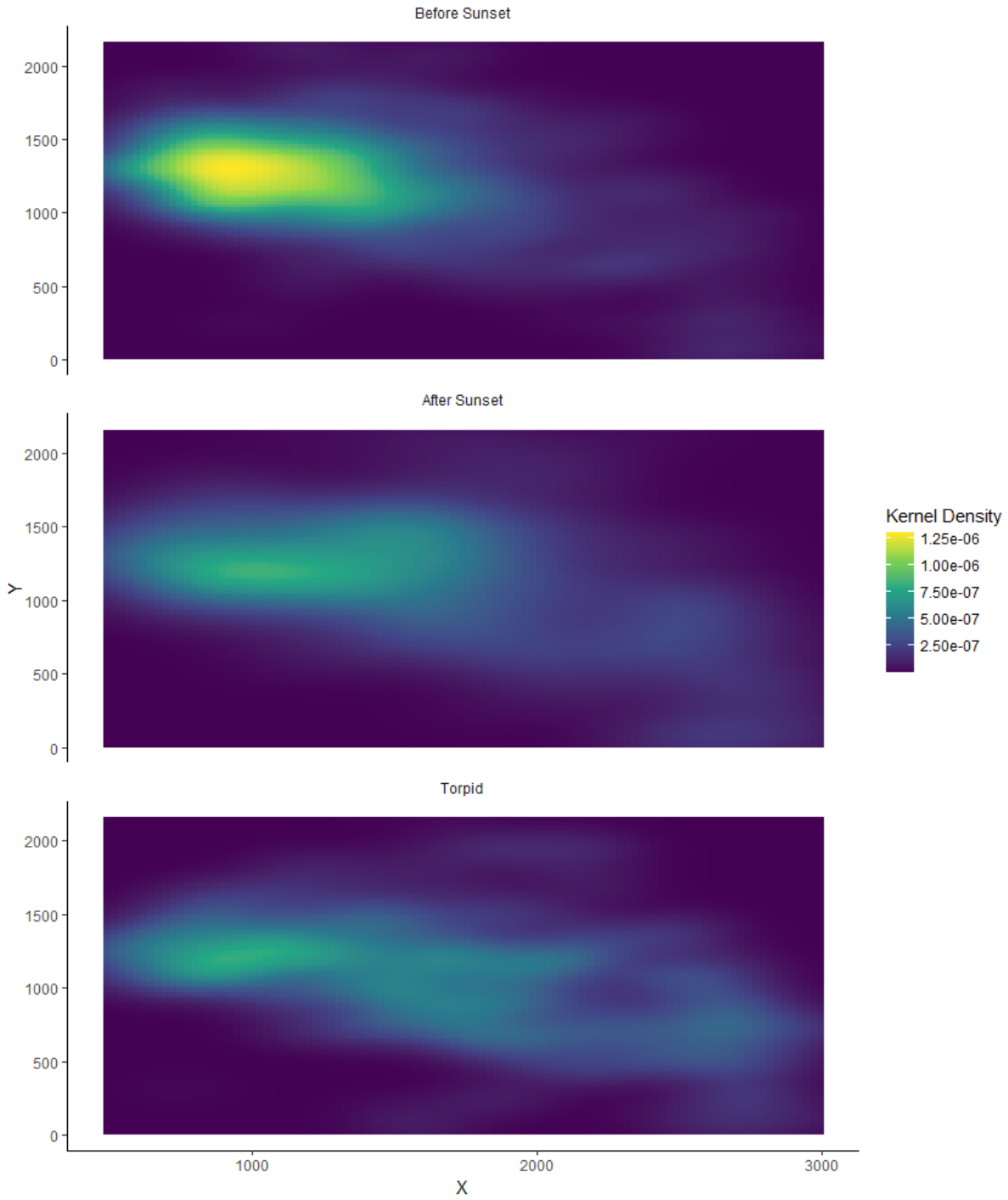

Figure S 3.8 *Density of roost positions of individual* R. hipposideros *that remained torpid (n=2173) or aroused from torpor before (n=685) or after sunset (n=348) during the study period (October 2020 - March 2021). 2D kernel density estimation performed using the kde2d function in the MASS R package and ggplot2.*

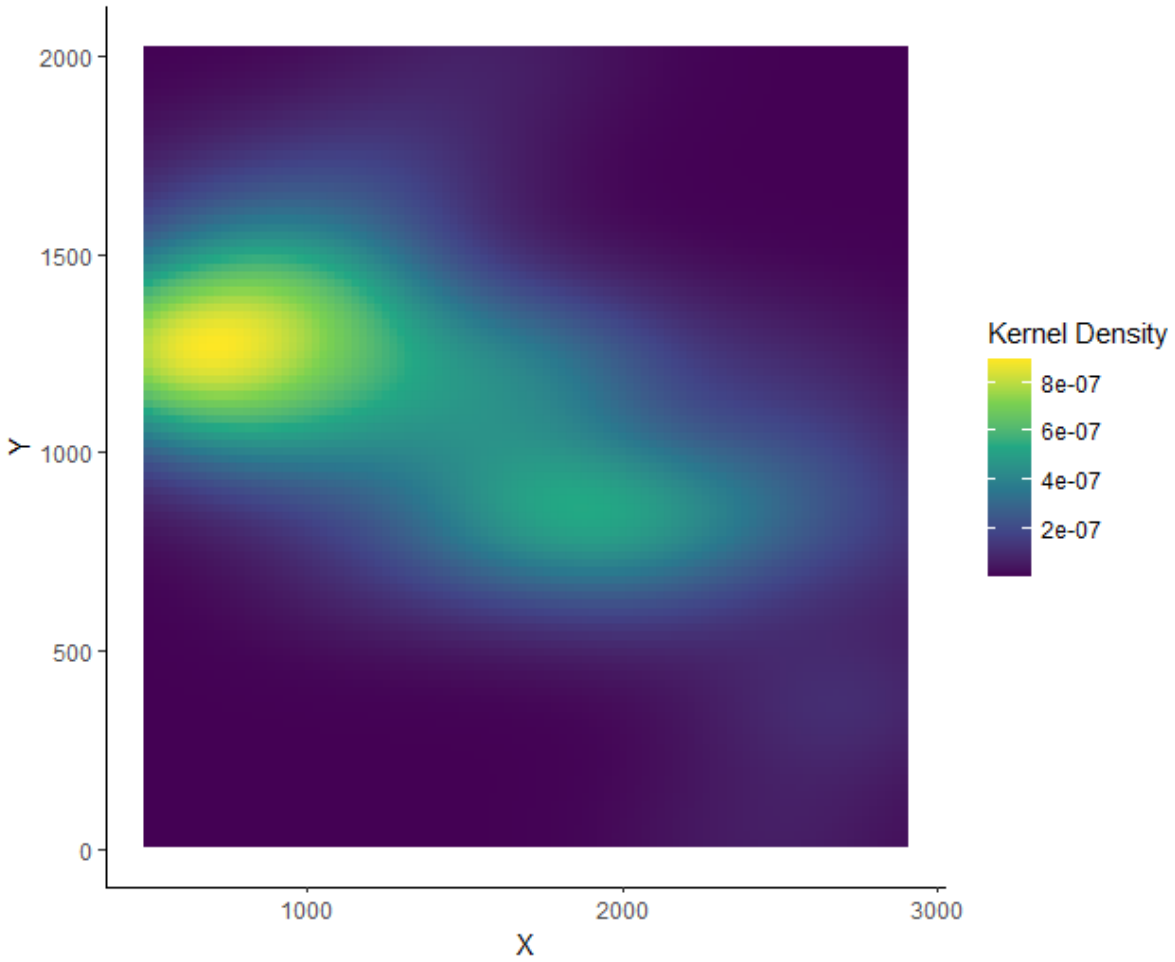

Figure S 3.9 *Density of roost positions of individual* R. hipposideros *that aroused first in their group (n=64) during the study period (October 2020 - March 2021). 2D kernel density estimation performed using the kde2d function in the MASS R package and ggplot2.*

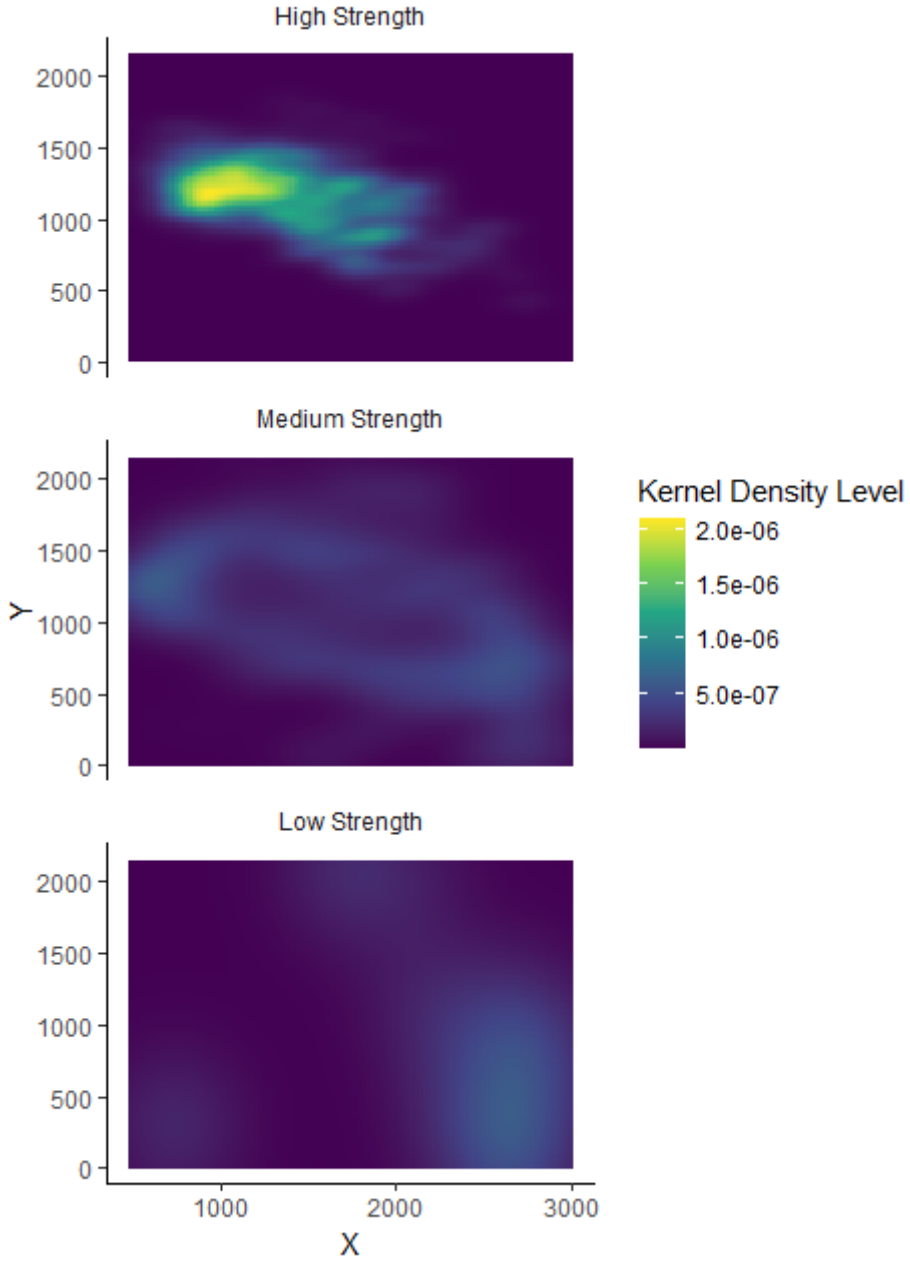

Figure S 3.10 *Density of roost positions of individual* R. hipposideros *with high (≥0.75, n=1407), medium (>0.25 & <0.75, n=1781), and low (≤0.25, n=18) relative strength centrality during the study period (October 2020 - March 2021). Strength centrality was calculated as the sum of an individual's edge weights in a spatial proximity network. 'Relative' strength centrality is relative to the individual with the highest centrality (1). 2D kernel density estimation performed using the kde2d function in the MASS R package and ggplot2.*

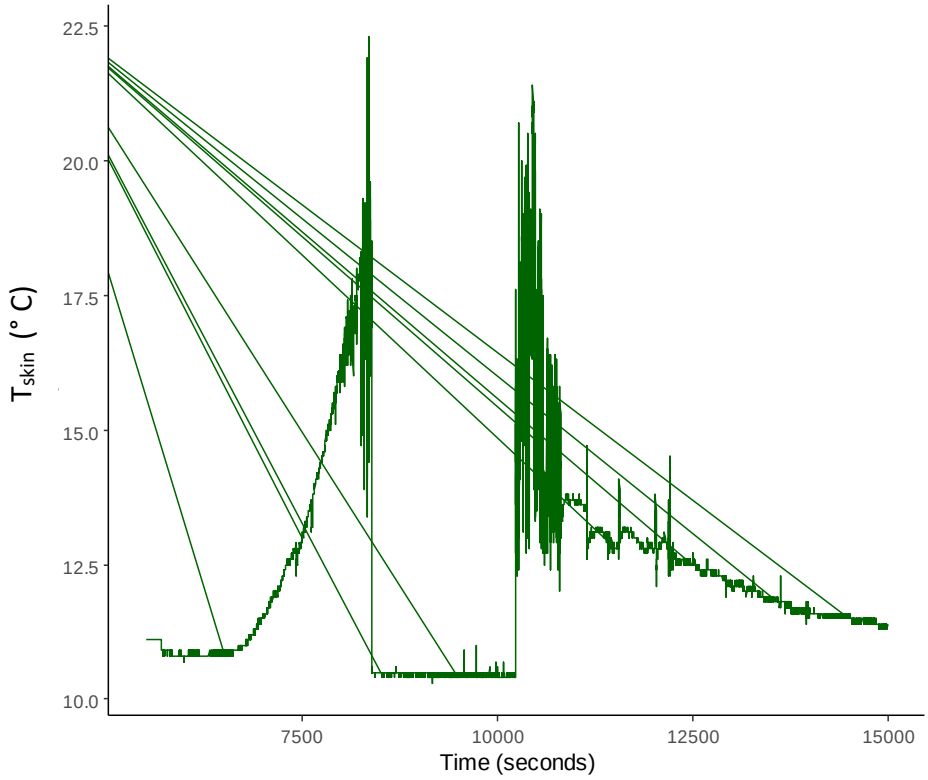

Figure S 3.11 *Example of a T_{skin} trace showing a bat arousing and leaving its post, before another (or the same) bat matches the original roost position*

Chapter 4

Components of variance, evolvabilities, and heritabilities in the life-history and morphology of a wild bat population

"Additive genetic variation in a bat colony in the style of JMW Turner". Created by DALL-E 2.

4.1 Abstract

The application of quantitative genetic analysis to long-term field studies of wild animal populations has revolutionised our understanding of how various traits evolve in nature. Long-term studies are rare in bats – a diverse and ecologically important group of mammals. One exception is the greater horseshoe bats (*Rhinolophus ferrumequinum*) at Woodchester Mansion, UK, which have been continuously monitored for 65 years. Here, we combine a 27-year pedigree, comprising ~1700 individual bats, with morphometric and life-history data. We apply 'animal models' to estimate the heritability and evolvability of morphological and life-history traits. We found high heritability (h^2= 0.666-0.576), but low evolvability (I_A= 0.0003-0.0002) for morphological traits (forearm and 5th finger digit length respectively). In contrast, we found low heritability (h^2= 0.024-0.083), but high evolvability (I_A= X, 0.052, and 0.006) for life-history traits (lifetime reproductive success, average reproductive success and parturition date respectively), indicating stronger environmental influences. We also detected significant birth year effects on parturition date, reflecting temporal variation in climate or food availability; however, maternal effects on all traits studied were weak, implying limited maternal influence on offspring phenotype for the traits analysed. Our results give insight into the factors shaping intraspecific variation in bats and how they respond to selection in the context of environmental change.

4.2 Introduction

Understanding the extent and genetic basis of variation in natural populations is a fundamental aim in evolutionary biology. This is because genetic variation provides a critical ingredient for evolution by natural selection. As populations endure rapid environmental change, adaptation becomes a necessity for long-term persistence *in situ* [505]. For this reason, researchers often examine neutral genetic variation e.g. heterozygosity, to assess the adaptive potential of threatened species [506–509]. High levels of standing genetic diversity improve the probability that individuals possess adaptive genetic variants under new environmental conditions. Indeed, many threatened species exist in fragmented populations, and are vulnerable to inbreeding and genetic drift, further eroding genetic variation [508,510]. Species with slow life histories may also suffer from low rates of recombination, thus a weaker ability to generate new variants [511]. However, the disposition of a population to undergo adaptive evolution - its adaptive potential - is dependent on whether the underlying genetic variation translates to phenotypic variation in fitness-related traits [510,512].

The proportion of phenotypic variation in a trait explained by additive genetic variation – it's narrow-sense heritability (h^2 or 'heritability' hereon) – is a key parameter in quantitative genetics, giving a direct measure of the extent of variation in phenotypes likely to be transferred to the next generation [88]. However, heritability alone is a poor indication of whether traits can mount an effective evolutionary response, as it provides little information on the absolute amount of genetic variation on which natural selection can act [513,514]. Instead, Houle (1992)[515] proposed evolvability (I_A) as a mean-standardised measure of additive genetic variation (V_A) underlying a trait. 'Evolvability' can be interpreted as the percent unit change in a trait per unit change in the strength of selection, allowing comparisons of adaptive potential among traits, populations, and species.

Several decades of quantitative genetic studies of wild animal populations have revealed a prevailing pattern of low heritability, but high evolvability in traits more closely related to fitness (e.g. life-history traits), compared to morphological traits [516]. Lower heritabilities in fitness-related traits are expected when their expression occurs over an extended period, when the environment becomes increasingly influential, leading to a lower contribution of additive genetic effects. High evolvability is counter to the traditional interpretation of

Fisher's fundamental theorem of natural selection that predicts low V_A in fitness-related traits due to erosion by selection [87,517]. One explanation is that the expression of fitness traits is influenced by a larger, more complex range of loci, providing more opportunities for mutation to counteract selection and loss of genetic variants [515,518].

Accurate quantification of heritability, and the additive genetic variation underlying it, relies on knowledge of genetic relatedness between individuals. Traditionally, longitudinal studies of wild animal populations have been essential to generating extensive pedigrees, allowing the expected genetic relatedness among relatives to be estimated [516]. Using statistical tools such as the 'animal model' allows the covariance of phenotypes among individuals to be dissected, uncovering the relative contributions of genetic and shared environmental influences [90,91].

Quantitative genetic studies are typically biased towards large mammals and diurnal birds [516], owing to practical difficulties in collecting long-term data on more cryptic taxa. Such studies [188,203,519] are rare in bats, a mammalian order of over 1400 species [104]. All species exhibit self-powered flight, and the vast majority are nocturnal, adding challenges to the collection of individualised data. Uncharacteristically of small-bodied mammals, many bat species show extreme longevity alongside comparatively low reproductive rates. The slow life-history of bats places them at a disadvantage in a rapidly changing world, where human-induced perturbations to population numbers are met with slow recoveries [520]. Effective conservation of this unique group of mammals requires sound predictions on how wild populations might adapt to future environmental change [521]. Answering this question in the long-term requires knowledge of the capacity for selection to act on traits [87].

Greater horseshoe bats (*Rhinolophus ferrumequinum*) are a good study system in which to investigate heritability and evolutionary potential in bats. *R. ferrumequinum* exhibits a slow life-history, where females produce a single offspring a year, with individuals capable of breeding up to 30 years [177,522]. Populations are particularly sensitive to anthropogenic disturbance e.g. land-use change and light pollution [177]. Previous work identified low genetic diversity in insular British populations relative to mainland Europe [245,300,301]. Less is known about the extent and basis of phenotypic variation and whether this translates to adaptive potential.

In the northernmost part of the species' range, pups are born in by late-June or mid-July and are weaned by approximately 50 days post parturition, at which point skeletal growth is largely complete [187]. Finger development – an important determinant of wing morphology and foraging niche [523]- does not conclude until juveniles begin exploratory foraging bouts. Therefore, provision from the mother and environmental conditions experienced during foraging are expected to have significant contributions to phenotypic variance in body size and finger length respectively. Importantly, body size (measured by forearm length) has been previously positively correlated with reproductive success in males [205].

Parturition date i.e. birth timing, reflects a mother's ability to track annual climatic variability, and is a key determinant behind her offspring's survival relative to the annual phenology of key prey resources [197,204]. For example, *Aphodius* spp. beetles are a critical food source for juvenile *R. ferrumequinum* post-weaning in August and those born later are less likely to survive the season [172,197]. Consequently, beyond additive genetic effects, maternal provision may be an important factor shaping variance in both morphological and life-history traits [99]. Interestingly however, paternal indirect genetic effects, mediated by the father's ejaculate, on offspring body size development have also previously been shown in mammalian taxa [103,524].

Here we use data from a 27 year study of a wild colony of *R. ferrumequinum* living at Woodchester Mansion, Gloucestershire, England. Applying the 'animal model' [90,91], we use a multigenerational pedigree to estimate quantitative genetic parameters in four commonly measured traits. Three are related to life-history (average and lifetime reproductive success, and parturition date), and two are morphological (forearm and fifth finger digit length). We assess this population's adaptive potential by applying Fisher's fundamental theorem of natural selection [517]. Accordingly, the proportional increase in a population's fitness per generation is equal to the additive genetic variance of fitness (I_{Aw}). We use average reproductive success (ARS) and lifetime reproductive success (LRS) as fitness proxies to give a direct measure of adaptive potential. The following predictions are made: 1) low heritability yet high evolvability in our set of life-history traits and vice versa in the morphological traits; 2) maternal (and potentially paternal) effects shape variation in morphological traits and consequently reproductive success; 3) maternal and year of birth

effects according to variability in prey provision and climatic conditions shape variation in parturition date.

4.3 Methods

4.3.1 Study population and pedigree construction

The resident population of *R. ferrumequinum* at Woodchester Mansion, Gloucestershire was the subject of this study. Dr Roger D. Ransome has been catching and ringing these bats in the Woodchester Mansion maternity colony for 65 years. Morphometric and phenological data are collected in regular censuses occurring every two – four days between late June and early July (depending on when births start), and three times over winter in local hibernacula (as detailed in Ransome 1989 [197]).

From 1993 to 2020, tissue samples were taken from every bat caught in the population. This included all breeding and non-breeding females, recent immigrants, transient males, alongside new-born pups. 1668 of the 1728 pups born during this 26-year span were assigned maternity, and 1227 were assigned paternity, with a confidence level of at least 80%, using microsatellite-based parentage analysis (**Figure S 4.1**). Methods detailing the genotyping and parentage analysis process are available in Chapter 2.

For 153 bats born between 1984 and 1993, additional maternity information was available because mothers have been matched to pups based on attachment during capture. As mothers only nurse their own pups, mother-pup pairs are easier to determine [274]. The full pedigree used for these analyses contained 2149 individuals with 1821 maternal links and 1227 paternal links (from 380 and 259 females and males respectively). ARS was then inferred for each individual by dividing the number of offspring assigned to an individual by their breeding span (the last year of known breeding minus the first year of known breeding).

We used ARS alongside LRS, which is commonly used in studies on wild populations [525,526]. The extended lifespan of this species[522] limits the sample of individuals from which LRS can be calculated accurately. ARS indicates an individual's typical reproductive output in a given year, allowing us to include data from bats that are still alive, thereby extending our sample size. However, calculations of ARS may introduce biased fitness estimates if a trade-off exists between lifespan and reproduction [308]. For example, individuals with long lifespans

but a low reproductive rate might achieve lower ARS despite having equivalent lifetime reproductive success to individuals with short lifespans and higher reproductive rates. LRS (the total number of offspring produced in a lifetime) was calculated for individuals (all female) for which we could be highly confident on both reproductive output and lifespan. As females in this species are highly philopatric [177], unsuccessful re-capture after a sustained period is a reliable indication of mortality. The year of death for each individual female was defined as the last year of capture after at least two years of unsuccessful re-captures. A further correlation test was performed to estimate the strength of relationship between LRS and ARS.

4.3.2 Animal models – general approach

To decompose the genetic and environmental basis of the following traits (forearm length, 5th finger digit, parturition date, ARS, and LRS), quantitative genetic "animal models" were fitted with pedigree-derived estimates of relatedness in the package MCMCglmm v2.32 [527]. This approach allows integration of pedigrees to include an additive genetic relatedness matrix to account for relatedness among individuals when partitioning phenotypic variance into environmental and genomic components [91]. Data types and distributions of each trait are available in **Table S 4.3**.

Unless otherwise specified, models were run for 10 million iterations, a thinning interval of 1000 and burn-in of 10% (1 million iterations). A sequential method in building models was chosen to examine changes in heritability with the addition of random effects. When possible, the final set of models were compared using the Deviance Information Criterion (DIC) – a measure that balances model fit and complexity - to select the best fitting model. and parameter estimates are expressed as posterior means and 95% highest posterior density (HPD) intervals. For all models, model convergence was checked through autocorrelation values (difference between chains were all < 0.1), Heidelberg & Welch and Geweke tests were passed and that all effective sample sizes (ESS) for fixed and variance components were ≥ 1000 [527].

4.3.2.1 Variance components

The variance components, including additive genetic effects (V_A), were determined by calculating the mean of the posterior distributions obtained from the MCMC sample, alongside the upper and lower 95% Bayesian credible intervals. The contribution of each variance component was calculated using the simple formula (V_X/V_P), where *Vx* is the component of interest e.g. additive genetic variance V_A divided by the phenotypic variance V_P. Narrow-sense heritability, therefore, was calculated as:

$$h^2 = \frac{V_A}{V_P}$$

Equation 4.1 *Narrow-sense heritability*

Beyond giving a measure of a trait's ability to respond to selection – heritability provides little information on adaptive potential, nor the means to compare levels of additive genetic variance between traits and populations [515]. For example, a trait may be highly heritable but with little additive genetic variance underlying it. As such we calculated an additional measure – evolvability. Evolvability (I_A) represents the expected percentage change in traits per generation under the unit strength of selection [513], and gives a standardised measure of adaptive potential [514,516]. Evolvability was calculated as the additive genetic variance (V_A) divided by the squared mean phenotype ($\bar{x}^2$):

$$I_A = \frac{V_A}{\bar{x}^2}$$

Equation 4.2 *Evolvability*

Given I_{Aw} (the additive genetic variance of fitness, where 'w' means fitness) , assuming it is constant, one can estimate the number of generations required for the average fitness to double under unit directional selection i.e. the rate of adaptive evolution, using the following equation:

$$t_2 = \frac{\ln(2)}{(I_{Aw} \times 100) \times 1}$$

Equation 4.3 *Population average fitness doubling time*

where "t_2" refers to the doubling time, and "1" as the mean-scaled selection gradient for fitness [528].

Heritabilities and evolvabilities for all traits were compared with those estimated via animal models from wild mammal populations, using supplementary data of Postma et al., (2014)[516] and Hendry et al., (2018)[512], as well as additional studies published as of February 2023 (**Table S 4.1**)

To avoid inflating estimates of heritability it is important to consider other sources of phenotypic covariance among individuals. These include common environmental conditions experienced by individuals born within the same cohort or born of the same mother [529]. Therefore, to partition phenotypic variance, beyond simple additive genetic effects (V_A), year of birth (V_Y), and mother (V_M) were included in the model structure to examine variance associated with an individual's cohort and maternal environment respectively. A maternal (V_{MG}) genetic effect was included to further partition potential maternal effects into non-genetic and genetic components. A paternal genetic effect (V_{PG}) was included to examine potential IGEs on offspring phenotype. A paternal environment effect was not included as fathers are not responsible for the rearing of young, and likely contribute nothing to phenotypic variance. Neither V_{PG} nor V_{MG} were examined explicitly in the model for parturition date as we saw no rationale for their addition. To account for environmental influences unique to each cohort, such as weather, we included a random effect of the offspring's year of birth ($V_{BIRTHYEAR}$) to estimate its contribution to phenotypic variance. In most cases, only single measurements per individual were available for each trait. However for parturition date, repeated records for each individual were available, allowing intra-individual variation (V_I) to be accounted for, as well as estimation of permanent environment effects.

4.3.2.2 Fixed effects

An additional consideration when constructing animal models is the effect of certain variables on the mean phenotype value, rather than the variance. For example, the effect of sex in the sexual dimorphism of forearm length. Such variables, where there is rational explanation for their inclusion are fitted as fixed effects. For each trait of interest, fixed effects were tested within either simple linear models in using the *stats* R package v 4.2.2, or generalised linear mixed models in *lme4* v 1.1-31 where repeated measures were available and included in the final animal model if they were significant. Rationale for testing each fixed effect is provided in **Table S 4.2**.

4.3.3 Animal model specification

4.3.3.1 Morphological traits

Univariate models were constructed for forearm length and 5th digit separately and modelled assuming a Gaussian error distribution. The fixed effects included sex and corrected date of birth (the number of days an individual was born minus the mean birthdate of the individuals cohort). In both models, random effects included additive genetic variance (using the pedigree information), cohort effects, maternal environment effects, maternal genetic effects and paternal genetic effects. As there is only one measure of each variable per individual, permanent environmental effects could not be fitted separately from residual effects. Default priors were used for fixed effects and inverse-Wishart (uninformative priors) were used for the random effects and residual variance (V = 1, nu = 0.002).

To determine if there is a genetic correlation between forearm length and 5th digit, a bivariate model was constructed with both variables as response variables with sex and corrected data of birth retained as fixed effects and run for 2 million iterations, a thinning interval of 200 and burn-in of 200,000. To avoid over-parameterisation, the random effect structure only contained additive genetic variance linked to the genetic pedigree in addition to residual environmental effects, as the main goal of this analysis was to estimate among-individual covariation. The genetic correlation (r_G) between forearm length and 5th digit was calculated using the formula in Wilson *et al*., (2010) [91]:

$$r_G = \frac{COV_{A_{Fore,DGT5}}}{\sqrt{V_{FORE} * V_{DGT5}}}$$

Equation 4.4 *Genetic correlation*

where $COV_{AFore,DGT5}$ represents the additive genetic covariance between forearm length and 5th digit while V_{FORE} and V_{DGT5} represent the additive genetic variances of each trait respectively.

4.3.3.2 Life-history traits

Parturition dates of females were fitted as a response variable, modelled with a Gaussian error distribution and priors as detailed above. Fixed effects include the sex of the offspring, age of the mother at time of parturition date and the female's pedigree-based inbreeding coefficient (IBC) (calculated as the relatedness between the parents). Random effects

included additive genetic variance linked to the pedigree, individual identity not linked to pedigree (to account for repeated measures and to model permanent environmental effects), maternal environment effects, offspring birth year and mother birth year.

Models of ARS were run for males and females separately, modelled with Gaussian error distributions and priors as detailed above. As raw data for males was right-skewed, a square-root transformation was applied to approximate normality. For both sexes, fixed effects included forearm length, IBC and corrected date of birth. Additive genetic variance, year of birth, maternal environment, maternal genetic and paternal genetic effects were fitted as random variables. The same structure of fixed and random effects was specified initially for the model of female LRS, with a Poisson error distribution and parameter-expanded priors.

4.3.4 Parent-offspring regression analyses

For comparison with animal models, and to assess the relative contribution of each parent to offspring variation, we conducted parent-offspring regression analyses for morphological traits. Relative to the Bayesian "animal model" approach used in this study, parent-offspring regressions are no longer the preferred method of measuring heritability [529] as they risk either inflating estimates (due to common environmental effects among relatives) or underestimating (e.g. taking different aged individuals but not taking age-related variation into account [530]). Nevertheless, parent-offspring analyses can report similar heritability estimates to animal models and provide a useful means to visualise phenotypic relationships between relatives as well as providing estimates of heritability in a "broader" sense. Mother-offspring and father-offspring regressions were applied using linear mixed effect models of forearm length and 5th digit between the respective parent and their offspring. For each model the proportion of phenotypic variance among offspring explained by either or both the parents' phenotype i.e. the narrow-sense heritability, is given by the slope. As the dataset contains forearm length and 5th digit measures from offspring of the same parents in multiple years, parent ID and year were included as random effects. Sex and corrected date of birth were also included as fixed effects. To test whether heritability differed according to offspring sex, heritability was also measured from mother-daughter, mother-son, father-daughter and father-son regressions.

4.4 Results

4.4.1 Morphological traits

For both morphological variables, individuals born later in the season were smaller, as were males relative to females (**Table S 4.4**). Heritability was high (Forearm length h^2: 0.666, 95% HPD 0.533 – 0.798; 5th digit h^2: 0.576, 95% HPD 0.419– 0.798, **Table S 4.4**, **Figure 4.1**, **Figure 4.2**), with low evolvability (Forearm length I_A: 0.0003, 95% HPD 0.0002 – 0.0003; 5th digit I_A: 0.0002, 95% HPD 0.0001 – 0.0003, **Figure 4.1**). Both heritabilities were higher than the median heritability for morphological traits reported in previous studies of wild mammals (median h^2= 0.340), but with relatively low evolvabilities (median I_A = 0.00152).

Additional components, including maternal effects, accounted for little of the total variance with the remaining residual variance accounting for most of the variance after additive genetic effects (**Table S 4.4**, **Figure 4.2**). In offspring forearm length regressions, mother-offspring and father-offspring regressions resulted in h^2 of 0.688 and 0.607 respectively with estimates for daughters and sons yielding similar values (**Table S 4.5**, **Table S 4.6**, **Figure 4.3**, **Figure 4.6**). For 5th digit, mother-offspring h^2 was 0.673 with father-offspring in an lower estimate of 0.471 (**Table S 4.7**, **Table S 4.8**, **Figure 4.4**, **Figure 4.5**). For both mothers and fathers, estimates for daughters and sons differed with heritability for sons being higher (mother-daughter h^2: 0.565; mother-son h^2: 0.715; father-daughter h^2: 0.406; father-son h^2: 0.553; **Table S 4.7**, **Table S 4.8**, **Figure 4.4**, **Figure 4.5**). When considering the bivariate model, the genetic correlation between forearm length and 5th digit was high (r_G: 0.677, 95% HPD 0.590 – 0.766; **Table S 4.9**), implicating the same or linked genes influence both forearm length and 5th digit. In addition, residual (environmental) correlation was high (0.640, 95% HPD 0.541 – 0.736; **Table S 4.9**), suggesting environmental conditions play an important role in shaping both traits.

4.4.2 Life-history traits

Parturition date was influenced by a female's age (older females giving birth earlier), yet there were no effects of offspring sex or inbreeding coefficient (**Table S 4.10**). Parturition date heritability was low at 0.083 (95% HPD 0.035 – 0.131; **Table S 4.10**, **Figure 4.1**) with moderate evolvability (I_A: 0.006, 95% HPD 0.003 – 0.007; **Figure 4.1**). Permanent environment effects, maternal environment and birth year effects were low, with offspring birth year accounting for 64.1% of total variance (**Table S 4.10**, **Figure 4.2**).

For females and males, the effects of forearm length, corrected date of birth and IBC on ARS were negligible (**Table S 4.10**). Similarly, for female LRS, corrected date of birth and IBC were also negligible (**Table S 4.10**). However, a positive correlation was observed between forearm length and LRS (**Table S 4.10**). The heritability of ARS for both sexes was low (Females: 0.028, 95% HPD 0.001 – 0.086; Males: 0.024, 95% HPD 0.001 – 0.074; **Figure 4.1**, **Figure 4.2**) with other random effects contributing little to the total variance (**Table S 4.10**, **Figure 4.2**). Both sexes displayed high evolvability with males demonstrating marginally higher evolvability (Females I_{Aw}: 0.038, 95% HPD 0.001 – 0.121; Males I_{Aw}: 0.052, 95% HPD 0.002 – 0.163; **Figure 4.1**).

The Pearson's product-moment correlation between female LRS and ARS was positive, statistically significant, and very large (r = 0.68, 95% CI [0.62, 0.73], t(367) = 17.78, p < .001). However, relative to ARS, the heritability of female LRS was lower (h^2: 0.011, 95% HPD 0.001 – 0.049) with year of birth accounting for 22% of the total variance and other random effects contributing little to the total variance (**Table S 4.10**). Female LRS displayed moderate evolvability (I_{Aw}: 0.009, 95% HPD <0.001 – 0.036)).

The reported heritabilities are comparatively lower to those previously reported for wild mammal life-history traits (median h^2 = 0.118), while the evolvabilities are higher (median I_A = 0.005). For traits specifically linked to fitness, our reported evolvabilities were comparatively higher for ARS, but not LRS (median I_{Aw} = 0.023).

The median evolvability for fitness of male and females in this study, calculated via ARS, was 0.045, giving a doubling time for individual fitness under unit directional selection of 15 generations, assuming evolvability is constant. The evolvability for female LRS (I_{Aw} = 0.009) had a longer doubling time of 77 generations. The meta-analytic median evolvability of 0.023 for wild mammals yields a doubling time of 31 generations.

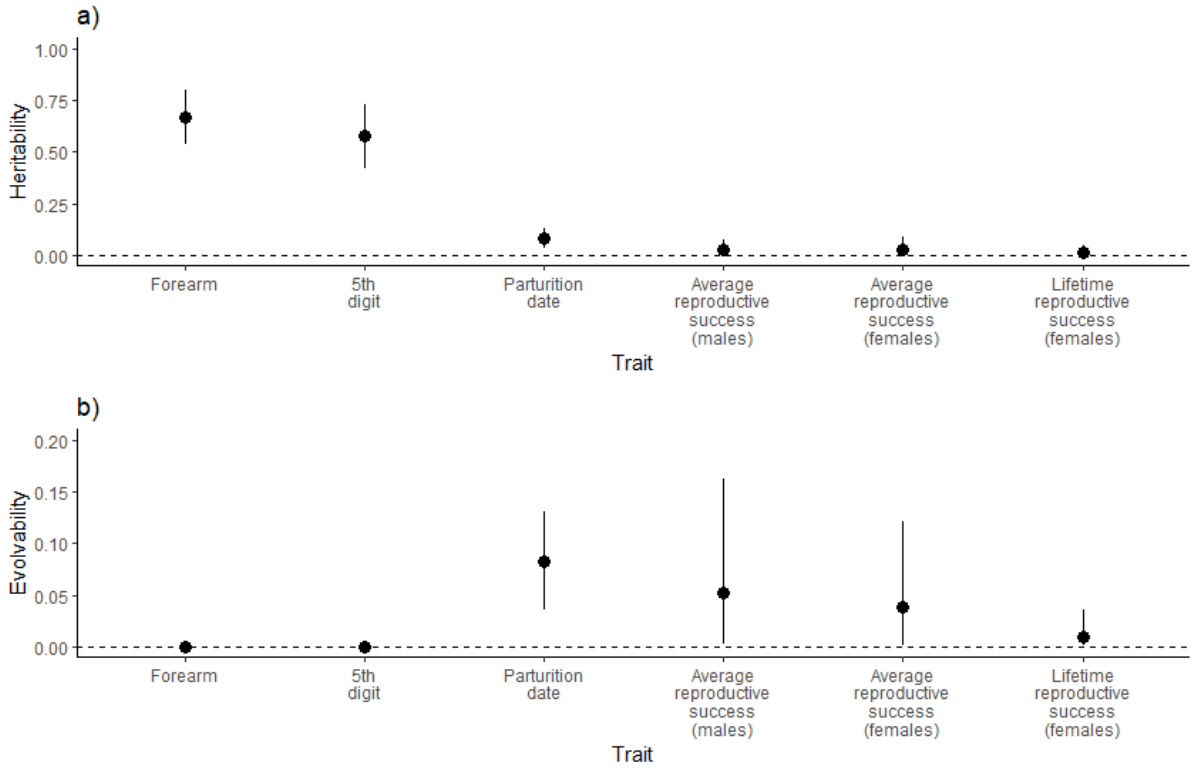

Figure 4.1 a) *Estimates of narrow-sense heritability (h^2) – the proportion of phenotypic variance explained by additive genetic effects (V_A) in each phenotypic trait. Estimates are given as the mean of the posterior distribution with lower and upper 95% highest posterior density (HPD) intervals.* ***b)*** *Estimates of evolvability (I_A) - the expected percentage change in traits per generation under the unit strength of selection, and gives a standardised measure of adaptive potential (Houle, 1992* [514]*). Evolvability was calculated as the additive genetic variance (V_A) divided by the squared mean phenotype ($\bar{x}^2$).*

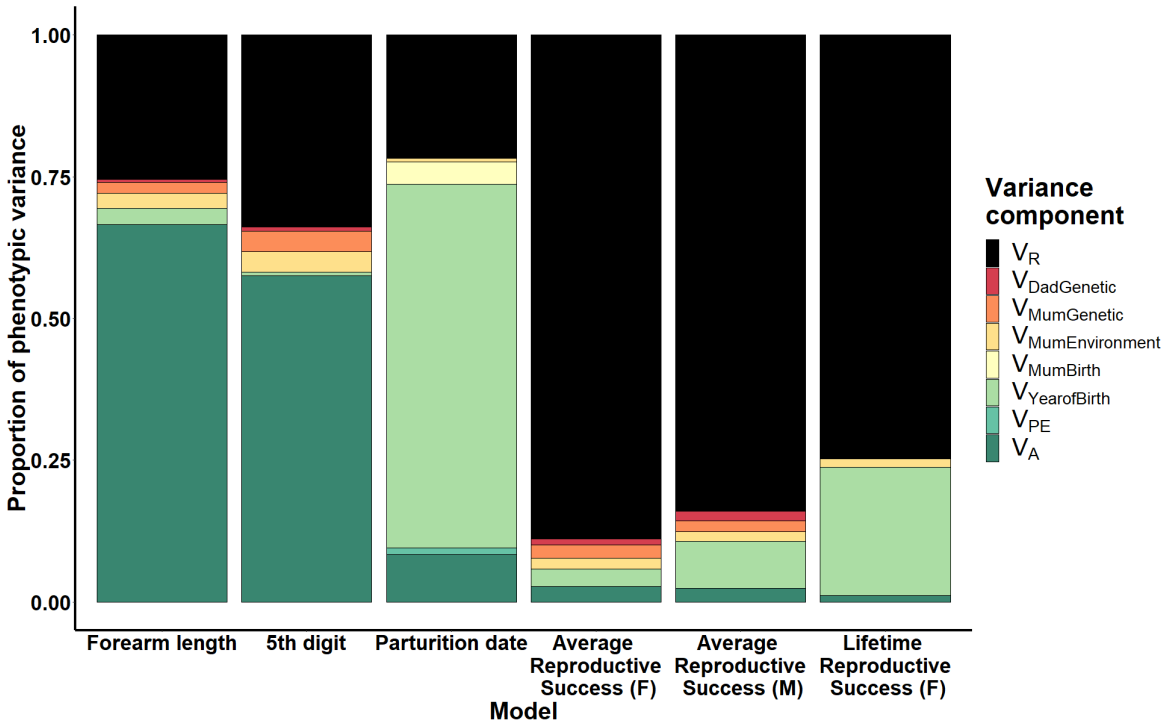

Figure 4.2 *Mean proportion of total phenotypic variance (V_P) explained by the different variance components of each trait (forearm, 5th digit, parturition date, average female reproductive success, average male reproductive success, female lifetime reproductive success). V_R gives the residual component, while V_A and V_{PE} give the additive genetic and permanent environment components respectively. 'YBIRTH' means year of birth.*

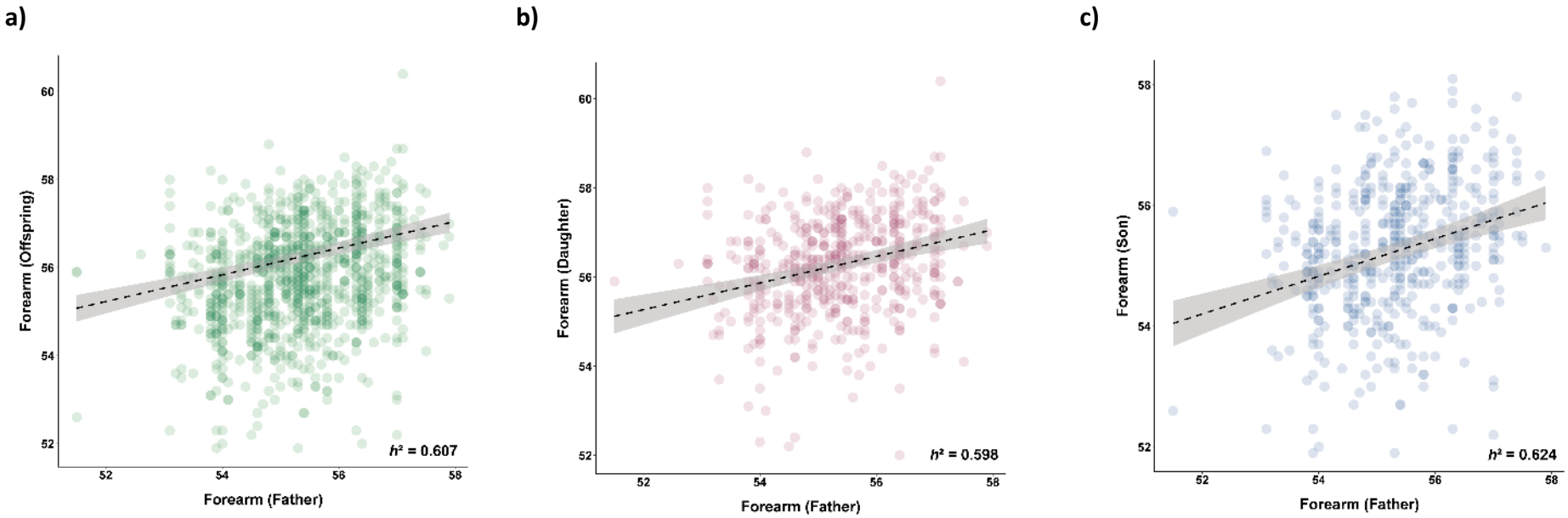

Figure 4.3 *Father-offspring regression of forearm length (mm).* ***a)*** *all offspring,* ***b)*** *female offspring,* ***c)*** *male offspring. The slope gives the heritability (h^2).*

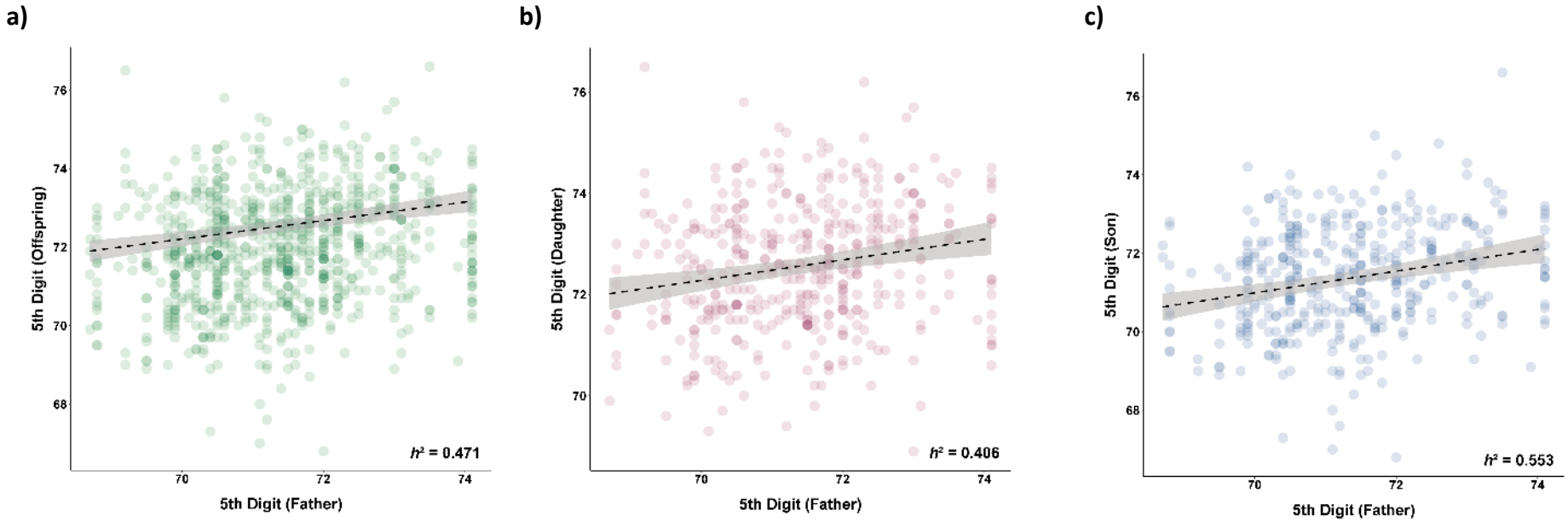

Figure 4.4 *Father-offspring regression of fifth finger digit (5th digit) length (mm).* ***a)*** *all offspring,* ***b)*** *female offspring,* ***c)*** *male offspring. The slope gives the heritability (h^2).*

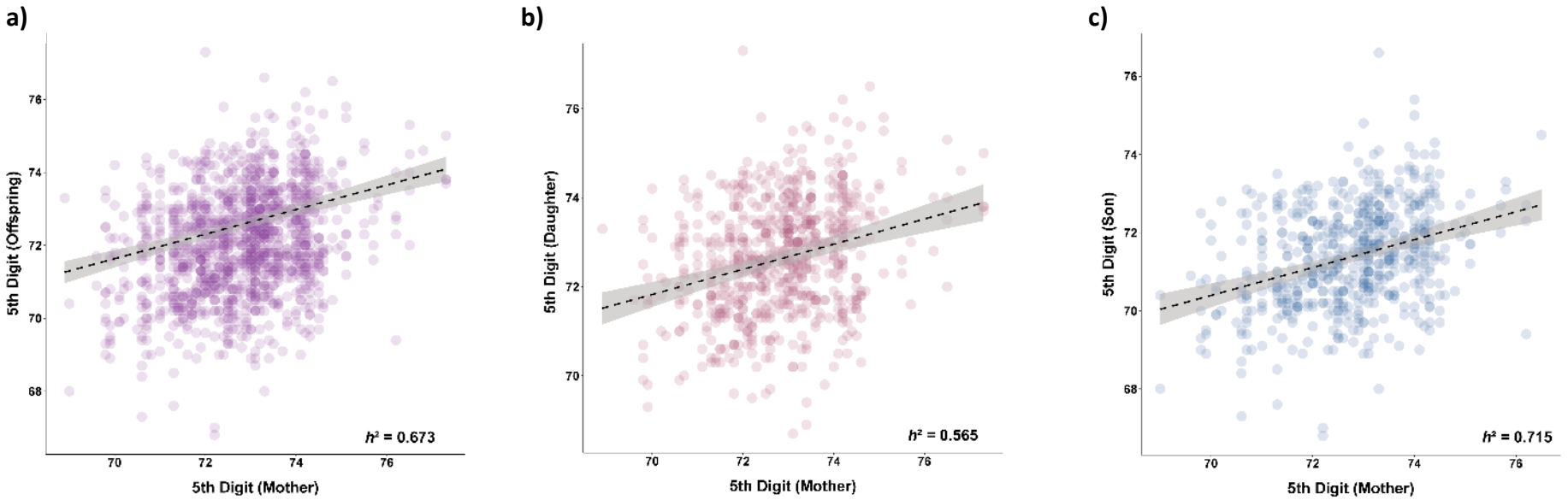

Figure 4.5 *Mother-offspring regressions for fifth finger digit (5^{th} digit) length (mm).* ***a)*** *all offspring,* ***b)*** *female offspring,* ***c)*** *male offspring. The slope gives the heritability (h^2).*

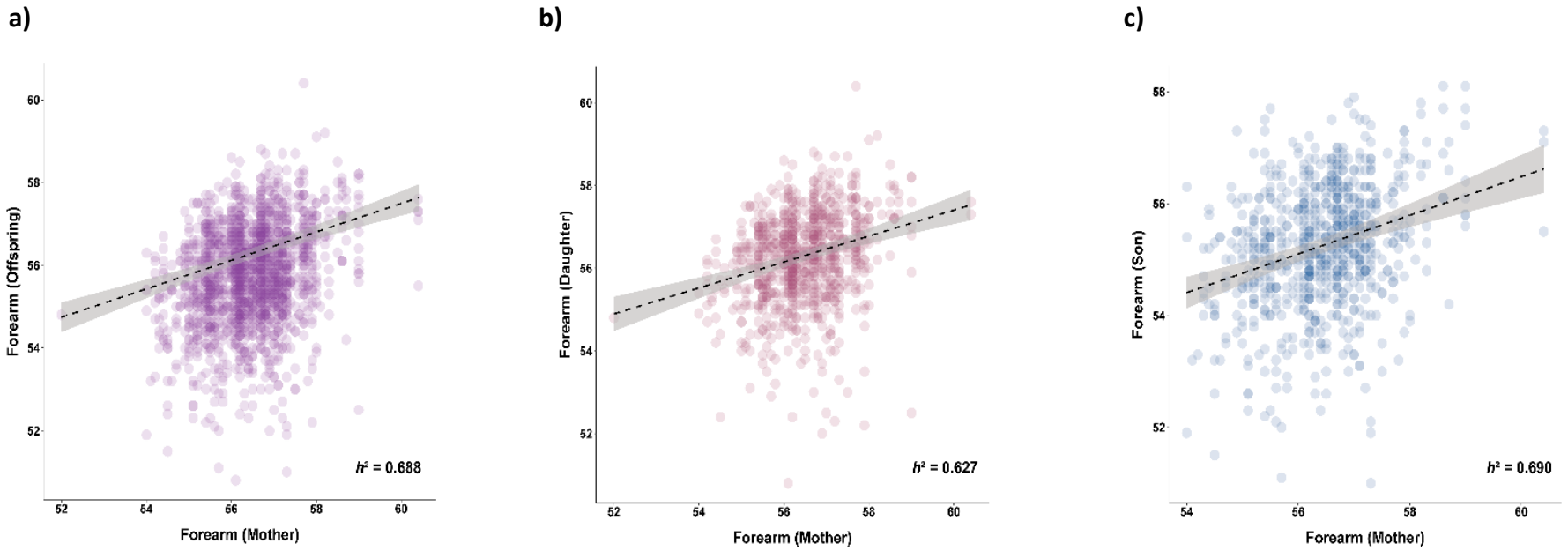

Figure 4.6 *Mother-offspring regressions for forearm length (mm).* ***a)*** *all offspring,* ***b)*** *female offspring,* ***c)*** *male offspring. The slope gives the heritability (h^2).*

4.5 Discussion

There is currently a lack of knowledge on intraspecific phenotypic variation and how it could impact evolution on bats. Using a 27-year dataset from a wild population of *R. ferrumequinum*, we applied a quantitative genetic approach to decompose variance in morphological and life-history phenotypes, to assess their adaptive significance and to compare genetic and environmental influences on expression. While estimates varied, all traits showed a degree of heritability and evolvability, and are thus amenable to selection - fulfilling the requirements for an evolutionary response to occur.

4.5.1 Additive genetic variance of fitness

Significantly, we found that individual fitness is heritable and evolvable, meaning that consistent directional selection can drive genetic changes in fitness-related traits in this population of *R. ferrumequinum* i.e. adaptive evolution. The male bias in I_{Aw} (additive genetic variance of fitness) could be attributed to greater error in paternity vs maternity assignments and/or sex-specific bias in reproductive success [512]. V_A may be easier to detect in whichever sex shows higher variance in reproductive success in a given year [531]. For example, a study of red deer (*Cervus elaphus*) showed greater evolvability for fitness in males, where females mate with a single male per year, and males compete for multiple females [532].

The median I_{Aw} of 0.045 from ARS, and the I_{Aw} of 0.009 from LRS, indicating a 4.5% and 0.9% increase in mean fitness per generation respectively, were well within the range reported for wild mammals (range = 0-0.852, SD = 0.175, median I_{Aw} = 0.023) [512,525]. Assuming constant evolvability, an I_{Aw} of 0.045 from ARS yielded a doubling time of mean fitness in 15 generations. In contrast, the I_{Aw} 0.009 from LRS gave a doubling time of 77 generations. These rates are faster and slower, respectively, than most previous wild mammal studies (median t_2 = 31 generations). Even so, some of these studies had estimates of zero for additive genetic variance, evolvability, and heritability, possibly due to small sample size. When excluding zero estimates, the median I_{Aw} was 0.099 (t_2 = 7 generations; Equation 3). I_{Aw} gives a reliable measure of a population's ability to address immediate selection pressures [507,512]. However, as this population is at the northern extreme of the species' range it may be more vulnerable to extinction [533]. To determine whether our observed I_{Aw} can adequately address future environmental changes, further research is needed. This

could include determining whether reproductive success is an accurate fitness proxy [534], and comparisons with colonies at the centre of the species' range.

4.5.2 High heritability and low evolvability in morphological traits

As predicted (1), both morphological traits (forearm and 5th digit length) showed higher heritabilities than life-history traits (ARS and parturition date), i.e. relatives are more likely to covary in morphology than they are in life-history. These results reflect the nature of ontogeny in the respective traits. Both forearm and 5th digit develop early during the juvenile stage, whereas both parturition date and reproductive success are expressed over the entire lifetime of the individual, exposing higher levels of residual variance. Indeed, low levels of environmental variance experienced during early life may explain why our estimates of h^2 for morphological traits were in the top 50% of estimates in wild mammal populations. A tubular heater was installed in the Woodchester Mansion roost in 1993 [265], producing stable temperature conditions for the maternity colony over the proceeding decades. In contrast, Mundinger et al., (2022)[519] report lower heritability for forearm length (h^2=0.46) in the long-lived[535] Bechstein's bat (*Myotis bechsteinii*), measured over the same period. Here individuals roosted in roost boxes that are vulnerable to dynamic temperature fluctuations, as are *R. ferrumequinum* in unheated roosts (see Dietz et al., 2007 [536] and Eghbali et al., 2019 [537]). Furthermore, studies in birds have demonstrated lower heritabilities (and higher VE) for morphological traits under more variable environmental conditions [538,539].

Evolvabilities for morphological traits were low, suggesting low standing additive genetic variation, indicating a negligible capacity to respond to selection, despite high heritability. Interestingly, Mundinger et al., (2022)[519] report an identical evolvability for forearm length (I_A = 0.0003) in *M. bechsteinii*. Yet, over a similar timeframe they found a substantial increase in forearm length, possibly driven by phenotypic plasticity in response to climate warming. In our *R. ferrumequinum* population, average forearm and 5th digit lengths have remained mostly stable over the last 27 years (**Figure S 4.3**). An interesting avenue of future study could be the heritability and evolvability of plasticity, given probable contrasts in thermal tolerance, in the respective populations [540]. It is possible that the stable conditions have led to the loss of standing genetic variation (and thus evolvability) [541,542]. Populations in

homogenous environments may risk losing extreme genotypes due to weak selection and genetic drift [543], although evidence of this effect on morphological traits is unclear [544].

4.5.3 High birth year effects on parturition date

Parturition date in females showed moderate evolvability (I_A = 0.006), commensurate to other life-history traits in wild mammal populations (I_A = 0.005). Traits exposed to strong temporal variation in natural selection are expected to hold higher levels of standing additive genetic variation [545–548]. This will be critical for an evolutionary response to the long-term advancement of phenology expected under future climate change scenarios [549,550].

Heritability was low (h^2 = 0.083), with the contribution of additive genetic variation masked by higher levels of environmental variation. Our value of h^2 is comparable to that obtained for red deer (*C. elaphus*) (h^2 = 0.09) (though see Bonnet et al., 2019 [551]), where the offspring birth year explained 13% of the phenotypic variance. In this study, birth year explained 64% of the variance, suggesting high levels of phenotypic plasticity. This is consistent with previous work in *R. ferrumequinum* [204,552] (but also *M. bechsteinii* [553] and *M. daubentonii* [554]) showing earlier parturition in years with warm springs. Higher spring temperatures probably advance the end of the hibernation period, and thus implantation of the fertilised embryo [536,554].

Phenotypic plasticity in parturition date is beneficial to females in tracking seasonal variation in prey availability. Abundance peaks in key prey species are tied to temperature [555], thus parturition date is likely to affect offspring growth [197,537]. Indeed, we found older females gave birth earlier, producing larger pups than younger mothers. Body mass tends to be higher in older females post-hibernation [183,199], thus potentially more resources can be diverted to gestation at an earlier date [556].

Spring temperatures are increasing in response to climate change [557]. As such, the ability for birth timing to respond to climate warming is a necessity for population viability [558]. In this population, parturition date has advanced by ~9 days over the past 4 decades (**Figure S 4.2**), following a phenological shift in prey availability [173]. Similar responses have been detected in mammal populations [551,559,560], including bats [553,554]. However, the existence of heritable phenotypic variation in this study does not necessarily suggest that adaptive evolution has

occurred. Studies on birds[561] and mammals[562] frequently show a plastic response[563] in phenological traits to climate change. This arises where the covariance between the phenotype and fitness is driven by environmental rather than genetic variance [564,565]. Matters are complicated where phenotypic changes are a combination of both plastic and genetic responses [551,562]. A recent meta-analysis covering five species of mammal (mainly ungulates) found plasticity for parturition date was insufficient in tracking the "optimum" phenotype [545]. While we show parturition date as a phenotype is evolvable in *R. ferrumequinum,* further study will be needed to ascertain whether the observed phenotypic shift is a sufficiently adaptive response.

4.5.4 Weak maternal effects

Using parent-offspring regressions, we find marginally higher heritability between mother and offspring than father and offspring in both forearm (body size) and 5th digit length. However, contrary to prediction (2), results from the animal models indicated only a minor influence of maternal effects on the development of morphological traits (**Figure 4.2**), suggesting the majority of maternal influence on offspring phenotype is conferred via the inheritance of a mother's genes, rather than through provisioning. We predicted a greater role for maternal effects considering the majority of skeletal development occurs pre-weaning. The contribution of maternal effects is typically stronger in the juvenile stage [99]. In mammals, differential resource allocation according to foraging ability could constitute a maternal environmental effect on offspring morphology of birth timing [101,566–570]. Likewise, variation among mothers in the expression of genes related to lactation could confer a maternal indirect genetic effect [102]. The potential for paternal genetic effects on offspring phenotype was also explored. Despite no paternal care, fathers could influence sex allocation (and thus body size)[571], or via the transgenerational inheritance of epigenetic markers, affecting offspring growth [103,524,572]. Such influences, if present, appear to contribute little to phenotypic variation in this population.

Interestingly, we identified a larger influence of birth year on variation of offspring forearm length (relative to other maternal variance components). Offspring are dependent on their mothers during forearm growth [187], thus any influence of the abiotic environment to offspring is largely transmitted through the mother e.g. through milk production. Therefore, in the forearm length model, birth year could represent a hidden maternal effect, where

variance in the external environment, such as prey availability, exceeds that of individual mothers [573]. Seasonal effects on maternal provision are prevalent in mammals [574–576], including bats. A similar birth year effect was identified in *M. bechsteinii*, where daughters grew larger in warmer summers, with truncated growth in cold summers [519]. Furthermore, given that body size in bats has been linked to the enhanced ability to fly with and nourish young [577], as reflected in the positive correlation between forearm length and female LRS, it is plausible that the birth year effect on forearm length, at least partially, explains the moderate birth year effect on female LRS.

At the same time, birth year had negligible influence on 5th digit length variation. 5th digit growth plateaus later in development than the forearm (**Figure S 4.4**), hence the potential to integrate additional environmental influences [187]. Hence one hypothesis for lower heritability of 5th digit in daughters than sons is that female offspring are weaned later than males due to their increased size [578,579] (**Figure 4.4**, **Figure 4.5**). The high residual variance relative to all other modelled environmental components suggests that, unlike the forearm, environmental influences on the individual exceed those for the cohort overall.

4.5.5 High genetic covariance in morphological traits

The evolvabilities presented in this study are conditional on the assumption that traits are expressed independently [513,580]. The fact that populations with heritable phenotypic variation frequently do not respond to selection as predicted, suggests this assumption rarely holds true [581]. The expression of multiple traits can be captured together through the effects of pleiotropy (shared genes) or linkage disequilibrium e.g. common developmental pathways [87,582]. This relationship between traits can be measured as genetic covariance. Consequently, depending on the direction of selection, a genetically correlated trait may either facilitate or constrain adaptive evolution of another [582]. Therefore, quantification of genetic covariance is vital to achieving reliable predictions of evolutionary responses. Here we estimated high, positive genetic covariance between forearm and fifth digit length. This result is not unexpected given the development of forelimb digits in mammals is regulated by a shared set of genes [583]. We hypothesise the evolutionary response of both forearm and 5th digit is highly correlated. Thus, selection is likely to act upon these traits as an integrated unit. Additionally, we identified a significant residual, non-heritable, component to the covariance between the two traits, demonstrating shared strong environmental influences

during development shape the phenotype. Strong seasonality, as experienced in the temperate zones, might be expected to constrain the range of viable phenotype combinations [584].

Further reliable predictions of adaptive potential rely on testing covariance among a large suite of putative traits and fitness. One avenue of interest is the investigation of trade-offs in the expression of life-history traits. Ransome (1995)[308] identified a trade-off between the age of first reproduction and longevity in *R. ferrumequinum*. Females that reached sexual maturity earlier, had shorter generation times, but experienced earlier mortality. Later breeders achieved equitable reproductive success over their lifetimes, but achieved extended lifespans. Therefore, providing both age of first reproduction and longevity are heritable, we might expect a negative genetic covariance, where alleles leading to early breeding dispose an individual to earlier mortality [585,586].

4.6 Conclusion

For this long-studied population of *R. ferrumequinum,* we have provided quantitative estimates for the heritability and evolvability of morphological and life-history traits for the first time. We have shown that genetic differences are largely responsible for the diversity of morphological phenotypes, while environmental conditions drive variation in life-history traits – aligning with our predictions and previous analyses of wild mammals. Importantly, we have found heritable genetic variation for fitness, demonstrating a pre-disposition for this population to respond to selection. Such knowledge is critical to predicting the future states of populations, particularly of those species with slow-life histories with increased vulnerability to extinction in rapidly changing environments. While all traits show capacity for an evolutionary response, determining if they are under selection is essential to determine if population-level genetic changes have occurred [587,588].

4.7 Supplementary Tables & Figures

Table S 4.1 *Studies of free-ranging wild mammals published 2012-February 2023 from which animal model-derived estimates of heritability (h^2) and evolvability (I_A) were extracted in addition to those available from the supplementary material in Postma et al., (2014)*[516] *and Hendry et al., (2018)*[512].

Author	Year	Title	Source	doi	Species (English)	Species (Latin)
Fletcher et al.,	2014	Daily energy expenditure during lactation is strongly selected in a free-living mammal	Functional Ecology	10.1111/1365-2435.12313	American red squirrel	*Tamiasciurus hudsonicus*
Huchard et al.,	2014	Additive genetic variance and developmental plasticity in growth trajectories in a wild cooperative mammal	Journal of Evolutionary Biology	10.1111/jeb.12440	Meerkat	*Suricata suricatta*
Logan et al.,	2016	Endocranial volume is heritable and is associated with longevity and fitness in a wild mammal	Royal Society Open Science	10.1098/rsos.160622	Red deer	*Cervus elaphus*
Bonnet et al.,	2017	Bigger Is Fitter? Quantitative Genetic Decomposition of Selection Reveals an Adaptive Evolutionary Decline of Body Mass in a Wild Rodent Population	PLoS Biology	10.1371/journal.pbio.1002592	Snow vole	*Chionomys nivalis*
Lane et al.,	2018	Phenological shifts in North American red squirrels: disentangling the roles of phenotypic plasticity and microevolution	Journal of Evolutionary Biology	10.1111/jeb.13263	American red squirrel	*Tamiasciurus hudsonicus*
Malenfant et al.,	2018	Heritability of body size in the polar bears of Western Hudson Bay	Molecular Ecology Resources	10.1111/1755-0998.12889	Polar bear	*Ursus maritimus*
Bonnet et al.,	2018	The role of selection and evolution in changing parturition date in a red deer population	PLoS Biology	10.1371/journal.pbio.3000493	Red deer	*Cervus elaphus*
Gauzere et al.,	2018	Between-population differences in the genetic and maternal components of body mass in roe deer	Evolution	10.1111/evo.14000	Roe deer	*Capreolus capreolus*
Rivrud et al.,	2019	Heritability of head size in a hunted large carnivore, the brown bear (*Ursus arctos*)	Evolutionary Applications	10.1111/eva.12786	Brown bear	*Ursus arctos*

Kimock et al.,	2019	Male morphological traits are heritable but do not predict reproductive success in a sexually-dimorphic primate	Scientific Reports	10.1038/s41598-019-52633-4	Rhesus macaque	*Macaca mulatta*
Sim & Coltman	2019	Heritability of Horn Size in Thinhorn Sheep	Frontiers in Genetics	10.3389/fgene.2019.00959	Thin horn Sheep	*Ovis dalli dalli*
Jamieson et al.,	2020	Heritability estimates of antler and body traits in white-tailed deer (*Odocoileus virginianus*) from genomic-relatedness matrices	Journal of Heredity	10.1093/jhered/esaa023	White-tailed deer	*Odocoileus virginianus*
Santostefano et al.,	2021	Indirect genetic and environmental effects on behaviors, morphology, and life-history traits in a wild Eastern chipmunk population	Evolution	10.1111/evo.14232	Eastern chipmunk	*Tamias striatus*
Peters et al.,	2021	Genomic analysis reveals a polygenic architecture of antler morphology in wild red deer (*Cervus elaphus*)	Molecular Ecology	10.1111/mec.16314	Red deer	*Cervus elaphus*
Bonnet et al.,	2022	Genetic variance in fitness indicates rapid contemporary adaptive evolution in wild animals	Science	10.1126/science.abk0853	NA	NA
Bubac	2022	Investigating the genetic basis of boldness and reproductive performance traits in the grey seal (Halichoerus grypus)	PhD Thesis	10.7939/r3-eqan-jj71	Grey seal	*Halichoerus grypus*
Huang et al.,	2022	Contemporary selection on MHC genes in a free-living ruminant population	Ecology Letters	10.1111/ele.13957	Soay sheep	*Ovis aries*
St. Lawrence et al.,	2022	Sex-specific reproductive strategies in wild yellow-bellied marmots (*Marmota flaviventer*)	Behavioral Ecology and Sociobiology	10.1007/s00265-022-03191-9	Yellow-bellied marmot	*Marmota flaviventer*
Mundinger	2022	Relative importance of plastic and genetic responses to weather conditions in long-lived bats	PhD Thesis	NA	Bechstein's bat	*Myotis bechsteinii*

Table S 4.2 *The set of models used to determine inclusion of fixed effects in subsequent animal models. Rationale behind the testing of each fixed effect are provided.*

Trait Type	Trait	Model type	Data Distribution	Fixed effects	Rationale
Morphological	Forearm	LM	Gaussian	Sex, Corrected birth date	Sexual dimorphism is present in this species with females being larger than males [206]. Birth date may affect the availability of insects and thus provision of milk by the mother during development [173,187].
	5th Digit	LM	Gaussian	Sex, Corrected birth date	Sexual dimorphism is present in this species with females being larger than males [206]. Birth date may affect availability of insects during a pup's initial foraging flights, affecting its development [173,187].
Life-history	Parturition date	LMM	Gaussian	Offspring Sex, Age	Female pups are larger than males and may take longer to come to term. Anecdotal observations suggest younger mothers give birth later in the season.
	Average reproductive success (males)	GLM	Poisson	Corrected birth date, Forearm length	Birth date and its potential influence on early morphological development may constrain reproductive success. Ward et al., (2014)[205] found males with longer forearms achieved more paternities.
	Average reproductive success (females)	LM	Poisson	Corrected birth date, Forearm length	Birth date and its potential influence on early morphological development may constrain reproductive success. A forearm of sufficient length may be essential to forage successfully and carry a pup to term (Ward et al., (2014)[205].

Table S 4.3 *Data types and distributions of trait data used in animal models.*

Trait type	Trait	Bats included	Measurement	N	Repeated measures from individuals
Morphological	Forearm	All bats	Length of radius bone (mm)	1038	No
	5th digit	All bats	Length of fifth finger digit (mm)	910	No
Life-history	Parturition date	All females	Determined directly if born on same day, otherwise calculated via growth curves.	1492	Yes
	Average reproductive success (males)	All males	Offspring fathered in a given year. Calculated as the total number of offspring produced between 1993 and 2020, divided by breeding span (the last known breeding year minus the first known breeding year).	480	No
	Average reproductive success (females)	All females	Offspring mothered in a given year. Calculated as the total number of offspring produced between 1991 and 2020, divided by breeding span (the last known breeding year minus the first known breeding year).	452	No
	Lifetime reproductive success (females)	Females with known lifespan	Total number of offspring produced over the lifespan. Individual death was defined as the last year of capture following at least two years of no re-captures.	369	No

Table S 4.4 *Results from the animal model for morphological traits (forearm length and fifth finger digit length, "5th digit") including variance components and the proportion of phenotypic variance in explained by each component. Estimates are given as the mean of the posterior distribution with lower and upper 95% highest posterior density (HPD) intervals. pMCMC values (testing significance away from zero) are given for fixed effects. Corrected date of birth is the number of days an individual was born minus the mean birthdate of the individuals cohort. Forearm length N = 1038. 5th digit N = 910.*

Type	Parameter	Posterior mean	Lower 95% HPD	Upper 95% HPD	Proportion of phenotypic variance	Lower 95% HPD	Upper 95% HPD	pMCMC
Forearm length								
	Additive genetic	0.792	0.566	1.020	0.666	0.533	0.798	
	Birth year	0.034	0.007	0.071	0.029	0.006	0.059	
Variance component	Maternal environment	0.031	<0.001	0.082	0.026	<0.001	0.069	
	Maternal genetic	0.023	<0.001	0.076	0.019	<0.001	0.063	
	Paternal genetic	0.008	<0.001	0.026	0.006	<0.001	0.022	
	Residual	0.298	0.165	0.415	0.254	0.127	0.370	
Fixed effect	Intercept	56.14	55.919	56.365				<0.001***
	Sex (male)	-0.978	-1.09	-0.866				<0.001***
	Corrected date of birth	-0.045	-0.055	-0.034				<0.001***
5th Digit								
	Additive genetic	0.965	0.637	1.315	0.576	0.419	0.727	
	Birth year	0.010	<0.001	0.032	0.006	<0.001	0.019	
Variance component	Maternal environment	0.060	<0.001	0.157	0.036	<0.001	0.094	
	Maternal genetic	0.062	<0.001	0.191	0.037	<0.001	0.112	
	Paternal genetic	0.012	<0.001	0.045	0.007	<0.001	0.027	
	Residual	0.560	0.367	0.760	0.339	0.207	0.486	
Fixed effect	Intercept	72.521	72.255	72.773				<0.001***
	Sex (male)	-1.171	-1.312	-1.026				<0.001***
	Corrected date of birth	-0.043	-0.057	-0.030				<0.001***

Table S 4.5 *Results of mother-offspring regressions for forearm length. Corrected date of birth is the number of days an individual was born minus the mean birthdate of the individuals cohort.*

	Estimate	**SE**	**t**	**p**
Mother-Offspring (n=1036)				
Fixed effects				
Intercept	36.847	2.149	17.144	<0.001
Mother forearm length	0.344	0.038	9.046	<0.001
Sex (Male)	-0.993	0.058	-17.251	<0.001
Corrected date of birth	-0.040	0.005	-7.332	<0.001
Random effects (variance ± SD)				
Mother	0.132 ± 0.363			
Father	0.164 ± 0.405			
Year of birth	0.031 ± 0.176			
Residual	0.692 ± 0.832			
Mother-Daughter (n=534)				
Fixed effects				
Intercept	38.600	2.540	15.199	<0.001
Mother forearm length	0.313	0.045	6.973	<0.001
Corrected date of birth	-0.052	0.007	-7.294	<0.001
Random effects (variance ± SD)				
Mother	0.057 ± 0.239			
Father	0.155 ± 0.393			
Year of birth	0.028 ± 0.167			
Residual	0.721 ± 0.849			
Mother-Son (n=502)				
Fixed effects				
Intercept	36.767	2.932	12.198	<0.001
Mother forearm length	0.345	0.052	6.644	<0.001
Corrected date of birth	-0.027	0.008	-3.388	<0.001
Random effects (variance ± SD)				
Mother	0.144 ± 0.379			
Father	0.164 ± 0.405			
Year of birth	0.023 ± 0.152			
Residual	0.735 ± 0.857			

Table S 4.6 *Results of father-offspring regressions for forearm length. Corrected date of birth is the number of days an individual was born minus the mean birthdate of the individuals cohort.*

	Estimate	**SE**	**t**	**p**
Father-Offspring (n=989)				
Fixed effects				
Intercept	39.469	1.990	19.747	<0.001
Father forearm length	0.303	0.036	8.397	<0.001
Sex (Male)	-0.972	0.059	-16.394	<0.001
Corrected date of birth	-0.045	0.006	-7.878	<0.001
Random effects (variance ± SD)				
Mother	0.277 ± 0.526			
Father	0.074 ± 0.272			
Year of birth	0.031 ± 0.176			
Residual	0.680 ± 0.824			
Father-Daughter (n=506)				
Fixed effects				
Intercept	39.756	2.667	14.905	<0.001
Father forearm length	0.299	0.048	6.199	<0.001
Corrected date of birth	-0.059	0.007	-7.666	<0.001
Random effects (variance ± SD)				
Mother	0.181 ± 0.426			
Father	0.086 ± 0.294			
Year of birth	0.027 ± 0.166			
Residual	0.705 ± 0.840			
Father-Son (n=483)				
Fixed effects				
Intercept	37.974	2.602	14.595	<0.001
Father forearm length	0.312	0.047	6.633	<0.001
Corrected date of birth	-0.031	0.008	-3.748	<0.001
Random effects (variance ± SD)				
Mother	0.327 ± 0.572			
Father	0.073 ± 0.270			
Year of birth	0.035 ± 0.187			
Residual	0.676 ± 0.822			

Table S 4.7 *Results of mother-offspring regressions for fifth finger digit (5th digit) length. Corrected date of birth is the number of days an individual was born minus the mean birthdate of the individuals cohort.*

	Estimate	**SE**	**t**	**p**
Mother-Offspring (n=904)				
Fixed effects				
Intercept	48.078	2.548	18.871	<0.001
Mother 5th digit	0.337	0.035	9.620	<0.001
Sex (Male)	-1.165	0.075	-15.597	<0.001
Corrected date of birth	-0.041	0.007	-6.029	<0.001
Random effects (variance ± SD)				
Mother	0.166 ± 0.363			
Father	0.184 ± 0.405			
Year of birth	0.012 ± 0.176			
Residual	1.037 ± 1.018			
Mother-Daughter (n=477)				
Fixed effects				
Intercept	52.048	3.103	16.771	<0.001
Mother 5th digit	0.283	0.043	6.627	<0.001
Corrected date of birth	-0.052	0.009	-5.682	<0.001
Random effects (variance ± SD)				
Mother	0.180 ± 0.424			
Father	0.171 ± 0.414			
Year of birth	0.049 ± 0.221			
Residual	0.898 ± 0.948			
Mother-Son (n=427)				
Fixed effects				
Intercept	45.418	3.644	12.465	<0.001
Mother 5th digit	0.356	0.050	7.124	<0.001
Corrected date of birth	-0.033	0.010	-3.285	0.001
Random effects (variance ± SD)				
Mother	0.136 ± 0.369			
Father	0.226 ± 0.475			
Year of birth	0.000 ± 0.000			
Residual	1.149 ± 1.072			

Table S 4.8 *Results of father-offspring regressions for fifth finger digit (5th digit) length. Corrected date of birth is the number of days an individual was born minus the mean birthdate of the individuals cohort.*

	Estimate	**SE**	**t**	**p**
Father-Offspring (n=835)				
Fixed effects				
Intercept	55.728	3.071	18.147	<0.001
Father 5th digit	0.236	0.043	5.474	<0.001
Sex (Male)	-1.138	0.081	-14.092	<0.001
Corrected date of birth	-0.043	0.008	-5.683	<0.001
Random effects (variance ± SD)				
Mother	0.355 ± 0.596			
Father	0.111 ± 0.333			
Year of birth	0.006 ± 0.075			
Residual	1.085 ± 1.042			
Father-Daughter (n=433)				
Fixed effects				
Intercept	58.100	3.823	15.197	<0.001
Father 5th digit	0.203	0.054	3.789	<0.001
Corrected date of birth	-0.056	0.001	-5.579	<0.001
Random effects (variance ± SD)				
Mother	0.297 ± 0.545			
Father	0.126 ± 0.355			
Year of birth	0.026 ± 0.160			
Residual	0.950 ± 0.975			
Father-Son (n=402)				
Fixed effects				
Intercept	51.626	4.283	12.055	<0.001
Father 5th digit	0.277	0.060	4.607	<0.001
Corrected date of birth	-0.033	0.011	-3.013	0.003
Random effects (variance ± SD)				
Mother	0.442 ± 0.665			
Father	0.135 ± 0.367			
Year of birth	0.000 ± 0.000			
Residual	1.127 ± 1.062			

Table S 4.9 *Results from bivariate animal model for forearm length and fifth finger digit (5th digit). R_G (genetic correlation), R_{Res} (residual correlation), Cov_A (additive genetic covariance), Cov_R (Residual covariance). Corrected date of birth is the number of days an individual was born minus the mean birthdate of the individuals cohort. N = 1310.*

Type	Parameter	Posterior mean	Lower 95% HPD	Upper 95% HPD	pMCMC
Variance components (forearm)	Additive genetic	0.688	0.550	0.822	
	Residual	0.327	0.254	0.407	
Variance components (5^{th} digit)	Additive genetic	0.999	0.765	1.237	
	Residual	0.646	0.509	0.791	
Fixed effects (forearm)	Intercept	56.140	55.955	56.239	<0.001***
	Sex (male)	-0.975	-1.069	-0.878	<0.001***
	Corrected date of birth	-0.044	-0.052	-0.036	<0.001***
Fixed effects (5^{th} digit)	Intercept	72.527	72.348	56.239	<0.001***
	Sex (male)	-1.196	-1.314	-1.070	<0.001***
	Corrected date of birth	-0.047	-0.052	-0.037	<0.001***
Correlations and covariances	R_G	0.677	0.590	0.766	
	R_{Res}	0.640	0.540	0.736	
	Cov_A	0.561	0.410	0.706	
	Cov_R	0.294	0.207	0.378	

Table S 4.10 *Results from the animal model for life-history traits (parturition date, average reproductive success for females and males) including variance components and the proportion of phenotypic variance in explained by each component. Estimates are given as the mean of the posterior distribution with lower and upper 95% highest posterior density (HPD) intervals. pMCMC values (testing significance away from zero). IBC is the pedigree-based inbreeding coefficient. Parturition date N = 1492. Average reproductive females N= 480 . Average reproductive success males N= 452. Female lifetime reproductive success = 541.*

Type	Parameter	Posterior mean	Lower 95% HPD	Upper 95% HPD	Proportion of phenotypic variance	Lower 95% HPD	Upper 95% HPD	pMCMC
Parturition date								
Variance component	Additive genetic	7.734	3.867	11.502	0.083	0.035	0.131	
	Permanent environment	1.138	<0.001	3.968	0.012	<0.001	0.043	
	Offspring birth year	62.160	32.471	95.784	0.641	0.519	0.756	
	Mother birth year	3.755	1.260	6.839	0.040	0.012	0.075	
	Maternal environment	0.503	<0.001	2.122	0.050	<0.001	0.023	
	Residual	20.202	18.640	21.877	0.218	0.146	0.291	
Fixed effect	Intercept	42.160	39.198	45.147				<0.001***
	Offspring sex (male)	-0.306	-0.775	0.199				0.231
	Age (years)	-0.594	-0.706	-0.487				<0.001***
	IBC	4.566	-5.463	14.943				0.384
Average female reproductive success								
Variance component	Additive genetic	0.007	<0.001	0.021	0.028	0.001	0.086	
	Birth year	0.007	<0.001	0.017	0.030	0.001	0.073	
	Maternal environment	0.005	<0.001	0.014	0.020	0.001	0.057	
	Maternal genetic	0.006	<0.001	0.016	0.023	0.001	0.066	
	Paternal genetic	0.003	<0.001	0.007	0.011	0.001	0.029	
	Residual	0.21	0.179	0.242	0.889	0.806	0.964	
Fixed effects	Intercept	-1.982	-4.465	0.464				0.117
	Forearm length	0.042	-0.001	0.086				0.057
	Corrected date of birth	0.006	-0.002	0.013				0.156
	IBC	-0.214	-1.017	0.639				0.605
Average male reproductive success								
Variance component	Additive genetic	0.006	<0.001	0.020	0.024	0.001	0.074	
	Birth year	0.022	0.005	0.046	0.083	0.020	0.162	
	Maternal environment	0.005	<0.001	0.014	0.017	0.001	0.051	
	Maternal genetic	0.005	<0.001	0.014	0.018	0.001	0.052	
	Paternal genetic	0.005	<0.001	0.014	0.017	0.001	0.052	
	Residual	0.223	0.189	0.257	0.840	0.741	0.930	
Fixed effect	Intercept	-1.633	-4.904	0.752				0.183
	Forearm length	0.035	-0.007	0.081				0.110

	Corrected date of birth	$-8.606*10^{-5}$	-0.008	0.008				0.986
	IBC	-0.105	-1.148	0.885				0.830
Female Lifetime Reproductive Success								
Variance component	Additive genetic	0.053	0.001	0.213	0.011	0.001	0.049	
	Birth year	1.018	0.327	2.057	0.226	0.090	0.377	
	Maternal environment	0.066	0.001	0.273	0.014	0.001	0.058	
	Residual	3.458	2.558	4.503	0.748	0.603	0.903	
Fixed effect	Intercept	-20.309	-32.243	-7.864				<0.001***
	Forearm length	0.340	0.111	0.544				<0.001***
	Corrected date of birth	0.030	-0.007	0.065				0.108
	IBC	-0.264	-4.421	4.359				0.910

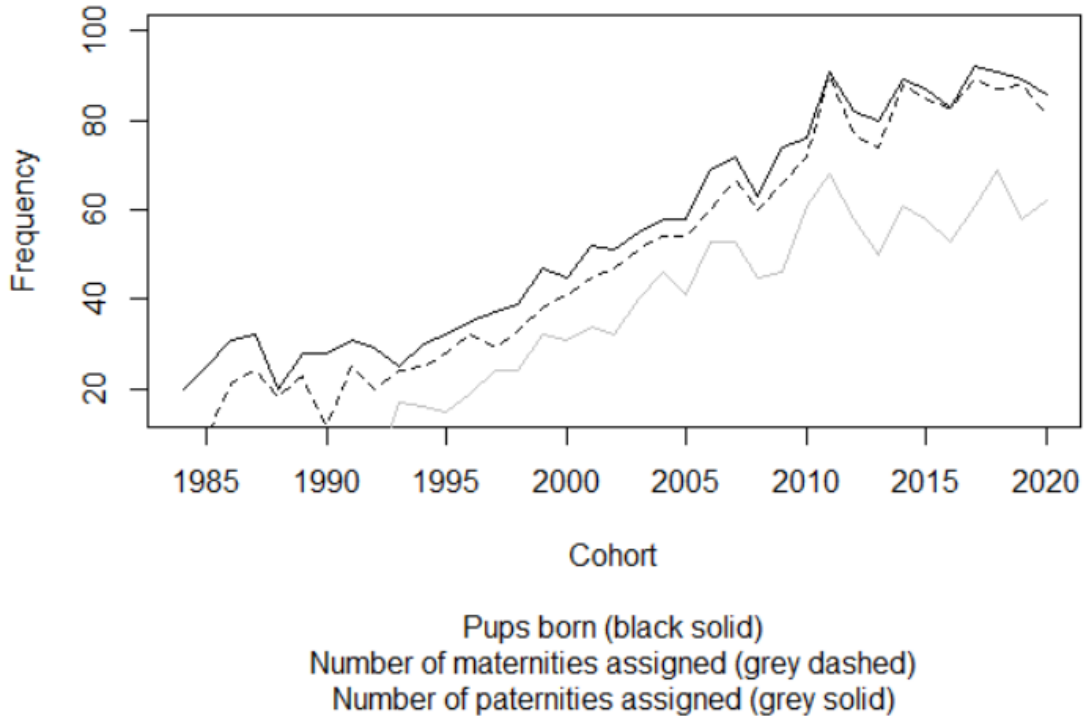

Figure S 4.1 *Parentage assignments between 1984 and 2020. Pups born 1993 onwards were tissue sampled allowing the assignment of paternities. Pups born 1984-1993 were assigned maternities based on attachment to their mother. The population birth rate (solid black line) is an indication of maternity colony size as breeding females produce a single pup per year.*

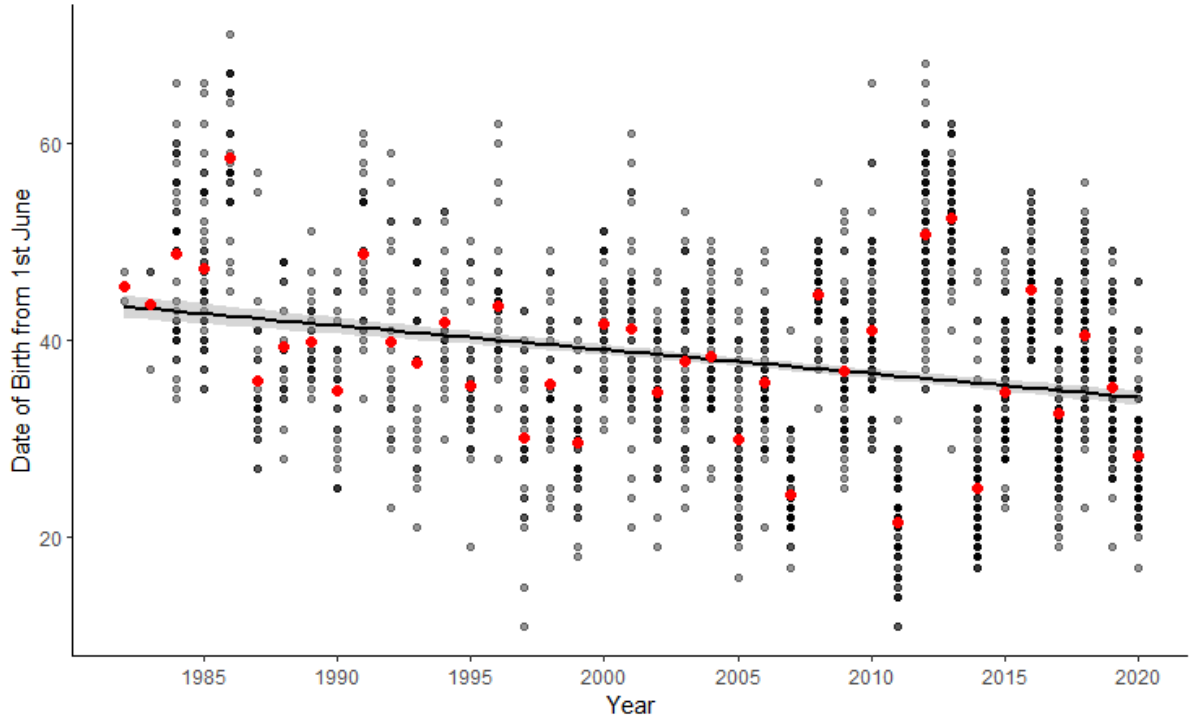

Figure S 4.2 *Phenotypic trend observed in parturition dates (1982-2020). 1 = 1st June. Figure adapted from Jones et al., (2015)* [173] *with additional data. The solid line represents the fitted linear regression model with 95% confidence intervals. Large red points indicate the mean parturition date of that cohort. Small grey points represent individual parturition dates, with darker shades showing greater numbers of offspring born on a given day.*

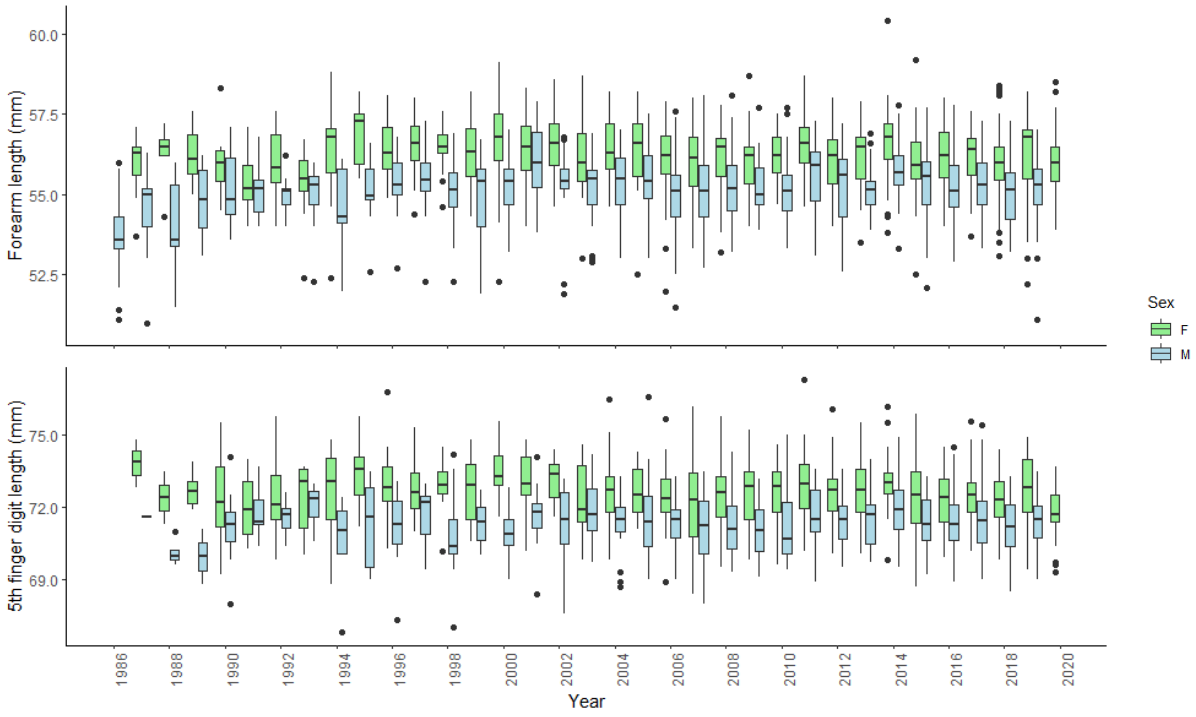

Figure S 4.3 *Boxplots showing variation in forearm length (mm) (top) and 5th finger digit length (mm) (bottom) over time (1986-2020). Females are shown in green, and males in blue. There were no data for 5th finger digit length (mm) in 1986.*

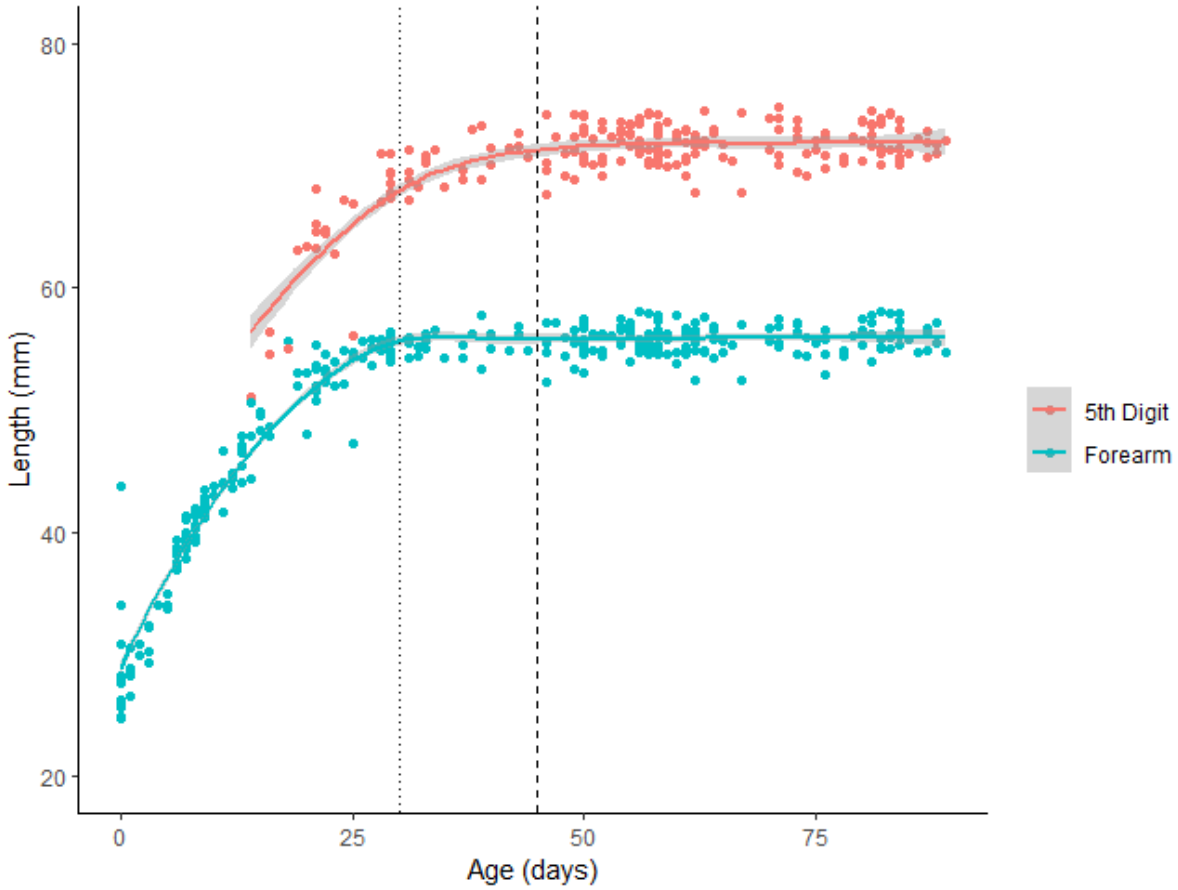

Figure S 4.4 *Growth in 5th digit and forearm length (mm) in juveniles in the first 90 days after birth. A smooth regression line is fit to the data using the locally estimated scatterplot smoothing method in ggplot2* [589]*, alongside the 95% confidence intervals. The first dotted line indicates the approximate age at which juveniles become volant and begin foraging flights (30 days). The dashed line indicates the approximate age at which lactation ends (45 days) according to* [187]*. Data are from 1998.*

Chapter 5

General Discussion

Light at the end of the tunnel. A sunbeam shines through the entrance of a hibernaculum near Nailsworth, Gloucestershire.

5.1 Overview

The central aim of this thesis was to explore the genetic and environmental basis behind how an individual makes resource-allocation 'decisions' throughout its life history. Specifically, I was interested in how an individual's social environment shapes these decisions. I made use of the gregarious nature of two species of hibernating rhinolophid bat as model systems.

In the temperate zone, annual cycles of ecosystem productivity place a strong selection pressure on allocation decisions [209,590], including shaping the costs and benefits of group living [24,26], which in turn determine an individual's social environment [337,591–595]. Sociality in temperate bat societies follows the seasons. Large all-female colonies form in the summer to socially thermoregulate during gestation and lactation, but dissolve in the winter as prey abundance wanes and becomes less predictable [185,596]. Intriguingly, individuals rarely hibernate alone. Are non-random decisions made in regard to who to hibernate with? Social structure emerges as the outcome of these decisions, with potential consequences for population gene flow [54,56]. In Chapter 2, integrating a multi-generational pedigree with long-term field data on associations, I report on the sociogenetic structure of these associations in *Rhinolophus ferrumequinum*. Does genetic relatedness drive spatial assortment in hibernating bats? Associations within hibernacula were not based on kinship between conspecifics. Instead, they were structured by age and, in adults, by sex.

Multi-day torpor bouts (hibernation) are critical for energy conservation over the hibernation period, but come at the cost of reproduction and foraging to accumulate energy reserves [213,373]. Does the social environment impact the decision to remain torpid or arouse? Could this partially explain why hibernating rhinolophid bats form groups? In Chapter 3, I explored the nature of social hibernation in rhinolophid bats in further detail. A thermal camera was positioned in a hibernaculum to record social interactions within a cluster of *R. hipposideros* and test the hypothesis that individuals form groups to induce arousals in neighbours when suitable foraging conditions arise ("the social alarm-clock" hypothesis). Support for the social alarm-clock hypothesis was identified, as bats with closer neighbours were more likely to arouse on average, particularly in late Autumn.

As winter's harsh conditions retreat, the females begin to form maternity colonies to share the mutual benefits of social thermoregulation and mitigate energy expenditure during gestation and lactation [115–118]. Offspring development is necessarily rapid within a restricted window of resource availability before winter returns [265]. Does a mother's behaviour influence the phenotype of her offspring? The decisions a mother makes regarding the timing and schedule of resource allocation to her offspring have the potential for long-lasting effects on an offspring's phenotype, including fitness, with potential consequences for phenotypic variation and evolution in the population [102,597]. In Chapter 4, I investigated the relative contribution of the maternal environment, relative to other components of variance, including additive genetic effects, to explain variation in morphology, birth timing, and fitness in a wild colony of *R. ferrumequinum*. Additive genetic effects explained the vast proportion of variance in morphological traits, with a comparatively weak contribution of maternal effects. Birth timing was largely explained by environmental conditions shared by the cohort. Residual environmental effects explained the largest proportion of variance in average reproductive success (fitness) for both sexes.

In this final chapter, I examine the key findings from Chapters 2 through 4 and discuss their implications for the behavioural ecology of rhinolophid bats. Finally, future avenues of investigation are proposed.

5.2 The nature of associations during the hibernation period

During the hibernation period, many rhinolophid bats will share hibernacula, with individuals often found close together in loose clusters. In Chapters 2 and 3, I sought to describe and explain these grouping patterns.

Individuals likely co-habit a hibernaculum by sharing a preference for its microclimate(s). Thermal conditions within a hibernaculum are a key driver of arousal frequency, as established in *R. ferrumequinum* by Ransome (1971)[233] and Park (1999)[327], and confirmed in *R. hipposideros* in Chapter 3. Ransome (1968)[199] observed that individuals of different age and sex may vary in body condition, subsequently selecting sites with ambient temperatures that might favour or disfavour arousals according to energetic requirements. My findings in Chapter 2 support these observations. I demonstrate positive assortment by age, sex (in adults), and body condition (in immature bats), extending and corroborating findings by

Finch et al., (2022)[306]. In addition, I demonstrate that kinship has little influence on roosting decisions. Further research will be required to deduce whether these associations constitute active social avoidance or preferences [598]. However, since approximately 80% of individuals are likely to be diffusely scattered within hibernacula on a given date (see Chapter 2), locating and recognising specific individual phenotypes represents a significant cognitive cost [186]. A more parsimonious explanation is that phenotypic assortment is shaped indirectly through space use. Whichever explanation is more likely, it is evident, the non-random structure of spatiotemporal associations determines an individual's opportunity to be social. In other words, by making decisions on where to roost, individuals can influence the social environment in which they hibernate.

In Chapter 3, I found evidence in support of the hypothesis that rhinolophid bats in clusters arouse by receiving social cues from active conspecifics. Consequently, an individual might join a group on the likelihood of receiving beneficial social information. Conceivably however, the first bat to arouse gains no immediate benefit, receiving no social information, and may face an increased predation risk upon leaving the roost alone [131,469]. Roosting with non-kin makes obtaining inclusive fitness benefits unachievable [17]. The cost can only be repaid if another bat arouses first at a later date. Does the "social-alarm clock" constitute non-kin cooperation? I hypothesise that this system might constitute "generalised" reciprocity provided group membership is stable and where there this no memory of the previous benefactor [599–601]. Requiring little cognitive demand, helpful acts are performed in the knowledge that the favour will be repaid anonymously at some point, given the benefactor has also been a previous recipient. How can an individual 'volunteer' to arouse first? I also show that roost position affects arousal time. Distinct spatial clustering of the first bats to arouse was observed. It is within reason, therefore, an individual can modulate their level of investment. Turnover of roost position [176,602], driven by competition, or leaving may limit defectors that never arouse first [603].

5.3 A mother's legacy?

In sexually-reproducing organisms, the mother-offspring bond is the first social connection an individual experiences. Even in species with limited, or no post-parturition maternal care, a mother can exert tremendous influence on her offspring's phenotype, for example by dictating the level of energetic investment *in utero,* affecting birth timing and morphology [99,117,265,567–569]. In matrilineal societies, such as many temperate bat species, female natal philopatry may facilitate reunification and potentially reinforce the maternal bond [109,244,318]. Such stable social connections can favour the evolution of cooperative behaviours [109,141], such as the exchange of social information [207,242]. In Chapter 4, I examined maternal effects on offspring phenotype variation in a wild horseshoe bat population. Contrary to expectations, I found that both indirect genetic and environmental maternal effects had minimal impact on offspring phenotypic variance, including fitness. This raises the question: do mothers invest little in their offspring? Maternity identity alone may be a poor approximation of maternal care. This is especially true if maternal provisioning varies, leading to phenotypic variation among offspring from the same mother [99]. For instance, my results showed that older females gave birth earlier and produced larger pups than younger mothers. Consequently, pups born to young mothers may exhibit different phenotypes compared to those born to the same mother at an older age. The larger post-hibernation body mass of older females may enable earlier allocation of resources to gestation [199]. Additionally, seasonal effects may mediate variability in maternal investment. I demonstrated that birth year significantly influenced parturition date variance, likely due to the impact of spring temperature on post-hibernation embryo implantation [204,536,554]. Phenological plasticity is crucial for a mother's (and father's) fitness [375] in adapting to rising spring temperatures caused by climate change [557]. There is also heritable, additive genetic variance for parturition date. The approach used by de Villemereuil et al., (2020) could reveal the extent to which this population uses either adaptive plastic or genetic responses (or both) to keep pace with climate warming [545].

Maternal investment can constitute sharing of ecological knowledge. In temperate bats, this can include guidance to new roosts [258,259] or foraging areas [207,242]. However, in Chapter 2, I did not find evidence consistent with maternal inheritance of hibernacula. Roost co-habitation nor cluster associations were influenced by matrilineal relatedness. Indeed, age-

structured associations suggest offspring and mothers (adult females) rarely share roosts. Nevertheless, the rate of association between offspring and mother was not assessed. Furthermore, integrating networks of mother-offspring associations into animal models (see Chapter 4) may provide greater insight into variation in maternal investment in this species. Indeed, residual variance represented the largest component of variance for reproductive success, indicating that many factors driving differences in reproductive success between individuals remain unknown.

5.4 Future investigation

5.4.1 Social inheritance in *R. ferrumequinum*

Rossiter et al., (2002)[207] found that matrilineal kin shared foraging grounds, suggesting a mechanism of social inheritance from mother to offspring and other close relatives. Social transmission of spatially-defined resources can be achieved through following [258,259] or by attraction to social calls [604] though neither were observed in the study. However, the use of traditional, hand-held radio telemetry, with its limited sampling intensity, may have prevented detection of following behaviour between matrilineal kin. Increasing sampling effort could be achieved through the use of next-generation sensor networks such as BATS [605,606], which do not rely on heavy Global Positioning System (GPS) tags that are less suitable for small vertebrates for long durations [607,608]. BATS employs a ground-based network of radio receivers that detect lightweight sensor nodes attached to individual bats. These sensor nodes weigh approximately 1-2 g and have the ability to record individual proximity at higher temporal and spatial resolution than what can be achieved through hand-held radio telemetry. The BATS system has already been used successfully to mother-infant guidance behaviour in common noctules (*Nyctalus noctula*) [259].

5.4.2 Social hibernation and longevity

Hibernation is linked to the evolution of slow life histories in some mammals, and is likely behind the extreme lifespans observed in many bats [234,235,609]. Long periods of torpor, by reducing metabolic rate and energy expenditure, may facilitate somatic maintenance [188,234,610], potentially contributing to the negligible rates of senescence seen in bats [609]. As demonstrated in Chapter 3, torpor is not consistent throughout the hibernation period, and the decision to remain torpid may depend on abiotic and biotic environmental conditions

[213,216,327,373,387,460]. Does the social environment during hibernation influence longevity in horseshoe bats?

A potential means to test this question is to investigate telomere length (TL) dynamics during hibernation in association with individual social position and/or changes to social structure. Telomeres – nucleoprotein structures capping eukaryotic chromosomes – prevent DNA degradation and rapidly shorten in response to reactive oxygen species (ROS) induced by environmental stress [611,612]. Consequently, TL has been proposed as a suitable biomarker to quantify physiological consequences of conditions experienced by individuals [612]. The rate of TL attrition has been linked to lifespan in a range of vertebrates [613,614], although lengthening can be achieved through the activity of the enzyme telomerase [615].

Power et al., *in review* [198] showed TL attrition in hibernating *R. ferrumequinum* during warm and wet nights, potentially linked to arousals combined with failed foraging bouts. In Chapter 3, I show that arousals in *R. hipposideros* can be triggered by conspecific disturbance, with bats positioned centrally in the cluster more likely to arouse on average. Consequently, individual sensitivity to disturbance according an individual's social position may mediate physiological costs. Furthermore, individuals employing cold arousals [391,392] on warm and wet nights might be expected to show slower rates of TL attrition relative to those that arouse fully. Multiple studies in non-human species have demonstrated social effects on TL length [616–620]. For example, socially-isolated African grey parrots (*Psittacus erithacus*) showed shorter TL than individuals housed in groups [620].

Recent genomic analysis has uncovered genes in hibernating bats related to longevity and stress regulation during torpor [621]. A quantitative genetic approach [90], as demonstrated in Chapter 4, could be used to study how phenotypic expression of such genes varies across individuals in accordance to social environmental influences.

An additional method to test the effects of the social hibernation on longevity could be to explore whether network metrics in a given winter influence recapture rates in the following summer. While not studied in Chapter 2, temporal changes in individual network position and group structure in response to environmental conditions are worth investigation.

5.5 Final remarks

This thesis contributes to our understanding of sociality and the nature of variation in wild bat populations. Utilising a unique set of longitudinal data, quantification of kinship and social network structure has provided new information on the drivers of social group formation and its function in the life history of hibernating horseshoe bats. In addition, an evolutionary quantitative genetic approach has revealed the additive genetic and environment components that underpin individual phenotypic variation. Combined, these data may provide a baseline against which future changes can be monitored [622].

My findings offer insight into how populations of wild bat persist amidst turbulent environmental change. Population resilience is a product of processes, e.g. gene flow [623] and cultural transmission [624], emerging through the aggregation of individuals [56,625]. Data on social structure are essential for designating conservation units [625]. Hibernacula of principal importance for social connectivity warrant additional protection. Additionally, I highlight the value of quantifying variation in fitness-related traits in the assessment of a population's evolutionary potential.

The elusive character of horseshoe bats provides an enigmatic, yet fascinating, model system. Many of the factors explaining sociality and its adaptive significance to their life history remain unknown. It is hoped that this body of work motivate future research that may ultimately uncover what determines the "struggle for existence" in these unique species.

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