SPATIAL ECOLOGY OF THE SEROTINE BAT (*EPTESICUS SEROTINUS*)

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I certify that all material in this thesis which is not my own work has been identified and that no material has previously been submitted and approved for the award of a degree by this or any other University.

Signature: .....

Summary

The spatial ecology of a species underpins an array of important questions about the ecology and evolution of a species, relating for instance to sociality, population dynamics, geographical distribution or genetic partitioning. Furthermore, the understanding of the spatial ecology of a species also has important implications for conservation and for disease management. It is in this context that I have examined the spatial ecology of the serotine bat (*Eptesicus serotinus*), in Europe, and especially at the northern limit of its distribution in England, using population genetic and stable isotope analyses.

Data from nuclear microsatellite markers indicated higher levels of gene flow on the continent than in England. Consistent with this, England was separated into three genetically distinct populations, inter-connected by male-driven gene flow. Substantial asymmetric gene movement over the English Channel was inferred, possibly indicating a northward and westward range expansion. Mitochondrial DNA revealed fast population expansion and strong female philopatry.

Moving to finer scale, $\delta^{13}C$ in wing tissue and fur revealed that the association of individuals at roosts is flexible and dynamic, possibly reflecting their membership to communities operating at larger geographic scales. Communities of *E. serotinus* seem therefore to create a network of roosts with individuals continually re-assorting among themselves both within and across breeding seasons.

The integration of the genetic and isotopic data highlighted complex spatial, social and temporal interactions in this species. While individual associations within communities seem to be flexible, communities were found to be strongly segregated over the breeding season. In contrast, genetic connectivity operates on a larger geographical scale than the local summer landscape.

Together, these findings help explain the apparent absence in the UK of the European Bat Lyssavirus 1 (EBLV-1) for which this species is the main host. Hence, the relative fragmentation of this low-density population may inhibit the virus' spread. However, with regular immigration from the continent, EBLV-1 could eventually enter the UK.

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Author’s declaration

During the research contributing to this thesis, Caroline Moussy was supported by a PhD studentship provided by the Food and Environment Research Agency (FERA). All of the chapters in this thesis were written by Caroline Moussy with comments from Prof David Hosken, Prof Stuart Bearhop and Dr James Aegerter.

Additional genetic data were obtained for Chapter 3 from previous samples collected in 2004-2006 by James Aegerter and genotyped by Helen Atterby at FERA.

Photographs in this thesis are taken by Caroline Moussy unless otherwise stated.

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CHAPTER 1: Introduction

Bats are the second largest Order of mammals and display an extraordinarily large ecological and behavioural diversity. For example, bats can be insectivorous, frugivorous, nectivorous, sanguivorous or piscivorous; they can forage by aerial-hawking or gleaning; roost in trees, foliage, crevices, caves, or man-made structures; display a broad range of mating (reviewed by McCracken & Wilkinson, 2000), migration and dispersal strategies (Moussy et al. 2013). All this allows bats to fill a broad range of ecological niches and as such, to provide valuable ecosystem services to humans (Kunz et al. 2011). For example, populations of insectivorous bats may consume tonnes of insects every night. Prey consumed include agricultural insect pests (Table 2: Kunz *et al.*, 2011) and the sheer biomass consumed probably contributes to regulating population cycles of pests. This effect could be directly translated in reduced agricultural production costs, and a recent study evaluated the value of bats to the American agriculture at $22.9 billion per year (range $3.7-53 billion/year) (Boyles et al. 2011). Another recognised service provided by bats occurs in tropical and subtropical habitats where nectivorous and frugivorous species act as pollinators and seed dispersers (Table 4: Kunz *et al.*, 2011). While the monetary value for this service is difficult to assess, many plants pollinated or dispersed by bats are of ecological or economical importance. Additional services include providing guano that is used as fertiliser on agricultural crops (bat droppings contain high concentrations of nitrogen and phosphorous) and the human consumption of bat meat in some tropical countries (Kunz et al. 2011).

The study of bats also has unexpected medical benefits, like the discovery of the anticoagulant compound found in the saliva of *Desmodus rotundus,* which has potential as a treatment for strokes (Schleuning 2001). Finally, bats inspire a wide range of human emotions from fear to fascination. By drawing such strong reactions, bats are of cultural importance, as demonstrated by appearance on artefacts from ancient civilisation, in books, films and nature documentaries. This cultural effect stimulates the public to discover more about these species by taking part in organised events such as bat walks, cave tours and educational activities.

For all these reasons, but also simply because they are part of our biodiversity and of the global ecosystem, conservation of bats is necessary. Land use change, resulting in habitat degradation, fragmentation and loss, is considered the greatest threat to bat populations (Racey & Entwistle 2003, Altringham 2011). The destruction or reduction of foraging habitats such as woodlands deprives bats of food, driving population declines. Fragmentation of habitat, through land use change or anthropogenic infrastructures like roads, and lack of connectivity, can result in isolated habitat fragments too small to support viable bat populations and thus to heightened risk of extinction through the deleterious effect of inbreeding and loss of genetic diversity (Frankham et al. 2004). Loss of roosts is another common threat to bats as is increased disturbance during key times in bats annual bio-cycle (Racey & Entwistle 2003, Altringham 2011).

Bats can be very flexible in their biology and many species now exploit human structures for roosting or foraging. However, the human environment is often dynamic, posing a serious threat to the more anthropogenic adapted species. For instance, increased economic and social pressures leading to a more efficient use of building result in new properties (or newly redeveloped properties) being almost devoid of potential roosting opportunities. In addition, pollution (chemical, noise or light), introduced species, persecution, hunting, climate change and diseases, have all been cited as potential and interacting threats to bats (Racey & Entwistle 2003, Altringham 2011).

Bats have also been identified as the reservoir hosts of a number of emerging infectious diseases (Messenger et al. 2003, Calisher et al. 2006, Kuzmin et al. 2011). Habitat destruction, agricultural intensification and hunting can disrupt the behavioural, social and population dynamics of bats and promote their interaction with humans or domestic animals, factors facilitating or exacerbating disease transmission. A number of viruses identified in bats cause zoonotic infections, particularly RNA viruses. Bats have thus been recognised as a significant human health threat and are thus of policy concern, especially in developing countries (Jones et al. 2008). The Ebola and Marburg viruses cause hemorrhagic fever with very high mortality rates in humans, almost all confined to Africa (Leroy et al. 2011), and a range of fruit-eating and insectivorous bats are thought to be reservoirs for them (Leroy et al. 2005, Pourrut et al. 2009). For

instance, the 2007 Ebola outbreak in the Democratic Republic of Congo was linked to the annual migration of fruit bats coinciding with hunting of bats as bush meat (Leroy et al. 2009). Paramyxoviridae is another viral family that includes viruses found in bats and has been found to be transmissible to domestic animals and humans; one of these, the Hendra virus, was identified in Australian *Pteropus spp* and caused the death of horses and two humans (Messenger et al. 2003, Calisher et al. 2006, Kuzmin et al. 2011). Its emergence was associated with habitat loss, urbanisation, ecological connectivity and seasonal variation in immunity (Plowright et al. 2011), and the viral transmission was indirect from bats to humans through horses. Similarly, the original outbreak of the Nipah virus (another paramyxovirus) in Malaysia in 1999, that affected 283 persons and resulted in 109 deaths, is thought to have been caused by a combination of regional drought and anthropogenic land use change that drove fruit bats into cultivated orchards located close to piggeries. This resulted in viral transmission from its reservoir host to domestic pigs and eventually to humans (Chua et al. 2002). Other viruses have been detected in bats, including Severe Acute Respiratory Syndrome coronavirus (SARS-CoV) in Chinese horseshoe bats (Calisher et al. 2006, Kuzmin et al. 2011), and lyssaviruses, causative agents for rabies (Calisher & Ellison 2012). Due to their fatal effect on humans and their broad geographical distribution, lyssaviruses are comparatively well studied (Messenger et al. 2003, Delmas et al. 2008, Freuling et al. 2009, Kuzmin et al. 2011, Calisher & Ellison 2012). At this time, at least 11 lyssavirus species have been formally recognised, all but one have been isolated from bats, and all cause rabies encephalitis (Calisher & Ellison 2012).

In Western Europe, large efforts to eradicate the rabies virus (RABV) in terrestrial species has led to significantly reduced rabies threat, however, other lyssaviruses remain, including European Bat Lyssavirus 1 (EBLV-1) and European Bat Lyssavirus 2 (EBLV-2), and have caused five documented human fatalities (Fooks 2005, Racey et al. 2013). European Bat Lyssavirus 1 is predominant, with over 800 records across north west Europe and mostly associated with *Eptesicus serotinus* and *E. isabellinus* (Harris et al. 2006, Racey et al. 2013). In contrast, EBLV-2 has been found only about 25 times in *Myotis daubentoni* and *M. dasycneme* (Racey et al. 2013). The effects of all

these viruses on bat health and behaviour have drawn only little attention compared to the risks of spill over to humans, although these viruses are not always fatal to bats (Johnson et al. 2008). In contrast, an emerging fungal disease of bats in North America, *Geomyces destructans* causing White Nose Syndrome (WNS), has been responsible for mass death in hibernating bats and is causing the most precipitous recorded decline in bat populations (Blehert et al. 2009). All these examples of diseases highlight the need to understand the behaviour, ecology and immunity of the host species as well as the environmental factors (natural or anthropological) driving any changes in these aspects in order to establish the epidemiology of a disease and model its transmission for effective control (Plowright et al. 2008, Kuzmin et al. 2011, Hayman et al. 2013). Social and population structure and connectivity are thus aspects of a species' ecology of special interest for species management in both conservation and epidemiological contexts.

Study context and aims

Eptesicus serotinus is a fairly large (20-30g, 32-38cm wingspan) and common vespertilionid species that is widely distributed across continental Europe; from northern Spain to Denmark and southern Sweden, and from the Atlantic coast to the Caspian Sea (Dietz, Von Helversen, et al. 2009) where it is the main reservoir for the viral bat rabies pathogen EBLV-1. In contrast, at the northern margin of its range, in England, the serotine is less common, and thought to be restricted to the southern counties. Furthermore, no live virus has so far been identified in the British Isles, and although one bat was found to be antibody sero-positive for EBLV-1, it appears unlikely that the disease is present in England (Smith et al. 2011). Many explanations exist for this important difference in disease dynamics, most of which involve either the large scale or fine scale spatial dynamics of its host, *E. serotinus*. While some aspects of this species' ecology have been studied, including diet, habitat use, foraging and roosting behaviour (Catto et al. 1994, 1995, 1996, Robinson & Stebbings 1997, Verboom & Huitema 1997, Harbusch 2003, Kervyn & Libois 2008, Zukal & Gajdosik 2012), others are mostly unknown, especially its hibernation ecology, mating system, dispersal and population structure. Basic research addressing

some of these information gaps is necessary in order to understand why English *E. serotinus* appear to remain free of EBLV-1, and to evaluate the risks for arrival, establishment or maintenance of the disease in England.

In addition, *E. serotinus* may be of conservation concern. Initial evidence suggests that the species may be in decline in its traditional heartland in the south east of England (Bat Conservation Trust 2011). In England roosts of the species are almost exclusively associated with occupied buildings. There is a general concern that synanthropic species such as *E. serotinus* may be under particular pressures from an increased rate of loss of existing roosts (especially maternity roosts), and from a decrease in the rate of accrual of new roosting opportunities. Both effects are thought to be a result of the increased economic and social pressures leading to a more efficient use of the nation's building stock, and modern building methods and regulations resulting in new properties (or newly redeveloped properties) being almost devoid of potential roosting opportunities. In these circumstances, some knowledge of the basic aspects of the spatial and social dynamics of *E. serotinus* is essential for an effective conservation management plan.

Therefore, the main aim of this thesis was to explore the individual movements, population and social structure and connectivity of *E. serotinus* in Europe, and more especially in England. This research used two forensic methods, population genetic and stable isotope analyses, and strategic and intensive sampling of individuals from maternity roosts across England to infer individual movements and behaviours and scale them up to community scale and population-level patterns. All procedures undertaken in the course of this thesis were carried out under the appropriate Home Office and Natural England licences and were approved by the Food and Environment Research Agency and the University of Exeter Biosciences ethics committees.

Thesis structure

In **Chapter 2**, I reviewed the current literature available on bat migration and dispersal and their effects on generating population genetic structure in order to establish if any general patterns arise and to document the variety and consequences of behaviours in bats.

In **Chapter 3**, I used ten nuclear microsatellites and a mitochondrial sequence to reveal the genetic structure and level of gene flow of *E. serotinus* across Europe. I investigated whether there were any differences in movements within both England and continental Europe that could explain EBLV-1 distribution and prevalence.

In **Chapter 4**, I investigated the coherence of the communities of bats sampled at maternity roosts over different time scales and the temporal variation in individual behaviour. To reach this aim, I explored fine scale movements and social dynamics of individuals within maternity roosts using $\delta^{13}C$ in two tissues that differed in their turn-over rates.

In **Chapter 5**, I assessed the performance of $\delta^{2}H$ in the study of animal movements at a small scale by examining the geographical distribution of $\delta^{2}H$ in bat fur and by linking $\delta^{2}H$ in fur and in precipitation. I then investigated sources of isotopic variation that could affect the use of this approach at a small scale.

In **Chapter 6**, I assessed the spatial organisation of *E. serotinus* at macro- and micro-geographical scales. As such, I integrated population genetic and stable isotope analyses to investigate the strength of maternity roosts as social units and the nature and level of connectivity among them.

Finally, in **Chapter 7**, I summarise the main findings of the thesis and assess the use of forensic methods in the study of animal movements and spatial and social organisation. I also discuss the implication of these results for conservation and disease ecology and introduce several lines for future investigations.

CHAPTER 2: Migration and dispersal patterns of bats and their influence on genetic structure

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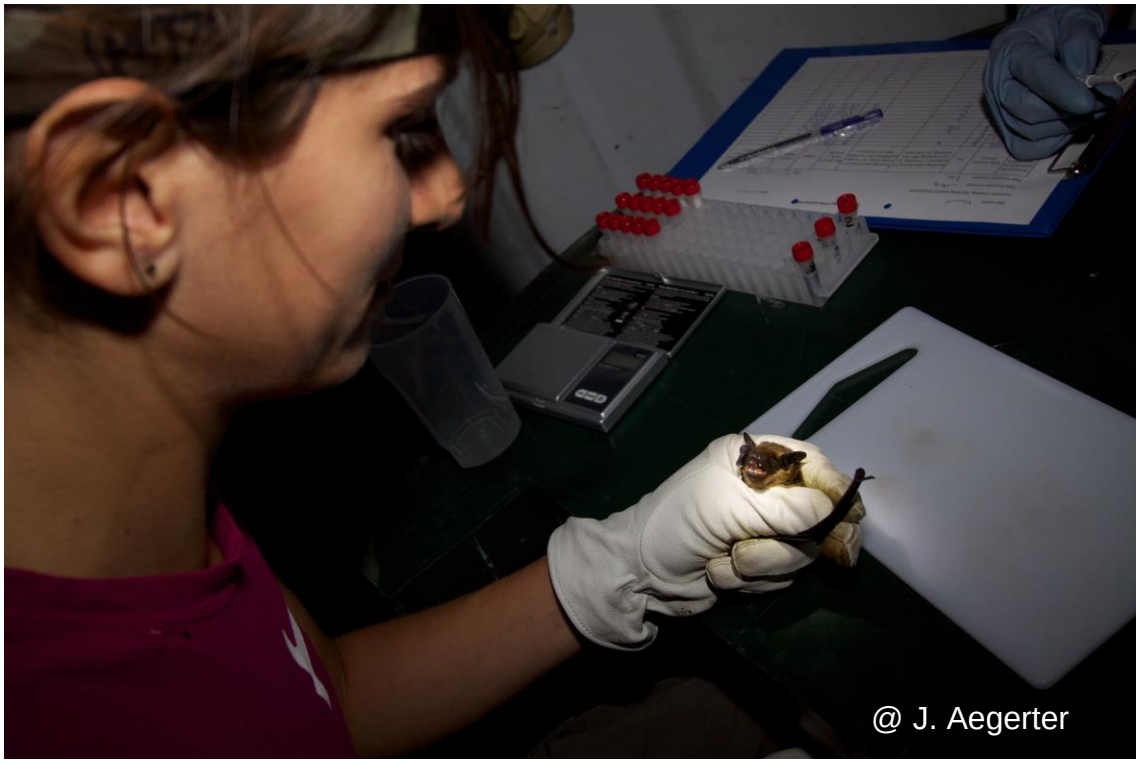

2.1 Abstract

Bats are important ecosystems service providers, make a significant contribution to biodiversity and can be important pests and disease vectors. In spite of this, information on their migration and dispersal patterns is limited.

In temperate bats, migration is most evident in females. This reflects seasonal differences in their habitat requirements, and the fact that seasonally suitable sites can be geographically distant. Tropical bats mainly migrate to track variation in food availability.

Little direct information is available on the patterns and drivers of bat dispersal, though drivers may include mate competition and inbreeding avoidance. In many temperate species, differential energy requirements and local resource competition among the sexes drive sexual segregation in the summer: females remain philopatric to their natal region, and frequently to their natal colony, while males disperse. In contrast, many tropical Pteropodidae form single-male/multi-female groups in which local resource defence contributes to female-biased or all-offspring dispersal from the natal site.

Population genetic studies are the most common source of evidence used to infer the spatial dynamics of bats. As expected, migratory species tend to have less genetically structured populations over large geographical scales due to mating outside of breeding areas, weak migratory connectivity and long-distance movements. In contrast and as expected, populations of sedentary species tend to be more differentiated at smaller geographical scales.

Despite this general pattern, a range of factors, including historical events, dispersal capabilities, and behavioural, ecological and geographical barriers, are implicated in the genetic partitioning of bat populations, irrespective of movement patterns. These factors limit the study of bat movements using only genetic methods.

Combining population genetics with other methods, such as mark-recapture, tracking or stable isotope analysis, should provide more insight into the movements of these ecologically and economically important species.

2.2 Introduction

Migration and dispersal are key events for many species. They play a significant role in shaping the genetic and demographic structure of populations, which in turn have implications for our understanding of population evolution and ecology. The management of pests and species carrying infectious diseases, and the conservation of endangered ones, also hinge on an accurate understanding of animal movement patterns. Distinguishing migration from dispersal has been the subject of considerable debate (Dingle & Drake 2007). For the purpose of this review, we define migration as a regular seasonal two-way movement of populations between regions, one of which usually includes the breeding site, where the young are born and raised, but where mating does not necessarily occur. In contrast, dispersal is a one-way movement from one location to another, often undertaken by juveniles or immature individuals (natal dispersal).

Migration can take several forms and occurs over a wide range of spatial scales (Dingle & Drake 2007), from a few metres for some newts (Jehle & Arntzen 2000) to thousands of kilometres for the arctic tern *Sterna paradisaea* (Egevang et al. 2010). Other forms of migration include vertical migration as seen in the Atlantic cod *Gadus morhua*, or trans-generational migration when the lifespan of an individual is shorter than the time needed to complete migration (e.g. monarch butterfly *Danaus plexippus*; Brower 1995). Not all populations or individuals of a given migratory species necessarily migrate: whilst obligate migrants always move, facultative migrants have more flexible strategies. Migration can also be partial when a portion of the population moves while another part remains sedentary, or differential when specific segments of a population behave differently (e.g. sex-specific movements). These different systems are not mutually exclusive, and the distinction between them is frequently blurred, leading to complex migration patterns. Despite this, migration is a general strategy to avoid unfavourable conditions. However, migration can be energetically costly, incur settlement costs, elevate predation risks, and increase the risk of not finding suitable new habitats or stop-over sites (Milner-Gulland et al. 2011). Migratory connectivity describes the link between breeding and non-breeding population-ranges provided by individual movements.

Populations in these ranges are composed mostly of the same individuals when migratory connectivity is strong. However, when migratory connectivity is weak, non-breeding populations are composed of individuals coming from different breeding populations (Webster et al. 2002).

The causes of natal dispersal are thought to include complex interactions between mating systems, sex-specific resource requirements, inbreeding avoidance and kin competition (Greenwood 1980, Perrin & Mazalov 2000, Handley & Perrin 2007). These interactions can result in sex-biased dispersal; birds tend to display female-biased dispersal, while terrestrial mammals usually show male-biased dispersal (Greenwood 1980). In contrast, philopatry, an individual's fidelity to its birth site or landscape, is favoured by kin cooperation, familiarity with natal habitat and mortality costs associated with movement (Handley & Perrin 2007).

One significant consequence of animal movement is the gene flow resulting from mating across populations. Factors potentially promoting gene flow include high dispersal ability (Bohonak 1999), long-distance migration when connectivity is weak (Webster et al. 2002), and the association of mating with movement. For instance, while birds typically copulate at their breeding sites, many temperate bats tend to copulate away from breeding sites when, or after, they move to their over-wintering sites. Geographical, ecological and behavioural barriers to movement are known to restrict gene flow and can generate structured populations.

Migration and dispersal are well studied in some taxa (e.g. birds), but there is a paucity of reliable data for other groups, including bats. Despite contributing greatly to biodiversity, and providing a range of ecosystem services (Kunz et al. 2011), many bat species are threatened by loss of foraging habitat and roosting sites, habitat fragmentation, hunting, persecution and other threats (Racey & Entwistle 2003). An understanding of their spatial ecology and behaviour is therefore essential for their effective management and conservation. Furthermore, bats are vectors for many diseases, especially zoonoses (Calisher et al. 2006), raising human health concerns, and some bat pathogens are also responsible for mass mortality, raising conservation concerns (e.g. white-nose syndrome; Lorch et al. 2011). Knowledge of the spatial and social dynamics of

bat populations is therefore essential for epidemiological predictions, risk assessment and management. The lack of information on bat movement is partly due to the difficulty in marking and tracking bats over long distances, as they are highly vagile and often too small to carry long-term tracking devices. Hence, though available data on the long-distance movements of larger bats, which can carry satellite trackers, are rapidly increasing (Epstein et al. 2009), similar information cannot be gathered from most species. With advances in molecular techniques however, population structure can be used to assess gene flow and, to some extent, individual movements. There have been reviews of bat migration (Fleming & Eby 2003, Popa-Lisseanu & Voigt 2009) and genetic structure and movement (Burland & Wilmer 2001, Fleming & Eby 2003), but recent additions to the literature, including most population genetics studies, have not been reviewed, hence a reassessment of bat movement and genetic structure is needed. We therefore summarise the current literature on bat migration and natal dispersal, as well as their influence on gene flow and on population structure.

2.3 Migration

2.3.1 Drivers of bat migration

Substantial mortality risks are associated with migration, especially over long distances (Milner-Gulland et al. 2011), because migration is energetically costly, requiring individuals to be in good condition to cope with its physiological demands. In addition, for bats, with their complex daily resource requirements, the hazards of inclement weather, predation and uncertainties in finding suitable temporary shelters during migration heighten mortality risks. Migration seems even more costly for juveniles because of their limited foraging experience and limited time post-weaning to lay down the fat stores needed for migration. For example, public recovery of banded *Myotis grisescens* in the USA occurs mostly at peak migration times, and juvenile mortality is significantly higher than that of adults (Tuttle & Stevenson 1977). Therefore, for migratory behaviour to have evolved, its benefits must outweigh these costs.

In temperate regions, roost requirements often differ between winter and the rest of the year. Hibernacula need to provide stable low temperatures, sufficient humidity to optimise evaporative water loss and protection from predators (Speakman & Racey 1989, Speakman & Thomas 2003, Neubaum et al. 2006). Summer roosts, especially maternity roosts, tend to exhibit specific microclimates with higher temperatures so bats can remain euthermic, but minimise energy expenditure in order to sustain pregnancy and lactation (Racey & Swift 1981, Tuttle & Stevenson 1982, Audet & Fenton 1988). Few sites could fulfil these very different requirements all year round, prompting seasonal relocation. The degree of roost selectivity varies between species, as does the distance between suitable seasonal sites. Such factors partly determine whether the individuals are sedentary (with very local relocation, if any), regional migrants, or long-distance migrants, although these distinctions are often unclear. In contrast to birds and other migrating mammals that move seasonally to escape unfavourable conditions, but remain active over the winter, migration in temperate bats is strongly associated with hibernation, although some species migrate to sub-tropical habitats and remain active over the winter (e.g. some migratory populations of *Tadarida brasiliensis*; Fleming & Eby 2003, Geluso 2008). Migration in tropical species has been little studied but is thought to be associated with fluctuations in food availability that probably result from variation in rainfall and season (Fleming et al. 1993, Moreno-Valdez et al. 2000, Tidemann & Nelson 2004, Richter & Cumming 2006).

2.3.2 Patterns of migratory behaviour

Bat migration seems to be a flexible behaviour that varies greatly among and within species. Many species appear to display partial migration, where migratory and non-migratory populations co-exist within a species' range, and share either hibernacula or maternity roosts (Fleming & Eby 2003, Ibáñez et al. 2009). Additionally, different migratory behaviours can be found intraspecifically in populations which are geographically isolated (Cockrum 1969, Fleming & Eby 2003). This suggests that migratory behaviour in bats is facultative or possibly even cultural (Rojas-Martínez et al. 1999, Popa-Lisseanu & Voigt 2009). Sex-biased migration is also common (Fleming & Eby 2003, Dietz et al. 2009, Ibáñez et al. 2009). Females are often more likely to migrate, or migrate longer

distances, than males. This sexual differentiation can be partly explained by the high energy requirements of females during pregnancy and lactation, which can result in them moving to higher latitudes where seasonal resources are more abundant during summer (Fleming & Eby 2003). Females have also more stringent roosting requirements than males in the summer (Racey 1982, Williams & Brittingham 1997, Speakman & Thomas 2003). Roost temperature is the main driver of the migratory behaviour of *Miniopterus schreibersii* in the Mediterranean (Rodrigues & Palmeirim 2008). In contrast, the lower energetic requirements of males can be sustained in habitats with fewer resources, and their summer roost selection may be less stringent, allowing greater flexibility in roosts used than females (Senior et al. 2005). This may reduce males' need to migrate.

2.3.3 Which species migrate?

Phylogenetic study of bat migration suggests it evolved independently in several lineages as the need to track resources, such as roosts or food, arose (Bisson et al. 2009). In temperate bats, migration is less likely to have evolved in cave-roosting species than in tree-roosting species (Fleming & Eby 2003, Bisson et al. 2009). Species using ephemeral or exposed roosts all year round might need to migrate to latitudes where conditions are milder so they can survive over winter (Fleming & Eby 2003, Dietz et al. 2009, Popa-Lisseanu & Voigt 2009). It has also been argued that species adapted for aerial hawking are more likely to migrate (Fleming & Eby 2003). Bat wing shape is influenced by foraging strategy and diet, and aerial hawkers are pre-disposed to efficient long-distance flight owing to their flight morphology (Norberg & Rayner 1987, Fleming & Eby 2003).

The number of bat species migrating and the scale over which they migrate is not known (Appendix 1). This is especially true for tropical species for which migration has only rarely been studied (six species are migratory, 12 non-migratory or putative non-migratory and 11 have unknown behaviour; Appendix 1). In temperate climates, some species such as *Plecotus auritus* and *Myotis bechsteinii* relocate within the same area, and seasonal movements are rarely more than 100 km. Regional migration, of up to a few hundred kilometres, is

recorded in several species (e.g. *Myotis brandtii, Myotis daubentonii and Myotis myotis;* Hutterer et al. 2005). Long-distance migration involving thousands of kilometres return flight is thought to be rarer. European examples of long-distance migrants include *Nyctalus noctula* and *Pipistrellus nathusii* (one-way maximum recorded distances 1600 km and 1905 km respectively; Russ et al. 2001, Fleming & Eby 2003, Hutterer et al. 2005). North American examples include tree roosting species in the genera *Lasiurus* and *Lasionycteris* which undertake complex migrations of up to 2000 km from temperate to sub-tropical habitats (Cryan 2003).

In the tropics, species affected by significant environmental fluctuations are expected to migrate. Migration could be particularly advantageous for bats feeding on nectar and fruits, since the phenology of both may mean that food is temporally and spatially patchy. Consistent with this, stable isotope analysis of the movements of the nectarivorous bat *Leptonycteris curasoae* indicate spring and autumn migration along a 'nectar corridor' determined by the flowering phenology of cactus and Agave (Fleming et al. 1993). Similarly, migration of *Eidolon helvum* in Zambia is driven by food supply (Richter & Cumming 2006). Unlike these plant-dependent species, insectivores are expected to be more sedentary since seasonal fluctuations in insect availability are relatively low (Fleming & Eby 2003).

2.4 Dispersal

Variation in bat dispersal has been linked to differences in mating systems, social structures and behaviours (McCracken & Wilkinson 2000). However, dispersal is difficult to study and remains poorly understood in most species. In the absence of direct evidence from mark-recapture studies, dispersal, or the lack of it, is often inferred from population genetic structure. However, some problems are associated with such inferences, including a lack of consistency in the definitions of population and philopatry (Waples & Gaggiotti 2006). Furthermore, migration, mating at hibernacula, and autumnal swarming (lekking behaviour; Parsons et al. 2003), can all increase gene flow without the need for dispersal. A genetic-only approach cannot differentiate between these potentially interacting agents. The time of year at which dispersal is investigated

also affects results. Most studies are carried out at summer sites, and especially at maternity roosts. As a result, there is a lack of quantitative information on the composition of populations at hibernacula and on how social structure may differ at different locations and times. Consequently, the winter ecology and dispersal of juveniles remains largely unknown for most bat species. In contrast, in some other taxa, for instance some species of snake, effective juvenile dispersal occurs in the summer, but juveniles are then recruited into the maternal hibernaculum (Reinert & Zappalorti 1988, Cobb et al. 2005, Brown et al. 2007, Clark et al. 2008). These issues and the data scarcity for many bat species restrict our understanding of dispersal patterns and what may drive them.

2.4.1 Female philopatry and male dispersal

In many temperate bat species, females segregate from males in spring and summer to form breeding colonies (McCracken & Wilkinson 2000, Senior et al. 2005, Safi et al. 2007, Ibáñez et al. 2009). Few researchers have rigorously evaluated female fidelity to their breeding colonies, but annual recapture of females in a few temperate species, including *Pipistrellus pipistrellus* (Thompson 1990, 1992), *Eptesicus nilssoni* (Rydell 1989), *Rhinolophus ferrumequinum* (Ransome 1989, Rossiter et al. 2002), and *Myotis bechsteinii* (Kerth et al. 2002b), suggest philopatry to their natal colony. The broad taxonomic and ecological nature of this list suggests that this may be a widespread strategy. Nonetheless some female dispersal, and in some cases recruitment to other breeding colonies, can occur (Thompson 1990, 1992). However, movement is frequently limited, both in the numbers of individuals moving and in the distances covered by them, indicating strong philopatry to natal areas. The degree of colony fidelity and recruitment varies interspecifically, and at one extreme can lead to the creation of matriarchal societies and virtually closed maternity colonies (e.g. in *Myotis bechsteinii* in Germany; Kerth et al. 2000, Kerth et al. 2002b, and in *Rhinolophus ferrumequinum* at the edge of its range in the UK; Rossiter et al. 2005). High female energy requirements during the breeding season may drive this sexual segregation, and females potentially benefit from philopatry since familiarity with their natal area could facilitate access to foraging and roosting sites. In addition,

philopatry could promote long-term social relationships and enhance cooperative behaviours such as information transfer, social thermoregulation, allogrooming and food sharing (Wilkinson 1985, Kerth 2008a, Kerth et al. 2011). In such systems, male dispersal could be driven by a combination of local resource competition with females (Senior et al. 2005, Safi et al. 2007), inbreeding avoidance (Kerth et al. 2002a, Kerth et al. 2002b), local mate competition, and possibly avoidance of kin competition (Kerth 2008a). Hence, in some species, dispersal may allow males to secure and defend mating territories or roosts, and thus provide them access to mates (Gerell & Lundberg 1985, Petri et al. 1997, Zahn & Dippel 1997, Petit & Mayer 1999, Castella et al. 2001, Petit et al. 2001). Male fidelity to a mating site has been reported (Gerell & Lundberg 1985, Zahn & Dippel 1997), but does not necessarily qualify as philopatry since males may have dispersed from their natal area to establish these mating territories. Unfortunately, the degree of dispersal and the distances over which males disperse are difficult to assess; they are often indirectly inferred through population genetics, by estimating the levels of male-biased and female-biased gene flow over geographical distances (Petit & Mayer 1999, Castella et al. 2001, Petit et al. 2001, Ngamprasertwong et al. 2008). However, some mating systems, such as autumnal swarming and mating at hibernation sites, can increase regional or national scale gene flow without the need for male natal dispersal, and may thus confound straight forward male-movement scenarios. This is thought to be the case in *Myotis bechsteinii,* where males disperse from the natal colony but remain philopatric to their natal area, and mating occurs at swarming sites, which genetically mixes colonies (Kerth et al. 2002a, Kerth & Morf 2004).

2.4.2 Female dispersal

Female dispersal and male philopatry have rarely been seen in bats, but in the few taxa that follow this pattern, drivers appear to be male resource acquisition and female inbreeding avoidance. For example, for males of the harem-forming species *Saccopteryx bilineata,* acquaintance with the natal colony facilitates territory take-over (Voigt & Streich 2003), thus driving male philopatry. Male length of tenure exceeds female age at sexual maturity, so selection has presumably favoured female dispersal as an inbreeding avoidance mechanism

(Nagy et al. 2007). In other species, despite the common pattern of male-biased dispersal, strict female philopatry has never been demonstrated. This is true even of the highly philopatric *Myotis bechsteinii,* where female dispersal rate is estimated to be approximately one individual in five generations (Kerth et al. 2000). Conversely, in *Eptesicus fuscus*, a high number of matrilines and low proportion of mother-daughter associations were reported in maternity colonies, prompting questions about the level of philopatry and dispersal of females (Vonhof et al. 2008). Some female dispersal occurs, but the extent and timing of this phenomenon and its importance and effects on social structure and population genetics are unknown. Similarly, it is unclear whether female dispersal is more generally restricted to colonisation of unoccupied locations. New colony foundation seems to result from the fission of a few closely related females from a common maternity colony (Kerth 2008b, Metheny et al. 2008), but more work is required to understand this crucial aspect of bat life-history.

2.4.3 Philopatry of both sexes

During the breeding season, colonies of some species, for example *Plecotus auritus* and *Myotis natterii*, can be composed of males and females, and both seemingly display high levels of philopatry (Burland et al. 1999, Entwistle et al. 2000, Burland et al. 2001, Veith et al. 2004, Rivers et al. 2005). The underlying reasons for this behaviour are complex and not well understood. The benefits of philopatry (such as familiarity with natal area, cooperative behaviour and sociality) presumably outweigh the costs (such as local resource competition and parasite transmission within roosts). Moreover, dispersal costs in these species (both of which are poorly adapted for efficient long-distance flight) might be high enough to select for fidelity to natal area. Low levels of within-roost paternity (Burland et al. 2001) indicate that inbreeding is avoided by extra-colony copulation (Burland et al. 1999, Burland et al. 2001), perhaps during swarming events (Veith et al. 2004, Rivers et al. 2005, Rivers et al. 2006). Within-species between-population variation in philopatry has also been reported. For instance, while philopatry of both males and females of *Plecotus auritus* is observed in northern parts of their range in Scotland, male dispersal occurs widely in central Europe (Entwistle et al. 2000). This behavioural

difference prompts questions about the causes of philopatry and the costs of dispersal in relation to the geographical distribution of species.

2.4.4 Dispersal of both sexes

Different drivers for male and for female dispersal can have an additive effect in some species like *Lophostoma silvicolum* and lead to dispersal of both sexes. In taxa with intense local mate competition, males often invest time and energy in resources used by females, for example excavating termite nests in *Lophostoma silvicolum* (Dechmann et al. 2005, Dechmann et al. 2007), or tent-making in *Cynopterus sphinx* (Storz et al. 2000). Competition for mates and the need to find these key resources may drive male dispersal. In addition, inbreeding avoidance is thought to be important because females usually reach sexual maturity before male tenure ends, and hence daughters are thought to disperse to avoid mating with their sires (Dechmann et al. 2007). However, unequivocal evidence for these potential drivers is lacking.

2.5 Influence of dispersal and migration on population genetic structure

Various migratory, dispersal and mating behaviours are displayed by bat species, so population genetic structure is also expected to vary widely. Migratory bat species, which are mobile and may mate at winter sites or on their way to or from them, are predicted to show higher levels of gene flow and thus lower levels of structuring among populations than non-migratory species. In contrast, the restricted movements of more sedentary bats are expected to promote greater genetic differentiation between populations (Burland & Wilmer 2001, Fleming & Eby 2003, Popa-Lisseanu & Voigt 2009). However, migration connectivity, sex-biased dispersal and mating systems, plus historical processes, including local extinctions and colonisation, generate substantial variation in population structure beyond this simplistic scenario (Appendix 1).

2.5.1 Non-migratory bat species

Genetic partitioning among populations of bats considered to be non-migratory or to be only regional migrants is a result of current gene flow through natal and mating dispersal, colonisation (Kerth & Petit 2005, Kerth 2008b) and historical processes (Burland & Wilmer 2001, Rossiter et al. 2007, Flanders et al. 2009). For example, the genetic differentiation in mitochondrial genes of *Macroglossus minimus*, *Ptenochirus jagori* and *Cynopterus brachyotis* in the Philippines is associated with Pleistocene aggregate island complexes rather than only with contemporary islands, and their mismatched distribution reflects past demographic events including population expansion and secondary contacts between lineages (Roberts, 2006a). Variation in dispersal and mating strategies, plus geographical or behavioural barriers, affect the level of gene flow within species, leading to significant heterogeneity in population structuring. Sex-biased dispersal is also reflected in genetic partitioning. Strong female philopatry is thus responsible for extreme population subdivision and isolation by distance in the *Macroderma gigas* in Australia and *Rhinolophus monoceros* in Taiwan. However, gene flow occurs through male dispersal in *Rhinolophus monoceros,* but is limited in *Macroderma gigas* (Worthington Wilmer et al. 1994, Worthington Wilmer et al. 1999, Chen et al. 2006, Chen et al. 2008). In the polygynous bats *Lophostoma silvicolum* and *Cynopterus sphinx,* low genetic structuring reflects dispersal of both sexes, although female dispersal is more limited (Storz et al. 2001, Dechmann et al. 2007, Karuppudurai et al. 2007).

Geographical barriers, such as open water or mountains, are expected to affect the dispersal of non-migratory individuals, restricting gene flow (Burland & Wilmer 2001). A number of studies have assessed the population structure of island and archipelago bats in order to estimate gene flow and individual movement across seas (e.g. Fleming et al. 2009, Schmitt et al. 2009). Many taxa including *Miniopterus* spp. in the Comores (Weyeneth et al. 2008), *Haplonycteris* spp. in the Philippines (Townsend Peterson & Heaney 1993, Roberts 2006b), *Myotis punicus* in the Mediterranean basin (Biollaz et al. 2010) and the Azorean bat *Nyctalus azoreum* (Salgueiro et al. 2004, Salgueiro et al. 2008) show significant genetic differentiation and/or haplotype segregation between islands or groups of islands but not within them. This suggests that

gene flow is restricted among islands, and that open water constitutes an effective barrier to movement. However, this is not the case for all non-migratory species, and less population subdivision between islands has also been reported (e.g. in *Artibeus jamaicensis* from the Lesser Antilles; Carstens et al. 2004, and in *Cynopterus* spp. in the Philippines; Townsend Peterson & Heaney 1993). In temperate bats, the English Channel seems to provide a substantial barrier to gene flow for *Eptesicus serotinus* (Smith et al. 2011) and *Rhinolophus ferrumequinum* (Rossiter et al. 2000, Rossiter et al. 2007), but not for *Myotis daubentonii* (Atterby et al. 2010). Bat population structure is also variable across the strait of Gibraltar; some species cross and mate either side of the strait (e.g. *Eptesicus isabellinus;* Juste et al. 2009), while dispersal is limited in others (The Straits of Gibraltar: barrier or bridge to Ibero-Moroccan bat diversity? -Mudarra et al. 2009). This suggests that factors in addition to geography can restrict gene flow. Behavioural and ecological barriers, such as habitat gaps and ecotones, limit gene flow in non-migratory birds (Harris & Reed 2002). Consistent with this, ecological barriers are likely to divide *Desmodus rotundus* into five mating populations, reflecting distinct ecodomains, despite the species' high dispersal ability (Martins et al. 2007, Martins et al. 2009). Similarly, low habitat connectivity may restrict gene flow among colonies of *Myotis bechsteinii*, *Myotis macropus* and *Carollia perspicillata*, resulting in high genetic differentiation between colonies (Kerth & Petit 2005, Campbell et al. 2009, Meyer et al. 2009). These species are able to disperse on the scale studied, but the limited gene flow indicates the importance of suitable habitat availability in promoting dispersal.

Although low juvenile dispersal can promote population differentiation, some mating systems facilitate genetic homogenisation, and sometimes this can occur over large geographical scales. Autumnal swarms involving bats from a number of spatially separated natal colonies may facilitate homogenization, and higher genetic diversity is reported at swarming sites for *Myotis bechsteinii* and for *Plecotus auritus* than at maternity roosts (Burland et al. 1999, Burland et al. 2001, Kerth et al. 2003, Kerth & Morf 2004, Veith et al. 2004, Furmankiewicz & Altringham 2007). As a result, these sedentary species show lower levels of differentiation than expected over large geographical scales, although female philopatry still increases population structure in mitochondrial genes (Kerth et al.

2002a, Veith et al. 2004). *Myotis natterii* and *Myotis daubentonii* are also considered to be swarming species (Parsons et al. 2003, Glover & Altringham 2008) and both maintain high gene flow between geographically isolated colonies (Rivers et al. 2005, Senior et al. 2005, Rivers et al. 2006, Ngamprasertwong et al. 2008, Atterby et al. 2010). However, in some cases, isolation by distance is still detected, indicative of limits to the distance bats cover during these mating movements (Ngamprasertwong et al. 2008), or of the rarity of potential mating sites between populations. The latter restriction has been implicated in the population structuring of non-migratory populations of *Miniopterus schreibersii* throughout its Portuguese range (Ramos Pereira et al. 2009, Rodrigues et al. 2010). Four sub-populations exist within which there is very low differentiation, indicating high gene flow. Ringing data suggest that autumn mating at communal sites rather than juvenile or adult dispersal is responsible.

2.5.2 Migratory bat species

Only a few researchers have investigated the genetic variation and population structure of migratory bats over large geographical scales (in 16 of the 59 species listed in Appendix 1). Some of the best studied long-distance migrants, such as *Nyctalus noctula*, *Tadarida brasiliensis* and *Pteropus* spp., display very low genetic differentiation over long distances despite philopatry to their summer and/or winter colonies (Svoboda et al. 1985, McCracken et al. 1994, Sinclair et al. 1996, Webb & Tidemann 1996, McCracken & Gassel 1997, Petit & Mayer 1999, 2000, Petit et al. 2001, Russell et al. 2005). This low population structure can be explained by the timing of mating relative to migration, weak migratory connectivity and dispersal. In contrast to many migratory bird species that may be monogamous and/or mate and breed on summer grounds after migration (Greenwood 1980, Fleming & Eby 2003), migratory bat species are often polygamous or promiscuous (Hosken 1997, 1998, McCracken & Wilkinson 2000) and mating, fertilization, and birth usually occur at different geographical locations. For example, temperate bats tend to mate on or near the wintering grounds before or during migration (Fleming & Eby 2003). In species displaying weak migratory connectivity, this means that individuals from different summer populations share mating sites, increasing gene flow if successful mating

occurs, as seen in *Nyctalus noctula* (Petit & Mayer 2000). The migratory connectivity in subspecies *Tadarida brasiliensis mexicana* and *Tadarida brasiliensis cynocephali* is less obvious: banding records indicate isolated migratory and non-migratory populations (Cockrum 1969). Genetic differentiation is, however, very low among all populations, even among the two subspecies. This indicates high levels of current gene flow, probably caused by mating during migration and juvenile dispersal (Cockrum 1969, Svoboda et al. 1985, McCracken et al. 1994, McCracken & Gassel 1997, Russell et al. 2005).

Other migratory species display significant population structure, reflecting limits in dispersal and migratory abilities, stronger migratory connectivity and/or the importance of some geographical or ecological barriers to gene flow. *Myotis myotis* for instance, displays a relatively high level of male-biased dispersal, but extreme female philopatry results in extreme population structure in the mitochondrial genome (Castella et al. 2001). Furthermore, geographical barriers constrain gene flow over distances that bats of this species could otherwise cover, resulting in isolated populations (Castella et al. 2000, Newton et al. 2003, Ruedi et al. 2008). The isolation of different populations has led to local adaptation in *Miniopterus schreibersii natalensis* in South Africa. Strong population sub-structure in both sexes is closely associated with four biomes and correlated with differences in wing morphology and migratory behaviour. Bats from the north-eastern population exhibit higher wing aspect ratio, more economical for long-distance flight, and tend to migrate over longer distances than bats from the other populations, perhaps because the biome they occupy is subjected to larger resource fluctuations (Miller-Butterworth et al. 2003).

In species displaying partial migration, migratory populations are often nearly panmictic, but non-migratory populations are more structured and genetically differentiated. This is the case in *Eidolon helvum*: the migratory population found throughout continental sub-Saharan Africa has no structure (Peel et al. 2010), but the sedentary populations on the islands of the Gulf of Guinea display varying degrees of genetic differentiation (Juste et al. 2000, Peel et al. 2010).

The effects of dispersal and migration on gene flow are difficult to disentangle, as seen in two cryptic European species *Pipistrellus pipistrelllus* and *Pipistrellus*

pygmaeus. Low genetic differentiation and lack of isolation by distance is found in these species in central Europe, indicating high levels of gene flow (Bryja et al. 2009) similar to those found in long-distance migrants such as *Nyctalus noctula*. However, extreme juvenile dispersal could also explain this genetic pattern. The lack of banding data (Hutterer et al. 2005) prevents further exploration of relevant hypotheses. Furthermore, population differentiation, which is found in another part of these species' ranges, is inconsistent with panmixia (Racey et al. 2007). However, this research was mainly based on samples from Great Britain, and although the English Channel was not found to be a significant barrier to gene flow, it is possible that these species exhibit partial migration behaviour. This inconclusive example stresses the importance of using methods other than just population genetics (e.g. mark-recapture or stable isotopes) to understand bat movement patterns.

2.6 Conclusion

We have explored bat movement through both migration and dispersal, and its influence on the genetic make-up of populations. A wide range of migratory and dispersal behaviours exist in bats, generating considerable variation in population structure. In general, populations of migratory species are less structured than populations of sedentary species, at least when assessed over longer distances (Appendix 1). However, this is only true when migratory bats mate during migration, hibernation or swarming, and when weak migratory connectivity is observed. Even spatially dynamic populations may show significant genetic structuring. In sedentary bats, although population structure can be seen at smaller geographical scales, varying degrees of gene flow can occur depending on the mating strategy, the dispersal ability of bats and their susceptibility to isolating barriers.

It is essential to understand a species' ecology, including its mating system, to interpret population genetics data accurately, and to distinguish between different mechanisms that could generate similar genetic patterns (e.g. autumnal swarming and male dispersal). Furthermore, most population genetics studies are not quantitatively comparable because of differences in the markers used (60 inferences from mtDNA, 59 from microsatellites, 14 from allozymes,

two from random amplification of polymorphic DNA and six from nuclear genes in Appendix 1), in the numbers of markers (range 1-2 for mtDNA, 3-15 for microsatellites, 4-19 for allozymes and 5-7 for random amplification of polymorphic DNA in Appendix 1), in statistical methods, in geographical scales (21 species studies at small scale, 34 at medium scale and 48 at large scale in Appendix 1), in defining a population (defined as bats in a colony, site, locality and region in Appendix 1), and in the season in which studies are carried out. The use of mtDNA as the sole genetic marker and its assumption of neutrality is frequently questioned, and is probably flawed (Ballard & Whitlock 2004, Balloux 2010).

Nevertheless, the boundaries of spatial ecology studies in bats are constantly being pushed by new developments and inter-disciplinary approaches. For instance, DNA can now be extracted from faecal samples, which offers the possibility of sampling larger numbers of individuals and colonies (Puechmaille et al. 2007), and advances in analytical methods, including Bayesian clustering models and coalescent methods (Beerli & Felsenstein 2001, François & Durand 2010), allow more powerful population genetics inferences. Inter-disciplinary approaches combining several methods to infer movements, including behavioural observations, banding data, radio-tracking, population genetics, and stable isotopes, can give rise to new disciplines, such as landscape genetics (Manel et al. 2003, Muscarella et al. 2011), which marries population genetics and geographic information systems.

Confidently quantifying bat movements is essential for several reasons. Many bat species, such as *Nyctalus azoreum* in the Azores, *Myotis sodalis* in the USA, and *Pteropus* spp. (Anonymous 2011), are threatened, and conservation plans are needed to sustain populations. Due to variation in their dispersal abilities, species may vary in their vulnerability to habitat fragmentation and roost loss. It is often assumed that colonies excluded from their roost will join other neighbouring colonies or colonise other habitats, but behaviourally limited dispersal may restrict such opportunities. Dispersal ability therefore needs to be assessed for management plans. Furthermore, it is essential to determine which species are migratory and where they migrate to, and to delimit flyways and migratory connectivity to conserve summer, winter and stop-over sites. Bat

mortality at wind farms has been linked to migration behaviour (Cryan & Brown 2007, Cryan & Barclay 2009), but further study is required to identify whether migration, dispersal or some other behaviour drives mortality elsewhere. In addition to conservation, studying bat movements facilitates the investigation of disease, particularly those caused by Lyssaviruses, which are important because of their effects on public health (Harris et al. 2006, Vazquez et al. 2006, Vos et al. 2007). Understanding the movement patterns of bats assists disease risk assessments, modelling of disease dynamics and planning effective emergency responses.

CHAPTER 3: Population genetic structure of *Eptesicus serotinus* across Europe and implication for EBLV-1 prevalence

3.1 Abstract

The genetic structure of populations of the serotine bat *Eptesicus serotinus* across Europe was described using ten microsatellite markers and a 424bp portion of the hypervariable region II of the mitochondrial D-loop. Significant population structure was found for both markers, although stronger differentiation in the mitochondrial data suggests a mating system driven mainly by the movement of males (nuclear: F_{ST} = 0.048, D_{EST} = 0.088, mitochondrial: φ_{ST} = 0.405). Multivariate and Bayesian analyses revealed that a higher degree of genetic structuring was found in England than on the continent, despite considerably smaller geographical distances and the presence of only one potential small geographic barrier. Nuclear data suggest a single continuous population on the continent with the presence of isolation by distance and the possible effect of mountainous region in limiting gene flow. Mitochondrial sequences indicate an east-west substructure across Europe, possibly reflecting post-glacial re-colonisation. In contrast, three distinct populations were found in England using microsatellite markers while mitochondrial diversity was too low to suggest structure. This pattern may be caused by combinations of factors operating on this species at the northern and western margin of its range: possible community or population behaviours, dislike for crossing the sea, post glacial history, inferred mating strategy and winter ecology. Whilst high admixture indicated strong male mediated gene flow among populations, it was not clear what the context of these movements was, non-dispersive out-breeding behaviours, dispersal or migration. While the English Channel restricts gene flow, relatively frequent movement from the continent to England was inferred, with the west of England having the most genetic immigration, suggesting a westward range expansion.

3.2 Introduction

Large scale studies of bat species are restricted by the high mobility, nocturnal behaviour and small size of these animals, which impede the use of mark-recapture techniques or direct tracking. Advances in molecular techniques and statistics, and their integration with spatial analysis (Storfer et al. 2010) provide new avenues to address an array of ecological and evolutionary questions (Burland & Wilmer 2001, Moussy et al. 2013). Molecular approaches have therefore been used to assess many aspects of bat biology including potential sex-biased dispersal and movements (Petit et al. 2001, Chen et al. 2008, Dixon 2011), mating systems (Rivers et al. 2005, Furmankiewicz & Altringham 2007), migration behaviour (Bryja et al. 2009), effect of landscape barriers (García-Mudarra et al. 2009), demography and history (Muscarella et al. 2011) and sociality (Kerth et al. 2000, Kerth & Van Schaik 2012). Gaining an understanding of such key components of bat ecology and behaviour is fundamental in a conservation context (Racey & Entwistle 2003), to improve our understanding of ecosystem services (Kunz et al. 2011), and disease epidemiology since bats are now thought to be the wildlife reservoirs for a range of important zoonoses and emerging diseases (Kuzmin et al. 2011).

European bat lyssaviruses (EBLVs) are zoonoses of significant policy concern, causing rabies and death in bats and humans. The diversity of known viruses and their hosts is expanding rapidly, and at least three viruses are known in western Europe; EBLV-1 mainly circulating in the serotine *Eptesicus serotinus*, EBLV-2 mainly known from the Daubenton's bat (*Myotis daubentonii*) and Bokeloh virus isolated from the Natterer's bat (*M. nattereri).* Other lyssaviruses are also being characterised in eastern Europe and central Asia (Calisher & Ellison 2012). EBLV-1 is predominant, with over 800 cases across north-western Europe (McElhinney et al. 2008). Over 95% of recorded EBLV-1 infections are associated with the common serotine, *E. serotinus*, mostly in Denmark, Germany and The Netherlands (Harris et al. 2006) and despite the proximity, no live EBLV-1 has been documented within England by either passive or active surveillance. In contrast, active surveillance in England has found a sero-prevalence of 1-4% for EBLV-2 (Harris et al. 2006) and it is

thought that the virus is maintained at low endemic level within *M. daubentonii* (Atterby et al. 2010).

E. serotinus is a fairly large (20-30g, 32-38cm wingspan) and common species within its core range and is widely distributed in Europe from northern Spain to Denmark and southern Sweden, and from the Atlantic coast to the Caspian Sea. In the UK it is less common, and is largely restricted to southern England (Dietz, Von Helversen, et al. 2009) with a population size estimated at 15,000 individuals (Bat Conservation Trust, 2011). Summer maternity roosts mostly occur in used buildings, such as houses and churches, and usually consist of small numbers of adult females and their young (10-50). Females seem to display considerable philopatry (Harbusch 2003, Harbusch & Racey 2006). Individuals typically forage between four to six kilometres from their roost in open and edge habitats such as pastures and woodland edges, though actual commuting distances are reported up to 14 kilometres (Catto et al. 1996, Robinson & Stebbings 1997, Verboom & Huitema 1997, Harbusch 2003). Adult females start to leave nursery roosts in August once young are weaned (Harbusch 2003), though it is unclear where the community moves to for six to nine months of the year, or even whether individuals remain associated throughout the winter. The species is considered sedentary (Hutterer et al. 2005) with recorded seasonal flights of under 100 kilometres, although ringing data are sparse and this restricts our understanding of any seasonal movements.

The winter ecology of *E. serotinus* is poorly known and only a small number of individuals have been found in hibernation in caves, rock crevices, and inaccessible fissures or hollow walls in houses (Robinson & Stebbings 1997, Harbusch 2003). Similarly, knowledge of the mating system and of the ecology of males is absent. No adult males are found within maternity roosts, indicating exclusion once they reach sexual maturity, and they are thought to roost singly or possibly in small groups (Harbusch 2003). Mating is hypothesised to take place in autumn or possibly in late summer. Information on whether males set up mating territories like *Nyctalus noctula* or mate at swarming sites like *M. nattereri* is still lacking although reports of multi-male and female aggregations at underground sites (Christé, pers. comm.) may indicate the latter.

These gaps in our knowledge of serotine ecology and movements undermine our understanding of how pathogens such as EBLV-1 circulate across their wildlife reservoirs, and limit predictions of whether EBLV-1 may either travel to the English population, spread across it, or be maintained once established. Currently the prevalence of EBLV-1 in bats in countries on one side of the English Channel contrasts strongly with its apparent absence from England. Whilst the Channel appears to act as a barrier to the disease, it is unclear if this is also true of its hosts. Furthermore, it does not appear to inhibit the gene flow in *M. daubentonii* (Atterby et al. 2010) or incidence of EBLV-2 (Harris et al. 2006). A small scale comparison of the genetic structure of these EBLV-1 and EBLV-2 hosts revealed more structure within England in *E. serotinus* than in *M. daubentonii* (Smith et al. 2011). This implies a lower mobility of the English serotines, which could prevent endemic maintenance of the EBLV-1, whilst higher vagility and mating at swarming sites in *M. daubentonii* (Parsons et al. 2003) might facilitate EBLV-2 transmission. However, despite the importance of understanding movement and mating patterns and social organisation of the serotine bat in light of potential disease transmission, no large-scale studies of serotine movement have been undertaken.

Here, we used ten microsatellites and a portion of the hypervariable region II (HVII) of mitochondrial DNA (mtDNA) to investigate the genetic structure of *E. serotinus* at multiple spatial scales. We hypothesised that high contemporary gene flow occurs over large scales on the continent, helping to maintain the prevalence of EBLV-1. In contrast, low gene flow across the Channel is predicted, restricting the passage of EBLV-1 into England. We also assess the population genetic structure of this bat within England. The use of both nuclear and mitochondrial markers allows the description of differential mating behaviour. Our default expectations are that *E. serotinus* will display male-mediated gene flow (possibly dispersal) and female philopatry, as is found to be common in temperate bats (Moussy et al. 2013).

3.3 Methods

3.3.1 Sample collection

A total of 593 individuals were captured at 28 maternity roosts in inhabited buildings across the English range in the summers of 2004-2006 and 2010-2011 (Table 3.1; Figure 3.1). All bats were described (sex, age and other biometry) and marked with a unique numbered forearm band for identification (Mammal Society, UK). A wing tissue sample was taken from each bat using a 3mm biopsy punch (Stiefel Laboratories, Wooburn Green, UK) and stored in 70% ethanol at +4°C until DNA extraction. All bats were released back into the wild within a few minutes of the procedure at their location of capture. All the procedures were performed under license from the UK Home Office [Animals (Scientific Procedures) Act 1986] and Natural England (Habitats Regulations, 2010). Wing biopsies were also supplied for 106 individuals from eight locations in six countries across continental Europe (Table 3.1; Figure 3.1). Museum samples were collected from France and their provenance was known to be local, as such they were pooled together as one location.

3.3.2 DNA extraction and Whole Genome Amplification

DNA was extracted from each wing biopsy following the method reported by Sambrook & Russel (2001) for the rapid isolation of nucleic acid from mammalian tissue. Ammonium acetate (10M; Sigma-Aldrich, Poole, UK) was used to precipitate proteins instead of potassium acetate. The DNA pellet was recovered in 1 x Tris–ethylenediaminetetraacetic acid buffer (TE) (Sigma-Aldrich) and stored at -20°C before further procedures or at -80°C for archival.

The whole genome of each individual was amplified using illustra GenomiPhi V2 DNA Amplification kit (GE Healthcare, Little Chalfont, UK) following the manufacturer's protocol. A subset of samples was genotyped for the microsatellites used before and after whole genome amplification to ensure the procedure did not affect typing success or quality. This additional step increased the DNA yield about 50 times compared to extraction alone

(approximately 0.2mg in DNA extract and 9mg after whole genome amplification).

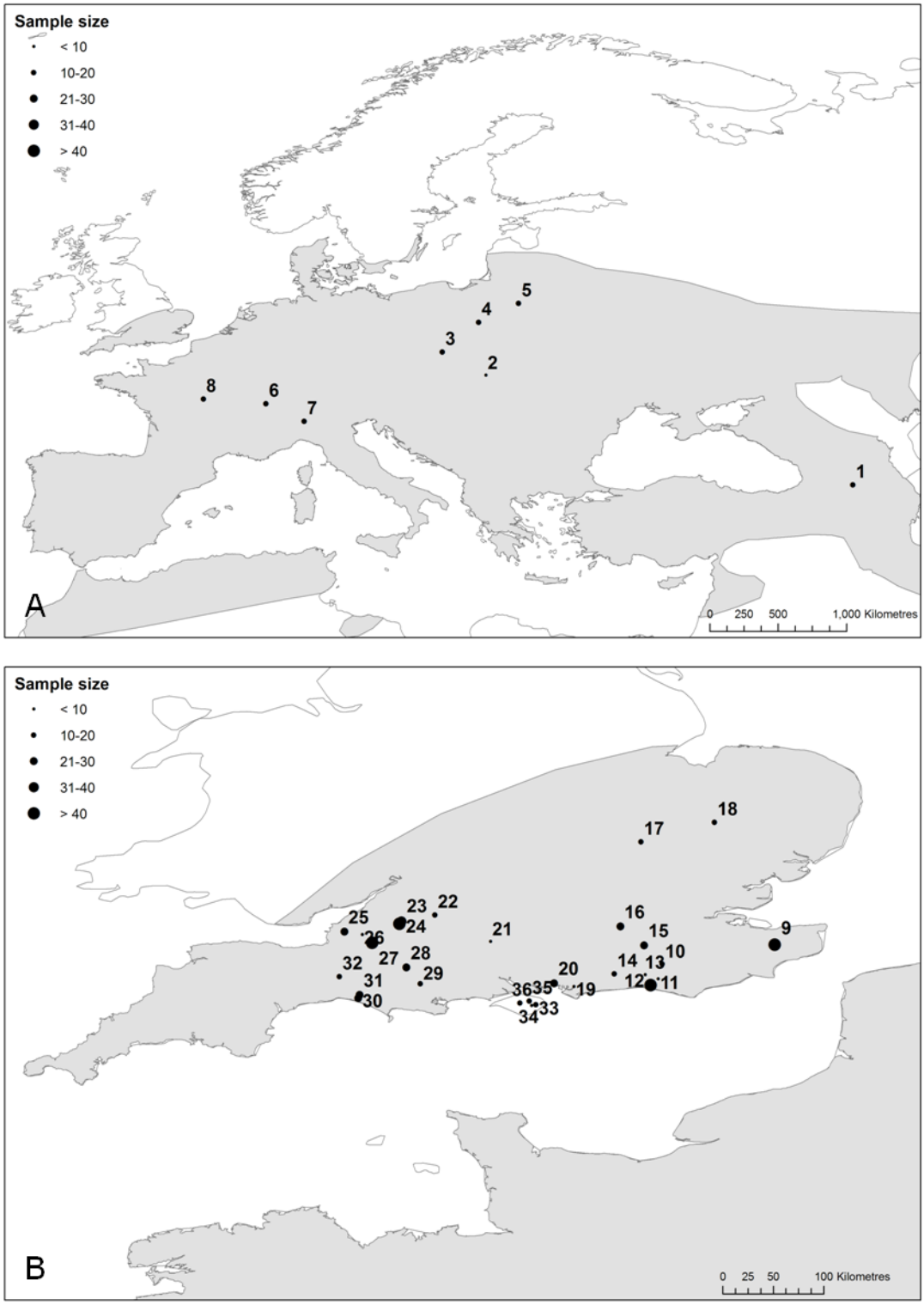

Figure 3.1: Location and sample size of the roosts sampled.
A. On continental Europe. B. In England. Shaded area indicates the species range (IUCN, 2012).

Some samples were washed with isopropanol and 70% ethanol before being resuspended in 100µl 1 x TE buffer while others were not purified and their volume made up to 100µl with 1 x TE. No difference was noted in further processing (microsatellite typing and mtDNA sequencing) although the omission of the purification steps yielded more DNA.

3.3.3 Microsatellites genotyping

Samples were amplified using a panel of 11 primers originally designed for other vespertilionid species, optimised for cross-species amplification in *E. serotinus* (Smith et al. 2011) and described in Table 3.2. Polymerase chain reaction (PCR) was carried out in 15µl, with 7.5µl 2x PCR mastermix (ABgene, Epsom, UK) containing 1.5mM $MgCl_2$, 1µM of each primer (Sigma-Aldrich/Applied Biosystems, Foster City, CA, USA), and 1.5µl DNA template (DNA extract or 1/10 dilution in 1 x TE of whole genome amplification product). The remaining volume was made up with molecular grade water. PCR reactions consisted of denaturation at 94°C for 2 min, followed by 35 cycles of 94°C for 1 min, 1 min at the annealing temperature (Table 3.2), 72°C for 1 min, and final elongation at 72°C for 5 min and 60°C for 1 h.

PCR products were diluted and mixed into four sets (Table 3.2) and run on an ABI Prism 3130xl genetic analyser (Applied Biosystems) with Genescan Rox 500 size standard (Applied Biosystems) following the manufacturer's instructions. Microsatellite alleles were sized using GeneMapper 3.7 software (Applied Biosystems). The genotyping error rate per loci was estimated by re-amplifying and re-genotyping between 11 to 24% of samples.

Table 3.1: Genetic diversity of each roost

Sample size (N), mean number of alleles (A), mean allelic richness (A_R), observed (H_O) and expected (H_E) heterozygosity, within sample gene diversity (H_S), and fixation indexes (F_{ST} and F_{IS}) (Weir and Cockerham 1984) over all microsatellite loci. Samples size (N), haplotypic (h) and nucleotide (π) diversities and fixation index (φ_{ST}) for hypervariable portion II of mitochondrial control region. * indicates significantly different from 0 at p<0.05 after 1000 bootstraps over loci.

	Microsatellites (10)								Mitochondrial DNA (HVII)						
	N	**A**	**A_R**	**H_o**	**H_E**	**H_S**	**F_{IS}**	**F_{ST}**	**N**	**Number of haplotypes observed**	**Polymorphic sites**	**Mean no. of pairwise differences**	***h***	**π**	**φ_{ST}**
Continental colonies															
1	15	5.3	3.1	0.511	0.569	0.598	0.146	-	0	0	0	-	-	-	-
2	4	3.3	2.9	0.425	0.538	0.646	0.342*	-	1	1	0	-	-	-	-
3	20	5.1	2.9	0.602	0.583	0.598	-0.006	-	3	2	5	3.333	0.667	0.00786	-
4	15	5.2	3.0	0.657	0.611	0.632	-0.039	-	6	3	6	2.667	0.733	0.00629	-
5	12	4.7	3.1	0.574	0.626	0.662	0.133	-	6	3	5	1.867	0.600	0.00440	-
6	9	4.0	2.9	0.395	0.573	0.627	0.369*	-	1	1	0	-	-	-	-
7	12	5.0	3.0	0.550	0.597	0.627	0.122	-	4	2	5	2.500	0.500	0.00590	-
8	19	5.8	3.1	0.623	0.608	0.625	0.004	-	11	5	8	3.091	0.782	0.00729	-
All Continental colonies	**106**	**4.8**	**3.0**	**0.542**	**0.588**	**0.627**	**0.081***	**0.037***	**32**	**17**	**24**	**3.702**	**0.948**	**0.00873**	**0.311***
English colonies															
East															
9	57	5.0	2.9	0.553	0.585	0.591	0.065*	-	36	3	3	1.005	0.510	0.00237	
10	14	4.4	2.8	0.636	0.592	0.613	-0.037	-	10	2	2	0.400	0.200	0.00094	
11	5	3.5	2.9	0.520	0.538	0.608	0.144	-	3	1	0	-	-	-	
12	68	5.6	2.9	0.606	0.606	0.611	0.008	-	39	5	7	1.425	0.436	0.00336	
13	8	4.0	3.0	0.575	0.587	0.635	0.095	-	5	1	0	-	-	-	
14	16	4.4	2.8	0.531	0.571	0.591	0.102	-	9	2	0	-	-	-	
15	22	4.3	2.8	0.579	0.574	0.589	0.016	-	14	2	3	0.429	0.143	0.00101	
16	23	4.8	2.8	0.594	0.583	0.596	0.003	-	20	5	6	2.453	0.726	0.00579	
17	11	3.7	2.5	0.418	0.483	0.510	0.180	-	11	1	0	-	-	-	
18	11	3.8	2.6	0.436	0.520	0.550	0.207*	-	11	1	0	-	-	-	

19	7	4.0	2.8	0.543	0.521	0.563	0.036	-	5	1	0	-	-	-	
20	26	4.5	2.8	0.530	0.560	0.572	0.073	-	15	2	1	0.533	0.533	0.00126	
All East colonies	**268**	**4.3**	**2.8**	**0.543**	**0.560**	**0.586**	**0.048**	**0.035***	**178**	**10**	**12**	**1.288**	**0.572**	**0.00304**	
West															
21	8	4.0	3.0	0.532	0.584	0.630	0.155	-	7	1	0	-	-	-	
22	14	4.2	2.8	0.493	0.566	0.591	0.165*	-	13	1	0	-	-	-	
23	39	5.2	2.8	0.529	0.588	0.597	0.114*	-	27	1	0	-	-	-	
24	48	5.0	2.9	0.601	0.617	0.624	0.037	-	42	3	2	0.141	0.138	0.00033	
25	21	4.7	3.0	0.599	0.624	0.640	0.064	-	19	1	0	-	-	-	
26	6	3.7	2.9	0.550	0.549	0.603	0.088	-	3	1	0	-	-	-	
27	42	5.1	3.0	0.608	0.642	0.651	0.066*	-	19	2	1	0.105	0.105	0.00025	
28	25	5.0	2.9	0.560	0.594	0.608	0.079	-	12	3	2	0.576	0.530	0.00136	
29	10	4.2	2.9	0.580	0.570	0.601	0.035	-	7	1	0	-	-	-	
30	24	4.7	2.9	0.519	0.581	0.596	0.130*	-	15	1	0	-	-	-	
31	30	4.5	3.0	0.545	0.621	0.634	0.140*	-	27	1	0	-	-	-	
32	15	4.2	2.8	0.520	0.578	0.601	0.134	-	7	1	0	-	-	-	
All West colonies	**282**	**4.5**	**2.9**	**0.553**	**0.593**	**0.615**	**0.091***	**0.014***	**198**	**6**	**5**	**0.084**	**0.079**	**0.00040**	
Isle of Wight															
33	13	3.9	2.7	0.500	0.523	0.546	0.084	-	6	2	2	0.667	0.333	0.00157	
34	7	3.7	2.8	0.543	0.522	0.565	0.038	-	4	2	2	1.000	0.500	0.00236	
35	11	3.9	2.6	0.509	0.500	0.524	0.029	-	11	2	2	1.018	0.509	0.00240	
36	12	3.9	2.6	0.505	0.509	0.533	0.053	-	12	2	2	0.970	0.485	0.00229	
All IOW colonies	**43**	**3.9**	**2.7**	**0.514**	**0.514**	**0.542**	**0.054**	**-0.002**	**33**	**2**	**2**	**1.008**	**0.504**	**0.00238**	
All English colonies	**593**	**4.2**	**2.8**	**0.537**	**0.555**	**0.581**	**0.070***	**0.038***	**409**	**14**	**15**	**0.753**	**0.372**	**0.00178**	**0.338***
All colonies	**699**	**4.5**	**2.9**	**0.540**	**0.572**	**0.604**	**0.072***	**0.048***	**441**	**26**	**30**	**1.061**	**0.458**	**0.00250**	**0.405***

Table 3.2: Microsatellites primers
A 'pig-tailing' modification (GTTTCTT) was added to the 5' end of D15, B22 and TT20 to reduce non-templated addition of primarily adenosine nucleotides (Brownstein et al. 1996), which could otherwise lead to problems in accurate sizing of the DNA fragment. TD indicates a touch-down PCR. PCR products were multiplexed into four sets for genotyping.

Set	Locus	Forward and reverse sequence 5' to 3'	Microsatellite repeat	Annealing temperature (°C)	Number of alleles	Allele size range (bp)	Reference
1	NN8 = Mu438+9	F: NED-TTGTGTTTTAAAGAAAATCC R: ATAGGTGATTTCCATTCCCA	$(GT/CA)_{21}$	44	4	141-163	(Petri et al. 1997)
	EF1	F: 6-FAM-ATCTGGGCAATGATACCTTT R: GCAGGCTGGGCTGAG	$(GT)_{22}CT(GT)_{17}$	50	3	177-194	(Vonhof et al. 2002)
	EF4	F: HEX-ATAGGCTCCCAGAAATAGC R: GATCACCACAAAATGTGC	$(CT)_4(GT)_{17}$	48	6	217-227	(Vonhof et al. 2002)
	EF14	F: HEX-ATCATATATTTGTGTTCTGG R: AAAATCAGCTATGTAGCAC	$(GT)_{19}$	43	12	107-129	(Vonhof et al. 2002)
2	Paur05	F: 6-FAM-GGACAGTATGCCATGTTATGCTG R: GCACTTTCACAAACCTAGATGG	$(GT)_{10}$	66-56 TD	11	231-253	(Burland et al. 1998)
	AF141650	F: HEX-ACAGGAACCCTCAGAAGTGG R: TGGTCTCCTTTTCTTCACTTTGT	$(TATC)_9$	52	11	265-311	(Petit & Mayer 1999)
3	EF6	F: HEX-ATCACATTTTTGAAGCAT R: ATCTGTTTTTCTCTCCTTAT	$(GT)_{20}$	41	16	161-197	(Vonhof et al. 2002)
	EF15	F: NED-AGCAGCAAAGGGGACTCAGA R: GAGAAGCAGGGAGGGCATTT	$(CA)_3GA(CA)_{20}$	55	18	107-147	(Vonhof et al. 2002)
4	B22	F: HEX-CTGATGCAAGACCCCTTACAAC R: GTTTCTTACGGCAGCAGTGAAATCAGA	$(GT)_x$	55	2	135-137	(Kerth et al. 2002)
	TT20	F: 6-FAM-TCTTACCTCTTTTCCTGC R: GTTTCTTTTTTTTTCTTCTGTGTTACC	$(TG)_{11}$	47	6	185-195	(Vonhof et al. 2002)
5	D15	F: 6-FAM-GCTCTCTGAAGAGGCCCTG R: GTTTCTTATTCCAAGAGTGACAGCATCC	$(AC)_{17}$	61	10	127-151	(Castella and Ruedi 2000)

3.3.4 Mitochondrial DNA sequencing

A 460bp portion of the hypervariable domain II (HVII) of the mtDNA control region was amplified in a subset of samples (Table 3.1) using primers for conserved sequence block F on L-strand 5'-CTACCTCCGTGAAACCAGCAAC-3' (Wilkinson & Chapman 1991) and for HVII on H-strand 5'-CGTACACGTATTCGTATGTATGTCCT-3' (J. Juste , pers. comm.). PCR was carried out in 20µl, with 10µl 2x BioMix Red (Bioline, London, UK) containing 1mM $MgCl_2$, 0.5µM of each primer (Sigma-Aldrich), and 1µl of amplified genomic DNA. The remaining volume was made up with molecular grade water. PCR reactions consisted of denaturation at 94°C for 5 min, followed by 30 cycles of 94°C for 1 min, 55°C for 1.5 min, 72°C for 2 min, and final elongation at 72°C for 5 min and 60°C for 1 h.

PCR products were purified by an enzymatic reaction to remove leftover primers and dNTPs. A 10µl mix of 0.5U Exonuclease I (Fermentas, Vilnius, Lithuania) and 0.25U Alkaline Phosphatase (Fermentas) in molecular grade water was added to each product before incubation at 37°C for 30 min and deactivation at 85°C for 15 min.

The purified DNA fragment was sequenced from both directions using the BigDye Terminator v3.1 sequencing kit (Applied Biosystems) in a 20µl reaction volume with 1.5µl BigDye Terminator mix, 3.5µl 5x sequencing buffer, 3.33pmol of primer and 1µl DNA template. The remaining volume was made up with molecular grade water. The sequencing reaction consisted of 25 cycles of 96 °C for 10 s, 50 °C for 5 s, and 60 °C for 4 min. The sequencing reactions were cleaned by precipitation with 100% ethanol and 20µg glycogen before a washing step with 70% ethanol. They were then reconstituted in 11µl HiDi formamide before being run on an ABI Prism 3130xl genetic analyser (Applied Biosystems) following manufacturer's instructions. The resulting sequences were assembled, aligned and trimmed into GENEIOUS 5.5.7 (Drummond et al. 2011) to create a 424bp consensus sequence for each individual.

3.3.5 Microsatellites analysis

Tests of assumptions, genetic diversity and basic statistics

The presence of scoring inconsistencies, null alleles, large allele dropout and stuttering was tested in MICROCHECKER (Van Oosterhout et al. 2004). Exact tests for departure from Hardy-Weinberg (HW) equilibrium were performed for each roost and each locus in GENEPOP 4.1.4 (Rousset 2008) under the hypothesis of heterozygote deficit. A sequential Bonferonni correction was applied to the p-values to account for multiple tests (Rice 1989).Tests of linkage disequilibrium were performed in FSTAT 2.9.3.2 (Goudet 1995, 2001) and the significance levels were adjusted by sequential Bonferonni correction. Genetic diversity indices were obtained for each roost from FSTAT and GENETIX 4.05 (Belkhir *et al.*1996-2005). Estimated F_{ST} averaged over loci (Weir & Cockerham 1984) and its 95% confidence interval (CI) after 1000 bootstrap over loci was calculated in GENETIX. An additional measure of population differentiation, Jost's D_{est}, was calculated in SMOGD 1.2.5 (Crawford 2010), based on allele identities rather than sample heterozygosity (Jost 2008) to avoid the underestimation of F_{ST} with highly polymorphic microsatellite markers (Hedrick 2005, Jost 2008, 2009). Finally, a log-likelihood G-test for population differentiation (not assuming random mating within roosts) was performed (Goudet et al. 1996) in FSTAT on 100,000 randomisations of complete multilocus genotypes.

Bayesian clustering analysis

The Bayesian clustering program STRUCTURE was used to investigate genetic structure (Pritchard et al. 2000, Hubisz et al. 2009). This method uses multilocus genotypes to assign individuals to distinct groups in the sample so departure from HW expectations is minimised within these groups. We evaluated the number of assumed clusters K from 2 to 6 using a burn-in of 5 x 10^6 iterations followed by 5 x 10^7 MCMC iterations. The admixture model with correlated allele frequencies was run with and without population information (roosts the individuals were sampled from) as a prior. Each K value was run 12 times to ensure stability and convergence of the chains. The simulations were

run on the computer cluster provided by the University of Oslo Bioportal (Kumar et al. 2009). The optimal number of clusters K was obtained from ΔK, based on the rate of change in the log probability of data in successive K values (Evanno et al. 2005) as implemented on STRUCTURE HARVESTER (Earl & VonHoldt 2012). The program CLUMMP with a Greedy algorithm was used to align and average membership coefficients across replicate runs (Jakobsson & Rosenberg 2007). Graphical display was obtained from the program DISTRUCT (Rosenberg 2003).

Distance-based and multivariate analyses

To visually assess the general pattern of genetic structuring among roosts, several genetic distances were calculated between each pair of roosts for principal coordinate (PCoA) and neighbour joining analyses. Pairwise F_{ST} and D_{est} were calculated in ARLEQUIN and SMOGD respectively. Cavalli-Sforza chord distance D_C (Cavalli-Sforza & Edwards 1967) was also computed as it does not rely on any biological assumptions. Pairwise D_C were calculated in R 2.15.1 (R Development Core Team 2011) by the HIERFSTAT 0.04-6 package (Goudet 2005). However, it has been argued that genetic distances calculated for pairs of populations independently of all others might be over-simplifying systems and might not directly reflect gene flow (Dyer et al. 2010). To account for genetic covariance associated with both direct and indirect gene flow among populations, a new measure, the conditional genetic distance $_C$GD, was developed as a component of Population Graphs (Dyer & Nason 2004). Pairwise $_C$GD were calculated in R by the GSTUDIO 0.8 package (Dyer 2012). Neighbour Joining analyses were carried out according to Saitou and Nei (1987) as implemented in PHYLIP 3.6 (Felsenstein 2005). The resulting output was visualised in Dendroscope 3 (Huson et al. 2007). The principal coordinate analyses were conducted to complement the output of the neighbour joining analysis. The package ADE4 1.5-0 (Chessel et al. 2004) was used for PCoA calculation and graphical display in R. The first three axes were kept in all four analyses.

Genetic structuring was further investigated by two multivariate methods applied to the individual genotypes: (1) a discriminant analysis of principal components (DAPC) (Jombart et al. 2010) and (2) a spatial principal component analysis

(sPCA) (Jombart et al. 2008). In contrast to Bayesian clustering, the strength of these approaches rests in their independence from any assumptions of HW equilibrium or linkage equilibrium between loci and inferences are therefore made only on the contribution of alleles within genotypes (Jombart et al. 2009). The two methods were implemented in the ADEGENET 1.3-4 package (Jombart 2008) in R. DAPC has been developed to summarise the overall genetic variability of individuals within groups while optimising discrimination between groups by transforming genetic data (alleles) into uncorrelated synthetic variables by a principal component analysis (PCA) as a prior step to discriminant analysis (DA). The assignment of individuals to clusters by DAPC requires a group prior. When this information is unknown, K-means clustering of principal components associated with Bayesian Information Criterion (BIC) is used. However, in the case of low population differentiation and high admixture as it is expected with bats, this method might struggle to find biologically relevant clusters with information from only ten loci. We therefore decided to use the roosts individuals were sampled from as prior. The first 30 principal components (PC) of PCA were retained in the data transformation step, covering 90.5% of genetic variance.

While DAPC can retrieve a range of population structures, including clines, and estimate individual membership to its assigned group, spatial information is only used *a posteriori* to interpret and display the genetic structure inferred. The interaction of genetic variation and geographical landscape is receiving increased attention (Manel et al. 2003, Sork & Waits 2010, Storfer et al. 2010) and methods that explicitly use spatial information to investigate spatial patterns of genetic variation are continuously being developed (Guillot et al. 2005, 2009, Balkenhol et al. 2009). However, most of these methods rely on assumptions of HW and linkage equilibrium that are rarely met in natural populations and the presence of isolation by distance (IBD) can lead to spurious results (Jombart et al. 2009, Guillot et al. 2009). sPCA is a recent approach designed to be independent from these assumptions and to explicitly incorporate spatial information in the investigation of genetic variability across landscapes (Jombart et al. 2008). We applied this method at the roost level. A connection network was constructed using K-nearest neighbour (Cover & Hart 1967), setting K to seven to reflect the number of sites sampled on the continent. This network was

used for the calculation of Moran's *I* (Moran 1948, 1950). sPCA then finds synthetic variables that optimise the product of the variance of individual scores, based on roosts' allelic frequencies, and of Moran's *I,* in order to summarise genetic variability in a spatial context. The resulting components are separated into positive (global) and negative (local) eigenvalues. Global scores retrieve global patterns including clusters and clines from random noise while local scores identify differentiated neighbouring roosts. These patterns were tested for significance by multivariate tests based on a Monte Carlo (MC) procedure of 10,000 permutations as described in (Jombart et al. 2008) and implemented in the package ADEGENET.

AMOVA and genetic differentiation among clusters

Consensus on the population structure was reached based on results from the Bayesian and multivariate analyses and on biological intuition and knowledge of the species. Roosts were assigned to one of the clusters (hereafter called populations) defined and this genetic structure was tested in an analysis of molecular variance (AMOVA) (Excoffier et al. 1992) in ARLEQUIN. Log-likelihood G tests for differentiation were performed between and within populations using 10,000 permutations in the HIERFSTAT package implemented in R. Pairwise F_{ST} and D_{est} between the populations were obtained from ARLEQUIN and SMOGD respectively to assess the level of connectivity. Log-likelihood G tests were performed for all pairs of populations on 10,000 permutations by the HIERFSTAT package.

Isolation by distance

Mantel and partial Mantel tests were performed between matrices of pairwise linearised F_{ST} (Rousset 1997) and geographical distances (log-transformed as a two-dimension stepping-stone pattern is expected for bats) among roosts. An indicator matrix was included to account for the presence of the most obvious geographical barriers including the English Channel (isolating the UK), the Solent (between the Isle of Wight and England), the Alps (isolating the Italian roost), the Carpathians (isolating the Slovakian roosts) and the Caucasus range (isolating Georgia). Tests were carried out on the full dataset, the continent only

dataset and on the English dataset in the program Isolation by distance web service 3.23 (IBDWS) (Jensen et al. 2005).

Contemporary gene flow

The magnitude and direction of contemporary gene flow occurring between the consensus populations were estimated using the program BAYESASS 3.0.1 (Wilson & Rannala 2003). This Bayesian method does not assume the populations to be in migration drift or HW equilibrium. It identifies first or second generation immigrants by the fact that they will show temporal linkage disequilibrium relative to the other individuals composing the hosting population. The simulation was run with 10^7 iterations, with the first 25 x 10^5 iteration discarded as burn-in. Samples were collected every 100 iterations. Allelic frequencies and inbreeding coefficients were set at 0.2 while migration rate was left at its default value of 0.1. These mixing parameters ensured the acceptance rates were between 0.2 and 0.4. The trace file was examined in TRACER 1.5 to ensure mixing and convergence of the chains (Rambaut & Drummond 2009).

3.3.6 Mitochondrial DNA analysis

Genetic diversity and differentiation

Haplotype (*h*) and nucleotide diversities (π), number of polymorphic sites and mean number of pairwise differences were calculated for each roost and each population in ARLEQUIN. Differentiation index for haploid data φ_{ST} (Weir & Cockerham 1984) was estimated in ARLEQUIN and tested for significant departure from panmixia after 10,000 permutations. Differentiation test with 100,000 MC iterations after 10,000 burn-in iterations was also performed in ARLEQUIN.

Population structure

Spatial structuring of genetic variation in the mtDNA sequences was investigated by sPCA, optimising both the variance of polymorphism frequency and spatial autocorrelation (Jombart et al. 2008). All roosts were considered as neighbours in this analysis, but their inter-connection was weighted by the inverse Euclidian distance separating the roosts. Global and local patterns

retrieved were tested for significance by a MC procedure of 10,000 permutations (Jombart et al. 2008). The analysis was undertaken in R with the package ADEGENET.

Geographical structuring of mtDNA variation was also investigated in an AMOVA. Roosts were grouped in various groups based on the sPCA results and on the structure observed at the nuclear level was tested in order to optimise the among groups variance component. The contribution of each hierarchical component (among groups, among roosts within groups and within roosts) was estimated and tested for significance after 10,000 permutations. Differentiation between identified clusters was inferred by pairwise φ_{ST} and tested by log-likelihood tests with 100,000 MC iterations after 10,000 burn-in iterations. All calculations were undertaken in ARLEQUIN.

Mantel and partial Mantel tests were performed between matrices of pairwise φ_{ST} and log-transformed geographical distances among roosts to test for isolation by distance. The same indicator matrix used to infer presence of geographical barriers in the microsatellite dataset was included. Tests were carried out on the full dataset, the continent only dataset and on the English dataset in IBDWS.

Phylogenetic analysis

Phylogenetic reconstructions were undertaken on unique haplotypes by maximum-likelihood (ML) (Beerli & Felsenstein 2001) criteria and a Bayesian approach. The K80+I model (Kimura 1980) of DNA substitution (transition/transversion ratio = 18.374, proportion of invariable sites = 0.817) was applied to the ML and the Bayesian analyses as determined by the program jMODELTEST 0.1.1 (Wu & Eisen 2008, Posada 2008) using Bayesian Information Criterion (BIC). The ML analysis was undertaken in PHYML 3.0 (Wu & Eisen 2008) and the Bayesian inference in MRBAYES 3.1 (Ronquist & Huelsenbeck 2003). For the Bayesian analysis, four chains of 10^7 iterations each were run, taking samples every 100 generations and the first 25% of trees were discarded as burn-in. The statistical support of the branching pattern obtained by ML approach was assessed by 1,000 bootstrap replicates. In addition, a haplotype network (Excoffier & Smouse 1994) was constructed using

ARLEQUIN and visualised in HAPSTAR (Teacher & Griffiths 2011). This method is especially suited for analysis of intra-species haplotypes.

3.4 Results

3.4.1 Microsatellites

Tests of assumption, genetic diversity and basic statistics

Of the 11 microsatellites genotyped, one (AF141650) demonstrated a high level of estimated null alleles, genotyping inconsistencies and significant deviation from the assumption of HW equilibrium in a large number of sampled roosts. It was thus removed from subsequent analyses. Of the remaining loci, four deviated from HW equilibrium in one to three roosts out of 36 and six showed low to moderate levels of estimated null alleles. Deviation from HW expectations was due to heterozygote deficiency and since it was noticed in only a few roosts, it could be caused by factors other rather than by selection on the markers. No significant linkage disequilibrium between loci was detected after sequential Bonferroni correction. Subsequent analyses were thus carried out on these ten loci. The mean genotyping error rate for the ten markers was 4.21% (± 2.50%). Mean observed and expected heterozygosity and within roost gene diversity were moderate and consistent across all roosts (H_o =0.540 ± 0.06; H_E=0.572 ± 0.04; H_S=0.604 ± 0.04). In contrast, the allelic richness was low but still similar across all roosts (A_R=2.9 ± 0.1). Descriptive statistics for each sampled roost are detailed in Table 3.1.

Estimated F_{ST} averaged over loci was low at 0.048 but significant (0.039 – 0.059 95% confidence interval). Similarly, D_{est} was low at 0.088 but significant at every locus. Roosts were found to be genetically differentiated by the randomization test ($p<0.001$), which in combination suggests that roosts sampled are not part of a single panmictic population and that some degree of structuring exists.

Bayesian modelling

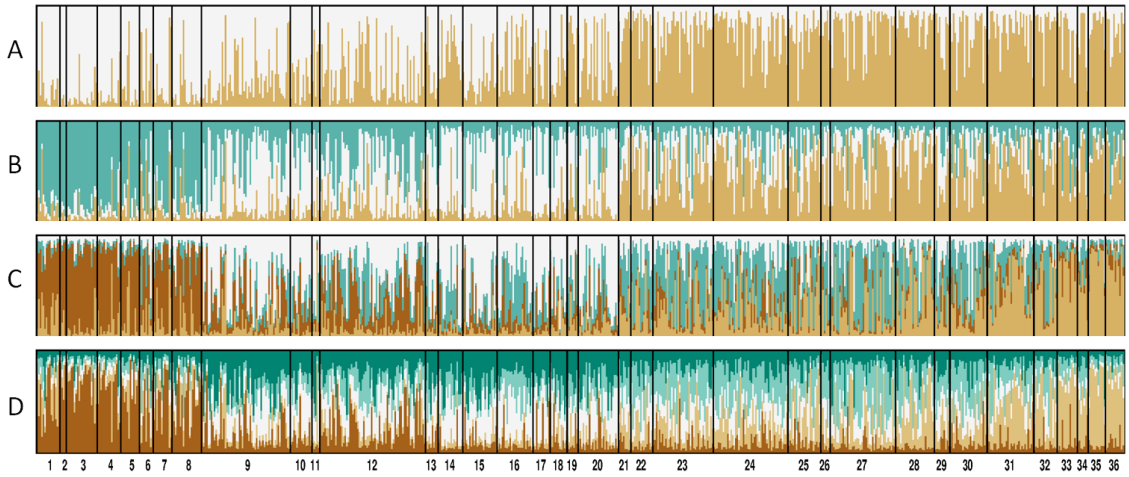

Figure 3.2: Posterior probabilities of Bayesian individual membership
To one of K populations obtained by the program STRUCTURE (Pritchard et al. 2000) using sampling roosts as prior and admixture setting. A. K = 2, B. K = 3, C. K =4, D. K =5. Each vertical line represents an individual. Solid lines separate roosts the individuals were sampled from. Numbers indicate the roost identifying number: 1 to 8 are continental roosts; 9 to 20 are east of England roosts; 21 to 32 are in west of England; 33 to 36 are located on the Isle of Wight.

Despite high levels of admixture, clustering was consistent with the sampling geograph. At K = 2, the sites on the continent and eastern England formed one group and those in western England and Isle of Wight (IOW) another. At K = 3, the continent was discriminated from eastern England. At K = 4 and 5, the IOW was distinguished as distinct (Figure2). The standardised second order rate of change ΔK indicated that the most likely number of clusters describing the data was three. Because this method only distinguished higher structuring level, each cluster was then run separately with the same settings. No further sub-structure was supported for the continent or for eastern England. In contrast, a sub-structure was revealed in the third cluster, with roosts on the IOW segregating from western England.

Multivariate analysis

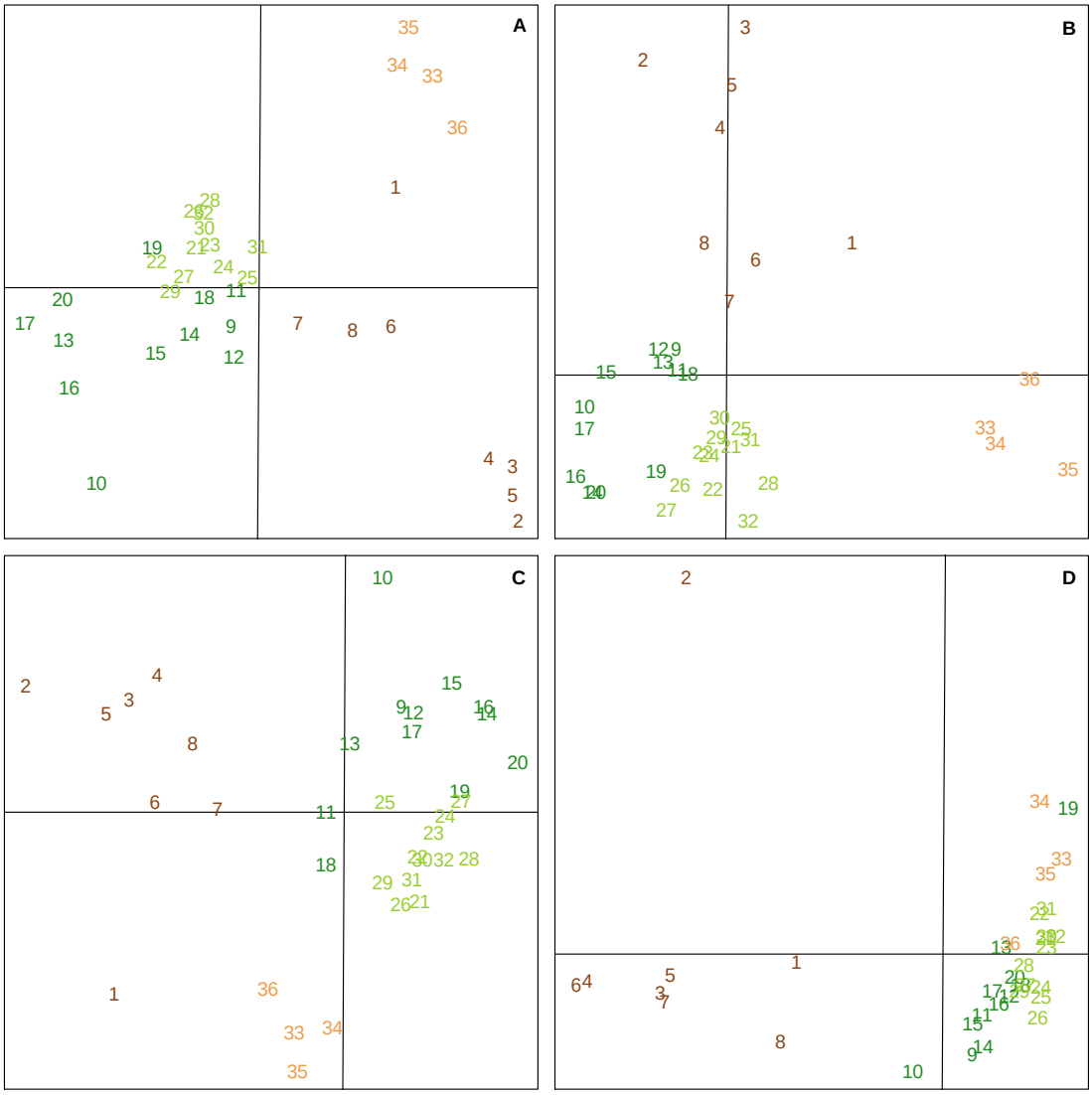

Figure 3.3: Principal coordinate analysis of the pairwise genetic distances among the 36 sampled roosts based on genotyping at ten microsatellites.
The first two axes are represented. A. F_{ST}; B. D_{EST}; C. Cavalli-Sforza chord distance D_C ; D. conditional genetic distance. Numbers indicate the roost identifying numbers: 1 to 8 are continental roosts (brown); 9 to 20 are east of England roosts (dark green); 21 to 32 are in west of England (light green); 33 to 36 are located on the Isle of Wight (orange).

While discrimination between roosts was not strong, reflecting low differentiation indices, some geographical patterns still appeared in both the neighbour joining and principal coordinate analyses of the four genetic distances computed (Figures 3 and 4). The roosts seemed to cluster into two to four main groups depending on the distance and analysis used. Continental sites clustered

together and some sub-structure seemed to occur between east and west Europe with the Slovakian and the Georgian sites as outliers for some of the genetic distances. However, care should be taken in interpreting this as the Slovakian site consists of only four individuals and the DNA extracts from the Georgian samples were of poor quality, resulting in failure to genotype a number of individuals at several loci. Up to three main clusters could be observed for the English roosts, with the IOW roosts often separated from the mainland populations that marginally clustered into east and west groups in the neighbour joining analyses.

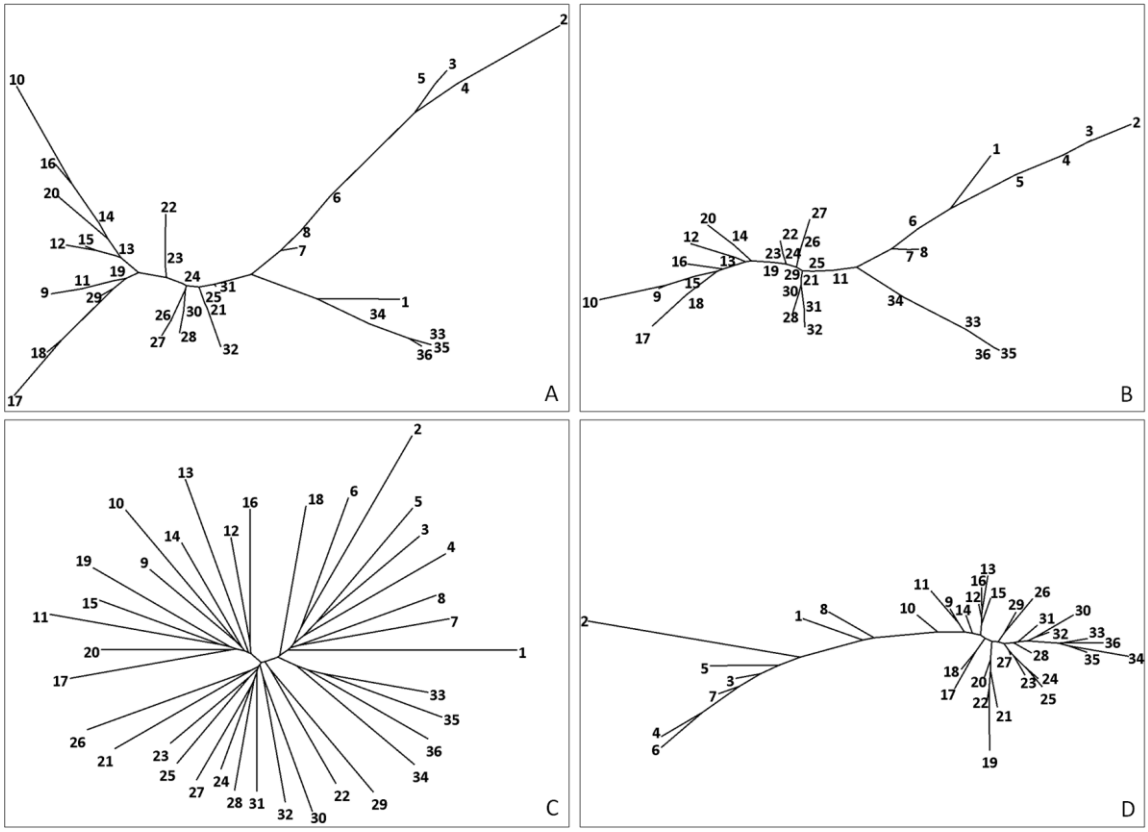

Figure 3.4: Neighbour joining analysis of the pairwise genetic distances among the 36 sampled roosts based on genotyping at ten microsatellites.
A. F_{ST}; B. D_{EST}; C. Cavalli-Sforza chord distance D_C ; D. conditional genetic distance. Numbers indicate the roost identifying numbers: 1 to 8 are continental roosts; 9 to 20 are east of England roosts; 21 to 32 are in west of England; 33 to 36 are located on the Isle of Wight.

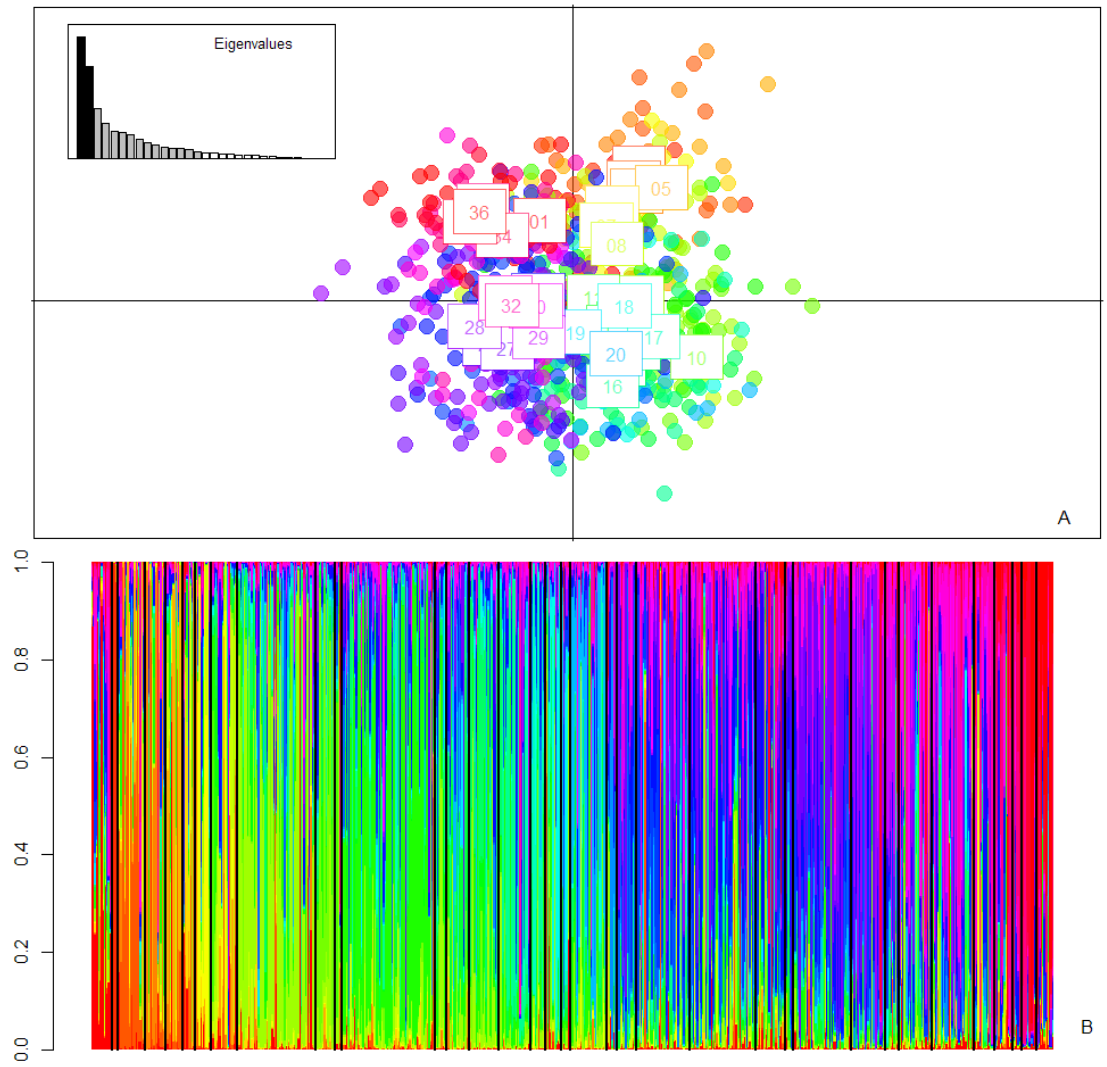

Figure 3.5: Results from the discriminant analysis of principal components (DAPC) from genotyping individuals at ten microsatellites using sampled roosts as a group prior.
A. Scatterplot of individual principal components on the first two axes. The labels indicate the roost identifying numbers and each roost is colour coded. 1 to 8: continental roosts (red to yellow-green); 9 to 20: east England roosts (light green to light blue); 21 to 32: west England roosts (blue to magenta); 33 to 36: Isle of Wight roosts (shades of dark pink). B. Posterior probability of membership to one of the roosts. Each vertical line represents an individual. Solid lines separate roosts the individuals were sampled from. The roosts are ordered according to their identifying number, from 1 to 36, and colour coded as in A.

The discrimination between roosts and clusters using individual-based multivariate analysis DAPC was poorer, reflecting high within roost genetic variability and high admixture levels. The eigenvalues (Figure 3.5A Inset) showed that the first two PCs captured 42% of the genetic structure. The first PC mostly segregated roosts from the continent and the IOW from those on mainland England whilst the second PC discriminated roosts from west of

England and the IOW from those on the continent and in east of England (Figure 3.5A). The individual membership probability obtained from this analysis indicated very high admixture (91.72% of individuals have membership probabilities lower than 90%, Jombart 2011) (Figure 3.5B) although a similar pattern of clustering into four main groups appeared.

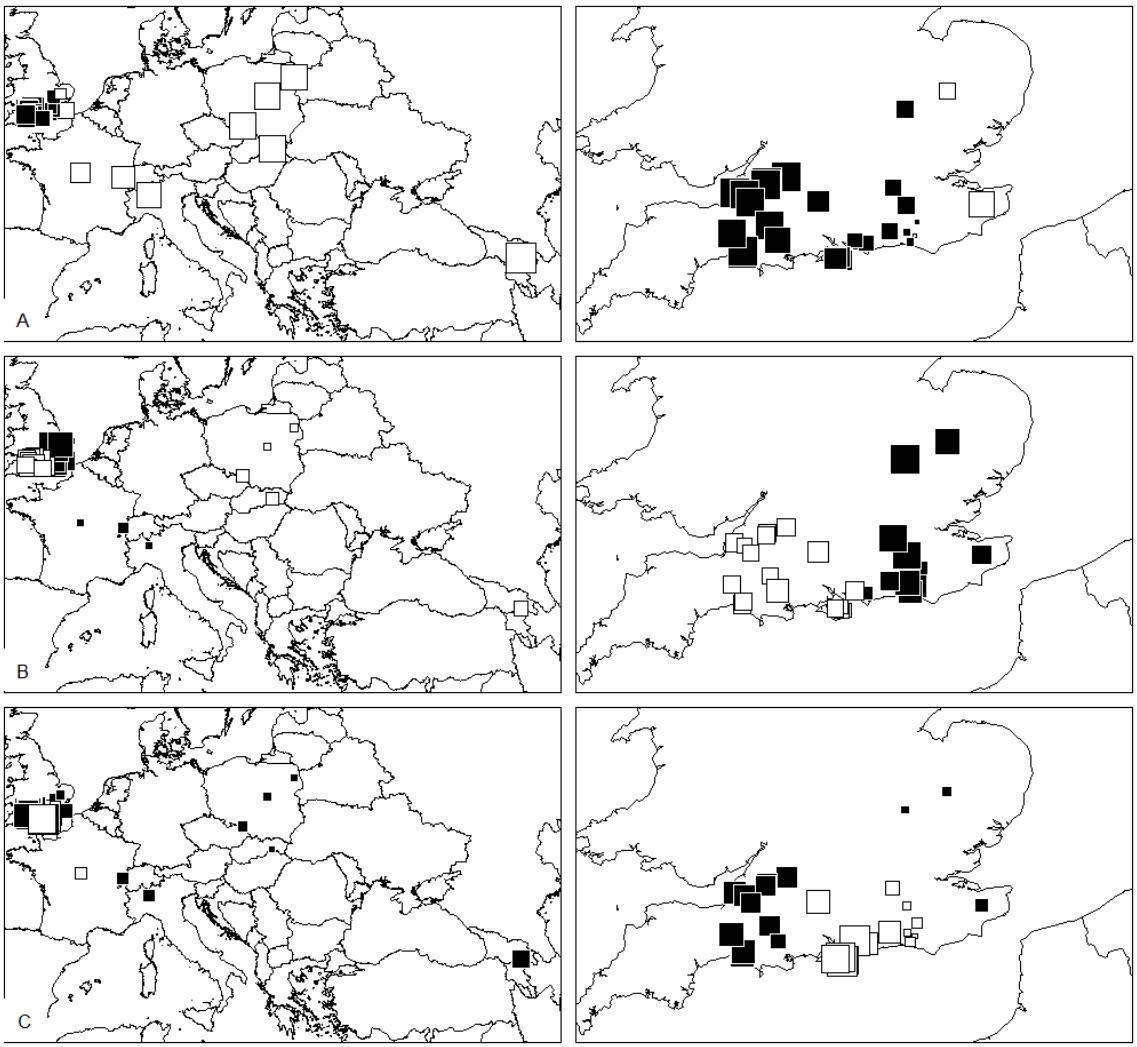

Figure 3.6: Scores from the spatial Principal Component Analysis (sPCA) on genetic data from ten microsatellites) for the three axes retained.
Each square represents a roost. White squares are positive eigenvalues, black squares are negative eigenvalues and their sizes are proportional to the absolute eigenvalues. Row A. First axis. Row B. Second axis. Row C. Third axis.

A spatial PCA analysis (sPCA) investigating both genetic variability and spatial autocorrelation was also performed using K nearest neighbours (K=7) as connection network between the roosts. The global test indicated a significant

structuring (Monte-Carlo test, 10^5 permutations, p = 0.038). The three first global axes had the highest eigenvalues and were therefore retained (62% of genetic variation). The first sPCA score mostly segregated England from the continent although it remained connected through some roosts in eastern England (Figure 3.6A). The second sPCA score differentiated eastern England from western England and western continental sites from Eastern Europe. The continental sPCA scores were low compared to English ones, suggesting that the continental structuring was weak compared to that across England (Figure 3.6B). The third and weakest sPCA score retrieved a final structure by clustering sites in France with the IOW and Central England (Figure 3.6C). Thus, the UK was clearly differentiated from the continent and subdivided into three groups representing the east, the west and the IOW (Figure 3.7). No local structuring was found (Monte-Carlo test, 10^5 permutations, p = 0.95).

AMOVA and genetic differentiation among clusters

Four populations were retained through consensus from the previous analyses and these consisted of continental Europe, eastern England, western England and the IOW (Table 3.1). Differentiation tests were significant both between and within populations (F_{CT} = 0.034, p = 0.0001; F_{SC} = 0.025, p = 0.0001), suggesting both the stratification of the genetic variation and the importance of the roosts as structuring units within those clusters. Pairwise F_{ST} and D_{est} suggested a closer relationship between the continent and eastern England than with the other clusters, a very close relationship between eastern and western England, and a stronger connection between the IOW and western England than to the other clusters. The IOW was also more connected to the continent than to eastern England (Table 3.3). Differentiation was significant for all pairwise connections with the exception of east-west England (p = 0.5095) and continent-IOW (p = 0.6521). Care should be taken interpreting this last result since these tests were performed while keeping the sub-structure (roost) intact, the continent-IOW test only had 12 units to be permuted.

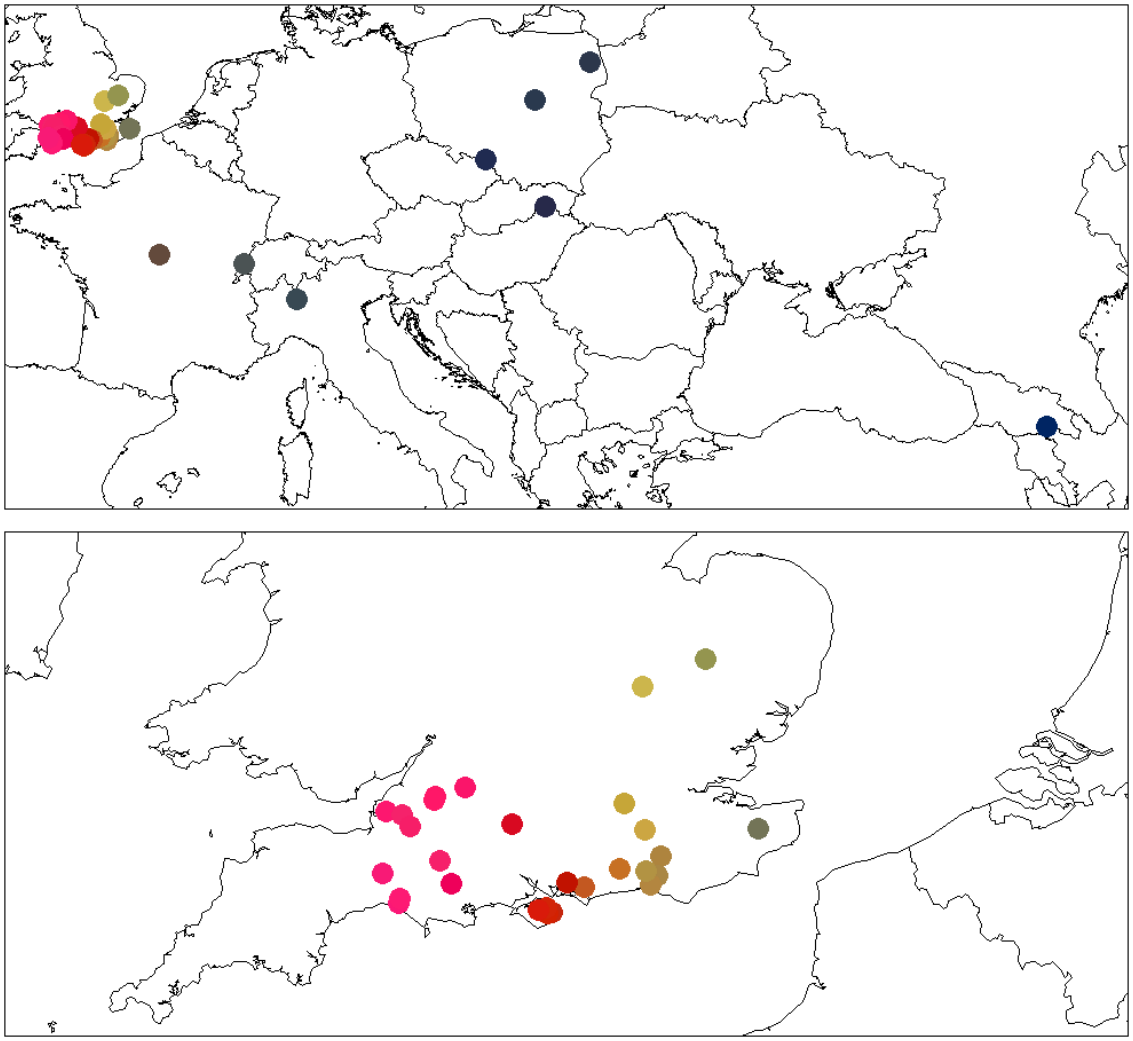

Figure 3.7: Scores from the spatial Principal Component Analysis (sPCA) on genetic data from ten microsatellites for the three axes retained.
Each roost was mapped by colour coding its three sPCA lagged scores as intensity of a given colour channel (first axis: red, second axis: green, third axis: blue).

Table 3.3: Pairwise F_{ST} (below) and D_{EST} (above) between populations.
* indicates significant differentiation (Goudet 1996)

	Continent	East	West	IOW
Continent		0.0684*	0.0748*	0.0755
East	0.0461*		0.0283	0.0883*
West	0.0463*	0.0207		0.0537*
IOW	0.0565	0.0764*	0.0478*	

Isolation by distance

Genetic and geographical distances were significantly correlated when controlling for the effects of physical barriers on the full dataset ($r = 0.210$, $p = 0.023$), on the continental dataset ($r = 0.463$, $p = 0.025$) and on the English dataset ($r = 0.323$, $p < 0.001$). The physical barriers tested also had a significant effect on genetic differentiation at all levels when controlling for geographical distance (Europe: $r = 0.373$, $p < 0.001$; Continent: $r = 0.511$, $p = 0.0245$; UK: $r = 0.585$, $p < 0.001$). Kernel density estimation was applied to the correlation plots, revealing three to four patches. This would indicate that the data do not follow a classical stepping stone pattern, since it would result in a continuous cline of genetic differentiation, but rather that three to four segregated populations are connected through gene flow (Figure 3.8), independently supporting the other results.

Contemporary gene flow

The roosts were pooled into the four populations retained from previous analyses and recent migration rates were estimated as the mean percentage of individuals moving between populations per generation (Table 3.4). The continental population displayed the highest emigration rate (11.4%) and the lowest immigration rate (1.6%), indicating that contemporary gene flow over the Channel is mostly biased toward England. Interestingly, the IOW seems to play an important role in mediating gene flow with immigration coming mostly from the continent (5.2%). Within England, most gene flow occurred between the east and west (5.3%) and between west and the IOW (4.03%) whilst the IOW was less connected to eastern England (1.9%). It is also worth noting that, within England, whilst the eastern population and the IOW were mostly source populations (higher genetic emigration than immigration rates), the western population was clearly a sink population with a genetic immigration rate nearly eight times as high as its emigration rate.

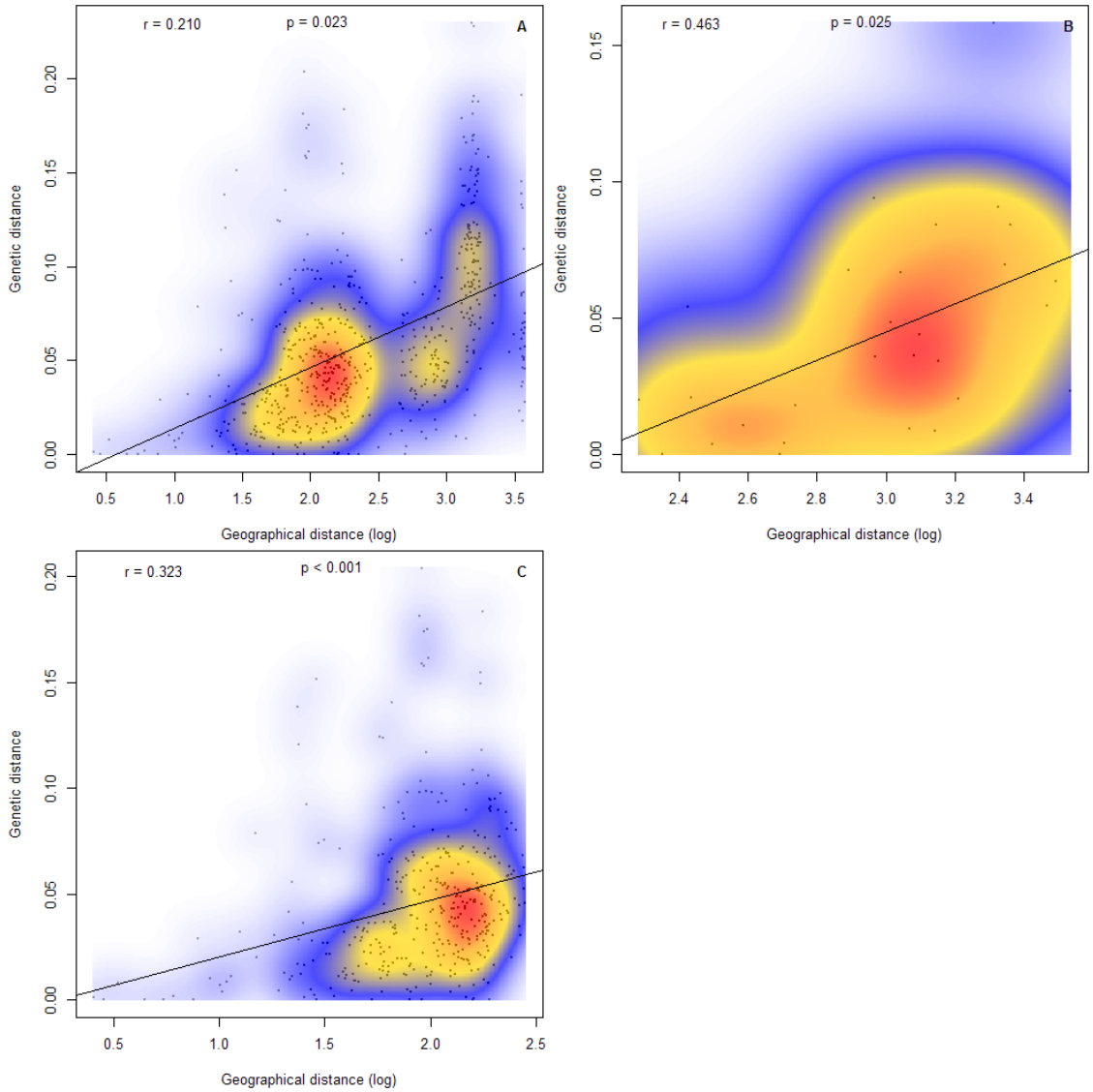

Figure 3.8: Mantel test for isolation by distance
Used pairwise Rousset genetic distances ($F_{ST}/(1-F_{ST})$) on microsatellite genotypes and pairwise log-transformed geographical distances (km) between roosts. Kernel density estimation was applied to the correlation plots. A. Full European dataset. B. Continental dataset. C. English dataset.

Table 3.4: Contemporary gene flow among populations.
Percentage of genetic migrants per generation (± 95% confidence interval)

		To			
		Continent	East UK	West UK	IOW
From	Continent	88.63 (±2.91)	3.47 (±2.20)	2.74 (±1.67)	5.16 (±1.88)
	East UK	0.36 (±0.34)	94.69 (±1.98)	4.55 (±1.97)	0.39 (±0.31)
	West UK	0.20 (±0.19)	0.72 (±0.63)	98.71 (±0.73)	0.37 (±0.34)
	IOW	1.03 (±0.99)	1.54 (±1.35)	3.66 (±2.70)	93.77 (±2.91)

3.4.2 Mitochondrial DNA

Genetic diversity and differentiation

A total of 26 haplotypes were identified among the 441 serotine samples from 35 roosts. The 424bp sequence contained on average A 27.3%, C 23.4%, G 23.6% and T 25.7%. A total of 30 polymorphic sites (7.79%) were recorded with a transition/transversion ratio of 18.37. Some clear patterns in diversity were observed (Table 3.1). Higher variability and diversity was observed in the continental sites than in the English roosts (mean pairwise differences: $F_{1, 16}$ = 34.037, $p < 0.01$; haplotype diversity: $F_{1, 16}$ = 7.855, $p < 0.05$; nucleotide diversity: $F_{1, 16}$ = 33.983, $p < 0.01$). Thus, all continental sites sequenced for more than one individual displayed several haplotypes. No continental haplotypes were shared among roosts, the exception being the French samples that shared all five haplotypes with several samples from eastern England and the IOW (Figure 3.9). In contrast, in England, one haplotype was shared by 322 out of the 409 English samples and all but one English roosts contained this haplotype. Furthermore, 13 out of the 28 UK roosts were fixed for this haplotype (Figure 3.9). More diversity was found in the roosts of eastern England and the IOW than in western England, which displayed only six haplotypes, two of them being shared with the rest of England. Estimated φ_{ST} was high at 0.405 and significant, indicating significant population structure at the mtDNA level.

Population structure

The sPCA investigated sequence variability in a spatial context and revealed significant global structuring (Monte-Carlo test, 10^5 permutations, p = 0.037) but no local patterns (Monte-Carlo test, 10^5 permutations, p = 0.815). The two first global axes had the highest eigenvalues and were therefore retained (80% of genetic variation). The first score separated France and England from the rest of the continent, although the boundary was not sharp and a slight cline across the whole of Europe could be noticed (Figure 3.10A). The second score created two clusters on the continent corresponding to eastern sites (Slovakia, Poland) and western sites (Italy, Switzerland, France). It also segregated the IOW from the rest of England, and eastern England from western England, although these last scores were very low, suggesting this structure is weak (Figure 3.10B).

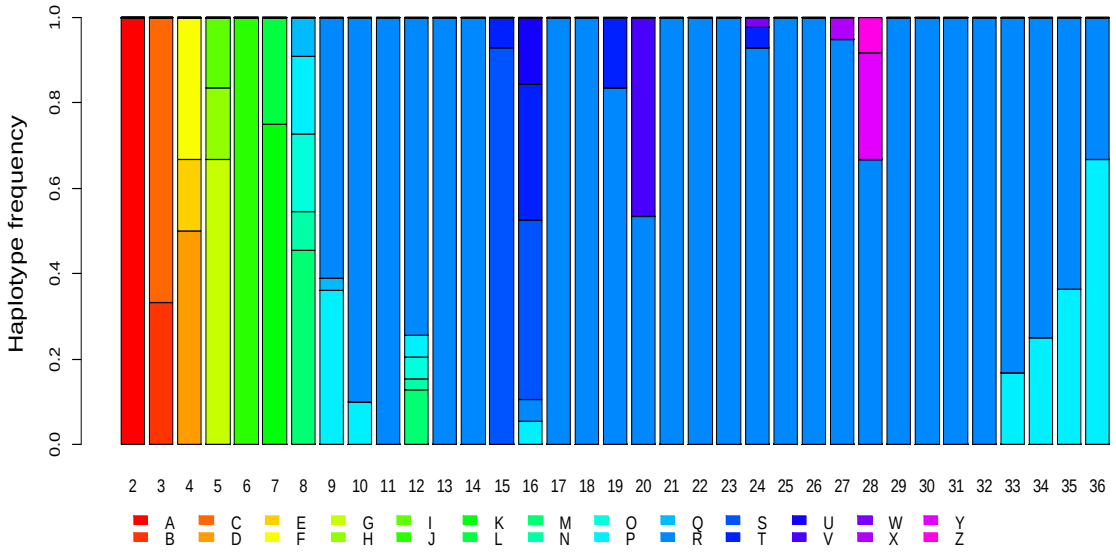

Figure 3.9: Frequency of mtDNA haplotypes among roosts. Each bar represents a roost. 1 to 8 are on the continent. 9 to 20 are in east of England. 21 to 32 are in west of England. 33 to 36 are on the Isle of Wight. Sample sizes are indicated in Table 3.1.

These patterns were mapped by colour coding the two sPCA scores as intensity of the red (score 1) and green (score2) channels (Figure 3.11). The longitudinal clustering on the continent can be clearly visualised, as well as the segregation of the IOW from the rest of the UK.

AMOVA produced a simpler structure with the among groups variance component optimised for three groups (48.08%): eastern continent, western continent and England (Table 3.5). All three hierarchical levels tested displayed significant genetic variance.

Table 3.5: Analysis of Molecular Variance on mtDNA sequences.
The populations are defined as Eastern continent, Western continent and England.

Source of Variation	Sum of Squares	Variance Components	Percentage Variation	F-statistics
Among populations	29.671	0.441	48.077	φ_{CT} = 0.481
Among roosts within populations	73.207	0.155	16.913	φ_{SC} = 0.326
Within roosts	130.536	0.322	35.009	φ_{ST} = 0.650

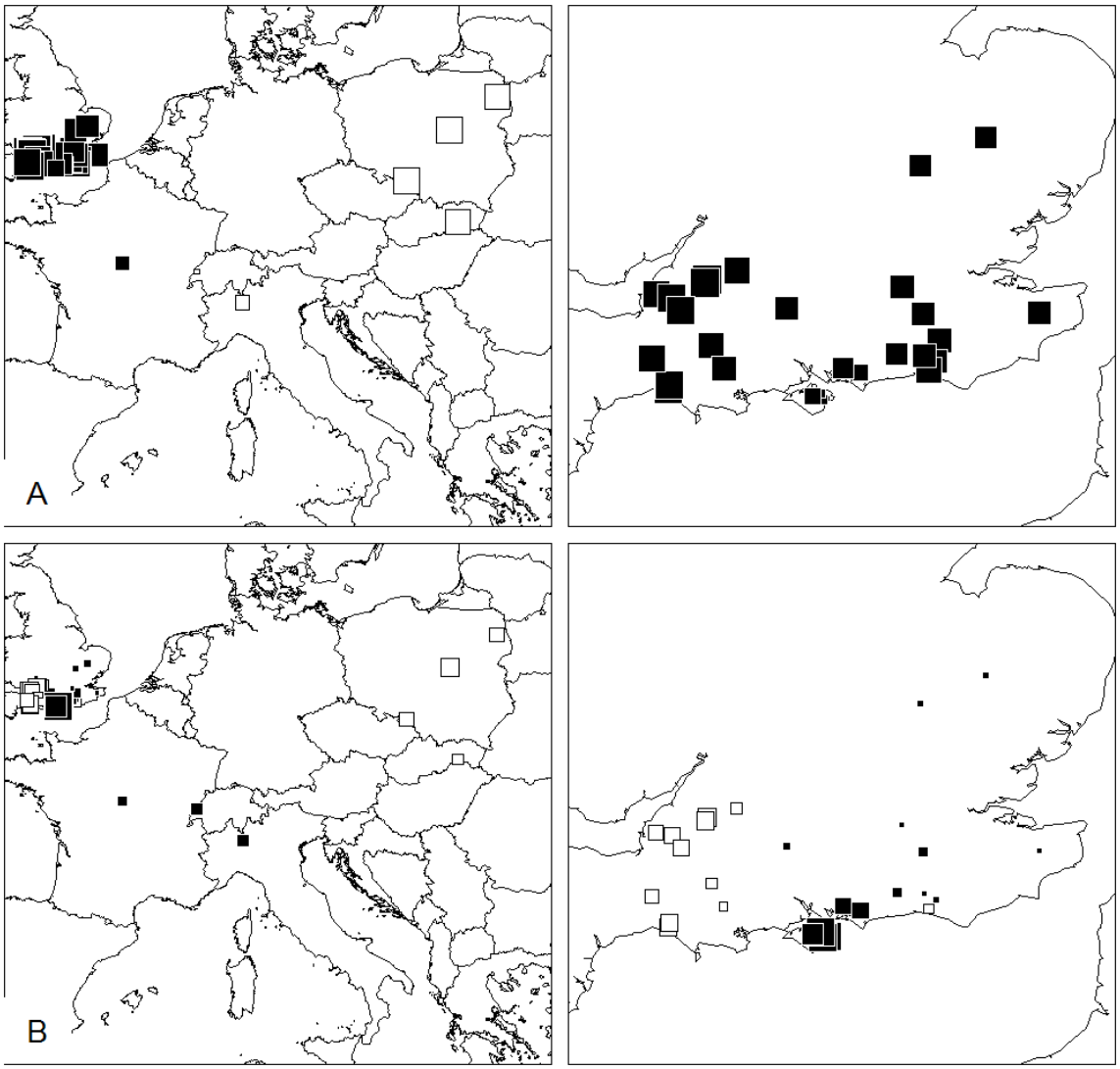

Figure 3.10: Scores from the spatial Principal Component Analysis (sPCA) on mtDNA sequences for the two axes retained.
Each square represents a roost. White squares are positive eigenvalues, black squares are negative eigenvalues and their sizes are proportional to the absolute eigenvalues. Row A. First axis. Row B. Second axis.

Table 3.6: Pairwise φ_{ST} between populations
* indicates significant differentiation (Goudet 1996)

	England	Eastern continent	Western continent
England	-		
Eastern continent	0.529*	-	
Western continent	0.532*	0.173*	-

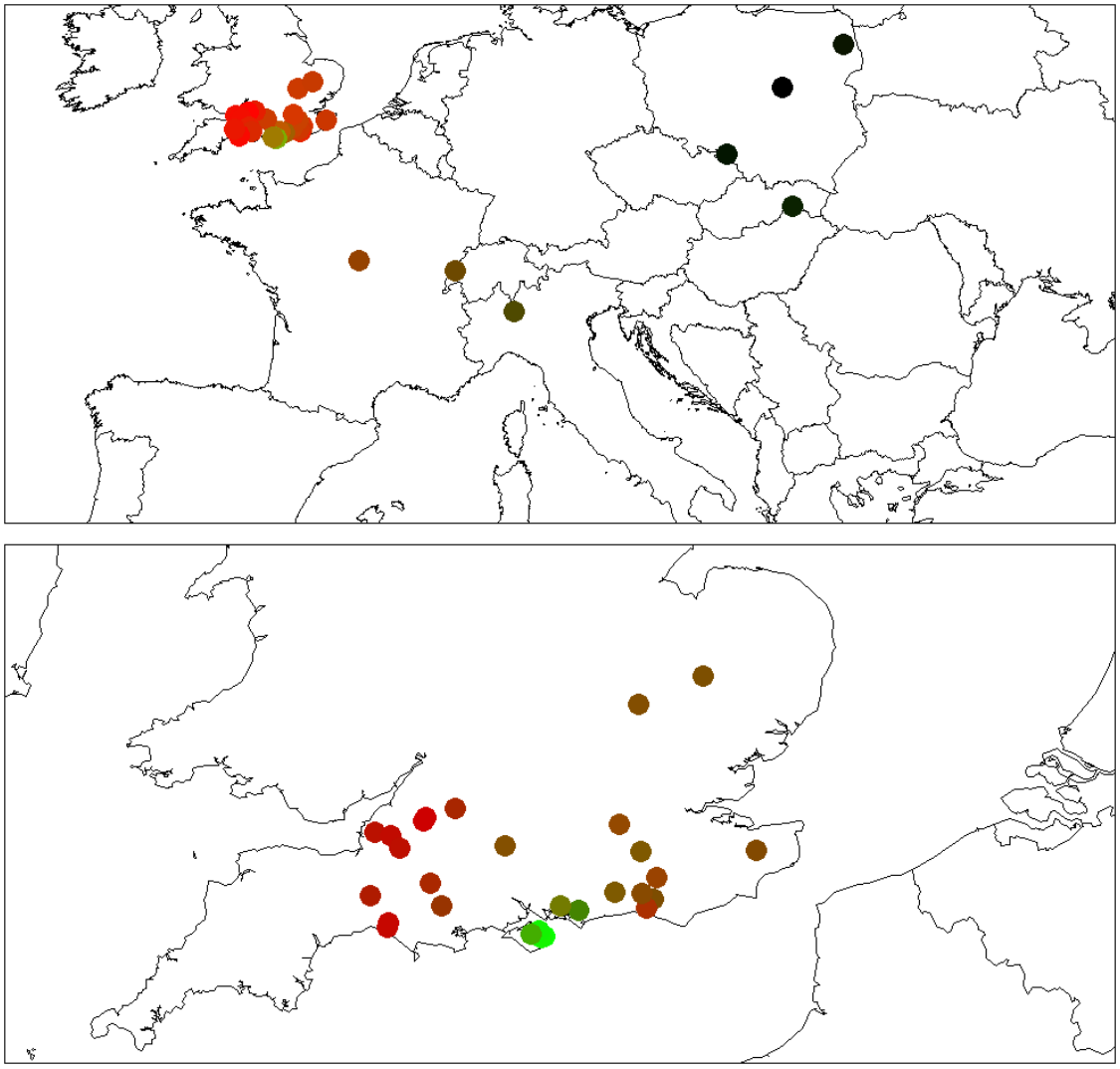

Figure 3.11: Scores from the spatial Principal Component Analysis (sPCA) on mtDNA sequences for the two axes retained.
Each roost was mapped by colour coding its two sPCA lagged scores as intensity of a given colour channel (first axis: red, second axis: green).

Pairwise φ_{ST} between the three groups (Table 3.6) indicated strong differentiation between England and both the eastern and western continental populations, and much weaker differentiation between the two continental populations. All pairwise differentiation tests were significant ($p = 0.008$).

A Mantel test revealed a significant correlation between genetic and geographical distances when controlling for the potential effects of geographical barriers ($r = 0.429$, $p < 0.001$). Physical barriers also contributed in structuring the genetic variation ($r = 0.333$, $p = 0.004$). In addition, Kernel density estimation applied to the correlation plot revealed two distinct patches, indicating that isolation by distance doesn't strictly apply (Figure 3.12). When

splitting the full dataset into a continental one and an English one, no cline in genetic differentiation or effect of geographical barriers were detected on the continent or in England (continent: geographical distance: r = 0.293, p = 0.068, barriers: r = 0.487, p = 0.102; UK: geographical distance: r = 0.119, p = 0.091, barriers: r = 0.246, p = 0.091).

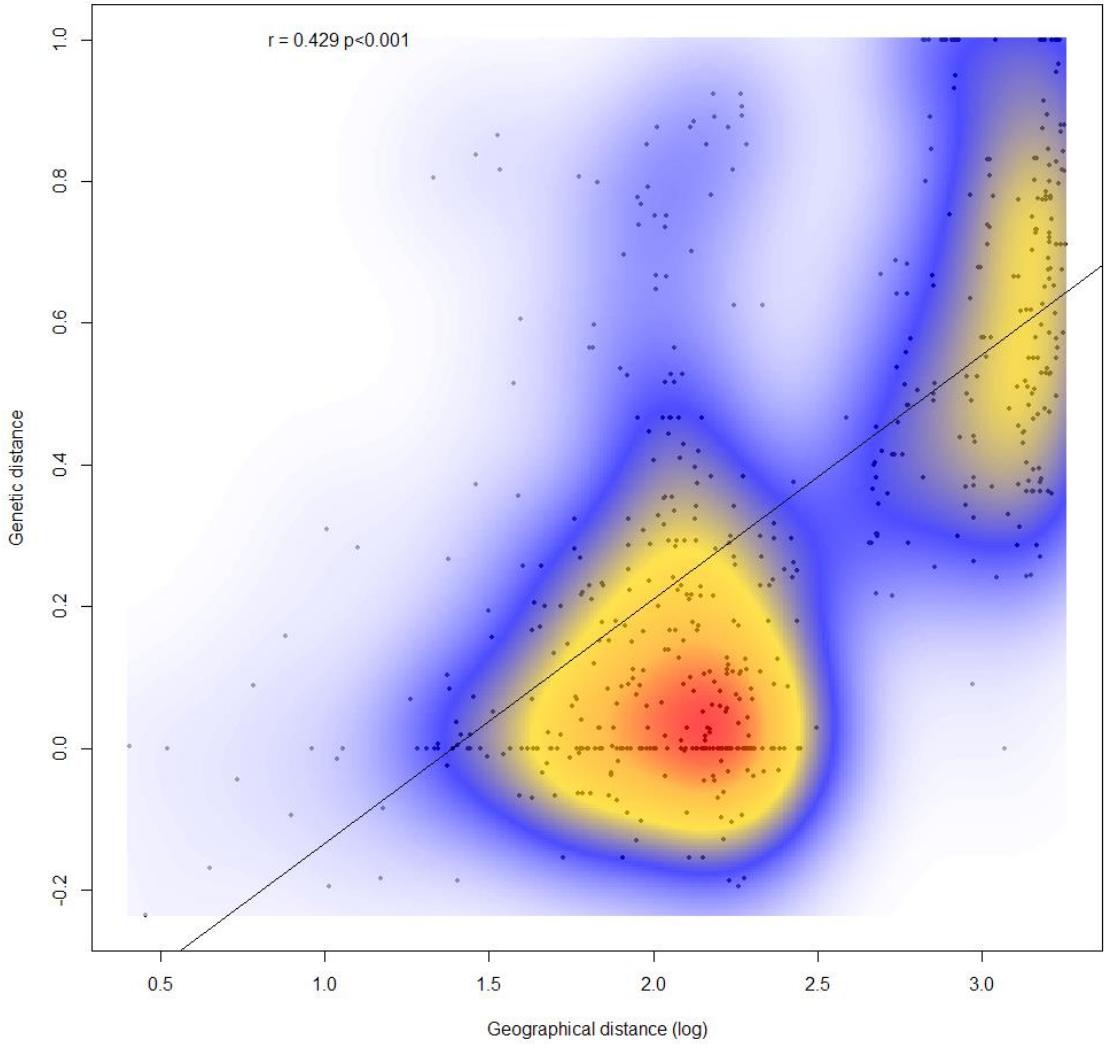

Figure 3.12: Mantel test for isolation by distance
Using pairwise Rousset genetic distances ($\varphi_{ST}/(1-\varphi_{ST})$) on mtDNA sequences and pairwise log-transformed geographical distances (km) between 35 European roosts. Kernel density estimation was applied to the correlation plots.

Phylogenetic analysis

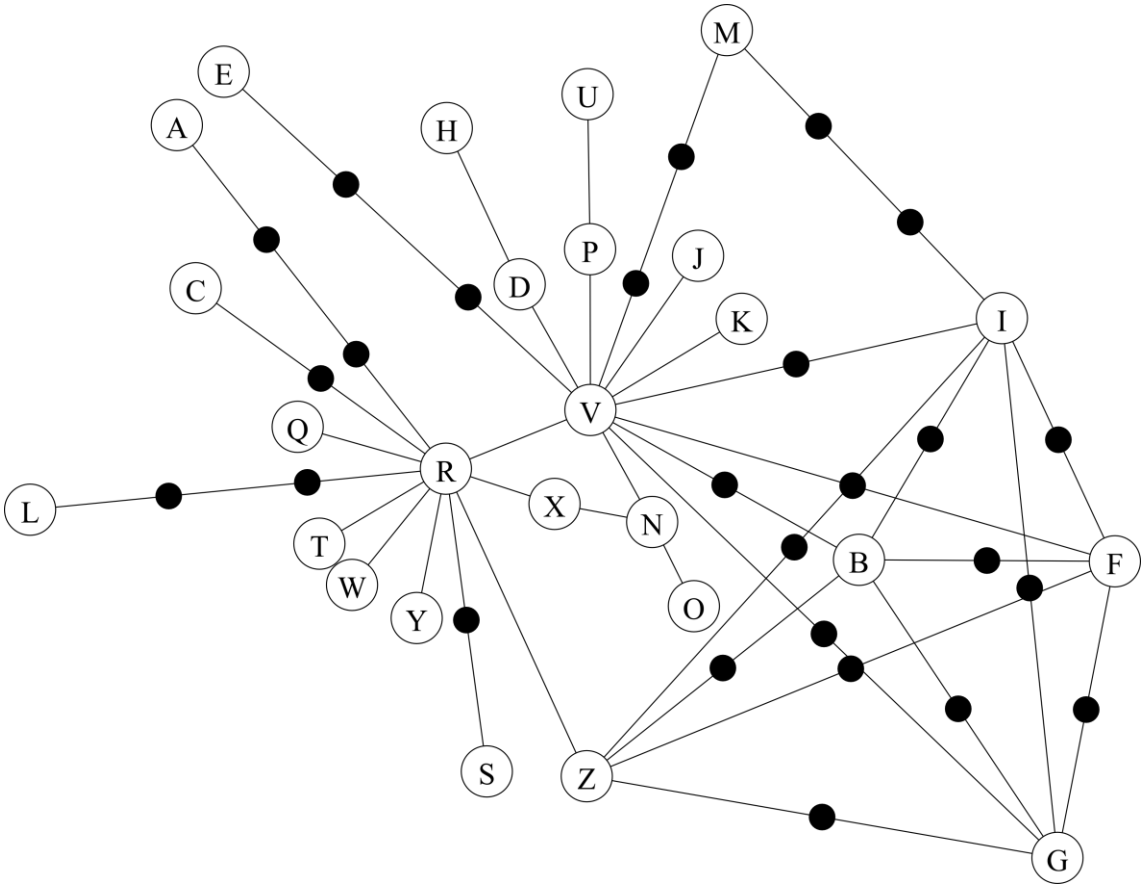

Figure 3.13: Haplotype network for the 424bp portion of HVII of mitochondrial D-loop. Each open circle corresponds to a sequenced haplotype. Haplotypes A to L are found on the continent only. M to Q are found in France, in east England and on the Isle of Wight. R is the main English haplotype, sequenced from all by one English roosts. S to V are found in east England and one western roost. W to Z are found in western England only. Filled circles correspond to the number of mutation steps between haplotypes.

The haplotype network displayed some highly connected star-shaped structure, indicative of rapid population expansion, with haplotypes sequences differing from only one to three base pairs (Figure 3.13). Some division within England was observed, although the two haplotypes responsible differed by only one base-pair, suggestive of the weakness of this structure. The topology of the ML and Bayesian analyses demonstrated a similar pattern (Figure 3.14), although it was not statistically supported by the bootstrapping scores or posterior probabilities. Overall there was a lack of obvious clustering or clear geographical patterns in the haplotype genealogies suggesting that all the

haplotypes belong to the same clade and indicating rapid population expansion following a demographic bottleneck.

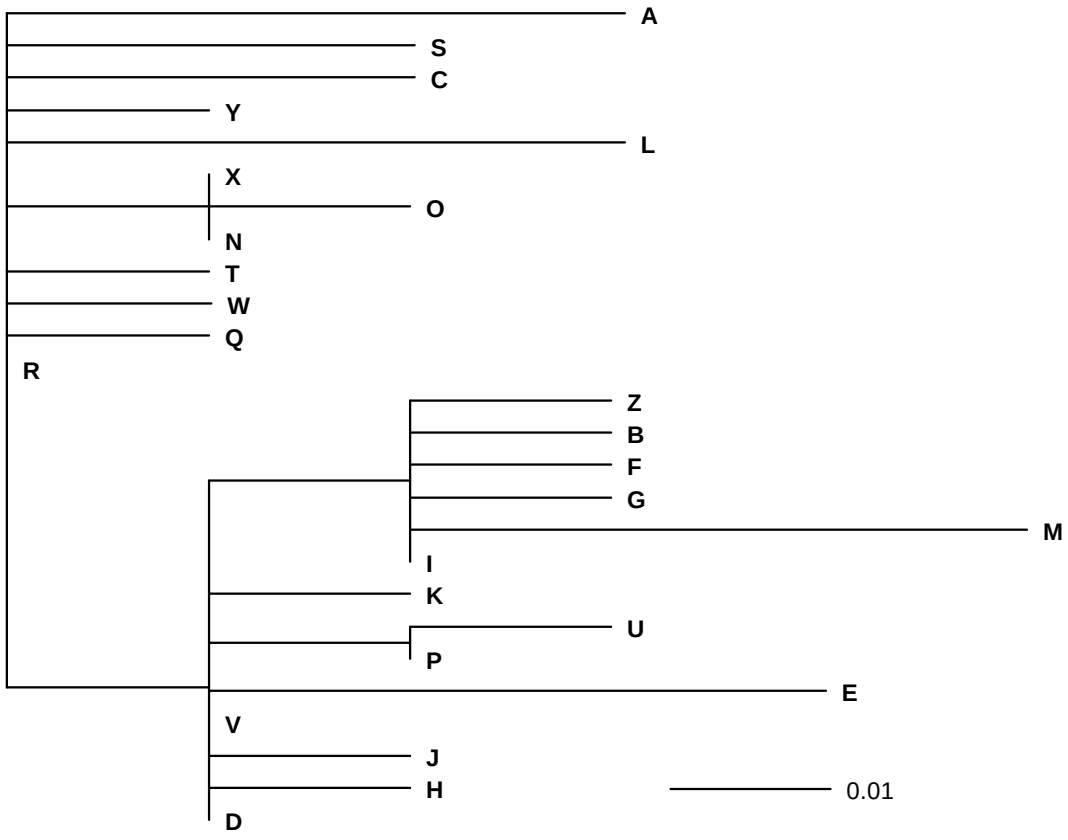

Figure 3.24: Maximum likelihood and Bayesian consensus tree
Built using K80+I model of DNA substitution rooted with the haplotype A. Nodes receiving less than 50% support by bootstrapping or posterior probability were collapsed. Haplotypes A to L are found on the continent only. M to Q are found in France, in east England and on the Isle of Wight. R is the main English haplotype, sequenced from all by one English roosts. S to V are found in east England and one western roost. W to Z are found in western England only.

3.5 Discussion

We used ten polymorphic nuclear autosomal markers and the mtDNA control region sequence to investigate the genetic structure of *E. serotinus* in Europe and infer levels of gene flow within the continent, within England and between the two. Analyses of the nuclear markers using methods based on genetic model assumptions (STRUCTURE, AMOVA), methods explicitly using spatial information (sPCA) and approaches not relying on any models or priors (DAPC, PCoA, NJ), converged on a similar pattern. Weak but significant population genetic structure was found at the largest geographical scale, partially caused by inferred barriers to gene flow, most notably the English Channel. As such, all analyses identified English *E. serotinus* as distinct from their European peers. Different patterns of genetic partitioning emerged within the two main populations with stronger genetic homogeneity on the continent than in England. The stronger differentiation advanced by the mtDNA data suggests some male-bias in mediating gene flow, consistent with patterns observed in many temperate bats (Moussy et al. 2013), and with previous inferences about serotine bat behaviour (Harbusch 2003, Harbusch & Racey 2006). This indicates a mating system driven by male movement to complement the apparent philopatry demonstrated by females.

Significant differentiation in the nuclear and the mitochondrial data occurred among sites at the European scale. However, estimates of genetic differentiation revealed that the level of population structure displayed by mtDNA haplotypes was ten times higher than that inferred by nuclear markers (mtDNA: $\varphi_{ST} = 0.4054$; microsatellites: $F_{ST} = 0.0477$). While the comparison of differentiation between different markers is difficult due to different rates and modes of mutations, with high migration, as expected for vagile species like bats, mutation is likely to contribute little to differentiation (Balloux & Lugon-Moulin 2002). Therefore the observed difference between biparental and maternal fixation indices would be more likely the result of differences in effective population size for the markers and in sex-biased gene flow. Conservatively assuming that sexual selection in *E. serotinus* is not strong enough to reduce the nuclear effective population significantly (Ballard &

Whitlock 2004), the contrast in differentiation measured between the two types of markers exceeds their expected four-fold difference in effective population. This suggests that gene flow in *E. serotinus* is mostly mediated by males, while higher structure in mtDNA sequences indicates female philopatry to their natal landscapes (assuming the demography of this marker is a true reflection of bat population demography: see (Balloux 2010)) which correlates with the qualitative experience of workers studying *E. serotinus* across Europe (Hutson, pers. comm.; Harbusch 2003; Harbusch & Racey 2006). Dispersal is an event poorly understood in this species due to a lack of direct evidence and to the difficulty in finding adult males (Dietz, Von Helversen, et al. 2009).The pattern of male-biased dispersal and female philopatry that we are inferring here is widely observed in mammals (Greenwood 1980, Handley et al. 2007), especially temperate bats (Moussy et al. 2013) such as *M. bechsteinii* (Kerth et al. 2000, 2002) and *Rhinolophus ferrumequinum* (Rossiter et al. 2005), which have virtually closed maternity roosts.

However female philopatry is not absolute as we observe some degree of female dispersal in *E. serotinus* with several mtDNA haplotypes shared among roosts. For example, all haplotypes found in French specimens are shared with roosts in east of England and in the IOW. The coexistence of these "foreign" haplotypes along with uniquely English haplotypes within roosts could be the results of past colonisation events, but could also suggest the contemporary recruitment of dispersed females into existing roosts or the formation of a new roosts by several matrilines. Similarly, one haplotype found more commonly in eastern England was also identified in a roost in the west, indicative of female dispersal and recruitment within England. However, the pattern of isolation by distance found over Europe suggests some limit to female dispersal, although this could also reflect local selection for different haplotypes (Balloux 2010; and see Arnqvist et al. 2010). Furthermore, no haplotypes are shared among the sites sampled on the continent. The geographical scale involved there is an order of magnitude larger than within England, and it therefore appears that female dispersal occurs over sub-continental spatial scales (e.g. <500km). This is further supported by the fact that with the exception of the main English haplotype that is widely distributed, most shared haplotypes in the UK are found in neighbouring roosts. Hence, whilst female dispersal can occur over longer

distances, most females apparently remain philopatric to their natal landscape if not to their natal roost. Movements of females to nearby roosts has been reported in other temperate species, such as *Plecotus auritus* (Entwistle et al. 2000) and *Pipistrellus pipistrellus* (Thompson 1990, 1992). Roost founding is another process that could facilitate female mediated gene flow, although it is still poorly understood (Kerth & Petit 2005, Kerth 2008b, Vonhof et al. 2008, Clutton-Brock & Lukas 2012).

Bayesian clustering and multivariate analyses of the nuclear markers failed to detect any significant sub-structuring within continental Europe. This suggests genetic homogenisation over a large geographical scale (>3,000 km) and high gene flow between continental sites. This is consistent with a recent study on the genetic structure of *E. serotinus* in Poland that revealed very high levels of gene flow among colonies (Bogdanowicz et al. 2013). Lack of information on the species' mating system and movements restricts our understanding of the mechanisms driving gene flow. However, bats are relatively mobile, and this facilitates gene movement across long distances compared with other mammals of similar size. This often results in lower levels of genetic structuring for bats (Ditchfield 2000), which are more similar to birds in this regard (Crochet 2000, Bicknell et al. 2012). However, beyond this generality, bat species display a wide range of migratory, dispersal and mating behaviours, leading to highly variable genetic structures (Burland & Wilmer 2001, Moussy et al. 2013). For example, migratory bats tend to display low differentiation since they mate on the way to, or at, hibernacula. Hibernal sites tend to contain individuals from more than one breeding roost, and are frequently separated by long distances. This pattern is encountered in *Nyctalus noctula,* which displays very low levels of genetic structuring across Europe (F_{ST} =0.006) (Petit & Mayer 1999, 2000, Petit et al. 2001). Similarly, the migratory behaviour of *P. pipistrellus* and *P. pygmaeus* on the continent was inferred from their low genetic differentiation (F_{ST} =0.005 and F_{ST} =0.006 respectively). The fixation index we estimated (0.038) for *E. serotinus,* while low, is relatively high for a bat and reflects values reported for non-migratory species (Moussy et al. 2013). However, because of differences in the properties of markers, including mutation rates and modes, differences in effective population size, in sampling regimes and in the geographical scales studied, fixation indexes are not necessarily easily

translatable across studies and species. Nonetheless, banding data for *E. serotinus* also indicates most movements occur over small scale, and it has been classified as sedentary in comparison to other European bat species (Hutterer et al. 2005). However, while these three approaches seem to be congruent, they all suffer from some limitations. Additionally, this species has mostly been banded at breeding sites whilst most hibernacula are unknown. In the absence of the recovery of banded individuals from winter sites, migratory behaviour cannot be fully ruled out. Therefore, while current evidence points out to relatively sedentary behaviour in *E. serotinus*, more work is needed to confirm this.

The mating behaviour of *E. serotinus* is not known, although it is strongly suspected to be promiscuous like many other temperate species (McCracken & Wilkinson 2000; and see Hosken 1997). Males of some temperate bats, like *P. pipistrellus,* establish mating territories that several females visit. Mating can also occur at hibernation sites as observed in *M. lucifugus* (Dixon 2011) or at swarming sites such as in *M. nattereri, M. bechsteinii* and *Pl. auritus* (Kerth et al. 2003, Parsons et al. 2003, Rivers et al. 2005, Furmankiewicz & Altringham 2007). These latter behaviours increase gene flow since individuals from different roosts across the breeding site catchment area inter-breed (Rivers et al. 2005). Unfortunately information on the winter ecology of *E. serotinus* is also lacking and this includes the possibility of autumnal swarming and the composition and catchment areas of hibernacula. Some individuals are however found in caves and crevices during winter (Dietz 2009; Dyer, pers. comm.).

While a low degree of structuring was found, the genetic differentiation detected was significant, with isolation by distance detected at the nuclear markers across Europe, and evidence for physical barriers to gene flow. Whilst our study finds weak evidence for the influence of mountain ranges as barriers, we find strong evidence for the sea to act as a obvious barrier to gene flow, even when limited to the narrow straits of water between England and France (English channel, 34km) or between England and the Isle of Wight (6km) which is intriguing as this is less than the mean commuting distance per night recorded for *E. serotinus* in England (Catto et al. 1996). Although this distance is small and could readily be covered by *E. serotinus,* open water still constitutes a

behavioural barrier to movements in many species (García-Mudarra et al. 2009, Moussy et al. 2013).

Within England, the fixation index was as high as within continental Europe, in spite of the smaller geographical scale. Bayesian clustering, multivariate analyses and AMOVA on the nuclear markers identified three populations: eastern England, western England and the Isle of Wight. Furthermore, isolation by distance was detected, although it was consistent with two populations geographically separated but connected by ongoing gene flow within mainland England. While initially surprising, this pattern of stronger genetic structure in English bats in contrast to continental bats has been observed in several species, including *M. bechsteinii* (Kerth et al. 2002, Durrant et al. 2009)*, R. ferrumequinum* (Flanders et al. 2009), *P. pygmaeus* and *P. pipistrellus* (Racey et al. 2007, Bryja et al. 2009). It is unclear what underlies these differences, although smaller effective population sizes in the UK than on the continent could contribute to generating these genetic patterns. Hence, the population size for *E. serotinus* in England is estimated at 15,000 individuals (Bat Conservation Trust, 2011) while it has not been estimated on the continent, although this species is considered abundant (Dietz, Von Helversen, et al. 2009). Furthermore, the UK represents the most northern limit of many bat species, including *E. serotinus*, and this could affect population behaviour and dynamics and thus genetic structure. Lower dispersal at range edges has been shown in other taxa (Travis & Dytham 1999) due to increased dispersal costs and reduced habitat density (Dytham 2009). In addition, the behaviour of bats can differ on islands. For example, continental populations of the tropical megachiropteran *Eidolon helvum* are migratory and display virtually no population structure, while island populations are sedentary and display varying degrees of genetic differentiation (Juste et al. 2000, Peel et al. 2010). Segregation of the IOW can be explained by the Solent restricting gene flow between the island and mainland England. No obvious physical or behavioural barriers, habitat fragmentation or lack of connectivity seem to explain what appears to be two partially isolated populations on the English mainland, although urbanisation in eastern England could constitute a potential resistance to movement. An alternative explanation is that the East/West structure in England represents two main catchments of gene circulation, possibly driven by

limited and geographically distinct swarming/hibernation sites with ongoing gene flow through male, and to a lesser extent female, dispersal.

Historical events, such as post-glacial recolonisation of Europe, certainly play a role in partitioning genetic variation of some species e.g. *M. bechsteinii* and *R. ferrumequinum* (Durrant et al. 2009, Flanders et al. 2009). On continental Europe, the mitochondrial data are structured into two significantly differentiated eastern and western continental clusters. This differentiation could be due to post-glacial recolonisation from separate refugia (Hewitt 1999) while the low structure in the nuclear markers reflects high contemporary gene flow. Furthermore, lower mitochondrial diversity was found in England compared to that of continental Europe, which could indicate that *E. serotinus* underwent past population bottlenecks during recolonisation of England before rapid population expansion. However, nuclear diversity levels are similar within England and within the continent and the nuclear structure is not reflected in the mitochondrial data. One interpretation of this is that England underwent several founder events from the same ancestral mitochondrial haplotype, leading to significant structuring in the nuclear gene pool but no structuring in mtDNA. Unfortunately, the occurrence of a single clade in Europe as recovered by the various tree topologies, the lack of geographical patterns in haplotype genealogies and the introgression of *E. nilssonii* mtDNA into *E. serotinus* during past hybridisation events (Artyushin et al. 2009) impede a full understanding of the contribution of past events to the genetic structuring of *E. serotinus.* Further studies involving systematic sampling across putative refugia in the Iberian, Italian and Balkan peninsulas and across the putative contact zone would be required to reconstruct the post-glacial history of this species and its contribution to current genetic structure. This is largely conjecture and other explanations for this pattern exist, including selection on mitochondrial haplotypes, which can be complicated by epistatic and environmental interactions (e.g. Arnqvist et al. 2010).

While significant genetic structure was found for *E. serotinus* with four main populations detected, there was nonetheless high admixture, as indicated by Bayesian and multivariate analyses of nuclear markers, and a number of first generation migrants were identified. This indicates strong ongoing gene flow

among populations rather than lack of drift effect due to recent population divergence, which in turn directly assumes the regular movement of individuals between populations, even across the English Channel. Specifically a notable immigration rate from continental Europe to the species' northern range, in England, was inferred both with nuclear data (11.4% estimated immigration rate from the continent) and directly demonstrated with mitochondrial data (shared haplotypes between France and England). Movements appear to be going mostly to IOW and eastern England, a pattern congruent at both markers although data are consistent with mostly male-mediated movement. Whilst eastern England is geographically close to continental Europe, the shortest distance to the IOW is ca. 150 km, it thus is less clear why the IOW appears so connected to mainland Europe.

Within England most bat movements seem to occur from east to west. This is suggested by the low biparental differentiation between these populations and by the recovery of eastern mitochondrial haplotypes in the west. Similarly, movements between the IOW and mainland England are mostly directed toward western England resulting in a net immigration rate higher than its emigration rate. This pattern could indicate a westward expansion of the species range margin, consistent with the lower haplotypic and nucleotide diversity in west than elsewhere in England. *E. serotinus* has indeed traditionally been mostly recorded in south eastern England (counties of Kent, E. & W. Sussex and Surrey) and its presumed absence from far west (especially Cornwall) has never been fully explained since habitat and roosting opportunities seem favourable. Furthermore, the constant immigration from the core population on the continent through eastern populations could be inhibiting local adaptation and thus range expansion due to the swamping effect of gene flow. However, since 2004, the National Bat Monitoring Programme (NBMP) has reported a significant regional difference in trends from their Field Survey with a steep increase in the southwest (Bat Conservation Trust 2011). This is consistent with reports from local bat workers and volunteers registering *E. serotinus* calls in western counties on a more frequent basis, prompting them to suspect the establishment of new communities in the west (Marshall, pers. comm.). Similarly, northward expansion is also suspected, especially with the recent discovery of a breeding colony in North Wales (Dyer, pers. comm.). This recent

range expansion could potentially be linked with climate change as it has been predicted for hibernating mammals (Humphries et al. 2002), but climatic and landscape modelling of the species distribution would be required to investigate the relationship between abiotic factors and range expansion.

3.6 Conclusion

Significant genetic differentiation was found at all spatial scales although stronger structure on a smaller geographical scale was detected in England, confirming and improving previous analysis of this species in England (Smith et al. 2011). Genetic structure could be attributed to a range of factors, including geographical barriers, differential behaviour between core and peripheral populations, post-glacial history including colonisation and demographic events, mating behaviour such as swarming, and winter ecology. Confirming and assessing the contribution of all these factors would require further genetic and behavioural studies, especially the direct observation of individuals. However, despite the presence of geographical barriers, considerable contemporary gene flow is still observed, mostly through a male-mediated mechanism, even across the English Channel.

In this context, the prevalence and scale of movements by continental bats suggest population behaviours that would facilitate the maintenance of endemic diseases such as EBLV-1. Conversely, the relative fragmentation of serotine populations in England may sufficiently alter host-virus epidemiology to maintain the apparent absence of this virus in England. Finally, the English Channel does not appear to offer a substantial barrier to gene flow and the eventual natural passage of EBLV-1 to England seems likely.

CHAPTER 4: Investigation of the social dynamics and fine scale individual movements in *Eptesicus serotinus* using a stable isotope approach

4.1 Abstract

Individual variation in behaviour and spatiotemporal dynamics contributes to shape the structure, strength and persistence of social units and as such influences the evolution of animal societies. Understanding fine scale individual patterns thus underpins important questions relating to the ecology and evolution of social species. The individual behaviour and movements of *Eptesicus serotinus* within summer maternity roosts was investigated using stable carbon isotopes in wing biopsies and fur in order to establish the social dynamics and persistence of maternity roosts over different time scales. Isotopic variation among fur samples within roosts was the highest in June and decreased over the summer. Since animals likely moult their hair during the summer months, this pattern suggested inter-annual variation in individual behaviour whereby some animals likely exhibit weak fidelity to the main maternity roost. In contrast, isotopic variation in wing samples was the lowest in June but increased in July and August. Wing tissue has a rapid turnover meaning that this result indicates changes in individual behaviour after parturition that could potentially be explained by changes in foraging behaviour and/or by increased movement among roosts later in the summer. We have thus demonstrated that the association of individuals at roosts is flexible and dynamic, possibly reflecting their membership to communities operating at larger geographic scales. Communities of *E. serotinus* seem to create a network of roosts with individuals continually re-assorting among them, both within and across breeding seasons. While the exact detail of these long- and short-term patterns cannot be pinpointed by stable isotope analysis alone, this technique provides us with unique insights into movements and social patterns of large numbers of animals that could not be obtained with traditional individual tracking or marking methods.

4.2 Introduction

The causes and consequences of group-living are central to our understanding of animal ecology (Parrish 1999). An important aspect of animal societies is the individual variation in behaviour and spatiotemporal dynamics that contributes to shape the structure, strength and persistence of social units. Of particular interest are individual patterns of movement, and the underlying phenomena that drive them, such as foraging, seasonal migration and/or dispersal. For example, individual foraging specialisation, in which individuals from a population exploit different prey and/or habitats, is a strategy encountered in some species to avoid intra-specific competition and can often result in spatial segregation of foragers (Bearhop et al. 2006, Votier et al. 2010). The level of fidelity and of philopatry to a social group, and the potential temporal variation associated with seasonality or breeding, also play important roles in the characterisation and persistence of a social unit. The study of individual movements associated with dispersal and migration is thus essential to provide insights into the formation and persistence of animal groups, but also to understand patterns of connectivity between different groups and across seasons (Webster et al. 2002, Hobson 2005). Furthermore, establishing patterns of animal movements and their causative agents is crucial for effective conservation, disease prevention and management.

Bats are an interesting taxon to study in this respect, as they display a wide range of social behaviours and strategies relating to roosting, foraging, mating, migration and dispersal (Hosken 1997, 1998, McCracken & Wilkinson 2000, Moussy 2011, Moussy et al. 2013). As such, the sociality, individual behaviour and movements of bats have increasingly been attracting interest (Willis & Brigham 2004, Kerth & Petit 2005, Safi 2008, Kerth & Van Schaik 2012, Moussy et al. 2013). Most temperate bats form breeding colonies in the summer (McCracken & Wilkinson 2000) and in general females are thought to be the philopatric sex, although the strength of this behaviour varies among species (Lewis 1995, Moussy et al. 2013). However, little is known about roost switching behaviour other than in a few examples of strong fission-fusion societies, where individuals from a community switch among multiple roosts (Willis & Brigham

2004, Popa-Lisseanu et al. 2008, Kerth et al. 2011). In addition, there is some understanding of resource partitioning, habitat use and home range within communities (Shiel et al. 1999, Kerth et al. 2001, Kusch & Idelberger 2005, Sullivan et al. 2006) and some recognition of dietary plasticity within species (Moosman et al. 2012). However, whilst it is recognised that communities may be both behaviourally flexible and dynamic in their choice of prey and foraging sites, the existence of individual variation (within-community) in these behaviours, including individual specialisation, has only recently begun to attract attention (Cryan et al. 2012). Similarly, information on bat dispersal and migratory movements remains scarce (Hutterer et al. 2005, Moussy et al. 2013), impairing our understanding of the extent to which summer and winter colonies are connected. There is some evidence for some migratory species pointing to weak migratory connectivity, as observed in *Nyctalus noctula,* with breeding females from spatially segregated maternity colonies sharing common hibernacula (Petit & Mayer 2000). An increased understanding of bat movements and sociality underpins their conservation as important ecosystem service providers (Kunz et al. 2011) and the management of diseases to which they may be hosts (Hayman et al. 2013).

The study of animal movement has traditionally employed mark-recapture and remote-sensing techniques, like radio telemetry and satellite tracking. However, these time-consuming approaches have limitations, including low or skewed recapture rates, the difficulty of distinguishing between migration and dispersal, lack of applicability for small animals, or high costs. Forensic methods are increasingly used in studies of animal movement (Crochet 2000, Burland & Wilmer 2001, Rubenstein & Hobson 2004, Crawford et al. 2008, Moussy et al. 2013). Hence, population genetics can reveal population structure, social organisation, dispersal and levels of connectivity between populations. Similarly, stable isotope analysis can be a powerful method to detect movement and explore population and spatial organisation. This technique is based on the incorporation of naturally occurring stable isotopes in animal tissues obtained from their diet. The natural abundances of a number of stable isotopes can vary predictably among different locations due to biological and biogeochemical processes. For instance, carbon stable isotopes (expressed as $\delta^{13}C$) vary in plant tissues depending on photosynthetic chemistry (C_3, C_4 or CAM) and these

differences are preserved throughout the food webs they fuel. Thus, the isotopic signatures in animal tissues can be used to infer the habitat or location in which they were grown. Differences in the rate of tissue turnover and the timing of tissue synthesis means an animal's diet and location can potentially be assessed across multiple temporal scales by comparing isotope ratios in different tissues. For instance, the isotopic information found in metabolically inert tissues like hair, feather or claw reflects the location in which they were grown and this is preserved over time. In contrast, metabolically active tissues like blood plasma and the liver keep such information for a few days, or in the case of whole blood and muscle, a few weeks. For example, carbon stable isotope $\delta^{13}C$ analysis of the muscle tissue of *Leptonycteris curasoae*, a nectarivorous bat with partial migration, was able to distinguish the resident population from the migratory population (Fleming et al. 1993).

The serotine bat, *Eptesicus serotinus*, is a medium sized vespertilionid bat distributed throughout Europe, but with its northern range restricted to southern England (Dietz, Von Helversen, et al. 2009). It is the host for one of the European Bat Lyssaviruses (EBLV-1), largely prevalent in continental Europe (Racey et al. 2013). The summer ecology of females is thought to be relatively well described. Adult females share a maternity roost, mostly in occupied buildings, and display relatively strong annual philopatric behaviour (Harbusch 2003, Harbusch & Racey 2006) to these sites, whilst adult males are almost entirely excluded. Radio-telemetry studies have revealed foraging flights to be typically four to six kilometres from their roost, and the use of open and edge habitats such as pastures and woodland edges (Catto et al. 1996, Robinson & Stebbings 1997, Verboom & Huitema 1997, Harbusch 2003) as well as locations such as urban parks and golf courses. While this species is not considered a migratory species across continental Europe (Hutterer et al. 2005), its winter ecology is largely unknown, especially in the UK which represents the very extreme of the northern and western distribution of this species. As such, aggregations of female *E. serotinus* at maternity roosts can be considered as social groups whose membership appears to persist throughout the lifetime of the female bats, a behaviour which is becoming increasingly evident across temperate bat species (Moussy et al. 2013). However, we currently do not understand the nature or strength of the social

interaction at other times of year after the females leave the maternity roost. Females might remain together (albeit at a single site unknown to us), be somewhat associated (i.e. at different roosts but in the same landscape) or disassociate completely in winter occupying roosts distant to one another.

Here we measure $\delta^{13}C$ in the hair and fur (synthesised at different times of year) of *E. serotinus* to detect changes in diet and explore individual movements and behavioural changes over different time scales. We hypothesised that isotopic variation in fur within roosts would be stable over time and more variable among roosts if bats remained faithful to their summer roost every year. We also reasoned that if summer social groups disassociate during winter with individuals moving to separate overwintering sites, then the isotopic variation in wing tissue would be higher early in the season, when social groups reform in spring/summer, compared to later in the season, after the bats have been together for some time and new hair and wing tissue is synthesised in the same location. Finally, we expected the isotopic variation in wing tissue to vary between classes within the community, especially those expected to have distinctly different energetic demands and foraging behaviour. Thus we anticipate differences in the patterns and variance of $\delta^{13}C$ between breeding and non-breeding females.

4.3 Methods

4.3.1 Tissue choice

The chronology of pelage moult is poorly known for most bat species, though studies agree that sub-tropical species appear to undergo a single annual moult over the summer (Constantine 1957, 1958, Alagaili et al. 2011, Fraser et al. 2012) and that this is similar in migratory species in the US (Cryan et al. 2004). The exact timing and length of this event in temperate species is unclear and most likely varies inter- and intra-specifically. However, like those species for which moult has been described, we anticipated that moult in the northern hemisphere would start soon after the energetically demanding reproductive period in July/August and consequently occur during and after August. Thus fur sampled in spring and early summer ought largely to reflect the previous

summer's diet, whereas fur sampled late in the summer will reflect the current year. In contrast, wing membranes are metabolically active tissue and are constantly replaced. Stable isotope signatures of the wings will therefore likely represent a shorter period dependent on the turnover rate of the tissue. In the summer, wing biopsies are reported to heal within 2-4 weeks (Worthington Wilmer & Barratt 1996). While this time-frame is associated with the healing process, the assumption of wing membrane turnover time of 4-6 weeks is reasonable and isotopes from wing samples therefore represent a short to medium-term marker.

4.3.2 Sample collection

A total of 219 adult females were captured at 20 maternity roosts across England during the summers of 2010 and 2011 (Table 4.1). The reproductive status of captives was determined, the forearm length and weight were recorded, and a unique forearm band was fitted for identification (Mammal Society, UK). A small amount of fur (less than 5mg) was clipped from the lower dorsal area and stored at ambient temperature until isotopic analysis. Two wing biopsies (circular punch ∅ 3mm: Stiefel Laboratories, Wooburn Green, UK) were collected and stored with silica gel at ambient temperature until isotopic analysis. All bats were released successfully within a few minutes of the procedure, which was performed under licence from Natural England and the UK Home Office following the Animals (Scientific Procedures) Act 1986. Bats were re-sampled in successive years if they were available.

4.3.3 Stable isotope analysis

Wing membrane samples were rinsed with distilled water and placed in a freeze-drier for 72 hours to remove moisture. The wing biopsies from each bat were weighed together on a micro-balance in a tin capsule (~0.2 mg). Fur samples from the 2010 season were cleansed of surface oils by soaking in 2:1 chloroform/methanol solution (hereafter solvent) for 24 hours, rinsed and left to air dry. Fur samples from 2011 were stored in solvent for fifteen days, rinsed and left to air dry. Clean fur samples were then weighed to 0.6 mg (± 0.05 mg) in duplicate in tin capsules (Elemental Microanalysis, Okehampton, UK).

Internal standards, calibrated against International Atomic Energy Agency reference materials (USGS 40 and 41), were matched to sample weight. Casein and collagen were used with wing samples, and horsehair from Bavaria and Paraguay were used as a keratin standard with fur samples. Carbon isotope analysis was performed by continuous–flow isotope-ratio mass-spectrometry on an elemental analyzer EA 1108 (Carlo Erba Instruments, Milan, Italy) coupled to an Isoprime IRMS (GVI, Manchester, UK). Carbon isotope ratios were reported in units per mill (‰) relative to Vienna-Pee Dee Belemnite following the standard equation: $\delta^{13}C = ((R_{sample}/R_{standard}) - 1) \times 1000$

where R is the ratio of the heavy: light stable isotope of the sample and standard, (i.e. $^{13}C:^{12}C$). Measurement precision based on the repeated analysis of the standards was 0.13‰ for fur and 0.29‰ for wing biopsies.

Table 4.1. Sampling period and sample size at each maternity roost.
Breeding ratio is the number of breeding individuals (pregnant, lactating or post-lactating) against non-breeding ones. Mean and standard deviation on $\delta^{13}C$ for wing tissues ($\delta^{13}C_W$) and fur ($\delta^{13}C_F$) were calculated per roost by combining multiple catching dates when appropriate.

Roost ID	Sampling months	Sample size	Breeding ratio	Mean $\delta^{13}C$ wing (‰)	SD $\delta^{13}C$ wing (‰)	Mean $\delta^{13}C$ fur (‰)	SD $\delta^{13}C$ fur (‰)
9	August	14	14/0	-24.40	0.55	-23.48	0.36
11	August	2	1/1	-24.84	0.06	-25.08	0.42
12	August	16	13/3	-25.47	0.40	-25.56	0.51
15	July/August	11	7/4	-24.55	0.81	-24.53	0.43
16	June/July/August	9	7/2	-25.37	0.55	-24.47	0.78
17	June/July	10	6/4	-24.37	0.37	-24.14	0.99
18	June/July	12	7/5	-24.70	0.47	-23.97	0.91
19	July	6	4/2	-25.10	0.22	-25.88	0.89
20	July/August	17	13/4	-24.66	0.35	-23.51	0.74
21	August	6	5/1	-24.68	0.19	-23.51	0.11
22	July	7	5/2	-24.39	0.47	-24.56	0.28
23	July	19	14/5	-25.31	0.20	-25.37	0.56
27	August	6	3/3	-25.08	0.89	-25.91	0.36
29	June/July/August	7	5/2	-24.95	0.37	-24.65	1.04
30	June/July	23	18/5	-25.31	0.33	-23.80	0.81
31	June/July	31	26/5	-25.59	0.33	-24.58	0.93
33	August	7	5/2	-24.09	1.09	-24.32	0.89
34	August	3	3/0	-24.98	0.10	-24.89	0.48
35	August	7	5/2	-23.84	0.83	-23.04	0.60
36	August	6	6/0	-25.01	0.47	-23.32	0.33

4.3.4 Statistical analysis

The analysis and interpretation of the data was based on the assumed and realised differences in the timing of wing and fur growth. Wing membrane turnover time being estimated at 4-6 weeks, so June samples should reflect events in late April-May, when the maternity roosts are being formed, while July and August samples provided information for June and July, before and after the pups were born (Figure 4.1). Moulting is thought to start in earnest during July, so fur samples from June should largely reflect events from the previous moult period, (i.e. the previous year), while July and certainly August samples should contain a $\delta^{13}C$ signature reflecting the current diet. To investigate the persistence of social groups over time, the correlation between the $\delta^{13}C$ measurements of both tissues was evaluated across months. Higher correlations within roosts were expected later in the summer when the two tissues are temporally matched, while earlier in the year, if bats have spent the winter and/or the previous summer apart there should be poorer spatio-temporal matching and correlations should be lower.

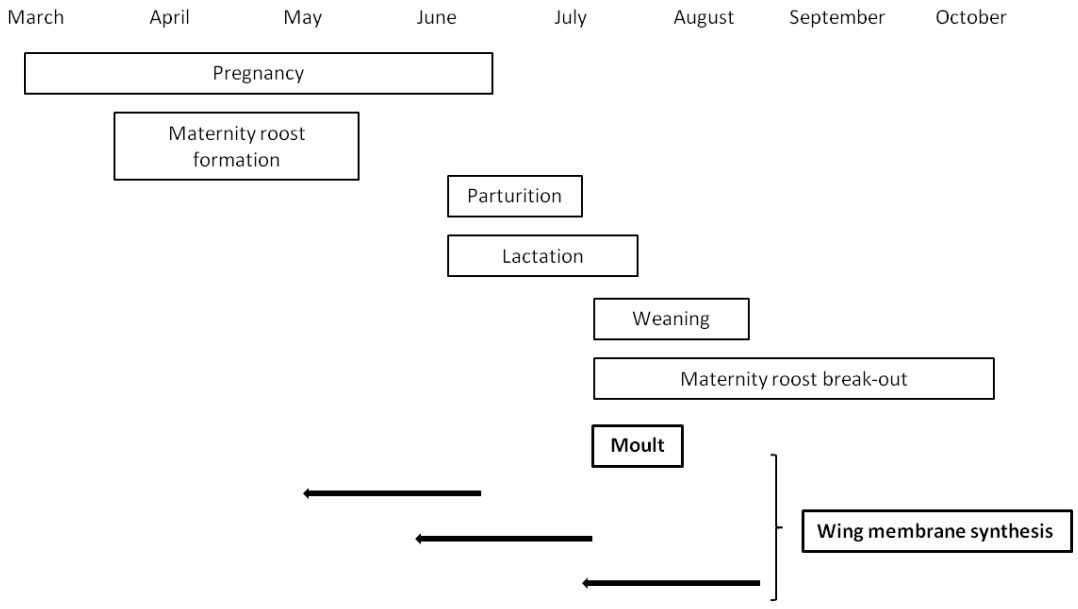

Figure 4.1. Approximate timeline of seasonal life history events in north hemisphere temperate bats.
The arrows represent the average turnover time of wing membrane.

Values of $\delta^{13}C$ from the fur samples were first regressed against those from the wing samples using a restricted maximum likelihood (REML) generalised linear mixed model fitted with a normal error structure and identity link. Individuals, nested within roosts, were fitted as a random intercept to account for repeat sampling of recaptured animals and for baseline $\delta^{13}C$ variation across roosts. Due to the different time scales over which fur and wing are synthesised, the $\delta^{13}C$ relationship between the two tissues could vary over the summer. The slope of the linear regression was thus allowed to vary between months nested into years, which in turn accounts for variation in $\delta^{13}C$ values between years. The residuals from this model represented the unexplained variation among individuals within roosts left in fur. The unexplained variation left in wing membrane was similarly obtained from the residuals of the linear mixed model regressing wing $\delta^{13}C$ values against those from fur using the same error structure and random factors. Since only the magnitude of these variations was of interest, absolute values were kept and transformed by a Box-Cox power transformation to ensure normality and homoscedacity.

To compare the amount of unexplained variation within each tissue across months and to determine whether this variation was due to differences in breeding condition, a generalised linear mixed model was built (normal error structure, identity link, REML). We therefore modelled the residuals obtained above as a function of time (month of sampling), tissue type (wing or fur), breeding condition, and the three-way interaction. The year of sampling and roost were used as random intercepts. Simplification was conducted using a backward stepwise procedure and maximum likelihood method, with non-significant terms removed until no significant change in deviance was observed. One of the two-way interactions was found to be significant. Post-hoc testing was carried out by merging pair-wise interaction levels and assessing the change in deviance from the minimum adequate model. All statistical analysis was carried out using R. 2.15.1 (R Development Core Team 2011).

4.4 Results

Fur and wing biopsies were collected from a total of 219 bats from 20 maternity roosts in 2010 (n = 93) and 2011 (n = 126), including 10 recaptures (Table 4.1). $\delta^{13}C$ values ranged between -26.9 and -22.05‰ in fur samples and between -26.79 and -22.55‰ in wing biopsies (Figure 4.2). Variance within a roost varied from 0.01 to 1.08‰ and from 0.003 to 1.19‰ in fur and wing respectively (Table 4.1). The correlation in $\delta^{13}C$ values between both tissues increased across the summer months, becoming significant in July and August (June: Pearson's r = 0.265, t_{48} = 1.906, p= 0.06; July: Pearson's r = 0.415, t_{77} = 4.000, p < 0.001; August: Pearson's r = 0.491, t_{89} = 5.321, p < 0.001), indicating that some synchronous tissue growth is occurring later in the summer, which is broadly consistent with our hypothesis about the timing of moult.

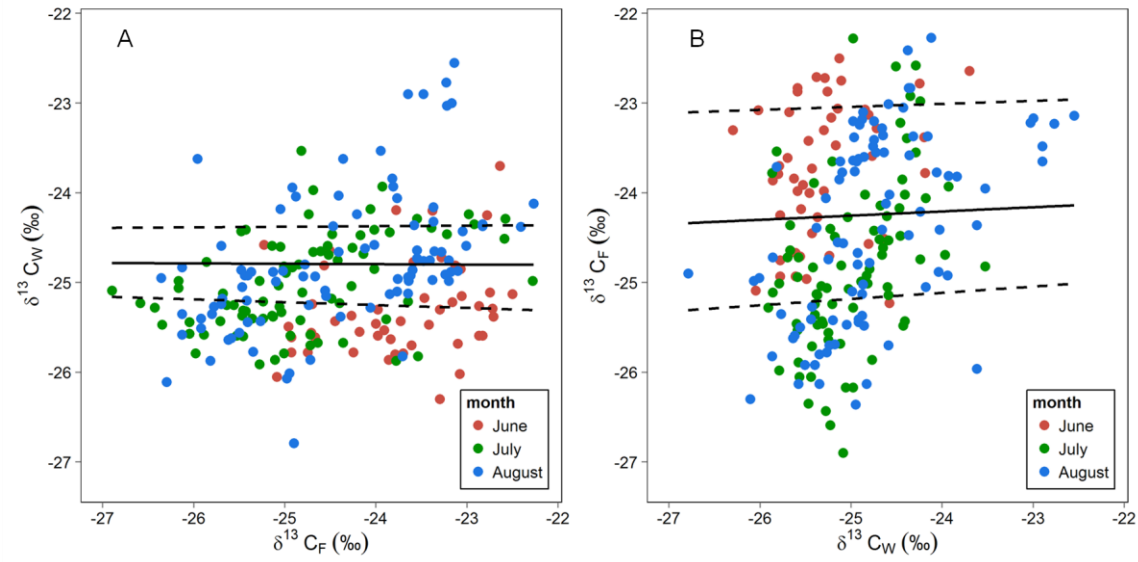

Figure 4.2. Relationship between A. $\delta^{13}C$ in fur ($\delta^{13}C_F$) and $\delta^{13}C$ in wing membrane ($\delta^{13}C_W$) and B. $\delta^{13}C$ in wing membrane ($\delta^{13}C_W$) and $\delta^{13}C$ in fur ($\delta^{13}C_F$).
The solid lines are the regression lines predicted by the appropriate linear mixed models and the dashed lines are the 95% confidence intervals.

The two linear mixed models establishing the relationship between isotopic values in wing and fur within each roost ($\delta^{13}C$ fur against $\delta^{13}C$ wing and $\delta^{13}C$ wing against $\delta^{13}C$ fur) were not significant ($X^2_{1, 11}$ = 0.3084, p = 0.308 and $X^2_{1, 11}$ = 0.2057, p = 0.650 respectively). This result indicates that there was a

mismatch in $\delta^{13}C$ signature between the two tissues at some point during the sampling season, likely in June, when the two tissues were not synthesised synchronously. The mean absolute residual variation in both tissues varied significantly with the sampling months ($X^2_{9,\ 5}$ = 70.172, $p < 0.001$), but not with breeding status ($X^2_{10,\ 1}$ =1.5487, $p = 0.664$). Thus the state of reproduction and the consequential variation in energetic demands on individuals does not appear to influence residual $\delta^{13}C$ variation in either fur or wing.

The temporal patterns were different between the two tissues (Figure 4.3). Fur variation was significantly higher in June than in July ($X^2_{9,\ 1}$ = 6.8629, $p < 0.01$) and in August ($X^2_{9,\ 1}$ = 10.4910, $p < 0.01$). Fur variation did not differ significantly between July and August ($X^2_{9,\ 1}$ = 1.0388, $p = 0.308$). Interpretation of the results from fur suggest that the variation in diet between individuals during the extended period before the bats arrive at the maternity roost is great, and larger than that seen at any other time when they are in the same roost. This suggests that, at the population level, they might have fed on a greater range of diets and/or in different landscapes during the previous moult period. Wing variation was significantly higher in August than in June ($X^2_{9,\ 1}$ = 10.395, $p < 0.01$) and July ($X^2_{9,\ 1}$ = 10.616, $p < 0.001$). No difference was observed between June and July wing variations ($X^2_{9,\ 1}$ = 0.2738 $p = 0.600$). Interpretation of the wing results suggests that individual diet within a community becomes more distinct (i.e. the samples have a more variable $\delta^{13}C$) as the summer progresses. In June and July, more variation was found in fur than in wing biopsies ($X^2_{9,\ 1}$ = 48.398, $p < 0.0001$ and $X^2_{9,\ 1}$ = 26.979, $p < 0.0001$ respectively), while in August, fur was found to be as variable as wing membrane ($X^2_{9,\ 1}$ = 0.7357, $p = 0.391$). The variation in fur in July was also comparable to the variation in wing in August ($X^2_{9,\ 1}$ = 1.0388, $p = 0.308$). Thus, variation in fur decreased over time, which coincided with variation in wing increasing until eventually they were both similar by the end of the summer (Figure 4.3). The correlation in the $\delta^{13}C$ variance for both tissues reduced throughout the summer such that by the end of the summer their $\delta^{13}C$ composition appears to have synchronised. This appears to be due to a period of rapid fur growth during the summer, incorporating $\delta^{13}C$ derived from summer forage during a period when the bats are sedentary at the maternity roost.

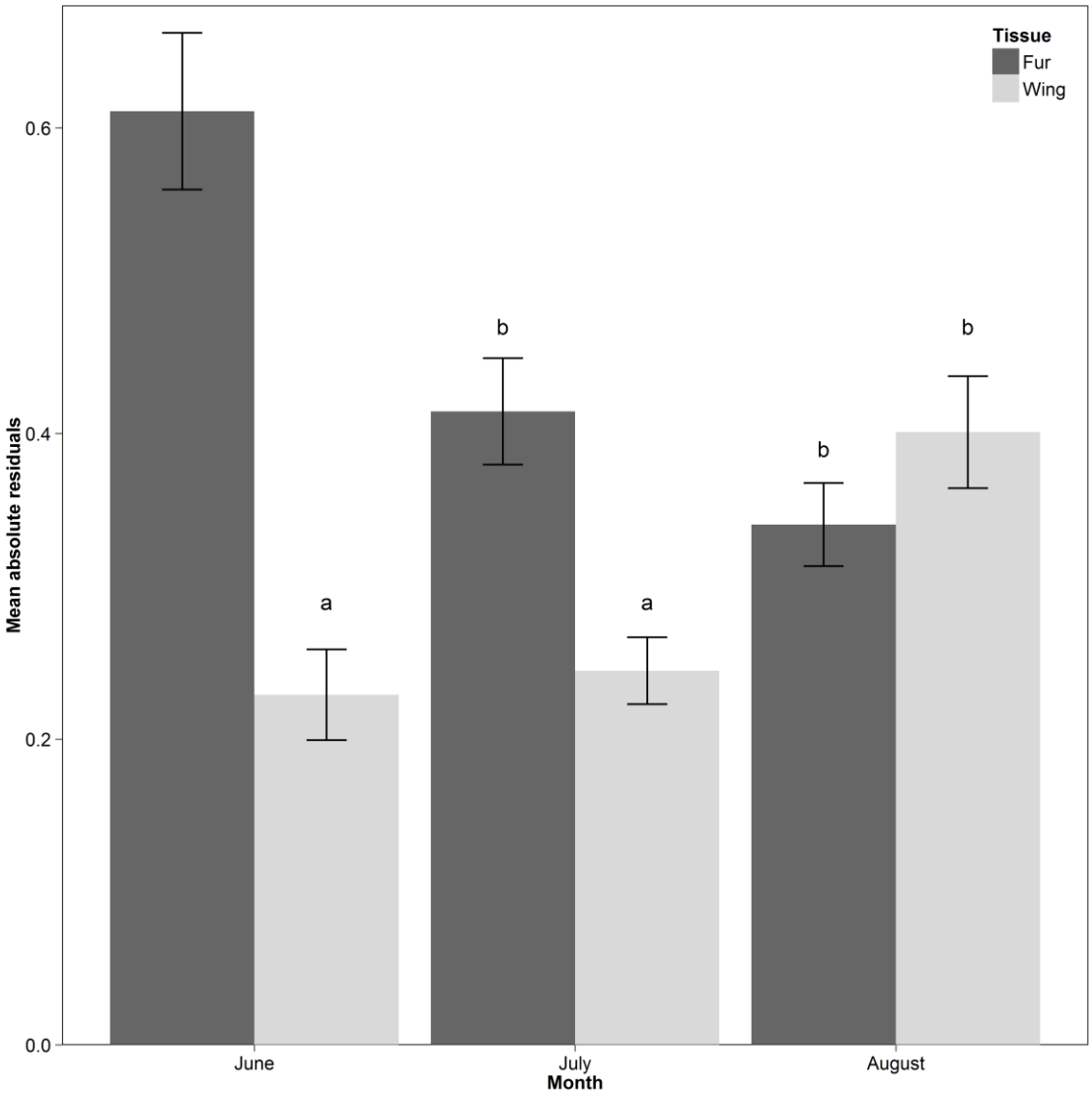

Figure 4.3. Mean absolute residuals in wing and fur tissues partitioned per month. Obtained from the two linear mixed models. The error bars are standard errors. The letters indicates non-significant pairwise comparisons.

In addition, these patterns suggest that moult starts slightly earlier than anticipated, i.e. in July rather than assumed post-reproduction in August and later. Furthermore, in this study, the only three bats sampled in early/mid-August in the first year and recaptured in mid-July of the second year still had visible fur clip marks from the previous year. In contrast, the other recaptured bats, sampled across a period of over one year showed no signs of clipping. Though these observations represent a very small sample size, interpreted alongside the isotopic pattern, it can be concluded that moult in *E. serotinus* starts in late July and the lower dorsal pelage is rapidly replaced (possibly over

less than a month). We anticipate that fur growth ceases at or before bats enter hibernation (at which point all extraneous metabolic processes appear to cease). Thus whilst a large part of the $\delta^{13}C$ signature of the fur may be established in a month long period in summer, it may be added to throughout the autumn.

4.5 Discussion

Here, we investigated female social communities over different time scales using $\delta^{13}C$ to define group associations. We show for the first time that adult associations in a community are flexible and dynamic and can vary both within the breeding season and inter-annually. In particular, we demonstrated that some individuals have spent the previous moult at sites other than the current maternity roost. This may represent a one-off lifetime dispersal event or alternatively it may reflect the scale at which communities create a network of roosts to which individuals continually re-assort. The results also suggest that summer communities disassociate at the end of the breeding period, possibly to spend the winter apart. We also found that individual diet within a community becomes more distinct as the summer progresses, indicating changes in foraging strategy, possibly in response to changes to reproductive status and to variation in resource availability. Finally, we demonstrated that the moult period starts slightly earlier than we expected, at the end of, or shortly after reproduction (late July-early August). We discuss each finding in turn.

$\delta^{13}C$ variation in fur was at its highest in June, indicating that individual diets were the most distinct, and then decreased over the summer as the moult started (Figure 4.3). This indicates that a number of individuals spent the previous moult period at different sites, possibly spending the previous summer at different roosts to those that they were sampled at. High levels of philopatry have been reported for many temperate bats, including *E. serotinus* (Moussy *et al.* 2013), but this is more linked to fidelity to a natal landscape rather than to a particular roost. Communities of many bat species in the UK appear to use a network of roosts within their natal landscape. Components of that community (i.e. non-reproductive or immature females) may constantly re-assort themselves across this network, spending the period of the previous moult away

from the community maternity roost, potentially at a significant distance but still within the natal landscape. This movement pattern has been reported in several species, for instance in *E. fuscus, N. leisleri, Myotis bechsteinii* (Willis & Brigham 2004, Kerth et al. 2011, Boston et al. 2012), with breeding females of the latter species preferentially roosting together, especially during lactation (Kerth & Konig 1999). Similarly, even though *E. serotinus* maternity colonies are strongly philopatric (Harbusch & Racey 2006; T. Hudson, pers. com; but see also Chapter 3), they have been reported to use multiple permanent roosts. This usually involves a principal roost used for most of the summer and several satellites roosts used for shorter periods (Catto et al. 1996, Harbusch 2003, Harbusch & Racey 2006). The increased $\delta^{13}C$ variation in wing membrane over the summer, coinciding with parturition (Figures 1 and 3), could be due to increased individual movements between roosts after the birth of pups, facilitating access to new foraging areas. Roost switching behaviour may also be a response to increased parasite burdens in primary roosts (Lewis 1995, Bartonička & Gaisler 2007, Reckardt & Kerth 2007). This behaviour may well increase in frequency later in the season, once the young are born, because hairless juveniles could be more prone to parasitism. Roost movements have also been suggested to help familiarise the newly volant juveniles with the variety of roosts and foraging habitats of their natal area (Lewis 1995).

While our data indicates that moult likely starts late July, it may continue throughout autumn, the isotopic variation observed in June fur samples could thus also be a result of sampling bats that had left at different times and/or to different locations the previous year and returned in the following spring for reproduction (Figures 4.1 and 4.3). In many temperate species, including *E. serotinus*, vacation of maternity roosts begins shortly after weaning of the juveniles, at the beginning of August, and are re-occupied in late spring, but their ecology at other times is unknown (Whitaker 1998, Harbusch 2003, Harbusch & Racey 2006, Rodrigues & Palmeirim 2008). Specifically, it is not clear if groups maintain associations throughout winter (coherent), or disassociate after reproduction before reforming the following year. Evidence from this study suggests that not only do they disassociate, but that their diet becomes so distinct that it seems unlikely that they were feeding within the same landscape. Thus whilst is possible that some summer roosts also provide

adequate conditions for hibernation and some bats may over-winter in their summer residence, this does not seem to be a joint higher-level behaviour. Anecdotally, some home-owners of roosts visited during this study have reported the presence of bats throughout the year, although we could not confirm whether these bats are part of the same *E. serotinus* community present in summer or even from the same species.

Finally, occasional natal dispersal and recruitment to a neighbouring community cannot be discounted. Movements between communities are not often recorded (A. Hutson, pers. comm.). However, they may occur, albeit infrequently, in female *E. serotinus*, as demonstrated by genetic studies (Chapter 3), and in other temperate species (Thompson 1990, 1992). Recruitment in spring of individuals from another community would result in larger $\delta^{13}C$ variation in fur early in the summer, as observed in June (Figure 4.3).

We interpret the variance in the isotopic composition of wing tissues to reflect differences in individual foraging behaviour and/or habitat use during the breeding season. *E. serotinus* is known for its broad diet (Catto et al. 1994, Kervyn & Libois 2008) and the diversity of habitats exploited (Catto et al. 1996). Whilst individuals from the same community have been observed sharing primary foraging sites in close proximity to their maternity roost (Catto et al. 1996, Robinson & Stebbings 1997), feeding areas used by individual bats at greater distances may become more distinct (Robinson & Stebbings 1997). Furthermore, while this species could be opportunistic with respect to habitat use or prey selection at the population level, some degree of individual specialisation might also occur, as demonstrated in *E. fuscus* (Cryan et al. 2012). Individual foraging behaviour could therefore result in $\delta^{13}C$ variation when different feeding areas are isotopically distinct.

This isotopic variation within a roost could also vary temporally, both due to individual behaviour and to resource variability. In this respect, the within-roost residual $\delta^{13}C$ variation in wing membrane was found similar in June and July, but increased significantly in August (Figure 4.3). Because of the turnover time of wing tissue, the isotopic values obtained in those samples are averages of isotope assimilation over four to six weeks prior to sampling. June and July variations therefore reflect activities occurring mostly before parturition while

residual variation partitioned in August is associated with post-parturition events (lactation and post-lactation). In several bat species, including *E. serotinus*, evidence of changes in the foraging behaviour of breeding females according to their reproductive status has been recorded. Hence, the time spent foraging and the number of flights increased after birth in some temperate species, while the distance to foraging sites decreased, corresponding to the rising energy demands of lactation and the need to visit the roost during the night to feed the young (Barclay 1989, Clark et al. 1993, Rydell 1993, Catto et al. 1995, Shiel et al. 1999, Henry & Thomas 2002). After weaning their young, foraging activities of adults may become more similar to those of pregnant or even non-reproductive females (Barclay 1989, Rydell 1993, Shiel et al. 1999, Henry & Thomas 2002) and it has been noted that females of some species in distinct postpartum reproductive status (lactating or post-lactating) do not forage in the same area (e.g. Barclay 1989). Furthermore, a shift in the foraging areas of adult females when juveniles fledged has been recorded in high density populations of *M. lucifugus*, possibly in response to increased competition for resources (Adams 1997). Differential physiological requirements between lactating and post-lactating females and competition with independent juveniles could also drive individual specialisation (Araújo et al. 2011).

In addition, insect density, diversity and distribution may differ over the summer, driving bats to exploit different habitats and sites to track optimal resources. *E. serotinus* has been found to eat small insects early in the summer and to shift to larger species like dung beetles later on as they emerged (Catto et al. 1994) and therefore changes its foraging sites accordingly (Catto et al. 1995, Robinson & Stebbings 1997). Lower number of resources patches early in the summer, in May-June, could constrain individuals to forage at limited sites and therefore share resources, reducing the within roost isotopic variation. In contrast, with higher insect biomass, diversity and distribution as summer develops, a wider range of sites and resource patches becomes available, allowing individuals from a same roost to forage separately, resulting in an increase in the residual $\delta^{13}C$ variation in wing membrane.

The isotopic patterns obtained initially seemed to contradict the initial assumption of a summer moult occurring after reproduction and suggested

moult initiating in July. However, the start of moult period is likely to vary inter-annually according to the reproductive status of individuals and thus of environmental conditions. For instance, bats gave birth at the end of June and finished lactating late July/early August in 2010, while reproduction was earlier in 2011, with pups already born in early June and lactation over by early/mid-July. As such, our findings do not necessarily contradict our initial idea of moult starting towards the end of the reproductive period. This period of the year cycle seems to be the best time for such metabolic process to facilitate balancing the tight energy budget of temperate bats (e.g. Hosken & Withers 1997). The energy expenditure associated with moult has, so far, not been investigated in bats. However, evidence in some bird and mammal species indicates that moult is energetically costly due to the energy expenditure associated with tissue synthesis, with increased thermoregulation and with increased flight cost in bird (Murphy & King 1992, Boyd et al. 1993, Swaddle & Witter 1997, Paterson et al. 2012). Therefore moulting is likely to be incompatible with other life-history events like hibernation and reproduction due to their high energetic costs (Kurta et al. 1990, McLean & Speakman 2000, Speakman & Thomas 2003, Speakman 2008). Optimisation of the energy budget could thus favour moult starting shortly after lactation. This pattern of delaying moult after reproduction has similarly been observed in avian and mammal species (Siikamäki et al. 1994, Stewart & Macdonald 1997, Flinks et al. 2008). We would have however expected more variation in the moulting timing of non-breeding individuals since they are not constrained by the physiological demands of reproduction. The lack of effect of breeding status in our results could possibly be attributed to the low number of non-breeding bats captured, although it could also mean that non-breeding bats start moulting in response to higher food availability occurring mid-summer.

4.6 Conclusion

The variation in $\delta^{13}C$ between two tissues of differential metabolic activity highlighted the temporally and spatially dynamic behaviour of this species at fine scale. We have demonstrated that adult association into a community is a flexible and dynamic behaviour. Communities of *E. serotinus* seem to create a network of roosts across which individuals continually re-assort, possibly in response to their reproductive status. This re-assortment occurs both within the breeding season, and especially after parturition, and for some individuals inter-annually. Furthermore, there is some suggestion that the community disassociates later in the summer and while we could not strictly conclude whether members of a maternity roost spend the winter apart, we believe that estimating the $\delta^{13}C$ variation in wing tissue very early in the season, in April, could give a more definitive indication as to the winter dynamics of this species. Finally, it appears that individual foraging strategies vary over the summer, possibly in response to changes to reproductive status and to variation in resource availability. We therefore revealed the complexity of individual behaviour in this species and advocate the need to study bat communities at a larger scale than at the roost level to fully understand their social and spatial dynamics. Such work is essential for bat conservation but also for the risk assessment and management of the zoonoses they may be the host for. We propose that stable isotope analysis could be a powerful method for such studies, especially in conjunction with other direct (ringing, tracking) or molecular approaches.

CHAPTER 5: Assessment of the use of hydrogen stable isotope at regional scale for the study of bat movement

5.1 Abstract

Understanding the movements of animals underpins a host of important questions about the ecology and evolution of species and is essential for conservation and for disease management. With the limits of traditional methods such as mark-recapture or direct tracking hindering research on broad-scale movements, forensic approaches, including stable isotope analysis, have been developing rapidly. In particular the use of stable hydrogen isotopes (δ^2H) to infer movement patterns has become increasingly popular over the past decade, helping establish population connectivity over large geographical scales, but its use at smaller scale has been limited. Here, we investigate the performance of a δ^2H approach to study the movements of a bat species at the regional scale. We measured δ^2H in fur of *Eptesicus serotinus* sampled extensively across its range in the south of England during the moult period. We examined the data for spatial autocorrelation and for its correlation with δ^2H in precipitation using linear mixed-effects models. A geospatial pattern in δ^2H in fur was revealed, including an interaction of latitude and longitude, as well as a relationship with δ^2H in precipitation. However both models performed poorly, due to high within-location variance relative to between-location variance. This variance could be related to ecological, physiological and environmental factors and hampers the application of this approach on its own for the geographical assignment of this species at small scale. Despite these limits, the geospatial pattern observed may be used in combination with other markers such as $\delta^{13}C$, $\delta^{15}N$ and genetics, to discriminate among potential sampled origins of bats.

5.2 Introduction

The knowledge of a species' ecology and behaviour at all stages of its annual cycle is essential to understand the ecological and evolutionary processes shaping its life history, at both the individual and the population levels. Of particular interest is the geographical connectivity of individuals between different stages of their annual cycle and its consequences at the population level. For example, the strength of migratory connectivity, describing the movement of individuals between summer and winter populations, influences the partitioning of genetic variation in some migratory species (Webster et al. 2002). Furthermore, it has conservation implications since the knowledge of year-round geographical ranges and habitat requirements of a species is necessary for its management. In addition to conservation, the study of an animal's annual cycle, movements and migratory connectivity is essential for epidemiological studies of wildlife and their diseases, especially those of policy and public health concern. Hence, direct animal movements can be responsible for disease spread, and this risk is heightened in migratory populations that could cover a large geographical range and come into contact with other migratory or resident populations, and other species. For instance it has been shown that the resident population of *Anas platyrhynchos* in California acted as a year-round reservoir for avian influenza virus, transmitting the virus to migratory populations over-wintering at this lower latitude, and thus contributed greatly to viral circulation at the inter-continental scale (Hill et al. 2012).

Extrinsic marking techniques, ranging from simple coloured tags to complex satellite devices or light-sensitive geolocators, have considerably advanced the study of animal movements. However, these methods have limitations, including bias in marking efforts, cost, physical restrictions (for example, small species cannot carry a large satellite device), and the often small sample size they are associated with (Hobson & Norris 2008). In contrast intrinsic markers do not require recapture, allowing cost-effective sampling of a large number of individuals that can be used to infer movements. For instance, genetics studies in bats have been exploring the effects of dispersal, mating systems and migration on gene flow, helping to understand the spatial ecology of these

species (Moussy et al. 2013). Likewise, the stable isotope ratios of some elements in the tissues of consumers have the potential to yield information on movement (Hobson 1999, Crawford et al. 2008). Of particular importance for the study of migration, connectivity and animal movement is the use of stable hydrogen isotope ratios (^{2}H: ^{1}H expressed as $\delta^{2}H$ or δD) that has been successfully applied to many species, including birds (Chamberlain et al. 1997, Hobson 2005, Kelly et al. 2005, Langin et al. 2007, Marquiss et al. 2012), mammals (Cryan et al. 2004, Britzke et al. 2009, Hénaux et al. 2011, Sullivan et al. 2012, Fraser et al. 2012) and insects (Wassenaar & Hobson 1998, Hobson et al. 1999, Miller et al. 2012). This method relies on the assimilation of naturally occurring isotopic markers from the environment to animal tissues through their diet and drinking water. A strong and consistent spatial pattern in $\delta^{2}H$ distribution is observed at the global scale with $\delta^{2}H$ varying latitudinally, altitudinally and with distance from the ocean. Geostatistical methods have been developed to describe and map the distribution of $\delta^{2}H$ in precipitation, creating precipitation isoscapes (Bowen 2003, 2010, Bowen et al. 2005).

$\delta^{2}H$ values in animal tissues reflect those in surface waters and precipitation at the location of synthesis, meaning that if the isoscape is well resolved, it is possible to use this signature to gain information on animal movement. Metabolically active tissues will retain this information according to their turnover rates, which range from a few days for blood plasma to months for bone collagen. Metabolically inactive tissues such as keratin-based fur, hair or feather, will “lock-in” this information in as they are grown. The steps for studying animal movement using stable isotopes therefore require to: a) choose a tissue that is temporally suitable for the study, b) describe and characterise the isotopic signature of these tissue samples and c) relate the isotopic composition of the tissue to a suitable isoscape to infer origin. Several approaches are being developed for the geographical assignment of samples based on their stable isotope values, ranging from the simple “map lookup” and regression, to highly complex probability surface techniques that incorporate several sources of variance and uncertainties (Wunder & Ryan Norris 2008, Wunder 2012). However, many improvements are still needed for a more accurate geographical assignment, and especially at small scale (Kelly et al. 2008) where physiological, ecological, behavioural and environmental sources

of isotopic variance can lead to deviation from the global isotopic average and hinder the performance of geographical discrimination (Smith & Dufty Jr 2005, Wunder & Ryan Norris 2008, Hobson, Van Wilgenburg, Wassenaar, et al. 2012, Popa-Lisseanu et al. 2012).

In this study, we aimed to investigate the use of the δ^2H approach to resolving animal movement on *Eptesicus serotinus* within its English range. The summer ecology of this bat species has been documented to some extent, including its feeding behaviour, habitat use and roosting ecology (Catto et al. 1994, 1995, 1996, Verboom & Huitema 1997, Harbusch 2003, Harbusch & Racey 2006). However, its winter ecology is still unknown and so are the movements connecting the summer and winter roosts. Genetic studies revealed female philopatry and limited movements at regional scale (Smith *et al.* 2011; Chapter 3) while $\delta^{13}C$ indicated both short- and longer-term individual movements at a finer scale (Chapter 4). The aims of this study were thus to investigate geographical patterns of δ^2H variation in fur samples from *E. serotinus* collected at maternity roosts throughout its range in south of England during the moult period, and to examine its correlation with δ^2H in precipitation. This would allow us to assess the potential of the δ^2H approach for the study of a bat species at regional scale.

Table 5.1: Roost location, geospatial information, sample size and δ^2H values in fur (δ^2H_F) and in precipitation (δ^2H_P)

Roost ID	Longitude	Latitude	Elevation (m)	Sample size	Mean δ^2H_F (‰)	SD δ^2H_F (‰)	Mean annual δ^2H_P (‰)	Annual growing δ^2H_P (‰)
9	0.98	51.21	76	23	-47.68	2.94	-54.47	-52.26
11	-0.06	50.91	115	5	-53.56	4.79	-53.77	-50.87
12	-0.13	50.85	76	19	-55.61	6.12	-53.77	-50.87
15	-0.19	51.20	69	19	-55.97	3.98	-57.79	-52.45
16	-0.40	51.37	25	21	-56.80	5.03	-54.84	-52.36
17	-0.22	52.13	60	9	-54.73	8.94	-56.55	-54.06
18	0.44	52.30	49	6	-55.63	6.44	-56.96	-54.43
19	-0.81	50.84	41	7	-57.44	3.57	-54.21	-50.84
20	-0.99	50.86	4	29	-53.18	5.30	-54.40	-50.84
21	-1.56	51.24	105	7	-52.46	2.42	-54.31	-51.88
22	-2.05	51.47	76	13	-59.79	5.08	-55.09	-52.23
23	-2.35	51.42	95	37	-65.85	3.96	-54.64	-51.94
26	-2.70	51.30	189	4	-63.10	6.88	-55.29	-50.82
27	-2.61	51.23	153	6	-60.07	4.02	-54.85	-51.71
29	-2.18	50.86	52	9	-58.41	3.76	-53.40	-50.42
30	-2.74	50.73	61	6	-55.93	5.09	-53.66	-50.19
31	-2.73	50.76	67	11	-60.56	4.60	-53.66	-50.19
33	-1.15	50.67	11	13	-51.30	6.53	-52.84	-50.48
34	-1.19	50.67	23	6	-57.50	4.81	-52.84	-50.48
35	-1.21	50.71	17	10	-44.90	4.80	-52.84	-50.48
36	-1.29	50.69	30	11	-45.92	2.82	-52.84	-50.48

5.3 Methods

5.3.1 Sample collection

Individuals were captured at maternity roosts across their known range in England from June to August 2010 and July to August 2011 (Table 5.1; Figure 5.1). The age class (juvenile or adult), sex and breeding condition (non-breeding, pregnant, lactating or post-lactating) of each individual were determined, their forearm length and mass were recorded and a forearm band

was fitted for identification (Mammal Society, UK). A small amount of fur (less than 5mg) was clipped from the lower dorsal area and stored at ambient temperature until further processing. All bats were released successfully within a few minutes of the procedure, which was performed in accordance with licenses under the Animals (Scientific Procedures) Act 1986 and from Natural England.

5.3.2 Stable isotope analysis

Fur samples from the 2010 season were cleansed of surface oils by soaking in 2:1 chloroform/methanol for 24 hours, rinsed and left to air dry. Fur samples from 2011 were stored in 2:1 chloroform/methanol for fifteen days, rinsed and left to air dry. Each cleaned fur sample was analysed in duplicate and 1 mg (± 0.2 mg) was weighed into a tin capsule (Elemental Microanalysis, Okehampton, UK). Weighed samples in open capsules were equilibrated for 96 hours in ambient laboratory air along with Bavarian and Paraguayan horse hair standards (Institut für Rechtsmedizin, University of Münich, Germany) before closure and analysis (Wassenaar & Hobson 2003). The values of these standards are consensus values obtained by the Institut für Rechtsmedizin (University of Münich, Germany), Bundeskriminalamt (Germany), Isolab GmbH (Germany), Bayerisches Landesamt für Gesundheit und Lebensmittelsicherheit (Germany), the Faculty of Earth and Life Sciences (University of Amsterdam, Netherlands) and the Food and Environment Research Agency (UK) over the last three years. δ^2H analysis was performed by continuous–flow isotope-ratio mass-spectrometry on a Thermo-chemical Conversion Elemental Analyser (TC-EA, ThermoFisher, Bremen, Germany) coupled to a Finnigan Delta Plus XP via a Conflo III interface (ThermoFisher, Bremen, Germany). Stable hydrogen isotope ratios were reported in units per mill (‰) relative to Vienna-Standard Mean Ocean Water (V-SMOW) following the standard equation:

$$\delta^2H = ((R_{sample}/R_{standard}) - 1) \times 1000$$

where R is the ratio of the heavy to light stable isotope of the sample and of the reference respectively (i.e. ^2H: ^1H). Data were also adjusted for the effects associated with exchangeable proteinaceaous hydrogen using the horsehair

standards. Measurement precision based on the repeated analysis of the standards was 2.65‰ (standard deviation SD).

5.3.3 Additional data

The altitude at each maternity roost was extracted from the GTOPO 30 global digital elevation model (USGS, USA) on ArcView 9.2 (Esri, USA) based on their geographical coordinates. The δ^2H values in mean annual precipitation (Bowen 2003) and in growing season precipitation (Bowen et al. 2005) were extracted from the ArcGIS rasters available on WaterIsotopes.org for the geographical coordinates of each maternity roosts. The δ^2H values in rainfall were not modelled for five locations due to their close proximity to the sea and the resolution of the water isoscapes. In these situations, the δ^2H value of the closest raster cell was assigned to these locations. Body condition was obtained from the residuals from ordinary least squares regression of mass against forearm length (Pearson $r = 0.47$, $p<0.001$) (Schulte-Hostedde et al. 2005).

5.3.4 Statistical analysis

We wanted to establish if δ^2H in bat fur could be modelled at a small scale for further studies on bat movements and assignment to origin. For this purpose, we aimed to determine if the δ^2H variation in bat fur (δ^2H_F) within the south of England was linked to geographical location and to individual variation. Furthermore, we explored the relationship of δ^2H_F with δ^2H in precipitation (δ^2H_P) to ascertain the use of isoscapes for the study of individual movements at a small scale. We therefore used 271 samples of known origin, i.e. sampled at maternity roosts during the moult period (July and August) (Chapter 4), for statistical modelling in R. 2.15.1 (R Development Core Team 2011).

To explore the contribution of geography, time and individual factors in generating δ^2H_F variation, three sets of generalised linear mixed models were used: a) geographical, b) temporal and c) individual variation, with year and maternity roost as random factors to account for the differential storage of the samples in 2010 and 2011 and for the effect of pseudo-replication. All models were fitted with a normal error structure after checking for the assumptions of

normality of residuals and homoscedasticity of variance. a) The geographical full model included longitude, latitude, elevation and the three-way interaction. Simplification was conducted using a backward step-wise procedure and maximum likelihood method, with non-significant terms removed until no significant change in deviance was observed. b) The temporal model examined the variation of δ^2H_F across months. c) Candidate models for individual variation were considered, based on a full model with age, sex, body condition and its three-way interaction as well as the two-way interaction of breeding condition and body condition index. This model set up was used since only adult females varied in breeding condition while the body condition could vary with age, sex or breeding condition. Since we wanted to explore the individual factors that could best describe the δ^2H_F variation, we used AIC_C (Akaike's Information Criterion adjusted for small sample size) and AIC model weight (Burnham & Anderson 2002) to select the most parsimonious model and identify the individual parameters the most important to δ^2H_F variation. Finally, a general model was built to integrate the best geographical, temporal and individual predictors of δ^2H_F revealed previously, and compared to the previous models and to an intercept only model using AIC_C in order to identify which factors and interactions are best at explaining the δ^2H_F variation.

It was observed that δ^2H_F was more strongly correlated with mean annual δ^2H_P (Pearson $r = 0.27$, $p<0.001$) (Bowen 2003) than with growing season δ^2H_P (Pearson $r = 0.12$, $p<0.05$) (Bowen et al. 2005). Mean annual δ^2H_P was thus used as a linear covariate in subsequent modelling while growing season δ^2H_P was not considered further. To investigate the relationship between δ^2H_F and δ^2H_P, a generalised linear mixed model was built with month and age and the three-way interaction as predictors (hereafter referred to as isoscape model). As previously, random factors included maternity roost and year, and the full model was fitted with a normal error structure. Simplification was conducted using a backward step-wise procedure and maximum likelihood method, with non-significant terms removed until no significant change in deviance was observed. The residuals from the minimum adequate model were tested for remaining spatial variation using Moran's index of autocorrelation. They were also examined to assess if remaining variation was related to body condition index, breeding condition and their interaction by a general linear model (normal error

structure, identity link). The isoscape model exploring the relationship between δ^2H_F and δ^2H_P was then compared to the previous models describing δ^2H_F variation using AIC_C and AIC model weight (Burnham & Anderson 2002).

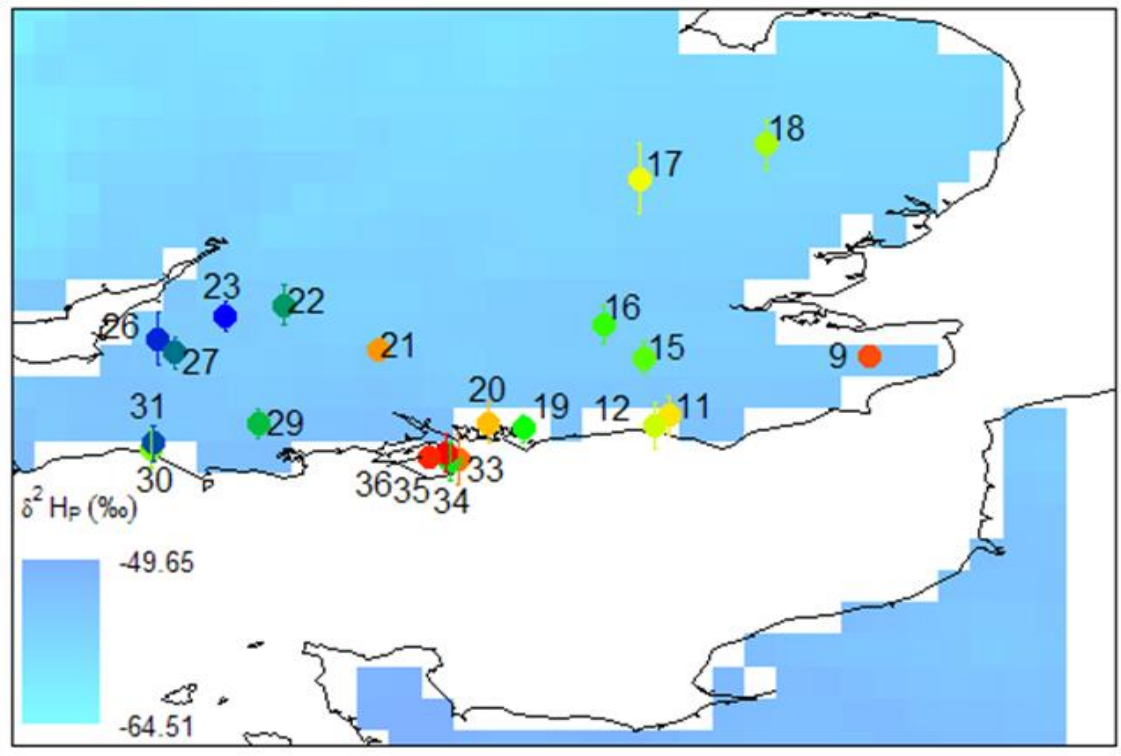

Figure 5.1: Geographical variation of mean δ^2H_F per roost in south of England.
The warmer the colour, the more enriched the δ^2H_F value (Table 1). Error bars are proportional to the standard deviation of δ^2H_F per roost. Numbers indicate roost identifiers. Mean annual δ^2H values in precipitations (Bowen 2003) are indicated as a colour gradient.

5.4 Results

We used fur samples from 271 bats captured at 21 maternity roosts (mean ± s.e. = 12.90 ± 1.91 individuals per roost) within south of England (Table 5.1; Figure 5.1). δ^2H_F ranged from -38.8‰ to -72.1‰ and the difference between two δ^2H_F measurements of the same individuals ranged from 0‰ to 9‰, but was ≤ 5‰ for 81% of all individuals sampled. Mean roost δ^2H_F varied from 44.90‰ to -65.85‰ and within roost standard deviation ranged between 2.42‰ and 8.94‰ (Table 5.1).

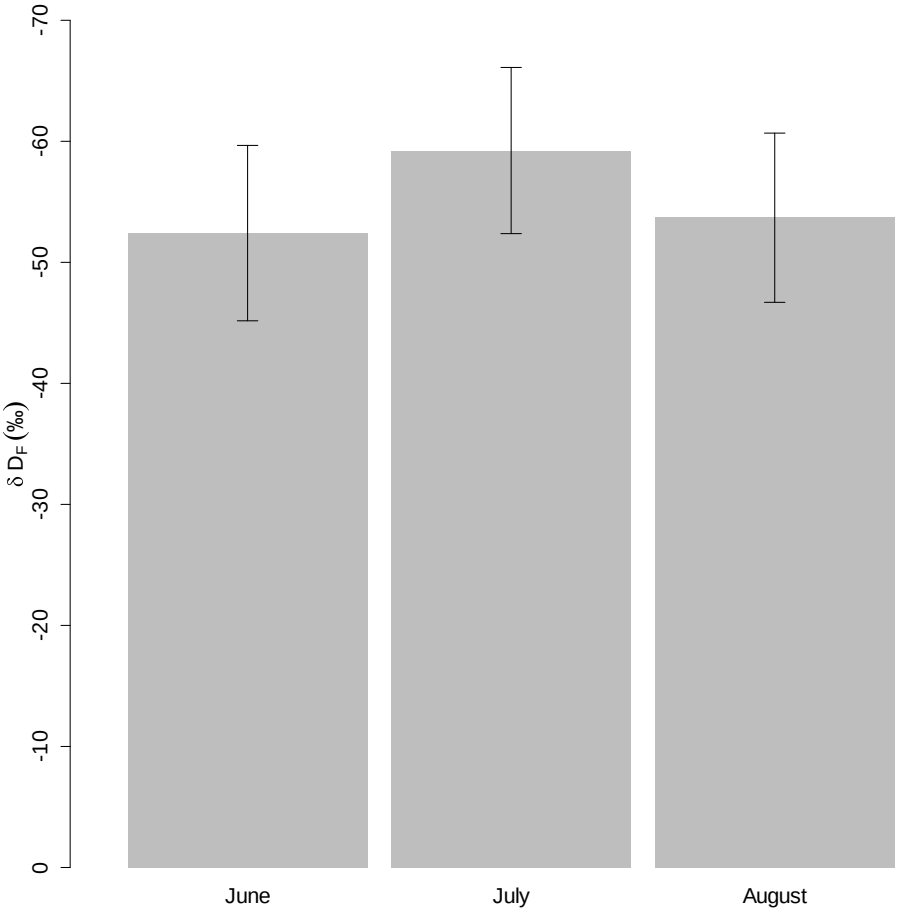

Figure 5.2: δ^2H_F variation over months.
The temporal model developed on the July and August dataset revealed no significant difference in δ^2H_F variation between these two months. Similarly, a model run on the full dataset (n = 306) confirmed that δ^2H_F did not vary significantly over the whole summer (X22, 6 = 0.784, p = 0.6758).

We examined the factors underpinning δ^2H_F variation using a range of generalised linear mixed models. The elevation term was dropped from the geographical model during simplification and δ^2H_F was found to vary with the interaction of latitude and longitude ($X^2_{3,\ 7}$ = 19.745, p <0.001, Nagelkerke pseudo-r^2 = 0.07; Figure 5.1). No temporal variation occurred between July and August ($X^2_{1,\ 5}$ = 0.333, p = 0.5638, Nagelkerke pseudo-r^2 = 0.001, Figure 5.2).

The most parsimonious individual variation model received 31.0% of the support based on model weights, and was separated from the next models by 2.08 AIC_C units (Table 5.2). This model included the age class as only predictor ($X^2_{1,\ 5}$ = 7.730, p <0.01) while the sex, body condition index, breeding condition

and all associated interactions were dropped. Juvenile samples were thus slightly more depleted in δ^2H than adult samples, by only -1.59‰ on average, and the low fit of this model (Nagelkerke pseudo-r^2 = 0.028) indicated that this age effect was only marginal.

Table 5.2: AIC table of individual models

Parameters	AICc	Δ AICc	Weight	Nagelkerke r^2
age	1619.789	0.000	0.310	0.028
age + sex	1621.867	2.078	0.110	0.028
age + body condition	1621.872	2.083	0.109	0.028
age * body condition	1623.037	3.248	0.061	0.032
age + breeding condition	1623.688	3.899	0.044	0.029
age * sex	1623.876	4.088	0.040	0.029
age + sex + body condition	1623.965	4.177	0.038	0.028
body condition	1624.101	4.312	0.036	0.013
age + sex * body condition	1624.449	4.660	0.030	0.020
breeding condition	1625.116	5.327	0.022	0.016
age * sex + body condition	1625.144	5.356	0.021	0.029
intercept	1625.442	5.653	0.018	0.000

The full model integrating all parsimonious predictors was therefore composed of latitude, longitude, age class and the three-way interaction. After model simplification, variation in δ^2H_F was found to vary with latitude, longitude, their two-way interaction and age class ($X^2_{4,\ 8}$ = 27.392, p <0.001; Nagelkerke pseudo-r^2 = 0. 096, Table 5.3). Hereafter this model will be referred to as the geospatial model.

Table 5.3: Geospatial model parameters

Fixed effects			
Parameter	Estimate	SE	t
Intercept	141.497	81.861	1.728
Longitude	-116.725	70.6294	-1.653
Latitude	-3.7724	1.5939	-2.367
Age-Juvenile	-1.5794	0.5659	-2.791
Longitude*Latitude interaction	2.326	1.380	1.686
Random effects			
Groups	Variance	SD	
Roost (n = 21)	4.6261	2.1508	
Year (n = 2)	26.5152	5.1493	
Residual	18.4478	4.2951	

Table 5.4: Isoscape model parameters

Fixed effects			
Parameter	Estimate	SE	t
Intercept	28.8321	37.8681	0.761
Mean annual δ^2H_P	1.5162	0.6959	2.179
Age-Juvenile	-1. 6044	0.5671	-2.829
Random effects			
Groups	Variance	SD	
Roost (n = 21)	9.9723	3.1579	
Year (n = 2)	14.0179	3.7440	
Residual	18.3851	4.2878	

δ^2H_F was found to vary significantly with δ^2H_P, confirming the relationship between δ^2H in rainfall and in animal tissues, and juveniles were slightly more depleted in δ^2H (-1.61‰ on average) than adults ($X^2_{2, 6} = 12.082$, $p < 0.01$; Table 5.4; Figure 5.3). The body condition index and breeding condition did not account for any of the residual variation ($X^2_{1, 5} = 0.0191$, $p = 0.8899$ and $X^2_{2, 6} = 0.1505$, $p = 0.9275$ respectively). Moreover, no spatial autocorrelation remained in the residual variation (Moran's I = 0.0014, p = 0.4448).

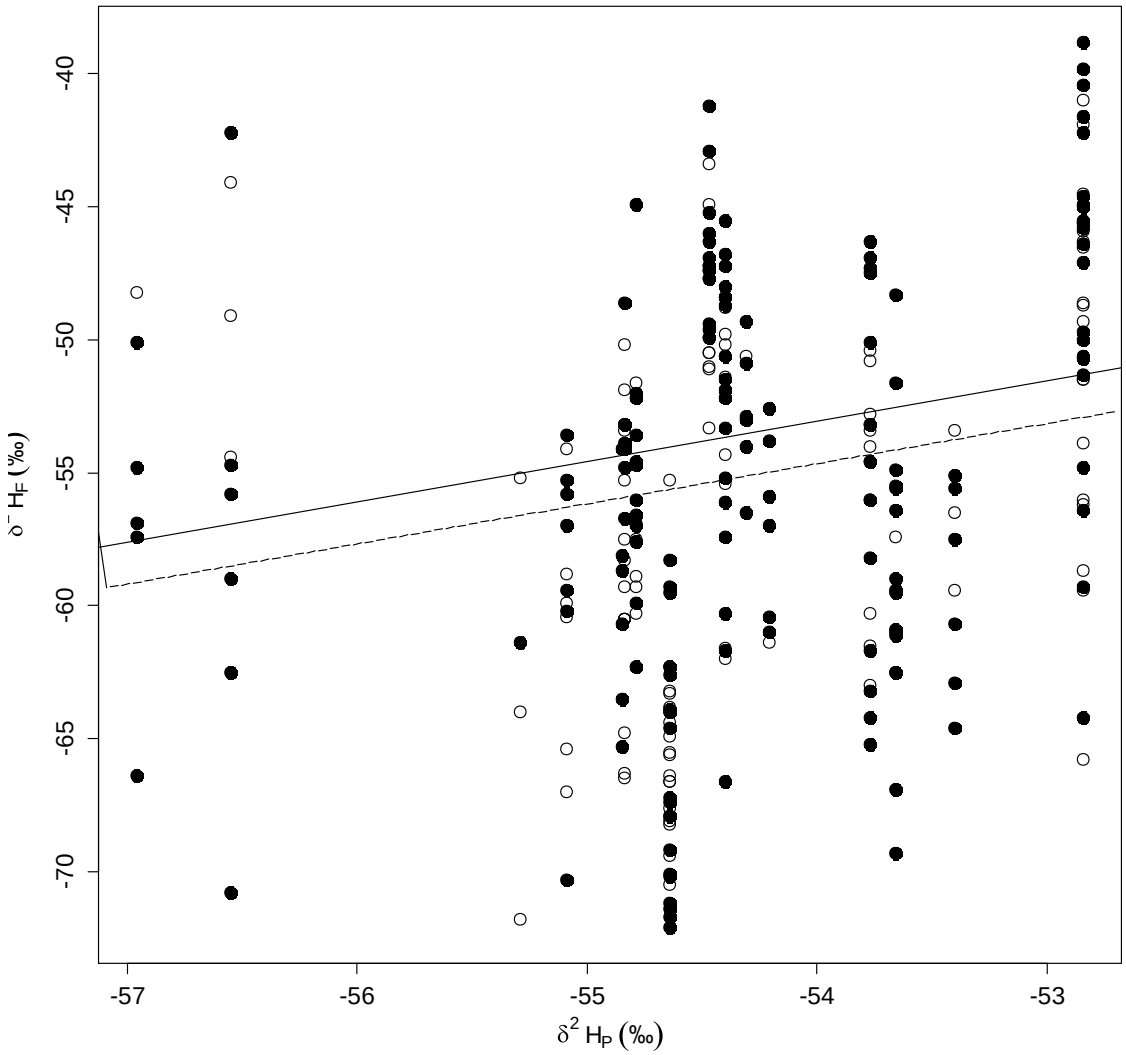

Figure 5.3: Relationship between δ^2H_F and δ^2H_P.
Closed and open circles represent adult and juvenile individuals respectively. Solid and dashed lines indicate the regression lines obtained from the isoscape general linear mixed model for adults and juveniles respectively.

Model selection based on AIC_C indicated that this isoscape model was less parsimonious than the geospatial model (Table 5.5) and its fit was poorer (Nagelkerke pseudo-r^2 = 0.043). In contrast, the geospatial model including longitude, latitude, their two-way interaction and the age class received 93.6% of support based on model weight and was separated from the next model, the geographical model, by 5.54 AIC_C units (Table 5.5). Although the geospatial model was found to be the most parsimonious at explaining δ^2H_F variation in south of England, its fit was poor (Nagelkerke pseudo-r^2 = 0.096). The large standard errors associated with the intercept and longitude (Table 5.3)

highlights the poor performance of this model and its lack of discriminatory power at this spatial scale.

Table 5.5: Comparison of the different models investigated for the description of δ^2H_F variation across England

Model	Parameters	AICc	Δ AICc	Weight	Nagelkerke r^2
Geospatial	latitude*longitude +age	1606.450	0.000	0.936	0.096
Geographical	latitude*longitude	1611.988	5.538	0.059	0.070
Isoscape	δ^2H_P + age	1617.590	11.140	0.004	0.043
Individual	age	1619.789	13.339	0.001	0.028
Precipitation	δ^2H_P	1623.373	16.923	0.000	0.015
Null	intercept	1625.442	18.992	0.000	0.000
Temporal	month	1627.185	20.735	0.000	0.001

5.5 Discussion

Naturally occurring δ^2H variation has been applied to the study of animal movement over large geographical scale (hundreds to thousands of kilometres) (Cryan et al. 2004, Kelly et al. 2005, Bowen et al. 2005, Langin et al. 2007, Hirt 2008, Marquiss et al. 2012, Sullivan et al. 2012), however this approach has seldom been used at smaller spatial scales. In this study we investigated the δ^2H variation in *E. serotinus* fur collected throughout its range in the south of England during the moult period for geospatial patterns, and examined its relationship with δ^2H in precipitation (Bowen 2003).

Despite the restricted regional scale studied, a significant relationship between δ^2H_F and δ^2H_P was revealed (Figure 5.3, Table 5.4), confirming that *E. serotinus* diet is directly linked to local precipitation-based food webs and thus that a δ^2H approach is applicable. Furthermore, a significant geospatial pattern of δ^2H_F distribution was found, with δ^2H_F varying according to the interaction of latitude and longitude (Figure 5.1, Table 5.3). Hence, samples collected in west England were more depleted than those collected in the east and samples were getting more depleted the northern their location. Such patterns are driven by a combination of global and regional climatic influences. As such, latitude is one

of the main geospatial parameters used to describe the global distribution of δ^2H in precipitation (Bowen 2003), and thus in our fur samples. Furthermore, global patterns such as depletion of heavy isotopes as distance to the coast increases also contribute to shape δ^2H distribution (Bowen 2003). In the south of England, the distance from the coast is highly correlated with latitude (Pearson $r = 0.85$, $p < 0.0001$) so its effect on δ^2H might strengthen the latitudinal effect. At the regional scale, the effect of the Atlantic Ocean would drive the longitudinal pattern observed by bringing higher amount of precipitation, δ^2H depleted, over south west England, while east of England remains drier and warmer during the summer (Darling & Talbot 2003, Darling et al. 2003).

A significant effect of age was also found for both the geospatial and the isoscape models, as juvenile bats were more depleted in δ^2H_F than adults. This age effect has been reported in other bat species such as *Lasionycteris noctivagans* and *Lasiurus cinereus* and nursing *Myotis lucifugus* pups were also found to be depleted in δ^2H_F compared to their mother (Fraser 2011). Juvenile bats grow their pelage when feeding exclusively on milk, and this isotopic depletion could thus be due to trophic discrimination occurring during milk production. However, some bird species also display this age effect while the diet of young birds is similar to their parents' (Meehan et al. 2003, Smith & Dufty Jr 2005, Langin et al. 2007). This would suggest that δ^2H_F depletion in young animals may be driven by physiological processes rather than differences in diet, although in mammals, a combination of both could be occurring.

We were also expecting other physiological processes, associated with sex, reproductive status or body condition, to drive δ^2H_F variation, but none of these parameters were retained in the individual model and they did not account for any remaining variation from the δ^2H_F - δ^2H_P relationship. For instance, the physiological burden of breeding could have had an effect on isotopic discrimination and influence individual variation in δ^2H_F. In our study however, bats were sampled during the moult period, occurring mostly post-partum (Chapter 4), and it is possible that the physiological state of post-lactating and non-breeding bats is similar enough to not affect isotopic discrimination. Furthermore, most adult bats sampled were in the post-lactating stage (107

individuals) and only few were lactating (13 individuals) or non-breeding (28 individuals). Therefore, the unbalanced sampling would lack statistical power to detect any effect of breeding condition on δ^2H_F variation. Similarly, body condition would have been expected to affect δ^2H discrimination through some physiological process. However, in this study, low variation in body condition index (mean = 0.39, SD = 0.053) was recorded.

Despite significant δ^2H_F relationships with the latitude-longitude interaction and with δ^2H_P, the fits across models were poor (Nagelkerke pseudo-r^2 = 0.096 and 0.043 respectively). Weak relationships between δ^2H in tissues and in precipitation have also been recorded at large scale in other species including in raptors (r = 0.30) (Lott et al. 2003) and in bats (e.g. *Lasiurus borealis* r = 0.26 and *Myotis lucifugus* r = 0.41 (Britzke et al. 2009)). In these studies, these weak correlations were attributed to the diet of these species potentially not directly linked to precipitation-based food webs (Lott et al. 2003) and to other factors including variation in moult timing and duration, movements, inter-annual variation, physiological differences among individuals and limitations of the current precipitation isoscapes (Britzke et al. 2009).

Similarly, in our study, the weak relationships between δ^2H_F and the latitude-longitude interaction or δ^2H_P were due to the high within-roost variance relative to the among-roosts variance (Table 5.5: within-roost SD = 4.85, among-roost SD = 5.24) and this local variance could be due to several non-measured factors, including individual and environmental variations. For instance, heat was found to increase body δ^2H values through elevated body evaporation in other taxa (McKechnie et al. 2004, Powell & Hobson 2006), so it wouldn't be surprising that variation in thermoregulatory behaviour among individual bats sharing a roost, through the use of torpor, could contribute to generating δ^2H_F variance.

Differences in diet and in the relative contribution of drinking water and diet in body water composition could also generate δ^2H variance (Kelly et al. 2008). In this respect, foraging substrate had a significant effect on δ^2H variation in bird feathers, mediated by different diet discrimination factors (Hobson, Van Wilgenburg, Wassenaar, et al. 2012). Similarly, resource partitioning in bats sharing a roost, through foraging in different areas, and/or feeding on different

prey species or even through individual specialisation (e.g. *E. fuscus* (Cryan et al. 2012)), or roost switching movements, exposing individuals to other foraging sites (Chapter 4), could result in δ^2H variation. Indeed, bats foraging at different sites could display different δ^2H_F due to environmental isotopic heterogeneity at local scale. This is a known challenge for the use of δ^2H in animal movement studies (Hobson 2008, Kelly et al. 2008, Wunder & Ryan Norris 2008) as local processes are not scaled up and incorporated in global isoscapes. For instance, variability in the source of water to local food webs driven by local climatic processes could generate small-scale isotopic variance. Unfortunately in England, only one collecting station is used monthly to monitor isotopes in precipitation (Bowen 2003, Darling et al. 2003) and this scarcity of data means that it is difficult to understand how δ^2H_P varies locally and how it influences local food webs.

Furthermore, isotopic values may vary over time, both seasonally and inter-annually (Darling et al. 2003, Wunder & Ryan Norris 2008) in relation to rainfall patterns and may thus depart from averages (Rubenstein & Hobson 2004). This temporal variation is thus expected to be more problematic at small scales due to local climatic influences. In this study, we found no significant isotopic variation over the summer (Figure 5.2), however, δ^2H_F depletion of nearly 10‰ between 2010 and 2011 was recorded. Such high isotopic variance among years has not been recorded in other species, whether from bats (Cryan et al. 2004) or from birds (Hobson, Van Wilgenburg, Wassenaar, et al. 2012). This inter-annual variation could be due to local climatic variation among the two years (Darling & Talbot 2003, Darling et al. 2003) and highlights the need for better continuity of isotopic measurements in space and time (Bowen 2010) to be able to build study-specific isoscapes explicitly incorporating time (Bowen et al. 2010, Hobson et al. 2010). However, differences in preservation methods between 2010 and 2011 could also have created this δ^2H_F shift. Supporting this, we have also noticed a significant shift in $\delta^{13}C$ values in fur between the 2010 and 2011 samples, but not in the wing samples that have been processed in the same manner during both years (data not shown). This study thus calls for more empirical evidence on sample preparation techniques and their potential effect on isotopic composition of tissues. Since we were unable to quantify the relative contribution of environmental and storage factors, this inter-annual variation

was absorbed by using year as a random intercept in the geospatial and isoscape models.

5.6 Conclusion

Using δ^2H to establish connectivity of animal populations in different stages of their annual cycle rests on the assumptions that a) δ^2H in tissue has a clear relationship with δ^2H in precipitation or geospatial parameters (e.g. latitude, longitude, elevation), b) there is relatively low δ^2H variance among individuals at the same location and, in the case of longer term studies c) δ^2H varies only little among years. Our study revealed that at the small geographical scale of south of England, most of these assumptions were not met, hindering the use of δ^2H to infer the breeding origin of bats. Indeed, while there was a significant relationship between δ^2H in fur and in precipitation, and between δ^2H in fur and geospatial parameters, the model fits were poor (Nagelkerke r^2 = 0.043 and 0.096 respectively). Furthermore, variance in δ^2H in fur among individuals at a same location was as high as the variance between locations. Lastly, δ^2H in fur varied strongly among sampling years, although this variation could be due to the differential storage of samples between the years. Improving the resolution of precipitation isoscape, gaining a better understanding of the mechanisms driving isotopic variation at local scale (physiological, ecological, behavioural or environmental) and incorporating this variation into statistic models would be the required steps to develop a δ^2H approach applied to smaller spatial scale. However, despite low resolution, spatial patterns in δ^2H variation and significant correlation with δ^2H in precipitation were revealed. Combining these data with other intrinsic markers including carbon and nitrogen stable isotopes, but also genetic data could help reveal spatial organisation and movements at small scale.

CHAPTER 6: Species organisation at a national scale revealed by an integrated triple isotope and genetic approach

6.1 Abstract

The spatial and social connectivity of populations greatly influence their demography and genetic variation and as such, has significant implications for species ecology and evolution. These properties are usually assessed over large geographical scales, but understanding connectivity at smaller scales is often just as important. Furthermore, population organisation and connectivity can vary over time, as social units fuse and undergo fission across seasons for example. Here we integrate genetic and stable isotope approaches to reveal that patterns organisation and connectivity of a bat species, *Eptesicus serotinus* vary markedly between macro- and micro-geographic scales. Discriminant function analyses were performed using allelic data from 10 microsatellites and values of $\delta^{2}H$, $\delta^{15}N$ and $\delta^{13}C$ in fur in 256 individuals captured at 21 maternity roosts across their northern range in England. The genetic and $\delta^{2}H$ data revealed strong long-term structure at large geographical scale with 81.64% of individuals correctly assigned to one of three genetically distinct populations. Within these populations, higher assignment of individuals to their known roost was achieved using the isotope only data, and especially $\delta^{15}N$ and $\delta^{13}C$, while the inclusion of the genetic data weakened roost discrimination. This result suggests relatively strong summer segregation of the different social groups, even when they are geographically close. In contrast, genetic connectivity operates on larger scale, probably through mating within populations, and through dispersal between populations. The use of multiple forensic markers highlights the complex spatial, social and temporal interactions in this species.

6.2 Introduction

The spatial and social organisation of animal populations has important implications for the ecology and evolution of species. The properties of local populations are largely determined by population connectivity, defined as the exchange of individuals among geographically separated subpopulations. Population connectivity influences a species' demography, including the size (over space and time), growth rate, structure and distribution of populations. It also impacts patterns of genetic variation through gene flow between populations (Slatkin 1987). Reductions in population connectivity, for instance because of landscape changes or habitat fragmentation, can lead to local extinctions and to population declines. In some cases, population declines and lack of connectivity lead to the loss of genetic diversity and to inbreeding, diminishing a population's ability to evolve in response to environmental change, thereby elevating extinction risks (Hedrick & Kalinowski 2000, Frankham et al. 2004). In other cases, reproductive isolation resulting from loss of connectivity can drive genetic divergence between populations, leading to local adaptation and ultimately speciation (Lenormand et al. 2002).

Knowing how animals move and understanding their population connectivity is also essential for a complete understanding of disease ecology, and this is important for many zoonoses. Many emerging infectious diseases have an animal origin, these include avian and swine flu, rabies, or plague (Kilpatrick & Randolph 2012). Knowledge of social and spatial organisation and connectivity of the vectors of these diseases is thus crucial for disease management. Defining populations and their connectivity usually occurs at large geographical scales, especially in the case of more abundant or widely distributed species (Waples & Gaggiotti 2006, Kool et al. 2012). However, within-population organisation and dynamics are also shaped by local interactive processes, such as resource partitioning, mating systems and sociality. Thus, a multi-scale approach to the study of animal populations and connectivity is likely to reveal more complex and meaningful information on species ecology than a focus at a single scale. Furthermore, changing environmental conditions, such as those associated with seasonality, drive temporal changes in population organisation

and connectivity. Hence, for social species, communities can change composition among seasons or years. Additionally, seasonal migration, dispersal and recruitment can all vary the strength of population connectivity. Therefore it is vital that both spatial and temporal variability are considered in evaluating animal population connectivity.

Bats are interesting from a population organisation and connectivity perspective due to their diverse social habits, high potential mobility, and diverse mating systems. Many temperate bats form maternity colonies over the summer where the young are born and raised. Individual loyalty to these colonies is thought to vary annually, although female philopatry is widely reported and could promote sociality (Kerth 2008a, Moussy et al. 2013). The level of connectivity between neighbouring colonies of the same species is, however, poorly understood for most species. Connectivity among neighbouring communities can also be confounded by the use of multiple roosts by the same colony and by a fission-fusion behaviour displayed by some species (e.g. *Eptesicus fuscus* (Willis & Brigham 2004) or *Myotis septentrionalis* (Patriquin et al. 2013)). Although both of these observations suggest the occurrence of communities placed across a wider spatial footprint (i.e. occupying a network of roosts within their natal landscapes), this concept has so far been unpopular because of the limited evidence available to support it.

In contrast, the organisation of bat colonies at higher hierarchical levels and larger geographical scales has received more attention, and especially by studies investigating population genetic structure (reviewed in (Moussy et al. 2013)). Mating and hibernation are often associated with movements away from colonies (Hutterer et al. 2005) and could promote connectivity between populations and colonies. Hence, swarming sites (where mating is often thought to occur) concentrate individuals from many neighbouring colonies along with solitary bats looking for mating opportunities (Parsons et al. 2003, Rivers et al. 2006, Glover & Altringham 2008). High genetic diversity has been recorded at such sites and could promote genetic connectivity among colonies, while differentiating genetic populations at larger geographical scales (Kerth et al. 2003, Veith et al. 2004, Rivers et al. 2005, Furmankiewicz & Altringham 2007). Similarly, hibernacula have been reported to be used by individuals from

several summer colonies and mating at those sites also results in gene flow among seasonally isolated colonies, and this occurs over large geographical scale with migratory species (Petit & Mayer 2000, Rodrigues & Palmeirim 2008, Sullivan et al. 2012, Angell et al. 2013). Natal dispersal and adult relocation are difficult to quantify, although individual movements among summer colonies and among hibernacula have been reported, contributing to population connectivity (Norquay et al. 2013).

Investigating population connectivity requires the definition of population units and the quantification of movements between them. The definition of a population is often debated and ultimately depends on the aims of the study and the spatial and temporal scale evaluated (Waples & Gaggiotti 2006). For instance, in the case of a temperate bat species, a population unit could be a summer colony, a winter colony, or a group of summer and/or winter colonies that are genetically similar. Evaluating individual movements among populations is also difficult (Nathan et al. 2003). Direct methods include mark-recapture or tracking (radio- or satellite telemetry). However, such approaches are time-consuming and thus often limited to small-scale studies. Furthermore, in the case of long-lived animals, such as bats, long-term datasets are often needed to fully characterise movements and avoid biasing records with shorter term movements.

In contrast, molecular genetics can be applied to a large number of individuals over a large geographical scale. This method can be used to investigate long-term genetic processes such as detecting patterns of population structuring and as such defining population units (Waples & Gaggiotti 2006), assessing levels of gene flow among populations (Lowe & Allendorf 2010), elucidating mating systems (Rivers et al. 2005) and quantifying effective population sizes (Wang 1997, Palstra & Ruzzante 2008, Charlesworth 2009). It is also possible to characterise more recent dynamics through individual assignment to populations and identification of recent migrants (Pritchard et al. 2000, Berry et al. 2004). Molecular genetics is thus a useful tool to define populations and establish long-term and short-term genetic connectivity among them. It is however limited to individual movements associated with mating. For example, the movement of an individual within a colony moving to a neighbouring colony

within the same genetic population is likely to remain undetected while it contributes to local connectivity and social dynamism.

Coupling genetic data with other ecological data has recently been used to overcome such limits. For instance, tagging data has been used in conjunction with microsatellites to infer dispersal in a salmonid species (Wilson et al. 2004), and demographic, environmental and genetic data have been combined to test metapopulation processes (Gaggiotti et al. 2004). Similarly, the integration of stable isotopes with genetic data has informed on population connectivity and migratory patterns in birds and fish (Wennerberg et al. 2002, Boulet et al. 2006, Lopes et al. 2006, Cook et al. 2007).

The stable isotope ratios of a number of elements in animal tissues reflect those of their diet in a predictable manner. Because these ratios can vary spatially as a function of biological and biogeochemical processes, they can be used to infer the broad location in which a tissue was grown, or to compare the isotopic signatures of different individuals to infer solitary or gregarious behaviour. Stable hydrogen isotope ratios (expressed as δ^2H) have traditionally been used to infer the spatial origin of individuals and establish migratory connectivity of diverse taxa over large geographical scales utilising a consistent continental-scale spatial gradient of isotopic values in precipitations (Cryan et al. 2004, Hobson 2005, Bowen et al. 2005, Britzke et al. 2009, Van Wilgenburg & Hobson 2011, Ossa et al. 2012). Stable carbon and nitrogen isotope ratios (expressed as $\delta^{13}C$ and $\delta^{15}N$) vary more locally according to plant photosynthetic chemistry and trophic level respectively. They have been used to describe food webs and infer habitat use, diet and foraging strategies (Fleming et al. 1993, Sullivan et al. 2006, Crawford et al. 2008, Cryan et al. 2012), but also to assign individuals to their foraging areas (Pajuelo et al. 2012) and to infer migratory connectivity and dispersal in birds (Marra 1998, Graves et al. 2002).

The combination of δ^2H, $\delta^{13}C$ and $\delta^{15}N$ signatures has previously been tested with variable success for the study of individual movements and connectivity of bird, mammal and insect species (Brattström et al. 2008, Hobson, Van Wilgenburg, Powell, et al. 2012, Hobson, Van Wilgenburg, Piersma, et al. 2012, Popa-Lisseanu et al. 2012). Similarly, δ^2H has been coupled to genetic data to investigate migratory connectivity of terrestrial species (Kelly et al. 2005, Boulet

et al. 2006) while $\delta^{13}C$, $\delta^{15}N$ and genetics were used to examine population structure and connectivity in aquatic species (Charles et al. 2006, Cook et al. 2007). However, so far no published work has combined a triple isotope approach with genetic data to investigate the spatial and social organisation and connectivity of animal species.

Here, we use information from $\delta^{2}H$, $\delta^{13}C$ and $\delta^{15}N$ and from microsatellites to investigate the organisation and connectivity of a bat species, *Eptesicus serotinus*, at macro- and micro-geographic scales in England. This vespertilionid species is widely distributed throughout Europe, but is less common in England where it is restricted to the southern part of the country. Nuclear DNA revealed three genetically distinct populations in England corresponding to an eastern one, a western one and the Isle of Wight with some gene flow occurring mostly from east to west and from the Isle of Wight to the west (Chapter 3). $\delta^{2}H$ in fur of individuals revealed a latitudinal and longitudinal pattern in distribution, however, local variation was high, hampering its performance as a stand-alone marker with which to study bat movement (Chapter 5). Additionally, $\delta^{13}C$ measured in fur and wing membrane demonstrated that although maternity roosts represent strong social units, individual movement occurs on both short- and long-term during the summer, and especially after parturition, but also potentially across years (Chapter 4). This behaviour could potentially indicate an increased connectivity between colonies within the three genetically distinct populations. To investigate the strength and connectivity of the three populations, and of colonies within each population, we performed a series of discriminant analysis using isotopic and genetic data, and quantified the rate of successful assignment of individuals to the populations or roosts in which they were captured. For each analysis we estimated the contribution of genetic and isotopic data to inform on the nature and the temporal element of these discriminations.

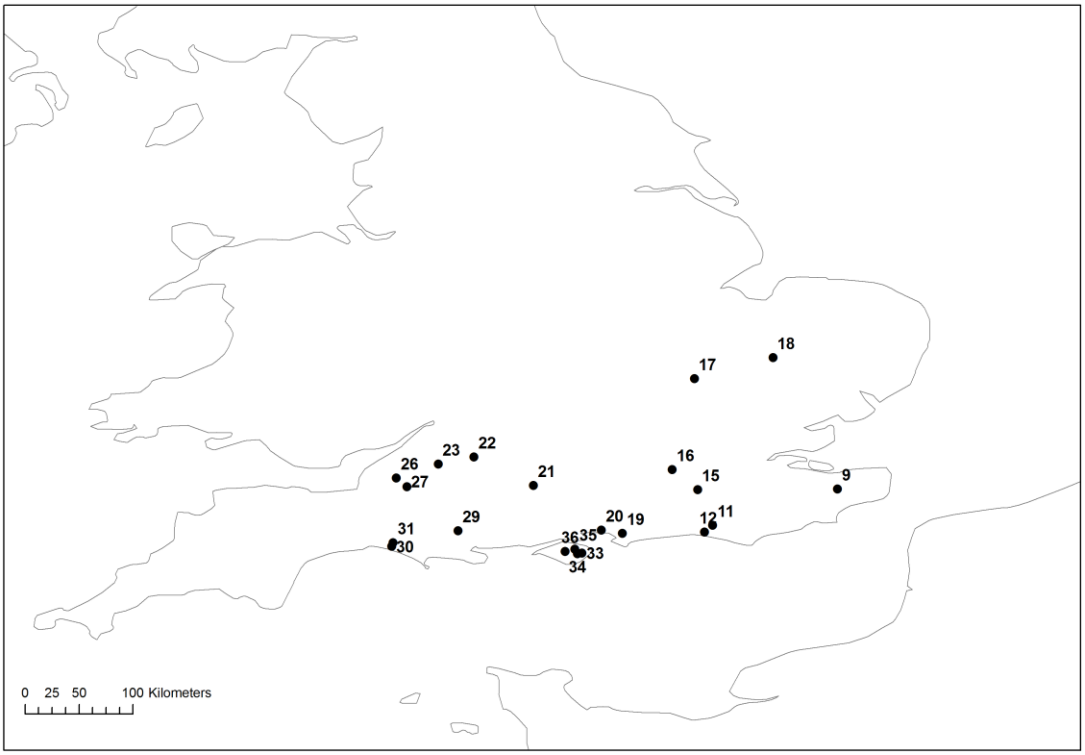

Figure 6.1: Location of the roosts sampled in south of England

6.3 Methods

6.3.1 Sample collection

We captured 290 serotine bats (*Eptesicus serotinus*) at 21 maternity roosts in the UK in the summers of 2010 and 2011 (Table 6.1; Figure 6.1). Sex, age, reproductive status and other biometric data were recorded and each individual was marked with a unique numbered forearm band for identification (Mammal Society, UK). A small amount of fur (less than 5mg) was clipped from the lower dorsal area and stored at ambient temperature until isotopic analysis. A wing tissue sample was using a 3mm biopsy punch (Stiefel Laboratories, Wooburn Green, UK) and stored in 70% ethanol at +4°C until DNA extraction. All bats were released back into the wild within minutes of the procedure at their location of capture. All the procedures were performed under license from the UK Home Office [Animals (Scientific Procedures) Act 1986] and Natural England (Habitats Regulations, 2010).

Table 6.1: Sample size and isotopic signature of each roost

Population	Roost ID	N (moult)	N (June)	Isotopic signature in fur during predicted moulting period (mean ± s.e.)		
				$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)	$\delta^{2}H$ (‰)
East	9	21	-	-23.42 ± 0.02	8.56 ± 0.04	-47.62 ± 0.15
	11	5	-	-24.92 ± 0.09	9.85 ± 0.22	-53.56 ± 0.96
	12	19	-	-25.73 ± 0.03	8.67 ± 0.04	-55.61 ± 0.32
	15	18	1	-24.95 ± 0.03	10.89 ± 0.08	-56.06 ± 0.22
	16	18	2	-24.61 ± 0.05	10.65 ± 0.04	-56.34 ± 0.29
	17	8	-	-24.23 ± 0.11	11.43 ± 0.07	-54.74 ± 1.20
	18	5	4	-24.19 ± 0.16	9.72 ± 0.22	-55.28 ± 1.43
	19	7	-	-25.75 ± 0.13	9.12 ± 0.19	-57.44 ± 0.51
	20	25	-	-23.72 ± 0.03	8.35 ± 0.03	-52.59 ± 0.21
West	21	7	-	-23.54 ± 0.02	7.23 ± 0.09	-52.46 ± 0.35
	22	13	-	-24.47 ± 0.03	10.76 ± 0.09	-59.79 ± 0.39
	23	35	-	-25.22 ± 0.02	8.83 ± 0.03	-66.14 ± 0.11
	26	4	-	-26.09 ± 0.14	10.01 ± 0.31	-63.10 ± 1.72
	27	8	-	-25.53 ± 0.10	11.32 ± 0.06	-59.31 ± 0.60
	29	8	-	-24.94 ± 0.05	9.31 ± 0.16	-58.83 ± 0.47
	30	6	8	-24.74 ± 0.06	9.98 ± 0.12	-55.93 ± 0.85
	31	11	19	-25.37 ± 0.04	9.47 ± 0.06	-61.38 ± 0.61
Isle of Wight	33	13	-	-24.36 ± 0.06	9.18 ± 0.06	-51.30 ± 0.50
	34	6	-	-25.01 ± 0.08	8.48 ± 0.16	-57.50 ± 0.80
	35	9	-	-23.16 ± 0.08	7.93 ± 0.10	-45.23 ± 0.55
	36	10	-	-23.27 ± 0.04	8.34 ± 0.04	-45.95 ± 0.30

6.3.2 Stable isotope analysis

Surface oils were cleansed from the fur samples by a 2:1 chloroform/methanol soak for 24 hours for the 2010 samples and for fifteen days for the 2011 samples. The samples were then rinsed with distilled water and left to air dry. Using a microbalance, clean fur samples were weighed in duplicate to 0.6 mg (± 0.05 mg) for $\delta^{13}C$ and $\delta^{15}N$ analysis and to 1 mg (± 0.2 mg) for $\delta^{2}H$ and placed in tin capsules (Elemental Microanalysis, Okehampton, UK). Internal standards, horse-hair from Bavaria and Paraguay (Institut für Rechtsmedizin, University of Münich, Germany), were matched to sample weight on a micro-scale.

Carbon and nitrogen isotopes analysis was performed by continuous–flow isotope-ratio mass-spectrometry on an elemental analyzer EA 1108 (Carlo Erba Instruments, Milan, Italy) coupled to an Isoprime IRMS (GVI, Manchester, UK). Carbon and nitrogen isotopes ratios were reported in units per mill (‰) relative to Vienna-Pee Dee Belemnite and atmospheric nitrogen respectively following the standard equation:

$$\delta^{Y}X=((R_{sample}/R_{standard})- 1) \times 1000$$

where R is the ratio of the heavy:light stable isotope of the sample and standard, (i.e. $^{13}C:^{12}C$ and $^{15}N:^{14}N$). The keratin standards were calibrated against International Atomic Energy Agency reference materials (USGS 40 and 41). Measurement precision based on the repeated analysis of the standards was 0.13‰ for. $\delta^{13}C$ and 0.14‰ for $\delta^{15}N$.

Prior to $\delta^{2}H$ analysis, weighed samples and standards in open capsules were equilibrated for 96 hours in ambient laboratory air before closure (Wassenaar & Hobson 2003). Deuterium analysis was performed by continuous–flow isotope-ratio mass-spectrometry on a Thermo-chemical Conversion Elemental Analyser (TC-EA, ThermoFisher, Bremen, Germany) coupled to a Finnigan Delta Plus XP via a Conflo III interface (ThermoFisher, Bremen, Germany). Hydrogen isotope ratios were reported in units per mill (‰) relative to Vienna-Standard Mean Ocean Water (V-SMOW). Data were also adjusted for the effects associated with exchangeable proteinaceaous hydrogen using the horse-hair standards. Their deuterium values are consensus values obtained by the Institut

für Rechtsmedizin (University of Münich, Germany), Bundeskriminalamt (Germany), Isolab GmbH (Germany), Bayerisches Landesamt für Gesundheit und Lebensmittelsicherheit (Germany), the Faculty of Earth and Life Sciences (University of Amsterdam, Netherlands) and the Food and Environment Research Agency (UK) over the last three years. Measurement precision based on the repeated analysis of the standards was 2.65‰.

6.3.3 Genetic analysis

DNA extraction was performed from each wing biopsy following the method reported by Sambrook & Russel (2001) for the rapid isolation of nucleic acid from mammalian tissue. Ammonium acetate (10M; Sigma-Aldrich, Poole, UK) was used to precipitate proteins instead of potassium acetate. The DNA pellet was recovered in 1 x Tris–ethylenediaminetetraacetic acid buffer (TE) (Sigma-Aldrich) and stored at -20°C. The DNA extract was then used for whole genome amplification using illustra GenomiPhi V2 DNA Amplification kit (GE Healthcare, Little Chalfont, UK) following the manufacturer's protocol.

Microsatellite genotyping was carried out using a panel of ten primers originally designed for other vespertilionidae species, optimised for cross-species amplification in *E. serotinus* (Smith et al. 2011) and described in Table 6.2. Polymerase chain reaction (PCR) was carried out in 15µl volumes, with 7.5µl 2x PCR mastermix (ABgene, Epsom, UK) containing 1.5mM $MgCl_2$, 1µM of each primer (Sigma-Aldrich/Applied Biosystems, Foster City, CA, USA), and 1.5 µl DNA template (DNA extract or 1/10 dilution in 1 x TE of whole genome amplification product). The remaining volume was made up with molecular grade water. PCR reactions consisted of denaturation at 94°C for 2 min, followed by 35 cycles of 94°C for 1 min, 1 min at the annealing temperature, 72°C for 1 min, and final elongation at 72°C for 5 min and 60°C for 1 h.

Table 6.2: Microsatellites primers
A 'pig-tailing' modification (GTTTCTT) was added to the 5' end of D15, B22 and TT20 to reduce non-templated addition of primarily adenosine nucleotides (Brownstein et al. 1996), which could otherwise lead to problems in accurate sizing of the DNA fragment. TD indicates a touch-down PCR. PCR products were multiplexed into four sets for genotyping.

Set	Locus	Forward and reverse sequence 5' to 3'	Microsatellite repeat	Annealing temperature (°C)	Number of alleles	Allele size range (bp)	Reference
1	NN8 = Mu438+9	F: NED-TTGTGTTTTAAAGAAAATCC R: ATAGGTGATTTCCATTCCCA	$(GT/CA)_{21}$	44	4	141-163	(Petri et al. 1997)
	EF1	F: 6-FAM-ATCTGGGCAATGATACCTTT R: GCAGGCTGGGCTGAG	$(GT)_{22}CT(GT)_{17}$	50	3	177-194	(Vonhof et al. 2002)
	EF4	F: HEX-ATAGGCTCCCAGAAATAGC R: GATCACCACAAAATGTGC	$(CT)_4(GT)_{17}$	48	6	217-227	(Vonhof et al. 2002)
	EF14	F: HEX-ATCATATATTTGTGTTCTGG R: AAAATCAGCTATGTAGCAC	$(GT)_{19}$	43	12	107-129	(Vonhof et al. 2002)
2	Paur05	F: 6-FAM-GGACAGTATGCCATGTTATGCTG R: GCACTTTCACAAACCTAGATGG	$(GT)_{10}$	66-56 TD	11	231-253	(Burland et al. 1998)
	AF141650	F: HEX-ACAGGAACCCTCAGAAGTGG R: TGGTCTCCTTTTCTTCACTTTGT	$(TATC)_9$	52	11	265-311	(Petit & Mayer 1999)
3	EF6	F: HEX-ATCACATTTTTGAAGCAT R: ATCTGTTTTTCTCTCCTTAT	$(GT)_{20}$	41	16	161-197	(Vonhof et al. 2002)
	EF15	F: NED-AGCAGCAAAGGGGACTCAGA R: GAGAAGCAGGGAGGGCATTT	$(CA)_3GA(CA)_{20}$	55	18	107-147	(Vonhof et al. 2002)
4	B22	F: HEX-CTGATGCAAGACCCCTTACAAC R: GTTTCTTACGGCAGCAGTGAAATCAGA	$(GT)_x$	55	2	135-137	(Kerth et al. 2002)
	TT20	F: 6-FAM-TCTTACCTCTTTTCCTGC R: GTTTCTTTTTTTTTCTTCTGTGTTACC	$(TG)_{11}$	47	6	185-195	(Vonhof et al. 2002)
5	D15	F: 6-FAM-GCTCTCTGAAGAGGCCCTG R: GTTTCTTATTCCAAGAGTGACAGCATCC	$(AC)_{17}$	61	10	127-151	(Castella and Ruedi 2000)

PCR products were diluted and mixed into four sets (Table 6.2) and run using POP-7 polymer in a 36-cm capillary array mounted on ABI Prism 3130xl genetic analyser (Applied Biosystems) with Genescan Rox 500 size standard (Applied Biosystems) following the manufacturer's instructions. Microsatellite alleles were sized using GeneMapper 3.7 software (Applied Biosystems).

6.3.4 Statistical analysis

A series of discriminant analyses was performed on the genetic and isotope data using 256 samples collected during presumed moult (in July and August; Chapter 4) and thus of known origin. A principal component analysis (PCA) with binomial scaling was used to reduce the number of genetic variables and to satisfy the requirement of independence among them. The package ADEGENET 1.3-4 package (Jombart 2008) in R 2.15.1 (R Development Core Team 2011) was used on the allelic dataset and 30 principal components (PC) were retained, representing 78.10% of genetic variance among individuals. Previous analyses of the microsatellite dataset revealed three distinct genetic populations in England, consisting of an eastern population, a western one and the Isle of Wight, despite strong gene flow connecting them (Chapter 3). PCA on the genetic data was therefore also performed on each three populations independently. For the Isle of Wight only ten PCs were retained because of the smaller sample size (n=38), representing 65.80% of genetic variance, while 30 PCs were kept for the east and the west populations (86.90% and 88.80% of genetic variance conserved respectively). The genetic information allows the evaluation of long-term patterns of connectivity (or lack of). In contrast, isotopic data inform on shorter-term spatial and social organisation of individuals, restricted to summer patterns. The combination of both types of data is expected to give some insight on the strength of individual associations at various spatial and temporal scales.

We first examined the organisation and connectivity of the species at a macro-geographical scale. Firstly, a generalised linear mixed model fitted with a normal error structure and identity link was used to determine whether the three populations had distinct isotopic signatures for $\delta^{2}H$, $\delta^{15}N$ and $\delta^{13}C$. The sampling years were fitted as random intercepts to account for isotopic variation

across years. A stepwise forward variable selection was then performed to determine which variables (genetic PCs and isotopic signatures) contributed to the highest discrimination of the three populations. The initial model was defined by starting with the best variable discriminating the populations and was then extended by selecting variables that significantly minimised the Wilk's lambda value of the model. The function greedy.wilks of the package klaR in R 2.15.1 (R Development Core Team 2011) was used for this procedure. The resulting model was used in a linear discriminant analysis with the genetic populations as group factor. Flat priors were used so the prior probability of class membership did not depend on the number of samples collected in each population. Leave-one-out cross-validation was performed to obtain the proportion of correct assignment of individuals to the population they were sampled in. The same methodology was applied to investigate the strength and persistence of maternity roosts as social units by using the roosts where individuals were sampled as grouping factors.

To understand the nature and level of connectivity at a micro-scale and whether it varies geographically, the variables contributing to the discrimination among maternity roosts and the strength of this discrimination were assessed for each of the three populations separately. The previous methodology of variable selection using Wilk's lambda criterion followed by linear discriminant analysis with flat priors and cross-validation was applied.

Finally, we assessed the inter-annual fidelity of individuals to roosts in order to assess the persistence of such social associations over longer periods. If animals were not faithful to the same roost sites across years, we hypothesised that adding individuals collected in June, whose fur was grown the previous year and potentially in other locations (Chapter 4), would lower the incidence of correct individual assignment. To test this hypothesis, we compared the results from the variable selection and discriminant analyses on the full dataset (290 individuals) and the moult-only dataset (256 individuals).

6.4 Results

There were significant differences among the isotopic signatures of the three populations for δ^2H ($X^2_{5,\ 2}$ = 91.028, p < 0.001, Nagelkerke fit = 0.300), $\delta^{15}N$ ($X^2_{5,\ 2}$ = 10.676, p < 0.005, Nagelkerke fit = 0.042) and $\delta^{13}C$ ($X^2_{5,\ 2}$ = 18.031, p < 0.001, Nagelkerke fit = 0.078) (Table 6.3), but notably the fits of the models for $\delta^{15}N$ and $\delta^{13}C$ were poorer than those of δ^2H. This is reflected in the discriminant analysis where the stepwise variable selection to optimise discrimination among the populations did not support the inclusion of $\delta^{15}N$ and $\delta^{13}C$ in the best discriminant model (Table 6.4). In contrast, δ^2H and five genetic PCs allowed high discrimination between the three populations with 81.64% of individuals assigned to the population they were sampled in (Table 6.4, Figure 6.2). Discriminant analysis using only the genetic data resulted in 75.78% of individuals assigned to their population of origin while this number dropped to 53.91% when using only isotope values (Table 6.4, Figure 6.2). When looking at the pattern of correct assignment rate among populations (Table 6.4), the isolation of the Isle of Wight from mainland England becomes apparent with the highest assignment rate (86.84%). The eastern population seems also clearly segregated with 83.33% of individuals sampled from and assigned to it. In contrast, this rate drops to 77.17% for the western population.

The roosts differed significantly in their mean δ^2H values ($X^2_{20,\ 23}$ = 183.83, p < 0.001, Nagelkerke fit = 0.513), $\delta^{15}N$ ($X^2_{20,\ 23}$ = 212.83, p < 0.001, Nagelkerke fit = 0.585) and $\delta^{13}C$ ($X^2_{20,\ 23}$ = 161.23, p < 0.001, Nagelkerke fit = 0.537) (Figure 6.3). Isotopic discrimination among roosts was reflected in the stepwise variable selection with the three isotopes retained along seven genetic PCs (Table 6.4). Using these isotopic and genetic variables resulted in 44.53% of individuals assigned to the roost they were sampled in while this assignment rate dropped to 21.09% with genetic-only information and to 33.59% with isotope-only values (Table 6.4, Figure 6.4).

Table 6.3: Isotopic values of the three populations (mean ± s.e.)

Population	Sample size	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)	$\delta^{2}H$ (‰)
East	126	-24.488 ± 0.008	9.477 ± 0.011	-53.802 ± 0.048
West	92	-25.013 ± 0.008	9.441 ± 0.015	-61.604 ± 0.067
Isle of Wight	38	-23.888 ± 0.025	8.553 ± 0.023	-49.434 ± 0.174

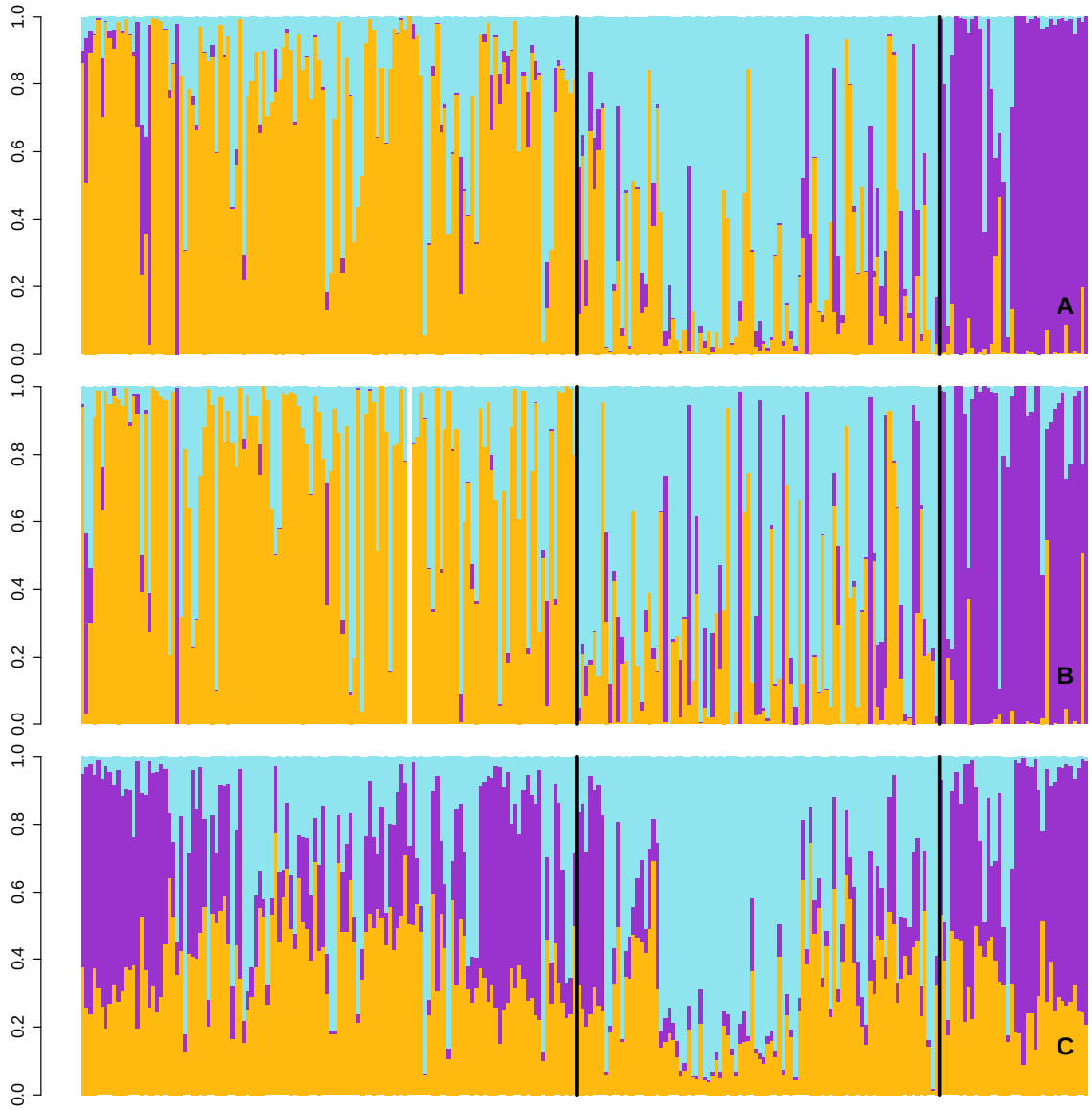

Figure 6.2: Individual posterior probability of assignment to one of the three populations. East (orange), West (blue) or Isle of Wight (purple). Solid lines partition the three populations. A- Optimal model. B- Genetic model. C- Isotopic model.

Table 6.4: Variable selection for optimal discriminant model
Percentage of correct individual assignment after discriminant analysis with leave-one out cross-validation is indicated.

		Variables selected	Percentage of correct individual assignment		
			Optimal model	Genetics (30 PCs)	Isotopes (δD, $\delta^{15}N$, $\delta^{13}C$)
Macro-geographical scale					
Among populations		δD+PC1+PC2+PC3+PC8+PC9	81.64	75.78	53.91
East			83.33	76.19	41.27
West			77.17	63.04	67.39
Isle of Wight			86.84	84.21	63.17
Among roosts		δD+$\delta^{13}C$+$\delta^{15}N$+PC1+PC2+PC7 +PC8+PC9+PC16+PC24	44.53	21.09	33.59
Micro-geographical scale					
Within east population	Moult period	$\delta^{13}C$+$\delta^{15}N$+PC1+PC4+PC7+ PC9+PC24	59.52	38.09	36.51
	Incl. June samples		59.4	36.84	36.84
Within west population	Moult period	δD+$\delta^{13}C$+$\delta^{15}N$	57.61	17.39	57.61
	Incl. June samples		47.06	20.17	47.06
Within Isle of Wight population	Moult period	$\delta^{13}C$+$\delta^{15}N$	55.26	15.79*	55.26

* Only 10PCs were included

We looked next at the organisation of *E. serotinus* at local scale within each population. At this smaller geographical scale, the isotopes $\delta^{13}C$ and $\delta^{15}N$ were always found to be important contributors to discrimination among roosts (Table 6.4). However there were differences among the three populations in the contribution of the genetic data to roost discrimination. Genetic information did not contribute to differentiation among roosts for the western and Isle of Wight populations. Hence, individual assignment rates were 57.61% and 55.26% for west and Isle of Wight respectively when using isotopic values while these rates dropped to 17.39% and 15.79% with the genetic data (Table 6.4, Figure 6.5). For the eastern population five genetic PCs and the $\delta^{13}C$ and $\delta^{15}N$ data

contributed to discrimination among roosts. This model led to an individual assignment rate of 59.52% while the genetic discriminant analysis only correctly assigned 38.09% of individuals to the roost they were sampled and this fell to 36.51% for isotopic-only discrimination (Table 6.4, Figure 6.5).

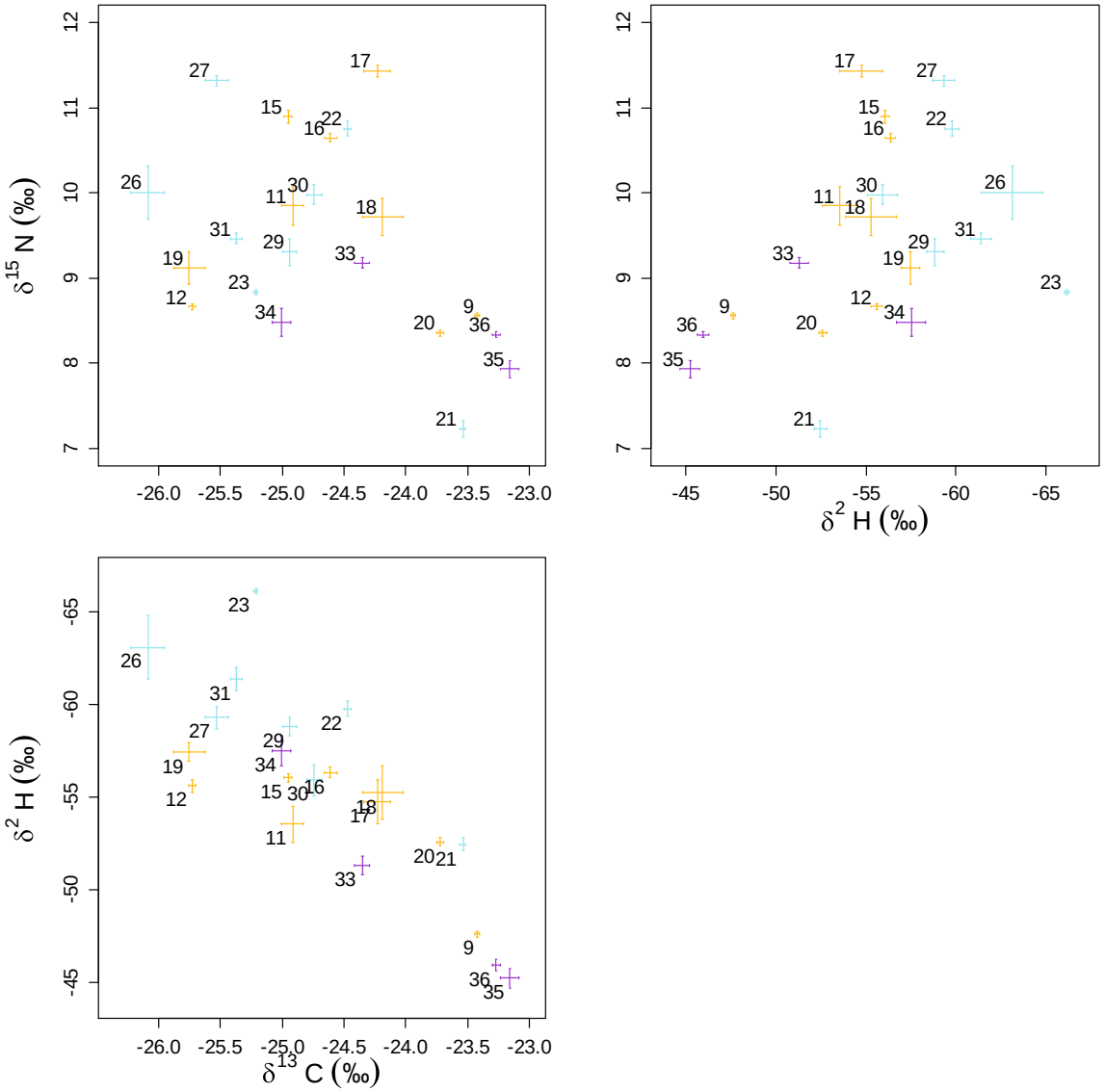

Figure 6.3: Isotopic signatures of each roost (mean ± s.e.).
Numbers indicate roost identifiants. Roosts have been assigned to the East (orange), West (blue) or Isle of Wight (purple) populations.

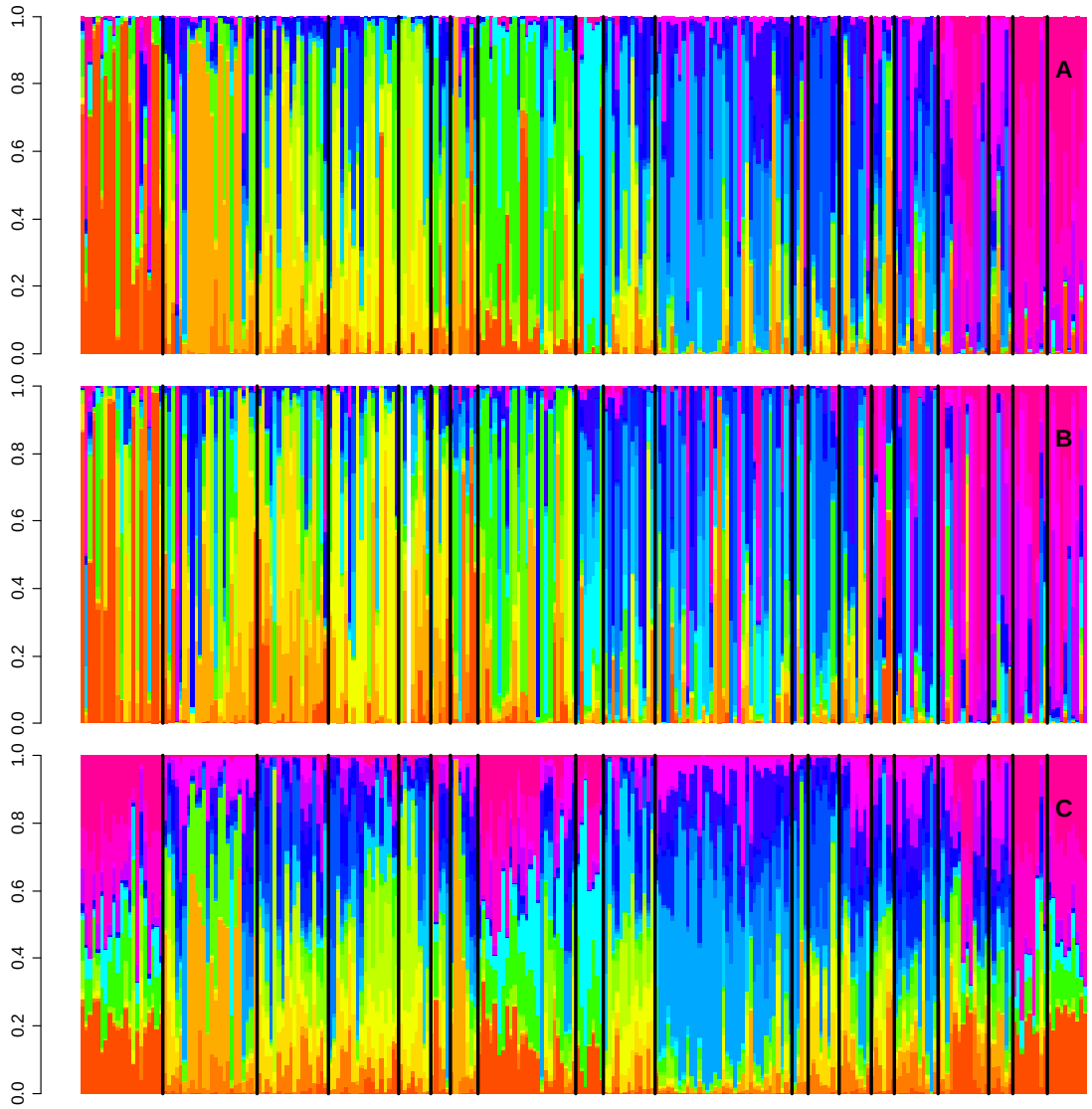

Figure 6.4: Individual posterior probability of assignment to one of 21 roosts, macro-scale analysis.
Discriminant analysis was performed at macro-scale. Each colour represents a single roost. Solid lines partition the roosts. A. Optimal model. B. Genetic model. C. Isotopic model.

Finally we assessed whether adding hair samples collected in June, and thus presumed to have grown the previous year, would result in lower correct individual assignment rates, an indication that these individuals might have spent some part of the previous summer away from their roost. Seven individuals sampled in June in the east population were added to the 126 individuals sampled during the moult period. The assignment rate did not differ for the selected model (59.40% vs. 59.52% for all samples and moult samples respectively, Table 6.4), likely because of the small sample size collected in June. In contrast, within the western population, these results were more

dramatic when adding 27 samples from June to the 92 moult samples. Isotopic discrimination in this population dropped from a 57.60% correct individual assignment to 47.06% (Table 6.4), which suggests that many individuals do not return to the same roosts in consecutive years.

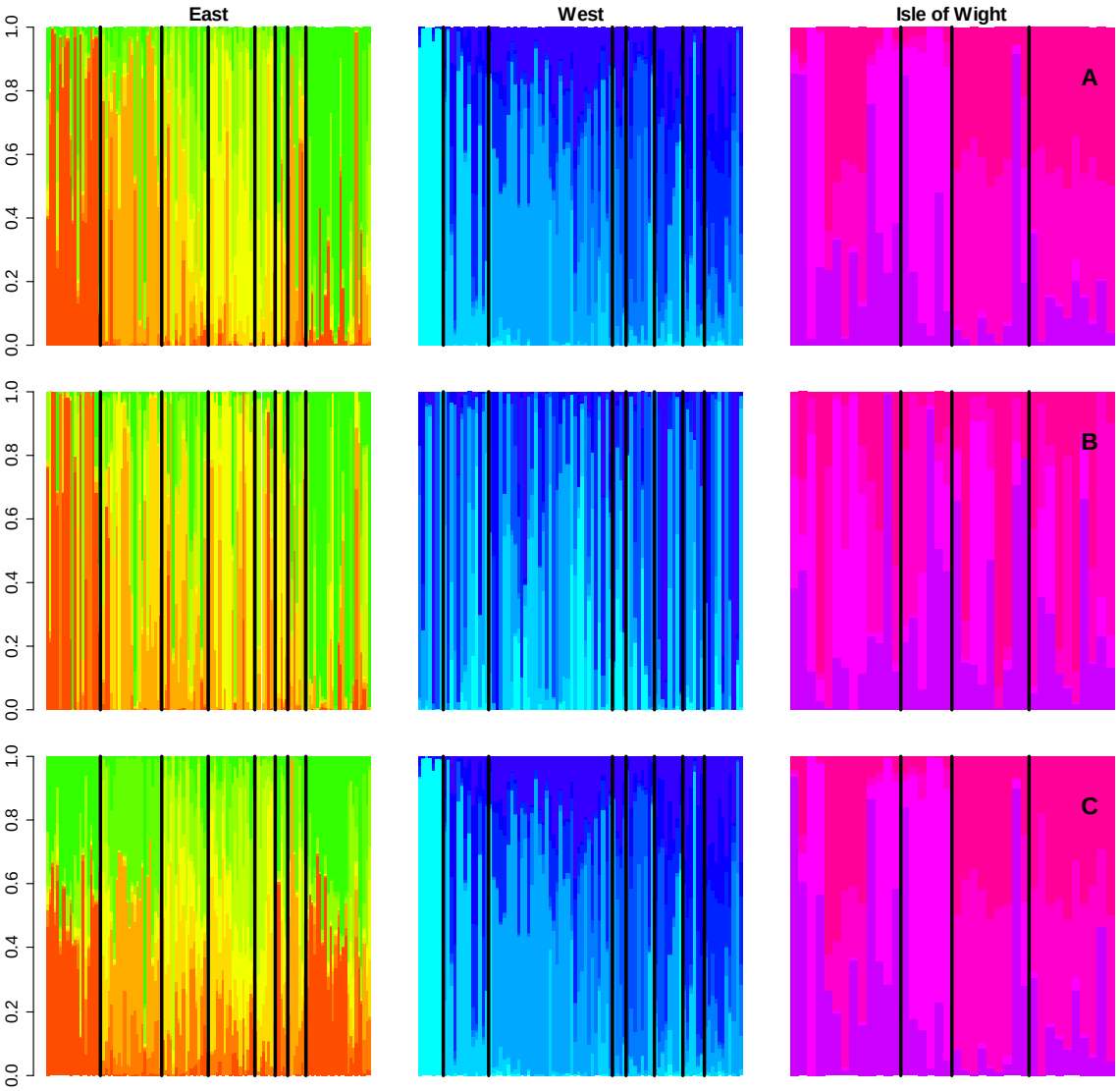

Figure 6.5: Individual posterior probability of assignment to one of 21 roosts, micro-scale analysis.
Discriminant analysis was performed at micro-scale, i.e. within each population. Each colour represents a single roost. Solid lines partition the roosts. A- Optimal model. B- Genetic model. C- Isotopic model.

6.5 Discussion

The integration of genetic and isotopic data allowed us to accurately reconstruct known spatial and temporal patterns of organisation of *E. serotinus* across its

English distribution. During the breeding period bats can be partitioned into a hierarchy with at least two levels driven by mating system, social structure and resource use. The highest level of organisation consists in three genetically differentiated populations coinciding with eastern and western English counties and with the Isle of Wight. Within these three populations, individuals form strong seasonal social units at maternity roosts over the breeding period. However not all individuals display inter-annual loyalty to these sites and a small proportion may choose to spend summers in other locations.

At this macro-scale, only five genetic PCs and δ^2H were necessary to allow the optimal discrimination among the three populations with a high correct individual assignment rate (81.64%). The inclusion of δ^2H in the model results from the longitudinal distribution in δ^2H ratios in England (as described in Chapter 5), with the eastern, western and Isle of Wight populations having significantly different δ^2H values (Table 6.3). The high correct individual assignment rate to one of the three populations using genetic data only (75.78%) indicates that this segregation is also persistent and influences mating among these populations and suggests the presence of a geographically constrained mating system which forms and maintains these genetically distinct populations. The genetic structure of *E. serotinus* in England has already been discussed in depth (Chapter 3) and could be summarised as being the product of historical events (e.g. post-glacial recolonisation, population bottlenecks), of current mating behaviour and of the effect of geographical features on individual movements. The mating system of this species is not known although it is strongly suspected to be promiscuous, consistent with a large number of temperate species (Hosken 1997, Wilkinson & McCracken 2003). Mating at swarming or hibernation sites as observed in *Myotis natteri*, *M. bechsteinii,* or *Plecotus auritus* (Kerth et al. 2003, Rivers et al. 2005, Furmankiewicz & Altringham 2007, Glover & Altringham 2008, Norquay et al. 2013), can result in a genetically homogeneous catchment area by facilitating inter-breeding among individuals that are otherwise segregated. This is very similar to the pattern observed by us in *E. serotinus*. Interestingly, the correct individual assignment rate using genetic data varied among populations, which provides information on potential movements between populations. Hence, the Isle of Wight displays the lowest genetic connectivity with 84.21% of individuals correctly assigned, and this

result may reflect reluctance to movement over open water, despite commuting to foraging sites over larger distances. In contrast, correct assignment to the eastern and western populations drops to 76.19% and 63.04%, showing not only higher genetic connectivity on the mainland, but some asymmetry in gene flow, with more movements towards the west. These results are congruent with previous findings (Chapter 3).

However, the temporal pattern of these movements cannot be strictly inferred from the genetic data. Indeed, mis-assigned individuals might not be recent migrants, but could have a strong admixed genotype. Comparative analysis of mis-assigned individuals using genetic data and δ^2H ratios could help identify recent migrants by using the different temporal properties of each marker, with genetics reflecting longer term processes and δ^2H in fur as a proxy for local signature. Hence, recent migrants could be defined as individuals whose genotypes are representative of one population but whose δ^2H signatures assign them to another. However, the genetic information used is bi-parentally inherited, so the genetic connectivity among populations could be driven by movements of both sexes. Unfortunately adult males could not be sampled and their ecology and locations are more or less unknown; it is thus not possible to infer the relative levels of male- and female-biased gene flow. Comparative analysis of nuclear and mitochondrial DNA was however undertaken (Chapter 3) and suggested gene flow was mostly driven by male movement. It is worth noting that despite the significant differences in $\delta^{15}N$ and $\delta^{13}C$ signatures among populations (Table 6.3), these isotopes did not appear in the optimal discrimination model. This could be because these markers are associated with local landscapes, and as such, are more useful at this scale.

When examining the organisation of individuals into maternity roosts at the macro-geographical scale, in addition to δ^2H and seven genetic PCs, $\delta^{15}N$ and $\delta^{13}C$ ratios were retained in the optimal discrimination model. This confirms these two isotopes are descriptors of local landscapes used by the bats rather than broad geographical areas. A much lower rate of correct individual assignment was retrieved (44.53%) at this level compared to assignment to populations, indicating potentially high individual movements. It could also be due to the fact that several roosts had similar isotopic signatures for $\delta^{15}N$, $\delta^{13}C$

or δ^2H (Figure 6.3). Nevertheless, a 44.53% rate of correct individual assignment can be regarded as high when considering this study followed a species with high mobility potential, and 256 individuals from 21 roosts separated by geographical distances of 2.86 to 280.69 kilometres. The low percentage of correct individual assignment to roosts using genetic data compared to assignment to its success at the population level (21.09% vs. 75.78% respectively) suggests that mating facilitates gene flow and homogenises gene pools at scales larger than the local summer landscape. This is consistent with a mating system that allows genetic mixing among otherwise segregated colonies.

The organisation of roosts at a micro-geographical scale (i.e. within populations) differed among populations. Only isotopic data were retained in the discriminant models for the west and the Isle of Wight populations. Furthermore, discrimination among roosts using only genetics resulted in low correct assignment rates (17.39% and 15.79% respectively). This suggests that individuals from different roosts within these populations share the same genetic make-up. In contrast, genetic information was included in the discriminant model for the eastern population, along with $\delta^{13}C$ and $\delta^{15}N$ ratios, and using genetics only, 38.09% of individuals were correctly assigned to the roost in which they were sampled. This implies that some genetic sub-structuring occurs within the eastern population. While the genetic analyses performed in Chapter 3 did not reveal further sub-structure within the east population, higher pairwise F_{ST} values among eastern roosts were noted there compared to western and Isle of Wight roosts. It would thus appear that roosts in the eastern population are more strongly segregated than roosts in the western and Isle of Wight populations. The drivers of such low connectivity are not clear. Larger pairwise geographical distances between roosts were seen in the eastern population (average pairwise distance among roosts: East =97.31 ± 48.31km; West = 57.21 ± 25.26 km; IOW = 6.10 ± 2.53 km) and this could contribute in restricting bat movement between roosts.

Isotopic ratios, especially of $\delta^{13}C$ and $\delta^{15}N$, increased discrimination among roosts for the western and Isle of Wight populations (57.61% and 55.26% correct assignment rates respectively), but were less effective for the eastern

population (36.51% assignment rate). The use of $\delta^{13}C$ and $\delta^{15}N$ rests on the principle that individuals incorporate these isotopes from their diet and their tissue signature should thus reflect isotopic composition of the local landscape. Assuming sufficient isotopic variation exists within the study population (in this study, $\delta^{13}C$ range = -26.9 to -22.2 ‰; $\delta^{15}N$ range = 6.21 to 13.63‰), individuals sharing resources are expected to have similar isotopic signatures. Bats from the same roost share the same local landscape so one could assume low isotopic variance within roost compared to among-roost isotopic variance, resulting in roost-specific isotopic signatures. The relatively high assignment rates suggest that maternity roosts constitute relatively strong, localised social units over the summer. This is consistent with radio-tracking and mark-recapture studies for the species (Catto et al. 1996, Robinson & Stebbings 1997, Harbusch 2003), and indicates that a colony might be using several roosts while displaying fidelity to a main roost and local landscape (Harbusch & Racey 2006).

While the mis-assignment of individuals could be indicative of movements to other roosts in the area for some period of time during the summer, individual variation in behaviours linked to foraging and roosting could drive isotopic variation and lead to individual mis-assignment. For instance, some species roost together but exploit different foraging sites or display individual specialisation in their foraging behaviour (Hillen et al. 2009, Cryan et al. 2012). This would increase within-roost isotopic variance and could reduce roost discrimination if among-roost isotopic variance is low. In contrast, other species form fission-fusion societies, frequently switching roosts and displaying dynamic individual associations, but remain faithful to their foraging sites (Metheny et al. 2007, Kerth et al. 2011). Roost switching behaviour would therefore not be recorded in the isotopic signature of individuals, although in this case, isotopes might help defining social units at broader scales. Some species have also been shown to partition resources among neighbouring colonies to avoid intra-specific colonisation, even when they share the same local landscape (see Chapter 4 in (Crawford 2008)) while individuals from other species display over-lapping home ranges among neighbouring colonies. Overlap has been observed in *E. serotinus* using radio-tracking indicating some level of resource sharing among colonies (Robinson & Stebbings 1997). However, it is worth

noting that the core areas of colonies overlapped only slightly and one colony was isolated. It therefore seems that while *E. serotinus* share some foraging sites among colonies (Robinson & Stebbings 1997), possibly opportunistically through the exploitation of temporary feeding sites (Catto et al. 1996), territoriality still occurs. It is also likely that species display a range of foraging and roosting behaviours according to habitat and resource availability, intra- and inter-species competition, environmental conditions and metabolic/reproductive status. This variation may explain the lower correct assignment rate using isotopic data within the eastern population. Therein lies a limit in using only genetic and isotopic data and the addition of direct tracking, GIS analysis and spatial and environmental modelling could improve our understanding of species' spatial and social organisation.

While relatively high levels of segregation over the summer have thus been demonstrated, inter-annual roost-switching was also suggested by the substantial drop in correct assignment rates when adding samples collected in June. As discussed in Chapter 4, fur has been found to start growing in July, so isotopic values in June samples reflect events from the previous year. The addition of June samples to the analysis reduced discrimination among roosts using isotopic data, but not when using genetic data. This difference could indicate that individuals sampled in June had been spending at least parts of the previous summer away from the main roost, although they are likely to be residing in the same local landscape. This result is consistent with the analysis of $\delta^{13}C$ variance in fur and wing biopsies (Chapter 4).

6.6 Conclusion

Comparative and integrated analyses of genetic and isotopic data provided insights on the spatial and social organisation of *E. serotinus* across its English range. Relatively strong summer segregation of the different social groups was inferred, with limited connectivity among them, even when roosts were geographically close. However, temporal patterns to connectivity were observed, with inter-annual roost switching behaviour likely. In contrast, genetic connectivity operated on larger scales, with genetic mixing of summer colonies into three genetically and geographically distinct populations. Asymmetric gene

flow connects these three populations, as the Isle of Wight is strongly isolated from mainland and the western population receives most immigration. Furthermore, it appears that segregation and resource use varies geographically with individuals from the eastern population potentially displaying different behaviour than individuals from the other two populations. These results suggest some flexibility in the social structuring of *E. serotinus* over time and space, and raise some interesting questions regarding the drivers, dynamics and consequence of sociality in this species. The use of multiple forensic markers highlighted the complexity of this species' spatial and social organisation and further investigations using an even broader array of methods is needed to gain further understanding of its basic ecology.

CHAPTER 7: Discussion

Knowledge on the spatial ecology of a species is critical for an understanding of their population dynamics, demography and evolution, as well for species management and conservation. In this thesis I explored the patterns of spatial organisation and movements of *Eptesicus serotinus*, at the northern limit of its distribution. I used population genetic and stable isotope analyses to reveal the hierarchical spatial organisation of this species during the breeding period, its connectivity, and individual and temporal variation in spatial use and social behaviour. Here I present a brief synthesis of the results from the previous chapters and discuss these findings and their implications.

Firstly, there was evidence that population structure varied across samples and scales. In England, maternity roosts were isotopically distinct, indicating strong segregation, even when individuals from neighbouring roosts shared the same landscape, indicative of the strength of these main roosts as social units (Chapter 6). Genetic structure revealed by the mitochondrial markers is consistent with this, but also indicated a degree of female philopatry over and above any between roost segregation (Chapter 3). A number of other lines of evidence support these inferences. I derived using genetic and isotopic data. For example, the inference of female philopatry is consistent with long-term mark-recapture data obtained from two of the sampled roosts. Individuals marked in their first year were re-captured in later, but not always successive, years, with some females being at least 14 years old and still using the same maternity roost (T. Hutson, pers. comm.). This pattern of female philopatry is extensively observed in temperate bat species (as reviewed in Chapter 2), for instance strong isolation of maternity colonies has been reported in *Myotis bechsteinii* (Kerth et al. 2000), *M. myotis* (Castella et al. 2001), and *Plecotus auritus* (Entwistle et al. 2000). The drivers that underlie this philopatry and social cohesion are still poorly defined although familiarity with roosting and foraging sites, information exchange, physiological constraints and kin selection have all been invoked as explanations (Kerth 2008a).

However, some temporal, geographical and individual variation in these patterns was also found, including variation in inter-annual fidelity to the maternity roost, temporal variation in fine-scale movements and differential behaviour across the species range (Chapters 4 and 6). The data suggested

that individuals might not occupy the maternity roost every year and could also switch roosts for short period of times within the breeding season (Chapter 4). This species could thus be organised in communities across a network of roosts during the breeding period, possibly a main maternity roost along with several satellite roosts. Radio-tracking studies on this species are consistent with this hypothesis as some individuals tracked from their primary roost used other day roosts in the vicinity, either temporarily or permanently (Catto et al. 1996, Harbusch 2003, Harbusch & Racey 2006). Similarly, long-term monitoring of two of maternity roosts sampled indicated that some individuals were not present every year during the capture session (T. Hutson, pers. comm.). Roost switching has been observed in many bat species, especially those that rely on more ephemeral roosts, such as tree-roosting *E. fuscus* and *M. bechsteinii*, and can lead to fission-fusion societies (Kerth & Konig 1999, Willis & Brigham 2004, Metheny et al. 2007, Kerth et al. 2011). Species using more permanent roosts that include a variety of microclimates, such as *E. serotinus* roosting in loft space of buildings, are thought to change roosts less often (Lewis 1995). In these cases, roost switching behaviour could be linked to parasite burdens (Reckardt & Kerth 2007), but also to the breeding status of individuals because of the high energetic demands of reproduction (Speakman & Thomas 2003). Non-breeding females might not need the same microclimate and choose roosts that facilitate torpor and/or are closer to foraging sites as energy saving strategy. Currently the reasons for roost switching behaviour remain elusive and further work is be required, however it is clear that sampling only within the primary maternity roost restricts a fuller understanding of the fine-scale social and spatial dynamics of bat colonies.

In contrast to these small-scale segregation patterns, the nuclear gene pool was largely homogenous over pan-European geographical scales, which is consistent with a mating system that promotes genetic connectivity, probably via males, which would explain the mtDNA data. This did not preclude some genetic structuring in England, which was found to have three distinct populations, east, west and Isle of Wight (Chapter 3). Despite the Channel acting as a potential barrier to movements, asymmetric gene flow connected the three English populations to continental Europe and indicated a northward and

westward range expansion, with the western most population acting as an effective gene sink.

Traditional methods for studying movements and spatial organisation are often restricted in their application as they are time-consuming or costly, and hence only a few individuals can be followed. Extrapolating the results of such studies to infer population patterns over large geographical scales is therefore problematic. This contrasts with the forensic approach applied here, which allowed me to more reliably scale information on individual movements up to population-level patterns. Hence, population genetics can reveal population structure, social organisation, dispersal and levels of connectivity between populations (Broquet & Petit 2009) while stable isotopes can be used as dietary and spatial markers (Crawford et al. 2008). However, these methods also have limits.

One of the challenges of using a molecular approach rests in assessing the contribution of both vicariant and contemporary events to an observed pattern of genetic partitioning over geographical scales. Using different markers as we have done in Chapter 3 helps clarifying some patterns, but further phylogenetic analyses of *E.* serotinus, including intensive sampling across putative refugia and contact zones, is necessary to shed light on the historical processes that influenced this species. Furthermore, genetic structure could also be attributed to mating behaviour (for instance mating at swarming or hibernation sites like *M. nattereri* (Rivers et al. 2005, 2006), or males establishing a mating roost visited by females like *Nyctalus noctula* (Ibáñez et al. 2009)) and dispersal, both of juveniles and adults. Interpreting the contribution of these factors and determining the mating system of this species would require the use of more traditional and direct methods, especially individual marking and tracking, as well as some novel approaches that are increasingly popular, such as social network analysis (e.g. (Rollins et al. 2012)).

A few caveats are also needed with stable isotope analysis (see for example (Crawford et al. 2008, Inger & Bearhop 2008)). Firstly, individuals with similar isotopic signature come from isotopically similar locations, but do not necessarily come from the same place. In our study, this means that we are unable to conclude that individuals from the same roost and with similar isotopic

signature share the same foraging habitat, and only direct tracking would confirm resource sharing within a roost. More generally, a large number of factors contribute variation to isotope measurements of individuals and populations (see (Rubenstein & Hobson 2004) for review). These include differences in diet (individual specialisation), behaviour or in metabolism and physiology among individuals, which increase isotopic variance among individuals in a location. Similarly, environmental effects can also increase isotopic variation at a local scale, and be confounded with any individual effects (Chapter 5). Furthermore, isotopic values in animal tissues can vary over time, both due to isotopic baseline shifts, or to changes in animal behaviour and physiology. As such, an important factor to know is the tissue turn-over time in order to understand the temporal scale over which the study applies and to explore the possible individual or environmental changes that could affect isotopic values. Unfortunately, this information is often difficult to obtain for free-ranging animals, and experience on captive animals can be time-consuming and unreliable, especially in the case of temperate bats whose metabolism is largely dependent on changing environmental and breeding conditions, factors difficult to replicate.

The findings of this research have profound implications for the conservation of this species and for the disease ecology of the virus it carries. The philopatry displayed by females indicates that such social units are of principal conservation concern. However, since a colony might be using a network of roosts, identifying satellite roosts and detailing their use is necessary to fully understand the dynamics of this species and provide protection to the whole colony. In England, population structure indicates some limits to dispersal movements, and as such, population declines in one area of the country might not be able to benefit from immigration for recovery. It would thus be judicious to treat each of the three genetic populations as a conservation unit. Finally, this thesis did not provide any strong insight on potential seasonal migration or hibernation, aspects essential to bat conservation. However, high summer segregation among roosts, limited dispersal and strong behavioural resistance to movements over geographical barriers (e.g. The Solent) point toward more sedentary behaviour. Efforts in locating hibernation sites are thus needed and

sampling individuals during the winter season would help establish population connectivity among seasons.

Over 800 cases of European Bat Lyssavirus 1 (EBLV-1) have been recorded in continental Europe (McElhinney et al. 2013) but none in the British Isles. The maintenance of EBLV-1 on the continent could be explained by wider mixing of *E. serotinus* communities on larger distances as indicated by extensive nuclear gene flow across vast distances (Chapter 3). In contrast, the relative fragmentation of a low-density population at the northern edge of its range in England may inhibit the viruses' spread. However, while the English Channel apparently limits movements, it does not fully prevent them. The apparent absence of the disease from English serotines (Harris et al. 2006) therefore remains unexplained given the opportunity for infection from the continent. It is possible that dispersal movements are facultative and are only undertaken by healthy individuals while infected animals displaying sub-clinical symptoms that include lack of coordination and muscle spasms (see (Fooks et al. 2003)) are unable to travel longer distances. However, tolerance to lyssavirus and "silent" infection with no clinical signs have also been reported (see Harris *et al.* 2006) and further work is necessary to understand the pathology of EBLV1 in *E. serotinus*. However, with regular immigration from the continent, associated with probable westward and northward range shift or expansion, EBLV-1 could enter the UK. Continued monitoring and further multidisciplinary investigations on bat movements and occurrence would therefore be required to assess the risk of disease introduction and spread.

Directions for future investigations

At the local scale, knowledge of fine-scale social and spatial dynamics could be gained by integrating several approaches such as genetic and stable isotope analyses, tracking, PIT-tagging and social network analysis. Identifying satellite roosts and foraging sites would gain an understanding of the dynamics of whole communities while individual marking combined with genetic data could reveal individual associations that would deepen our understanding of sociality in bats. Resource partitioning within and among colonies as well as individual specialisation in foraging strategy could be investigated by combining radio-

tracking with stable isotopes including prey sampling. The addition of genetic data could uncover evidence of kin-biased foraging behaviour or information transfer.

At a broader scale, this thesis revealed differential dispersal behaviour across the species range resulting in contrasting patterns of population structure between England and continental Europe. Comparative analyses of phenotypic characteristics, roosting, foraging, hibernating and mating behaviours and movements among the core population and the peripheral ones would be especially interesting to understand the effect of environmental conditions on generating ecological and phenotypic variation. The advance of genomics could be applied to this system to investigate potential local adaptation and selection.

Lastly, exploring the effect of disease on the ecology and population dynamics of the species would be especially important. It would be particularly interesting to establish whether infected individuals display different behaviours to those of uninfected ones and to understand the potential consequences of variation in behaviour, such as assortative mating.

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Appendix 1: Genetic structure in bat species displaying various migratory behaviours

The category ‘migratory’ includes all types of migratory behaviour as defined in the main text, and also includes facultative and partial migration. ‘Non-migratory’ includes sedentary behaviour or very short-distance seasonal relocation (typically under 100km). Putative migrants and non-migrants are classified as such when evidence is missing to confirm their migratory behaviour. Unknown covers all other species for which seasonal movement has never been studied. Sampling units include colonies when the social structure of the species is known, sites for discrete sampling locations, localities for more than one sampling site or colony within a 30km radius, regions for more than one sampling site/colony within a 30km+ radius, groups for more than one sampling site or colony grouped according to other criteria than geography. Sampling scale corresponds to the maximum Pair-wise distance between sampling units: under 100km for small, 100 – 1000km for medium and over 1000 km for large. Measures of population differentiation used are F-statistics (Weir & Cockerham 1984; F_{ST} for microsatellites and allozymes, Φ_{ST} for mitochondrial DNA), R-statistics (Slatkin 1995), and Analysis of Molecular Variance (Excoffier et al. 1992). Unless specified otherwise, population differentiation corresponds to genetic differentiation among the smallest sampling units. Isolation by distance is present when there is significant correlation between the matrices of pair-wise population differentiation and pair-wise geographical distances as tested by Mantel test and absent when the correlation is not significant. Marginal isolation by distance covers cases when there are discrepancies in the significance of the Mantel test according to which measure of differentiation has been used for the same dataset.

RAPD = random amplified polymorphic DNA. PAICS = Pleistocene Aggregate Island Complexes

Family/Species	Location	Marker (Number of Polymorphic Loci)	Sampling Unit and Number	Sample Size per Sampling Unit (Total Sample Size)	Scale	Population Differentiation	Range Pair-wise Population Differentiation	Isolation by Distance	Reference
					Migratory				
Molossidae									
Tadarida brasiliensis mexicana	Continent	Mitochondrial DNA (Control Region)	11 Summer Colonies from 3 Groups (2 migratory and 1 non-migratory)	4-12 (94)	Large	Among Groups: Φ_{ST} = 0.004 Migratory vs Non-Migratory: Φ_{ST} = 0.021, 2.1% of variation	Among Colonies: F_{ST} = -0.0137 - 0.128 Among Groups: Φ_{ST} = - 0.011 - 0.024	Absent	Russell et al. 2005
Tadarida brasiliensis mexicana	Continent	Allozymes (5)	3 Summer Colonies	non migratory: 9-26 (35) migratory: - (171)	Large	F_{ST} = 0.014	-	-	McCracken & Gassel 1997
Tadarida brasiliensis mexicana	Continent	Allozymes (6)	8 Summer Colonies	20-171 (406)	Large	F_{ST} = 0.008	F_{ST} = 0.004 - 0.008	-	McCracken et al. 1994
	Continent		4 Winter Colonies	Males: 25-28 (105) Females: 7-28 (89)	Medium	F_{ST} = 0.016 F_{ST} = 0.021	-	-	
Tadarida brasiliensis mexicana	Continent	Allozymes (6)	5 Summer Colonies (2 migratory Groups)	7- 50 (164)	Medium	F_{ST} = 0.052	-	-	Svoboda et al. 1985
Phyllostomidae									
Leptonycteris curasoae	Continent	RAPD (7)	6 Summer Colonies	19-26 (137)	Large	Φ_{ST} = 0.608 Among Colonies: 60.84% of variation	-	Present	Morales-Garza et al. 2007
Leptonycteris curasoae	Continent and Islands	Mitochondrial DNA (Control Region)	15 Sites (7 Localities)	- (42)	Medium	Among Localities: F_{ST} = 0.167 After removing outlier: F_{ST} = 0.058	-	All Localities: Present Without Outlier: Absent	Newton et al. 2003
Pteropodidae									
Eidolon helvum	Islands	Allozymes (15)	5 Islands	9-27 (74)	Medium	F_{ST} = 0.153	F_{ST} = 0.029 - 0.187	Present	Juste et al. 2000

Family/species	Location	Marker used (number of polymorphic loci)	Sampling unit and number	Sample size per sampling unit (total sample size)	Scale	Population differentiation	Range pair-wise population differentiation	Isolation by distance	Reference
Pteropus alecto	Island	Allozymes (9)	5 Localities	15-29 (114)	Large	F_{ST} = 0.023	-	-	Webb & Tidemann 1996
Pteropus poliocephalus	Island	Allozymes (9)	4 Localities	20-64 (156)	Large	F_{ST} = 0.014	-	-	Webb & Tidemann 1996
Pteropus scapulatus	Island	Allozymes (4)	6 Sites	17-20 (116)	Large	F_{ST} = 0.28	-	Absent	Sinclair et al. 1996
		RAPD (5)	4 Sites	16-20 (74)		Among Sites: 5.01% variation	-	-	
Vespertilionidae									
Miniopterus (schreibersii) natalensis	Continent	Microsatellites (6)	8 Maternity Colonies 1 Winter Colony 1 Transient Roost (3 Regions)	- (307)	Large	-	All Colonies: ρ = 0.152 - 0.686 Between Regions: ρ = 0.351–0.623 Among Colonies within Regions: ρ = -0.005 - 0.068	Absent	Miller-Butterworth et al. 2003
		Mitochondrial DNA (Control Region)		6-8 (78)		Φ_{ST} = 0.620	-	Present	
Miniopterus schreibersii	Continent	Microsatellites (5)	7 Maternity Colonies	24-52 (313)	Medium	-	F_{ST} = 0.01 - 0.09	Present	Rodrigues et al. 2010
		Mitochondrial DNA (Control Region)				-	Φ_{ST} = 0.07 - 0.43	Present	
Miniopterus schreibersii schreibersii	Continent	Mitochondrial DNA (Control Region)	13 Colonies (2 Regions)	5-14 (110)	Medium	Among Colonies: Φ_{ST} = 0.417, 41.7% of variation Among Regions: Φ_{CT} = 0.210, 33.6% of variation Among Colonies within Regions: Φ_{SC} = 0.335, 17.0% of variation	-	Present	Furman et al. 2009
Miniopterus schreibersii pallidus	Continent	Mitochondrial DNA (Control Region)	4 Colonies	8-9 (33)	Medium	Among Colonies: Φ_{ST} = 0.134, 13.4% of variation	-	Absent	Furman et al. 2009

Family/species	Location	Marker used (number of polymorphic loci)	Sampling unit and number	Sample size per sampling unit (total sample size)	Scale	Population differentiation	Range pair-wise population differentiation	Isolation by distance	Reference
Miniopterus schreibersii	Continent	Microsatellites (5)	11 Maternity Colonies (4 Regions)	17-55 (407)	Medium	Among Colonies: F_{ST} = 0.038 Among Regions: 3% of variation Among Colonies within Regions: 0.5% of variation	F_{ST} = -0.01 - 0.11	Present	Ramos Pereira et al. 2009
		Mitochondrial DNA (Control Region)				Among Regions: 17% of variation Among Colonies within Regions: 6% of variation	Φ_{ST} = 0.01 - 0.43	Present	
Miniopterus schreibersii	Continent	Microsatellites (6)	34 Sites (2 Groups)	1-14 (121)	Large	Among Groups: F_{ST} = 0.023 R_{ST} = 0.037	-	-	Bilgin et al. 2008a
		Mitochondrial DNA (Control Region)				Among Groups: 91.45% of variation Among Sites within Groups: 2.76% of variation	-	-	
Myotis blythii	Continent	Microsatellites (5)	2 Maternity Colonies	40 (80)	Small	F_{ST} = 0.017	-	-	Berthier et al. 2006
Myotis capaccinii	Continent	Microsatellites (7)	14 Sites	3-17 (36)	Large	F_{ST} = 0.000 Among Sites: 0% of variation	-	Absent	Bilgin et al. 2008b
		Mitochondrial DNA (Cytochrome B)				All: Φ_{ST} = 0.974 Among 2 clades: Φ_{ST} = 1.000	-	Absent	
Myotis daubentonii	Islands and continent	Microsatellites (8)	32 Colonies (3 Regions)	>9 (734)	Large	Among Regions: Φ_{CT} = 0.056 Among Colonies within Regions: Φ_{SC} = 0.012	F_{ST} = 0.000-0.159	Over the whole geographical range: Absent Within Northern UK: Present	Atterby et al. 2010

Family/species	Location	Marker used (number of polymorphic loci)	Sampling unit and number	Sample size per sampling unit (total sample size)	Scale	Population differentiation	Range pair-wise population differentiation	Isolation by distance	Reference
Myotis daubentonii	Island	Microsatellites (8)	27 Sites	14-56 (671)	Medium	All: F_{ST} = 0.017 Males: F_{ST} = 0.017 Females: F_{ST} = 0.023	F_{ST} = –0.007 - 0.061	Present	Ngamprasertwong et al. 2008
Myotis goudoti	Island	Mitochondrial DNA (Control Region and Cytochrome B)	41 Sites (15 Regions, 3 Groups)	- (195)	Large	Among Regions: Φ_{ST} = 0.170 Among Groups: Φ_{CT} = 0.19	Among Regions: Φ_{ST} = 0.00 - 0.73	Present	Weyeneth et al. 2011
Myotis myotis	Continent	Microsatellites (11) Mitochondrial DNA (Control Region)	29 Maternity Colonies	16-21 (460)	Large	Between 2 Groups: F_{CT} = 0.165 Among 9 Groups: 71.7% of variation	- -	- -	Ruedi et al. 2008
Myotis myotis	Continent	Microsatellites (11) Mitochondrial DNA (Control Region)	6 Maternity Colonies	16-21 (115)	Large	F_{ST} = 0.039 Between 2 Groups: F_{CT} = 0.12 Φ_{ST} = 0.47 Among 4 Groups: 52.8% of variation	F_{ST} = 0.013 - 0.067 -	Absent Absent	Ruedi et al. 2008
Myotis myotis	Continent	Microsatellites (5)	2 Maternity Colonies	40 (80)	Small	F_{ST} = 0.023	-	-	Berthier et al. 2006
Myotis myotis	Continent	Microsatellites (10) Mitochondrial DNA (Control Region)	24 Maternity Colonies	20 (480)	Large	F_{ST} = 0.035 Φ_{ST} = 0.641	Mean Pair-wise: F_{ST} = 0.009 Mean Pair-wise: Φ_{ST} = 0.400	Present Present	Ruedi & Castella 2003
Myotis myotis	Continent	Microsatellites (15) Mitochondrial DNA (Control Region)	13 Maternity Colonies	20 (260)	Medium	F_{ST} = 0.022 Φ_{ST} = 0.540	F_{ST} = -0.001 - 0.084 Φ_{ST} = -0.053 - 0.722	- -	Castella et al. 2001

Family/species	Location	Marker used (number of polymorphic loci)	Sampling unit and number	Sample size per sampling unit (total sample size)	Scale	Population differentiation	Range pair-wise population differentiation	Isolation by distance	Reference
Myotis myotis	Continent	Microsatellites (6)	3 Maternity Colonies	20 (60)	Medium	F_{ST} = 0.004	F_{ST} = –0.002 - 0.009	-	Castella et al. 2000
	Continent	Microsatellites (6)	3 Maternity Colonies	19-20 (59)	Medium	F_{ST} = 0.011	F_{ST} = –0.002 - 0.019	-	
	Across Gibraltar Strait	Microsatellites (6)	6 Maternity Colonies (2 Regions)	19-20 (119)	Large	Among Regions: Φ_{CT} = 0.333, 33.33% of variation Among Colonies within Regions: Φ_{SC} = 0.007, 0.5% of variation	F_{ST} = –0.002 - 0.019	-	
		Mitochondrial DNA (Cytochrome B)		- (28)		Among Regions: Φ_{CT} = 0.991, 99.1% of variation	-	-	
Myotis nattereri	Island	Microsatellites (8)	7 Swarming Caves (2 areas) 11 Maternity Colonies	10--192 (803)	Small	Among Maternity Colonies: F_{ST} = 0.017 Among Swarming Areas: 0.47% of variation Among Caves within Swarming Areas: 0.15% of variation	-	All Maternity Colonies: Present Without Outlier: Absent	Rivers et al. 2005
Nyctalus noctula	Continent	Mitochondrial DNA (Control Region)	5 Winter Colonies	10-38 (138)	Large	Among all Colonies: 2.3% variation	-	-	Petit & Mayer 2000
Nyctalus noctula	Island and Continent	Mitochondrial DNA (Control Region and ND1)	13 Maternity Colonies 5 Winter Colonies (4 Regions)	~20.2 (364)	Large	Among Regions: Φ_{CT} = 0.17, 17.32% of variation Among Colonies within Regions: Φ_{SC} = 0.06, 5.33% of variation	-	-	Petit et al. 1999
Nyctalus noctula	Continent	Microsatellites (8)	13 Maternity Colonies (4 Regions)	8-44 (259)	Large	Among all Colonies: F_{ST} = 0.006 Among Regions: 0% of variation Among Colonies within Regions: 0.42% of variation	F_{ST} = 0.000 - 0.028	Absent	Petit & Mayer 1999
Pipistrellus pipistrellus	Continent	Microsatellites (11)	11 Maternity Colonies	20-45 (274)	Medium	F_{ST} = 0.005	F_{ST} = -0.003 - 0.015	Absent	Bryja et al. 2009

Family/species	Location	Marker used (number of polymorphic loci)	Sampling unit and number	Sample size per sampling unit (total sample size)	Scale	Population differentiation	Range pair-wise population differentiation	Isolation by distance	Reference
Pipistrellus pipistrellus	Islands and Continent	Microsatellites (6)	32 Maternity Colonies	4-28 (382)	Large	F_{ST} = 0.044	F_{ST} = 0.001 - 0.238	Present	Racey et al. 2007
	Island		20 Maternity Colonies	4-20 (215)	Medium	F_{ST} = 0.029	F_{ST} = 0.001 - 0.116	Present	
Vespertilio murinus	Continent	Microsatellites (3)	3 Female Colonies	Female Colonies: 10-48 (82)	Medium	-	F_{ST} = 0.00 - 0.04	-	Safi et al. 2007
		Mitochondrial DNA (Control Region)	2 Male Colonies	Male Colonies: 69-72 (141)		-	F_{ST} = 0.00 - 0.16	-	
Vespertilionidae				**Putative Migratory**					
Antrozous pallidus	Continent	Mitochondrial DNA (Control Region and Cytochrome B)	36 Sites (3 Regions)	1-35 (194)	Large	Among Regions: 23.95% of variation	F_{ST} = 0.2340 - 0.2527	-	Weyandt & Van Den Bussche 2007
Pipistrellus pygmaeus	Continent	Microsatellites (11)	10 Maternity Colonies	12-30 (233)	Medium	F_{ST} = 0.006	F_{ST} = -0.002 - 0.017	Absent	Bryja et al. 2009
Pipistrellus pygmaeus	Islands and Continent	Microsatellites (6)	50 Maternity Colonies	6-67 (904)	Large	F_{ST} = 0.024	F_{ST} = 0.001 - 0.105	Present	Racey et al. 2007
	Island		34 Maternity Colonies	6-67 (702)	Medium	F_{ST} = 0.023	F_{ST} = 0.001 - 0.084	Present	
Hipposideridae				**Non-Migratory**					
Hipposideros armiger	Continent	Mitochondrial DNA (Control Region)	5 Regions (14 Colonies)	5-36 (115)	Large	Among Regions: Φ_{CT} = 0.564, 56.43% of variation Among Colonies within Regions: Φ_{SC} = 0.648, 28.22% of variation	Among Regions: Φ_{ST} = 0.415-0.899	Absent	Xu et al. 2010
Hipposideros turpis turpis	Islands	Microsatellites (6)	7 Maternity Colonies (3 Islands)	17-90 (266)	Small	Among Islands: Φ_{CT} = 0.034, 3.46% of variation Among Colonies within Islands: Φ_{SC} = 0.004 , 0.39% of variation	F_{ST} = 0.00037 - 0.09336	-	Echenique-Díaz et al. 2009

Family/species	Location	Marker used (number of polymorphic loci)	Sampling unit and number	Sample size per sampling unit (total sample size)	Scale	Population differentiation	Range pair-wise population differentiation	Isolation by distance	Reference
Megadermatidae									
Macroderma gigas	Australia	Microsatellites (6) Mitochondrial DNA (Control Region)	9 Localities	9-37 (217)	Large	All: UR_{ST} = 0.337 Northern territory: UR_{ST} = 0.024 All: Φ_{ST} = 0.804 Northern territory: Φ_{ST} = 0.393	UR_{ST} = 0.033 - 0.631 Φ_{ST} = 0.047 - 1.000	Present Present	Worthington Wilmer et al. 1999
Molossidae									
Tadarida brasiliensis cynocephala	Continent	Allozymes (5)	3 Summer Colonies	18-30 (68)	Large	F_{ST} = 0.038	-	-	McCracken & Gassel 1997
Phyllostomidae									
Ardops nichollsi	Islands	Mitochondrial DNA (Cytochrome B)	3 Islands	- (14)	Small	Φ_{ST} = -0.093	-	-	Carstens et al. 2004
Artibeus jamaicensis	Islands	Mitochondrial DNA (Cytochrome B)	6 Islands	- (49)	Small	Φ_{ST} = -0.044	-	-	Carstens et al. 2004
Artibeus jamaicensis	Continent	Microsatellites (14)	12 Harems (2 Caves)	- (124)	Small	Among Caves: F_{ST} = 0.008 Among Caves: 0.31% variation Among Harems within Caves: 0.31% variation	-	-	Ortega et al. 2003
Brachyphylla cavernarum	Islands	Mitochondrial DNA (Cytochrome B)	5 Islands	- (32)	Small	Φ_{ST} = -0.035	-	-	Carstens et al. 2004
Desmodus rotundus	Continent	Nuclear gene (DRB) Nuclear gene (RAG2) Mitochondrial DNA (Cytochrome B)	3 Groups (defined by mtDNA phylogenetics) 54 Sites (from 5 phylogenetic Groups)	- (62) - (88) - (118)	Large	Among Groups: 7.65% of variation Among Groups: 5% of variation All Localities: F_{ST} = 0.16 Among Groups: 15.85% of variation	- F_{ST} = 0.009 - 0.036 -	- - -	Martins et al. 2009

Family/species	Location	Marker used (number of polymorphic loci)	Sampling unit and number	Sample size per sampling unit (total sample size)	Scale	Population differentiation	Range pair-wise population differentiation	Isolation by distance	Reference
Desmodus rotundus	Continent	Mitochondrial DNA (Cytochrome B)	19 Sites	- (50)	Large	F_{ST} = 0.86	-	-	Martins et al. 2007
Glossophaga longirostris	Continent and Islands	Mitochondrial DNA (Control Region)	16 Sites (8 Localities)	- (41)	Medium	Among Localities: F_{ST} = 0.725	-	Absent	Newton et al. 2003
Lophostoma silvicolum(Females)	Continent and Island	Microsatellites (10) Mitochondrial DNA (Control Region)	14 Harems	4-10 (75)	Small	- -	F_{ST} = −0.073 - 0.061 F_{ST} = −0.176 - 0.166	Present Absent	Dechmann et al. 2007
Rhinolophidae									
Rhinolophus ferrumequinum	Continent and Islands	Mitochondrial DNA (ND2)	27 Sites (5 Regions)	1-26 (161)	Large	Among Regions: Φ_{CT} = 0.680, 93.44% of variation Among Sites within Regions: Φ_{SC} = 0.979, 4.46% of variation	-	Present	Flanders et al. 2009
Rhinolophus ferrumequinum	Continent and Islands	Microsatellites (17)	56 Sites	1-29 (516)	Large	Within Great Britain: F_{ST} = 0.051 Across Europe and Middle East: F_{ST} = 0.043 UK/France: F_{ST} = 0.112 Japan/Eat China: F_{ST} = 0.157 UK/Japan: F_{ST} = 0.320 Japan/all others: F_{ST} = 0.277 Northeast China/all others: F_{ST} = 0.266	Within Great Britain: F_{ST} = -0.001 - 0.153 Across Europe and Middle East: F_{ST} = -0.0185 - 0.099	Present	Rossiter et al. 2007
Vespertilionidae									
Corynorhinus townsendii ingens	Continent	Microsatellites (5) Mitochondrial DNA (Control Region)	5 Summer Colonies	2-20 (63)	Small	F_{ST} = 0.00 Among Colonies: 0% of variation F_{ST} = 0.0526 Among Colonies: 5.26% of variation	- -	- -	Weyandt et al. 2005

Family/species	Location	Marker used (number of polymorphic loci)	Sampling unit and number	Sample size per sampling unit (total sample size)	Scale	Population differentiation	Range pair-wise population differentiation	Isolation by distance	Reference
Corynorhinus townsendii pallescens	Continent	Microsatellites (6)	5 Localities	- (98)	Medium	-	F_{ST} = 0.00 - 0.09	All: Present Without Outlier: Absent	Piaggio et al. 2009
		Mitochondrial DNA (Control Region)				-	Φ_{ST} = 0.13 - 0.93	Absent	
Corynorhinus townsendii townsendii		Microsatellites (6)	2 Localities			F_{ST} = 0.03	-	-	
		Mitochondrial DNA (Control Region)				Φ_{ST} = 0.10	-	-	
Corynorhinus townsendii virginianus		Microsatellites (6)	5 Localities	- (69)		-	F_{ST} = 0.00 - 0.16	Present	
		Mitochondrial DNA (Control Region)				-	Φ_{ST} = -0.02 - 0.98	Absent	
Eptesicus fuscus	Continent and Islands	Nuclear loci (nCR)	5 Regions	- (226)	Large	-	Φ_{ST} = 0.000 - 0.500	-	Turmelle et al. 2010
		Nuclear loci (Enol)				-	Φ_{ST} = 0.001 - 0.753	Absent	
Eptesicus fuscus	Continent	Nuclear loci (nCR)	3 Regions	- (100)	Large	-	Φ_{ST} = 0.000 - 0.063	-	Turmelle et al. 2010
		Nuclear loci (Enol)				-	Φ_{ST} = 0.000 - 0.041	-	
		Mitochondrial DNA (Control Region)				-	Φ_{ST} = 0.034 - 0.217	Present	
Eptesicus fuscus	Continent	Microsatellites (9)	6 Maternity Colonies (3 Groups)	20-121 (309)	Medium	Among Colonies: 0.5% of variation	F_{ST} = -0.0001 - 0.012	Absent	Vonhof et al. 2008
		Mitochondrial DNA (Control Region and Cytochrome B)				Among Groups: 21.2% of variation Among Colonies within Groups: 1.5% of variation	Φ_{ST} = -0.007 - 0.491	Present	
Myotis bechsteinii	Island	Microsatellites (11)	7 Maternity Colonies	10-40 (175)	Small	F_{ST} = 0.0407 R_{ST} = 0.0479	F_{ST} = 0.0156 - 0.0776 R_{ST} = -0.0015 - 0.1346	Marginal	Durrant et al. 2009
Myotis bechsteinii	Continent	Microsatellites (7)	10 Maternity Colonies	- (196)	Small	F_{ST} = 0.015	-	Absent	Kerth et al. 2002
		Mitochondrial DNA (Control Region)				AT-1: Φ_{ST} = 0.658 AT-2: Φ_{ST} = 0.961	-	Absent	

Family/species	Location	Marker used (number of polymorphic loci)	Sampling unit and number	Sample size per sampling unit (total sample size)	Scale	Population differentiation	Range pair-wise population differentiation	Isolation by distance	Reference
Myotis bechsteinii	Continent	Mitochondrial DNA (Control Region)	10 Maternity Colonies	> 10 (232)	Small	F_{ST} = 0.68	-	Present	Kerth et al. 2000
Plecotus auritus	Continent	Microsatellites (8)	2 Swarming Sites 6 Maternity Colonies	6-111 (227)	Small	Among Maternity Colonies: F_{ST} = 0.024 Among Swarming Sites: F_{ST} = -0.001 Among all Sites: F_{ST} = 0.013	-	Absent	Furmankiewicz & Altringham 2007
Plecotus auritus	Continent	Microsatellites (6)	3 Swarming Sites 14 Maternity Colonies (5 Localities)	Swarming Sites: 5-18 (30) Maternity Colonies: 3-31 (198)	Small	Among Localities: F_{CT} = -0.0001, -0.01% of variation Among Colonies within Localities: F_{SC} = 0.00043, 0.04% of variation Among Swarming Sites: F_{ST} = -0.02544, 2.99% variation	-	-	Veith et al. 2004
		Mitochondrial DNA (Control Region)				Among Localities: Φ_{CT} = 0.59391, 59.39% of variation Among Colonies within Localities: Φ_{SC} = 0.62305, 25.30% of variation Among Swarming Sites: Φ_{ST} = -0.30600, 30.60% variation	-	-	
Plecotus auritus	Island	Microsatellites (5)	20 Maternity Colonies	10-31 (364)	Small	F_{ST} = 0.019	-	Present	Burland et al. 1999
				Putative Non-Migratory					
Mystacinidae									
Mystacina tuberculata	Island	Mitochondrial DNA (Control Region)	8 Sites (2 Regions)	6-39 (167)	Medium	Among Regions: 16.8% of variation Among Sites within Regions: 7.9% of variation	-	Present	Lloyd 2003

Family/species	Location	Marker used (number of polymorphic loci)	Sampling unit and number	Sample size per sampling unit (total sample size)	Scale	Population differentiation	Range pair-wise population differentiation	Isolation by distance	Reference
Phyllostomidae									
Carollia perspicillata	Continent and Islands	Mitochondrial DNA (Control Region)	5 Islands 3 Mainland Sites	1-17 (81)	Large	All: F_{ST} = 0.06 Among Islands: F_{ST} = 0.10 Within Mainland: F_{ST} = 0.00	-	Present	Meyer et al. 2009
Uroderma bilobatum	Continent and Islands	Mitochondrial DNA (Control Region)	9 Islands 3 Mainland Sites	1-17 (151)	Large	All: F_{ST} = 0.01 Among Islands: F_{ST} = 0.01 Within Mainland: F_{ST} = 0.01	-	Absent	Meyer et al. 2009
Rhinolophidae									
Rhinolophus monoceros	Island	Microsatellites (6)	18 Colonies	14-40 (455)	Medium	F_{ST} = 0.009 0.91% of variation	-	Present	Chen et al. 2008
		Mitochondrial DNA (Control Region)		5-14 (187)		Φ_{ST} = 0.303 30.29% of variation	-	Present	
Rhinolophus monoceros	Island	Mitochondrial DNA (Control Region)	26 Colonies (5 Regions)	1 - 62 (203)	Medium	Among Regions: Φ_{CT} = 0.373, 37.34% of variation Among Colonies within Regions Φ_{SC} = 0.072, 4.51% of variation	-	Present	Chen et al. 2006
Vespertilionidae									
Eptesicus isabellinus	Continent	Mitochondrial DNA (Control Region)	12 Maternity Colonies (4 Regions)	~20 (254)	Large	Among Regions: 14% of variation Among Colonies within Regions: 44.93%	Φ_{ST} = 0.000 - 0.919	Absent	Juste et al. 2009
Myotis davidii	Continent	Microsatellites (7)	17 Sites (3 Regions)	2-17 (126)	Large	Among Regions: Φ_{CT} = 0113, 14.45% of variation Among Sites within Regions: Φ_{SC} = 0.124, 16.57% of variation	F_{ST} = 0.004 - 0.276	Absent	You et al. 2010
		Mitochondrial DNA (Control Region)				Among Regions: Φ_{CT} = 0.771, 64.82% of variation Among Sites within Regions: Φ_{SC} = 0.349, 12.28% of variation	Φ_{ST} = 0.007 - 0.988	Present	

Family/species	Location	Marker used (number of polymorphic loci)	Sampling unit and number	Sample size per sampling unit (total sample size)	Scale	Population differentiation	Range pair-wise population differentiation	Isolation by distance	Reference
Myotis macropus	Island	Microsatellites (6)	5 Sites	15-50 (173)	Medium	F_{ST} = 0.221 Among Sites: Φ_{ST} = 0.216, 22% of variation	F_{ST} = 0.0210 - 0.2393	Present	Campbell et al. 2009
Myotis punicus	Islands and Continent	Microsatellites (7)	10 Maternity Colonies (3 Regions)	10-19 (182)	Large	Among Regions: Φ_{CT} = 0.050 Among Colonies within Regions: Φ_{SC} = 0.002	F_{ST} = -0.01 - 0.08	-	Biollaz et al. 2010
		Mitochondrial DNA (Control Region)		7-10 (79)		Among Regions: Φ_{CT} = 0.66, 66.35% of variation Among Colonies within Regions: Φ_{SC} = 0.66, 22.2% of variation	Φ_{ST} = 0.00 - 1.00	-	
Myotis punicus	Islands	Allozymes (9)	4 Colonies	4-17 (36)	Small	F_{ST} = 0.272	-	-	Baron & Vella 2010
Nyctalus azoreum	Islands	Microsatellites (8)	14 Maternity Colonies (6 Islands)	32-66 (280)	Medium	Among all Islands: FST = 0.036 Central Group vs Outlier: Among Groups: 5.87% of variation Among Islands within Groups: 0.91% variation	Among Islands: FST = 0.006 - 0.076	All Islands: Present Central Group Islands: Absent	Salgueiro et al. 2008
Nyctalus azoreum	Islands	Mitochondrial DNA (Control Region)	14 Maternity Colonies (6 Islands)	6-21 (159)	Medium	All Islands: Among Islands: 23% of variation Among Colonies within Islands: 15% variation Central Group vs Outlier: Among Groups: 30.52% of variation Among Islands within Groups: 10.22% variation	Among Islands: ΦST = 0.02 - 0.50	Present	Salgueiro et al. 2004

Family/species	Location	Marker used (number of polymorphic loci)	Sampling unit and number	Sample size per sampling unit (total sample size)	Scale	Population differentiation	Range pair-wise population differentiation	Isolation by distance	Reference
Hipposideridae					**Unknown**				
Hipposideros speoris	Continent	Microsatellites (6) Mitochondrial DNA (16S rRNA)	11 Colonies (2 Regions)	12-20 (186)	Medium	Among Colonies: F_{ST} = 0.650 Among Colonies: Φ_{ST} = 0.710 Among Regions: 64.75% of variation Among Colonies within Regions: 6.34% of variation	F_{ST} = 0.0079 - 0.1202 Φ_{ST} = -0.0243 - 0.8364	Present -	Chinnasamy et al. 2011
Mormoopidae									
Pteronotus davyi	Continent	Mitochondrial DNA (Control Region)	18 Sites (2 Regions)	- (105)	Large	Among Sites: F_{ST} = 0.301, 30.1% of variation Among Regions: Φ_{CT} = 0.423, 32.7% of variation Among Sites within Regions: 10.2% of variation	-	-	Guevara-Chumacero et al. 2010
Pteropodidae									
Cynopterus brachyotis Forest	Continent and Island	Microsatellites (6) Mitochondrial DNA (Control Region)	9 Sites 10 Sites	- (133) - (101)	Large	- -	F_{ST} = 0.001 - 0.125 Φ_{ST} = 0.000 - 0.350	Present Present	Campbell et al. 2006
Cynopterus brachyotis Sunda	Continent and Island	Microsatellites (6) Mitochondrial DNA (Control Region)	9 Sites 10 Sites	- (99) - (55)	Large	- -	F_{ST} = 0.000 - 0.092 Φ_{ST} = 0.004 - 0.29	Absent Present	Campbell et al. 2006

Family/species	Location	Marker used (number of polymorphic loci)	Sampling unit and number	Sample size per sampling unit (total sample size)	Scale	Population differentiation	Range pair-wise population differentiation	Isolation by distance	Reference
Cynopterus brachyotis	Islands	Allozymes (12)	6 Islands (5 Pleistocene Aggregate Island Complexes)	6-34 (99)	Medium	Among Islands: F_{ST} = 0.044 Among PAICS: Φ_{CT} = 0.044 Among Islands within PAICS: Φ_{SC} = 0.000	-	Absent	Townsend Peterson & Heaney 1993
Cynopterus horsfieldi	Continent and Island	Microsatellites (6)	8 Sites	- (93)	Large	-	F_{ST} = 0.000 - 0.230	Absent	Campbell et al. 2006
		Mitochondrial DNA (Control Region)	8 Sites	- (51)		-	Φ_{ST} = 0.000 - 0.320	Present	
Cynopterus sphinx	Continent	Microsatellites (6)	8 Sites	14-64 (218)	Large	F_{ST} = 0.0235 Among Sites: 3.38% of variation	F_{ST} = 0.0090 - 0.0801	Present	Chen et al. 2010
		Mitochondrial DNA (Cytochrome B)		6-8 (59)		All Sites: Among Sites: 55.11% of variation Without Outlier: Among Sites: 3.04% of variation	-	-	
Cynopterus sphinx	Continent and Island	Microsatellites (6)	8 Sites	- (111)	Large	-	F_{ST} = 0.000 - 0.100	Absent	Campbell et al. 2006
		Mitochondrial DNA (Control Region)	8 Sites	- (47)		-	Φ_{ST} = 0.000 - 0.490	Present	
Cynopterus sphinx	Continent	Microsatellites (10)	27 Harems from 10 Colonies	Pups: (185)	Small	1997: F_{ST} = 0.123 1998: F_{ST} = 0.008	1997: F_{ST} = 0.095-0.152 1998: F_{ST} = 0.000-0.017	-	Storz et al. 2001
				Adult Females: (209)	Small	1997: F_{ST} = 0.002 1998: F_{ST} = 0.001	1997: F_{ST} = -0.013-0.018 1998: F_{ST} = -0.003-0.005	-	

Family/species	Location	Marker used (number of polymorphic loci)	Sampling unit and number	Sample size per sampling unit (total sample size)	Scale	Population differentiation	Range pair-wise population differentiation	Isolation by distance	Reference
Haplonycteris fischeri	Islands	Allozymes (12)	9 Islands from 5 PAICs	1-39 (123)	Medium	Among PAICs: 78.16% of variation Among Islands within a PAIC: 12.38% of variation Within an Island: 9.46% of variation	-	-	Roberts 2006
Haplonycteris fischeri	Islands	Mitochondrial DNA (Cytochrome B and ND2)	6 Islands (5 PAICs)	2-13 (46)	Medium	Among Islands: F_{ST} = 0.583 Among PAICS: Φ_{CT} = 0.537 Among Islands within PAICS: Φ_{SC} = 0.100	-	Absent	Townsend Peterson & Heaney 1993
Pteropus conspicillatus	Islands	Microsatellites (6)	11 Colonies (3 Regions)	9-319 (718)	Large	Among Regions: Φ_{ST} = 0.043, 4% of variation	Between Colonies: F_{ST} = 0.000 - 0.094	Absent	Fox 2006
		Mitochondrial DNA (Control Region)	5 Colonies (3 Regions)	7-12 (50)		Among Regions: Φ_{ST} = 0.053, 5.3% of variation	Between Colonies: F_{ST} = -0.058 - 0.076	-	
Rousettus madagascariensis	Island	Microsatellites (6)	17 Sites (7 Groups)	- (193)	Medium	F_{ST} = 0.004	F_{ST} = -0.025 - 0.0312	-	Goodman et al. 2010
Rousettus obliviosus	Islands	Microsatellites (6)	3 Islands	- (43)	Small	F_{ST} = 0.009	F_{ST} = 0.0053 - 0.1322	-	Goodman et al. 2010
Rousettus leschenaulti	Continent	Microsatellites (8)	5 Sites	20-41 (157)	Large	FST = 0.0067 Among Sites: 0.52% of variation	-	Absent	Chen et al. 2010
		Mitochondrial DNA (Cytochrome B)		5-6 (29)		Among Sites: 3.90% of variation	-	-	

Family/species	Location	Marker used (number of polymorphic loci)	Sampling unit and number	Sample size per sampling unit (total sample size)	Scale	Population differentiation	Range pair-wise population differentiation	Isolation by distance	Reference
Rhinolophidae									
Rhinolophus affinis (himalayanus, macrurus and *hainanus* subspecies*)*	Continent and Island	Microsatellites (11) Mitochondrial DNA (Control Region)	17 Sites (3 sub-species)	*R. a. hainanus*: 3-20 (124) *R. a. himalayanus*: 3-21 (17) *R. a. macrurus*: 7-17 (31)	Large	Among Subspecies: Φ_{CT} = 0.100, 10.00% of variation Among Sites within subspecies: Φ_{SC} = 0.022, 2.02% of variation Among Subspecies: Φ_{CT} = 0.508, 50.88% of variation Among Sites within Subspecies: Φ_{SC} = 0.110, 5.43% of variation	Between sub-species: F_{ST} = 0.093 - 0.130 Between sub-species: Φ_{ST} = 0.476 - 0.789	- -	Mao et al. 2010
Rhinolophus affinis	Islands	Allozymes (19)	22 Sites (11 Islands)	1-32 (119)	Medium	Among Sites: F_{ST} = 0.389 Among Sites within Islands: F_{ST} = - 0.013 Among Islands: F_{ST} = 0.397	-	Present	Maharadatunkamsi et al. 2000
Rhinolophus cornutus pumilus	Island	Microsatellites (6) Mitochondrial DNA (Control Region)	11 Colonies (2 Regions)	9-34 (257)	Small	Among Regions: Φ_{CT} = 0.015 Among Colonies within Regions: Φ_{SC} = 0.011 Among Regions: Φ_{CT} = 0.150 Among Colonies within Regions: Φ_{SC} = 0.053	F_{ST} = -0.006 - 0.051 Φ_{ST} = -0.040 - 0.370	Present Present	Yoshino et al. 2008

Family/species	Location	Marker used (number of polymorphic loci)	Sampling unit and number	Sample size per sampling unit (total sample size)	Scale	Population differentiation	Range pair-wise population differentiation	Isolation by distance	Reference
Vespertilionidae									
Myotis muricola	Islands	Allozymes (16)	10 Islands	2-41 (218)	Large	Intra-Island: F_{ST} = 0.05 Inter-Islands: F_{ST} = 0.36	-	Present	Hisheh et al. 2004
Myotis septentrionalis	Continent	Microsatellites (6)	5 Maternity Colonies (22 Sites)	13-33 (92)	Medium	F_{ST} = 0.003	-	-	Arnold 2007
Myotis vivesi	Islands	Microsatellites (6) Mitochondrial DNA (Control Region)	11 Islands	- (257) - (134)	Medium	F_{ST} = 0.002 Φ_{ST} =0.150	F_{ST} = -0.029 - 0.028 Φ_{ST} = 0.000 - 0.763	Marginal Absent	Floyd et al. 2010
Pipistrellus abramus	Continent	Microsatellites (8) Mitochondrial DNA (Cytochrome B)	17 Maternity Colonies (3 Regions)	- (286) 5-9 (102)	Large	- Among Regions: 88.32% of variation, Φ_{CT} = 0.820 Among Colonies within Regions: 3.87% of variation, Φ_{SC} = 0.309	F_{ST} = 0.000 - 0.151 Φ_{ST} = 0.000 - 1.000	Present Present	Wei et al. 2010
Scotophilus kuhlii	Islands	Allozymes (8)	10 Islands	1-83 (189)	Large	Intra-Island: F_{ST} = -0.01 Inter-Islands: F_{ST} = 0.07	-	Absent	Hisheh et al. 2004

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Appendix 2: Haplotype sequences

>A

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