CONSERVATION GENETICS AND ECOLOGY OF THE HAZEL DORMOUSE

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Declaration

This dissertation is the result of my own work and includes nothing, which is the outcome of work done in collaboration except where specifically indicated in the text. It has not been previously submitted, in part or whole, to any university of institution for any degree, diploma, or other qualification.

Signed:__Fraser Combe_____

Date:_____28/11/2018_____

Abstract

Woodland species face a challenging future because of human activity and global climate change, as such it is vital to understand the ecology of species that inhabit these habitats in order to conduct effective conservation management. I describe the phylogeographic structure of the hazel dormouse within the UK, to identify post-glacial dispersal routes and to describe population units for conservation. I provide evidence for a single post-glacial colonization coincident with the start of the Holocene period, 7.5-11 Kya. I also demonstrate the utility of novel occupancy modelling techniques to determine dormouse presence with high probability of detection. I provide optimal survey guidelines for ecological practitioners to conduct rigorous statistically significant surveys. This species is known to be susceptible to habitat loss and fragmentation limiting dispersal and functional connectivity. I use a landscape genetics approach to characterize the genetic structure of populations of dormice from seven regions around the UK to explicitly test for the role of landscape heterogeneity and barriers to dispersal. The results suggests build-up of genetic structure amongst islands of fragmented habitat that is explained in part due to isolation-by-distance, and in part due to specific landscape features between different regions. The isolation-by-resistance analysis allows us to identify the landscape features, such as land cover, hedgerows and roads that facilitate or inhibit gene flow. Results suggest that dispersal in hazel dormice is strongly influenced by barriers in the landscape, with our main findings being that urban areas and roads are associated with decreased gene flow while habitat features such as hedgerows and forest cover are associated with increased gene flow. Finally, I investigated the effect of density dependence and climatic factors on the population demography of five marked hazel dormice populations in Europe (four in the UK and one in Lithuania). The results from the chapter have identified the environmental drivers of these population parameters on hibernating mammals whilst providing evidence that density dependence has the greatest effect on population dynamics in this species. The results provide information that variability in winter conditions can have serious consequences for individual fitness, decreasing the dormancy season and leading to an increased extinction risk in this species. I discuss the implications for hazel dormouse conservation in the UK, and make recommendations for conservation practitioners to ensure the future persistence of this species.

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Contents

1 Chapter 1: General Introduction

1.1 Introduction: Habitat fragmentation and genetic diversity in natural populations

This chapter provides a broad context and review of relevant topics that will be addressed again through-out the following chapters of the thesis. After introducing relevant thematic and conceptual topics I will discuss the ecology and conservation management of the study species, the hazel dormouse, *Muscardinus avellanarius*, within the UK and in context to continental Europe. Finally, the aims and objectives of each of the chapters are summarised.

1.2 Habitat fragmentation and conservation genetics

Habitat Loss and fragmentation are the primary cause for global biodiversity loss and degradation of ecosystem services, often regarded as the primary driver of species extinction (Baillie, Hilton-Taylor and Stuart, 2004; Schipper *et al.*, 2008), but the decline in species persistence may be accelerated by resource overexploitation, species invasions and climate change (Thomas *et al.*, 2004; Brook, Sodhi and Bradshaw, 2008). To buffer this loss of biodiversity, there is a global effort to identify and conserve species and habitats using a range of tools, including both political and biological approaches. While many conservation programmes are conceived as a "crisis response" to biodiversity loss, the science of conservation has matured to aim for biological outcomes that are sustainable without extraordinary human intervention. This can be achieved through explicit consideration of ecological, demographic and genetic factors (Redford *et al.*, 2011). Ecological and demographic aspects of conservation are prominent in modern conservation practice, however genetic considerations have only relatively recently become widely acknowledged (Hedrick, 2001). While genetic factors are widely accepted as being important, for example recognizing the importance of inbreeding in *ex situ* populations, some genetic considerations are much less common in conservation management, such as identifying or restoring adaptive genetic variation in wild populations (Kohn et al., 2006; Ouborg et al., 2010). Recent and ongoing advancement of genetic tools means that it is now relatively inexpensive and fast to genotype many individuals, making the application of these tools practical where just a few years ago they may have been impossible in conservation applications. Thus, unless we incorporate genetic goals into standard conservation practice, such as the identification and management of

adaptive variation in natural and *ex situ* populations, we may not be doing the best we can to preserve biodiversity (Redford *et al.*, 2011).

Genetic management in conservation has three general aims: measuring genetic exchange between populations, identifying both evolutionary significant units (ESUs) and management units (MUs) for conservation, and genetic restoration of populations by replacing animals into the natural environment. One of the basic genetic tools in conservation planning is the measurement of genetic exchange (i.e., gene flow). Understanding gene flow allows for the planning of population management goals on a landscape level and provides basic information about population size and extent, but also allows monitoring of the effectiveness of management practice, for example to determine whether wildlife corridors are functioning (DeSalle and Amato, 2004). It is expected that populations with significant gene flow have relatively high adaptive potential to respond to environmental change and have a lower chance that specific negative genetic processes (such as inbreeding depression) will impact them (Allendorf et al,. 2013). The results of inbreeding are declines of individual survival and reproduction (reproductive fitness) within populations which, in small populations tends to lead to an increased risk of extinction (Hedrick, 2000; Frankham, 2005).

Another concern associated with introducing individuals to other populations is outbreeding depression, which can result in reduced reproductive fitness i.e. reduction in offspring fitness when spatially separated or genetically differentiated populations are mixed (Edmands, 2007; Frankham *et al.*, 2011). When individuals within populations are highly locally adapted to their environment inter-population hybridization may result in offspring not adapted to their environments or co-adapted gene complexes that can disrupt the selective advantage of their adaptation to an environment (Templeton *et al.*, 1986). As a result, threatened species in conservation management programs face a conundrum; should we maintain small fragmented populations at risk of inbreeding depression, or should we actively allow genetically distinct populations to interbreed that may increase the probability of outbreeding depression? It has been suggested that a scenario of mixing inbred populations that are ecologically and genetically similar as possible may reduce the risk of inbreeding depression and outbreeding depression (Edmands, 2007). A problem in conservation management of threatened species however, is the initial or continued evaluation of inbreeding and outbreeding in wild populations is necessary to ensure population viability, a rarity in conservation programs due to resources.

1.3 Conservation units or ESU's for species management

Conservation genetics uses genetic tools to assess and reduce the risk of extinction in populations that are vulnerable (Allendorf and Luikart, 2007; Frankham, Ballou and Briscoe, 2010). There is an established role for genetic tools in conservation, such as resolving taxonomic uncertainties and defining conservation management units (MUs) or 'Evolutionary Significant Units' (ESUs) (Ryder, 1986; Moritz, 1994), that may focus on the identification of new species or establish the identity of regional variants within species (Fraser and Bernatchez, 2001). While the ESU concept is in wide practice, there is some debate about which specific theoretical and practical criteria are sufficient to warrant specific conservation management. This is especially important when populations of species have become isolated or divergent to the point they represent unique units of genetic diversity (Moritz, 1994). In practice, the difference between the two concepts of conservation units is ESUs represent populations separated by evolutionary history, whereas MUs relate to populations isolated by lack of gene flow, regardless of their evolutionary history. The primary outcome of using the ESU concept in conservation is to recognize populations for conservation protection in accordance with local, national or international laws (Moritz, 1994). In conservation genetics the definition proposed by Moritz (1994) is commonly used, which defined an ESU as ''populations that are reciprocally monophyletic for mtDNA alleles and demonstrating significant divergence of allele frequencies at nuclear loci''.

Rigorous assessment of species delimitations is vital in order to prioritise conservation management efforts in order to restore population connectivity or reintroduce species to their once historic ranges. A major breakthrough in molecular ecology and evolution has been the realised usage of molecular tools such as phylogenetic marker genes (i.e mitochondrial genes) for the assessment of species diversity and differentiation and to unravel the within-species population structure. Mitochondrial DNA evolves at a much faster rate (5–10 fold) and as such mtDNA is more suitable for resolving contemporary events and defining evolutionary significant units (Wan *et al.*, 2004). Alternatively, this information can be used to inform the genetic captive management, reintroduction or augmentation of species. A second consideration is to explicitly consider the genetic biodiversity, represented by regional genetic structure of ESUs, to understand the possible impacts of gene flow between recognised populations managing the risk of losing adaptive genetic variation via reintroductions or captive breeding itself.

1.4 Detection of species using presence/absence

Understanding change in the abundance or distribution of species across heterogeneous and fragmented habitats is vital in order to effectively protect species at risk from anthropogenic activities and environmental change (Fischer and Lindenmayer, 2007). This requires monitoring effort sufficient to identify trends in the presence or abundance of a species at variable temporal and spatial scales. While many aspects of such conservation monitoring are influenced by the biological characteristics of focal species, others are influenced purely by the sampling design being suited to answer a specific question (e.g. is the species present? is the population changing in size?). While this appears straightforward, there is evidence that much conservation monitoring suffers from sampling design limitations, despite imperative for success, perhaps arising from limitations in time or human and economic resources (Legg and Nagy, 2006; Lindenmayer, Piggott and Wintle, 2013). Aside from ethical and conservation reasons, monitoring is often motivated by legal or practical imperatives. One example of this is the mitigation of sensitive species to development, where populations that would be impacted by human activities are identified and translocated as a method of preservation. There is evidence that mitigation translocation practices have increased rapidly recently (e.g., Ewen et al. 2012). However, the practice of mitigation is prone to the same limitations and imperatives as conservation monitoring and, unfortunately, has a similarly equivocal record of success (Germano *et al.*, 2015). Thus, there appears to be a general and widespread need to improve the relationship between sampling design methods and conservation monitoring.

The concept of site occupancy is increasingly being applied in ecological monitoring to detect the presence of species and to identify population change (Bailey, Mackenzie and Nichols, 2014). Detection probability for rare or elusive species can be low, which can lead to site occupancy underestimation or a false conclusion that a species is absent (Storfer, 2003). However, methods exist to account for imperfect detection and, using presence and absence data, occupancy modelling can be used to detect and monitor species of conservation concern (MacKenzie *et al.*, 2003; Mackenzie and Royle, 2005). Many studies have shown how detectability varies among species due to survey methodology, observer experience, habitat and temporally over the survey season (Bailey, Simons and Pollock, 2004; Pellet and Schmidt, 2005; Petitot *et al.*, 2014). There is also a substantial literature on the theoretical sampling designs for occupancy modelling (e.g., MacKenzie et al. 2002; Mackenzie & Royle 2005; Bailey et al. 2007). There are several empirical examples of the required survey effort or intensity required to reliably infer absence or to determine the precision of estimates for presence however, these tend to focus on species-specific problems (Barata, Griffiths and Ridout, 2017). On the other hand, variance in detectability, for example during the breeding season or over a year, might be

of special importance for species that are found in a variety of different habitats and are active in different seasons, especially if the pattern of activity may influence detectability. However, few studies have investigated how occupancy estimates may be influenced by survey intensity or duration.

1.5 Landscape genetics

Landscape modification and fragmentation are believed to cause reduction of habitat into smaller and spatially isolated patches with loss of connectivity, influencing population abundances and disrupting population dynamics (Wiegand, Revilla and Moloney, 2005). This often results in the reduction of gene flow and genetic diversity of populations (Frankham, 2005; Allendorf and Luikart, 2007) that can decrease the future persistence of species especially in the face of global climate change (Willi, Van Buskirk and Hoffmann, 2006) and anthropogenic landscape changes (Fischer and Lindenmayer, 2007). This often results in lower genetic diversity due the reduction of gene flow or increasing the levels of genetic drift and inbreeding due to non-random mating and population sub division (Reed and Frankham, 2003; Frankham, 2005) that can increase the likelihood of local extinction of species especially in the face of global climate change and anthropogenic landscape changes (Willi, Van Buskirk and Hoffmann, 2006).

The field of landscape genetics aims to understand the relationship between landscape features and population genetic structure (Manel *et al.*, 2003; Storfer *et al.*, 2007; Balkenhol *et al.*, 2009). Landscape features may influence landscape connectivity by acting as barriers or promotors to dispersal that can directly influence migration and gene flow, and thus impact the spatial genetic structure of species (DeSalle and Amato, 2004). Landscape genetics also allows for the assessment of functional connectivity between habitat patches, quantification of dispersal and gene flow between habitat patches, accounting for both distance and specific landscape features (Spear *et al.*, 2010). For many species, direct observation of dispersal or movement is not logistically feasible; as such, landscape genetics allows the identification of specific habitat variables or features that impact on gene flow. Assessments on the effect of landscape variables on movement can reveal important ecological and evolutionary insights, such as predicting local adaptation and evolutionary divergence when gene flow is restricted (Manel *et al.*, 2003; Storfer *et al.*, 2007; Segelbacher *et al.*, 2010).

Landscape resistance

Typically, to describe the relationship between geographic distance and genetic variation an isolation-by-distance (IBD) model is used, but this can have limitations in describing population genetic structure or genetic processes. However, many studies have used modern tools to map landscape resistance surfaces which can quantify the 'effective' distance between populations to test the effect of landscape characteristics on the dispersal or gene flow that exists between populations (Adriaensen *et al.*, 2003; McRae, 2006; Spear *et al.*, 2010). A resistance surface is a hypothetical representation of the cost (or resistance value) that reflects the effect that landscape characteristics have on the ability for species to disperse and migrate, which is represented by cells or pixels within a GIS raster map (Sawyer, Epps and Brashares, 2011). Least-cost modelling and circuit theory are commonly used in the field of landscape ecology (Adriaensen et al. 2003; Holderegger & Wagner 2008; Segelbacher et al. 2010) to model animal dispersal and gene flow within and between populations considering the influence of the landscape make-up in the form of landscape resistance. Least-cost pathways estimate the least cost distance, whereas circuit theory (McRae, 2006) estimates distance between focal nodes (populations) using resistance within the landscape. Such isolation-by-resistance (IBR) models evaluate the relationship between the landscape composition and the genetic differentiation that exists (McRae, 2006). IBR is based on the theory of electrical circuits where gene flow is analogous to an electrical current, for example current flows more easily across pixels with wide areas of low resistance and reduced flow is observed across areas of high resistance (McRae, 2006; McRae and Beier, 2007). A distance matrix outlines the total resistance value between all nodes within the entire surface. IBR offers advantages in that it ranks potential corridors and allows modelling of alternative linkages (Sawyer, Epps and Brashares, 2011) and reflects patterns of gene flow or dispersal within a landscape. However, both LCP and IBR rely on biologically correct estimation of resistance within surfaces, based on subjective decisions (researchers' or 'expert opinion') to parameterise resistance values (Zeller, McGarigal and Whiteley, 2012).

A way to overcome this limitation is to conduct validation with several resistance surfaces with differing values for landscape features and select variables which show good fit with genetic or observational data (such as capture-mark-recapture) (Garroway, Bowman and Wilson, 2011; Shirk *et al.*, 2015). Use of both IBD and IBR methods are of particular importance for populations of threatened species, which suffer from the effects of small population sizes (i.e.

Allee effects and inbreeding) further exacerbated by fragmented habitats and human-induced disturbances which can interfere with connectivity between populations or limit dispersal to new areas. These methods allow reliable conclusions to be inferred on the spatial genetic variation and effect of key landscape determinants on habitat connectivity and restrictors to gene flow.

1.6 The hazel dormouse: current status and conservation

The hazel dormouse (*Muscardinus avellanarius*) provides an ideal study system to investigate the effect of habitat fragmentation on genetic variation and population demography under a regime of climate change and human anthropogenic influences.

Distribution and phylogeography

The dormouse is a member of the Gliridae family (synonym Myoxidae), within the order Rodentia (Daams and de Bruijn, 1995). It is widely distributed across Europe and into North Asia minor (Hutterer et al. 2016) and is the only extant member within the genus *Muscardinus* (Daams and de Bruijn, 1995). The hazel dormouse is relatively common and widespread throughout its known range, and as such is listed as Least concern on the IUCN red list of threatened species (Hutterer, et al. 2016). Phylogeographic data for dormouse within

continental Europe have suggested that genetic lineages split dormice into Western (Western Europe and Italy) and Eastern clades (Central Europe, Balkan peninsula and Turkey) that are genetically divergent and allopatric, but that within these clades there is low genetic diversity (Mouton *et al.*, 2012). These genetic lineages in Europe are further subdivided into five genetically distinct and geographically separate sublineages (Mouton *et al.*, 2012).

Figure 1. Geographical range of *Muscardinus avellanarius* within continental Europe and northern Asia Minor (Turkey). Data from http://dx.doi.org/10.2305/IUCN.UK.2016-3.RLTS.T13992A22222242.en.

While it is understood that genetic data are sometimes critical to delineate units for conservation (Moritz, 1994; Crandall *et al.*, 2000; Funk *et al.*, 2012), there is no available information available to describe the phylogeography of the common dormouse in the UK. Due to population in the UK being isolated from conspecifics in the European mainland, a regional classification is appropriate and useful (Mace *et al.*, 2008). Thus, while there is a long-term decline in dormouse populations in the UK precipitating a national monitoring programme, habitat restoration efforts and population reintroductions (Bright *et al.*, 2006), information about regional genetic variation is lacking to inform these efforts or to monitor the effects, if any, of reintroduction projects.

Life history and Ecology

The hazel dormouse has been described as a "small, elusive nocturnal mammal" (Bright *et al.*, 2006), which naturally occurs at low population densities and exhibits arboreal behaviour (Bright, Mitchell and Morris, 1994; Juškaitis, 2003). It is a nocturnal species that inhabits areas of deciduous forest with a thick understory, but is also found in mixed deciduous and coniferous forests with a well-developed understory (Sanderson, 2004; Bright *et al.*, 2006; Juškaitis, 2007). Hedgerows are found to be good habitats for dormice, given that sufficient shrub diversity is present and can act as connective routes between isolated populations (Hurrel & Mcintosh 1984; Bright et al. 1994; Chanin & Woods 2003). Dormice are thought to have narrow habitat requirements related to woodland habitat complexity, for example, features such as mature shrubs or hazel coppice stands, and food availability such as the presence of hazel, honeysuckle, and bramble (Bright *et al.*, 2006). Dormice also lack a caecum required to digest cellulose, resulting in dormice sustaining themselves on a diet of highly nutritious food such as nuts, fruits, flowers and insects (Juškaitis and Büchner S, 2013). As such, it is a highly ecological sensitive species, vulnerable to habitat loss and fragmentation (Morris, 2003) and are regarded as a 'Bioindicator Species' of animal and plant diversity within woodlands (Bright *et al.*, 2006).

They exhibit a long hibernation period (~6 months) across their range, typically dormice active season is between April and November based on nest box and nest tube usage (Bright, Mitchell and Morris, 1994; Juškaitis, 2014). In spring and summer dormice also use torpor bouts as a method to save energy reserves during cold days (below 15 degrees), however, this was found to be more frequent in males (Juškaitis, 2005). In the UK population density ranges from up to 8-10 individuals per ha in optimal habitat to 1-2 adults per ha in suboptimal habitat (i.e. coniferous forest), the average population density in the UK was found to be 3.5 adults/ha (Bright and Morris, 1996; Bright *et al.*, 2006). Dormice fecundity is considered to be low in comparison to other rodent species with litter sizes between 2-9 individuals (average 4.6), however there are reported occasions of dormice producing two litters during the breeding season in the UK and other countries within Europe (Bright et al., 2006; Juskaitis, 2014).

1.7 Conservation status and management

Decline in population sizes has been reported in the northern range of dormice, as such the species is strictly protected in Europe (Habitat 103 Directive Annex IV, Bern Convention Annex III) and the UK given protection under the Wildlife and Countryside act 1981, and considered a priority under the UK Post-2010 Biodiversity Framework (UKBAP) (Bright et al. 2006). Within

the UK the hazel dormouse was widespread (Rope, 1885), however it has suffered a major historical decline in England since the late 19th Century (Hurrel & McIntosh 1984) and may still be in decline (Goodwin *et al.*, 2017). The reasons for the decline observed in dormice within the UK have been little explored, however the main driving factors are considered to be due to habitat loss and change of woodlands within the UK, poor dispersal capabilities, and changes in climatic conditions being faced by populations (Bright and Morris, 1996; Büchner, 2008). As such, this species is the focus of national monitoring programs and several conservation management plans, including the restoration of habitat connectivity, breeding and reintroduction programs. The National Dormouse Monitoring Programme (NDPMP) was initiated due to the ongoing declines observed. It is a volunteer based, nationwide monitoring scheme that utilises dormice nest boxes in order to gather long-term data on abundance, breeding success and population densities within different habitats and geographical areas. This scheme is managed by the People's Trust for Endangered Species (PTES) and covers 400 known sites of dormouse occupation throughout Britain (White, 2012). Such data provides a valuable resource in investigating the reasons behind the long-term decline observed in dormice and the impact of habitat fragmentation.

The hazel dormouse is especially important, due to its public appeal and is often regarded as a flagship species for conservation of woodland habitat (Morris, 2003). Due to the narrow habitat requirements related to to woodland habitat complexity and food availability they are regarded as a bio-indicator of ancient woodland health and as such, are ecologically sensitive to habitat loss and fragmentation (Morris, 2003; Wuttke *et al.*, 2012). Dormice are also considered to be affected by climate change, through changes in seasonal weather (temperature and precipitation) altering the activity patterns, onset of breeding, survival rates and indirect effects on the phenology of food plants (Bright and Morris, 1996). Such changes can affect the population levels and population dynamics of dormice populations having a direct impact on the future population persistence.

1.8 Thesis Aims

The overall aim of this PhD thesis is to understand how habitat variables associate with dormouse presence and influence gene flow and functional connectivity between populations at several spatial scales in order to inform future sustainable conservation management of this species in the UK. The specific chapters of the thesis are:

Chapter 2 investigates the phylogeographic pattern to assess the geographical and temporal pattern of genetic variation observed within the UK, and between the UK and continental European populations. It will also assess competing postglacial expansion hypotheses and estimate the timing of dispersal into continental Europe in this species.

Chapter 3 will investigate the use of occupancy modelling as a tool to identify the presence or absence of species under variability in detection probability during a season. The chapter aims to optimise survey start time and duration to inform governmental guidelines on survey methodology for this species.

Chapter 4 will investigate patterns of spatial population genetics and woodland connectivity using a landscape genetics approach. Results from microsatellite data will reveal the population genetic structure of populations of dormice within the UK regions of Suffolk, Somerset, North Wales, Essex and Cumbria. Landscape data (habitat cover and remotely sensing) will be collated and analysed to inform the relative importance of a range of landscape features on the genetic variability of populations and conduct least cost path analysis to identify connectivity routes based on landscape resistance and genetic data. Specifically it will investigate the correlation between genetic population structure and historical landscape configuration. The results will be discussed in the context of population genetic theory and current research in conservation genetics.

Chapter 5 will investigate the importance of density-dependence and climate variation in regulating population dynamics in a hibernating mammal. The research harnesses long-term capture mark-recapture data and Bayesian population modelling techniques (IPMs) to estimate demographic vital rates such as age-specific survivorship, population growth and fecundity. The findings are discussed in the context of conservation and woodland management for hibernating mammals.

Finally **Chapter 6** considers the findings of each chapter together and discusses these in context with the current literature. Further, it discusses the implications for conservation management of hazel dormice within the UK and directions for future research are proposed.

1.9 References

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2 Chapter 2: After the ice age: the impact of post-glacial dispersal on the phylogeography of a small mammal, *Muscardinus avellanarius.*

2.1 Abstract

We used genetic tools to assess phylogeographic structure of the common dormouse (*Muscardinus avellenarius*) since the end of the last glacial maximum, to identify post-glacial dispersal routes and to describe population units for conservation. Comparative analysis of mitochondrial genes (Cytochrome *b,* 704 bp, D-loop, 506 bp) and one nuclear gene (Beta-Fibrinogen, 550 bp) was conducted to reconstruct the recent demographic history within and between UK and continental European populations. Our analysis indicated phylogeographic variation in the UK is similar in magnitude to that found in other regions of continental Europe and suggests a recent population expansion. We present evidence which supports a single post-glacial colonization into the UK. Dispersal time calculations, calibrated with geophysical events, are coincident with the start of the Holocene period, 7.5-11 kya, a time when geological evidence suggests temperatures were stable, woodland habitat was prevalent and a land bridge was present to allow the dispersal of small mammals into the UK. We discuss our findings in the context of the extant geographical genetic structure described here and in relation to conservation management of this threatened species.

2.2 Introduction

The phylogeographic structure of many species within the northern hemisphere is thought to have been shaped by climatic changes and glacial episodes 126-25kya, during the Late Pleistocene (Webb and Bartlein, 1992; Hewitt, 2004). This period was characterised by extreme climatic fluctuations, which had a major role in shaping contemporary biogeography (Emerson and Hewitt, 2005). There is evidence that when climatic conditions were relatively extreme during the Pleistocene ice ages, temperate species in continental Europe were displaced to southern areas, for example to refugia in the Iberian and Apennine peninsulas, and the Balkan regions (Hewitt, 2004; Teacher, Garner and Nichols, 2009). In order to understand how evolutionary processes have shaped biodiversity, it is useful to study how historical range contractions and expansions, potential admixture between lineages, and associated genetic processes have influenced current spatial patterns of this diversity. A modern approach to investigate the evolutionary history of species is to interpret molecular phylogenetic data in the context of climatic and environmental changes (Franks and Hoffmann, 2012).

While the impact of post-glacial colonization routes from refugia are thought to have defined the biogeography of most extant species in temperate regions, for the majority of species the details remain debated or merely unknown (Hewitt, 1996, 2004; Willis and Whittaker, 2000). Phylogeographic studies allow us to make inferences on historical dispersal routes; in the case of many European species, the hypothesis of post-glacial expansion from refugia found in southern areas of continental Europe is generally supported (Hewitt, 2004). Fossil records indicate that many species were found in lower latitudes where climatic conditions were not as extreme (Provan and Bennett, 2008). However, there are several competing hypotheses proposed for the specific details and timing of post-glacial expansion of plant and animal species during the last glacial maximum (LGM), specifically in relation to colonization of the UK. There is evidence that species only dispersed from lower latitudes of continental Europe as glacial ice retreated, at the end of the Pleistocene (e.g. the timing of dispersal of cold-tolerant Lusitanian plants is coincident with this period (Beatty and Provan, 2013, 2014)). As such there is consensus that species dispersed into northern regions and the UK from 23 kya onwards as glacial ice caps retreated and tundra steppes were no longer frozen. The Early Migration Hypothesis (hereafter, EMH) posits dispersal prior to the Younger Dryas period (12.6-11.7 kya) (Herman and Searle, 2011). Species migrating at this time must have been more cold tolerant and able to survive the colder conditions of the Younger Dryas period (Stewart *et al.*, 2010; Montgomery *et al.*, 2014). The Late Migration Hypothesis (hereafter, LMH) predicts that species may have not dispersed and colonized northern regions of Europe until a warmer time period, the Holocene (11.5 kya). Evidence is equivocal for LMH and EMH predominantly affecting modern European biodiversity for species studied so far (Montgomery *et al.*, 2014).

Regarding the UK, there is evidence a land bridge connecting continental Europe and the UK was present during the Holocene period (23 kya to approximately 7.5 kya) until sea levels rose significantly and separated the UK from continental Europe (Sturt, Garrow and Bradley, 2013). This land bridge is thought to have acted as an important mechanism for the dispersal of different species into the UK (Montgomery *et al.*, 2014). The specific ecological requirements of different species (e.g., food resources and suitable habitat) are thought to have driven the route and timing of expansion into new areas (Lesbarrères, 2009). Range expansion by many modern species has been proposed to have occurred during a single post-glacial event. However, there is not clear evidence supporting a single source of the hypothesized dispersal. For example, there is evidence that some species share a most recent common ancestor with populations in Eastern Europe, such is the case in the pygmy shrew (*Sorex minutus*;Vega et al., 2010) and the pool frog (*Rana lessonae*; Zeisset and Beebee, 2001), while other species appear to have originated from Western European populations, such as the common frog(*R. temporaria*;

Teacher et al., 2009). Alternatively, water voles (*Arvicola terrestris*) show English and Welsh haplotypes nested within Eastern European clades from Iberian refugia and Scottish haplotypes nest within a Western European clade (Piertney *et al.*, 2005), suggesting multiple episodes of colonization. While the timing of these recolonization events are poorly understood, advances in phylogenetic methods allow us to calibrate species or population divergence, in conjunction with historical geological and fossil data, with much greater precision (Hewitt, 2000; Herman *et al.*, 2014). Thus, justifying the fossil record with estimated divergence time of genetic lineages is a powerful approach for understanding modern biogeographical patterns.

Here, we investigate the phylogeographic pattern of dispersal of the common dormouse, *Muscardinus avellanarius,* into the UK. The common dormouse has been described as a "small, elusive nocturnal mammal" (Bright et al., 2006), which naturally occurs at low population densities and exhibits arboreal behavior (Bright, Mitchell and Morris, 1994; R. Juškaitis, 2003). Dormice are thought to have narrow habitat requirements related to woodland habitat complexity, for example, features such as mature shrubs or hazel coppice stands, and food availability such as the presence of hazel, honeysuckle and bramble (Bright et al., 2006). As such, they are important as a bio indicator of ancient woodland health (Morris, 2003). Because of the empirical observation that dormice have a limited range associated with such habitat requirements, it is thought they have a relatively small ecological window for dispersal (Mortelliti et al., 2010). Therefore, the hazel dormouse is a compelling species to investigate the effects of the LGM on geographical expansion under a regime of climate change.

Phylogeographic data for the common dormouse within continental Europe have suggested that lineages from western and eastern populations are relatively divergent, but that within these clades there is low genetic diversity (Mouton et al., 2012). While it is understood that genetic data are sometimes critical to delineate units for conservation (Moritz, 1994; K. A. Crandall *et al.*, 2000; Funk *et al.*, 2015), there is no available information available to describe the genetic biogeography of the common dormouse in the UK. Thus, while there is a long-term decline in dormouse populations in the UK precipitating a national monitoring programme, habitat restoration efforts and population reintroductions (Bright et al., 2006), information about regional genetic variation is lacking to inform these efforts or to monitor the effects, if any, of reintroduction projects. Our objectives were to (1) provide phylogeographic coverage of common dormouse populations in the UK relative to its continental European range; (2) assess the geographical and temporal patterns of genetic variation within the UK, and between the UK and continental European populations, and; (3) assess competing post-glacial expansion hypotheses (EMH vs. LMH) by estimating the timing of dispersal in this species.

2.3 Material and methods

Sampling Collection

Non-invasive genetic sampling of hair was conducted during summer nest-box surveys in 2014 and 2015. Samples were stored in sterile tubes at -20°C. A total of 125 samples were collected from 25 populations around the UK (Table 1, Figure 1) and stored under licenses from Natural England and the UK National Trust. This sample size is commensurate with current standards for biogeographical genetic studies with the goal of estimating long-term divergence of lineages (e.g., see Gillespie, 2004; Mouton et al., 2012). Study sites were chosen to represent the current natural range of the common dormouse in the UK. Three of our study sites were from reintroduced populations within the UK (Table 1) as part of the dormouse reintroduction programme, to enable us to quantify genetic differentiation, if any, between these and natural populations. We analysed UK data we produced along with previously published sequences available on GenBank from continental Europe (Mouton et al., 2012) and outgroup taxa.

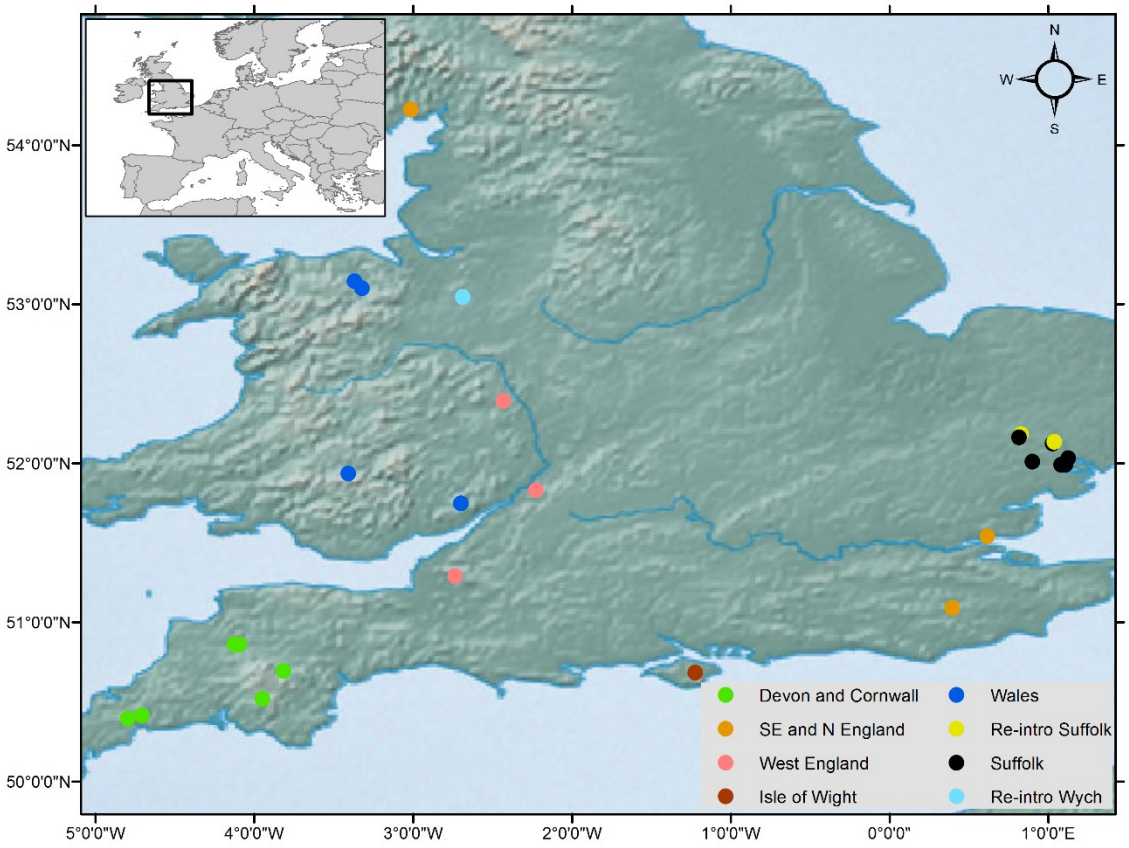

Figure 1. Geographical distribution of the common dormouse, *Muscardinus avellinarius,* and samples collected around the UK from 25 sites (125 samples) and haplotype distribution for concatenated sequences in fig 2.b. Key indicates the genetic grouping of populations and three reintroduced populations.

DNA extraction and sequencing

Total genomic DNA was extracted from hair roots using a Quick DNA extraction kit (Zymo research, USA) following the manufacturer's protocol with the addition of 20µl of 1M dithiothreitol during lysis. A 704 bp fragment of the mitochondrial Cytochrome *b* (Cyt*b*) gene was amplified and sequenced using primers LMA14255 and RMA15192 (Mouton *et al* 2012). DNA degradation was an issue affecting amplification using these primers. Consequently,

modified internal primers from Bentz and Montgelard (1999) were used (primers MUSCAR_RINTERN/MUSCAR_LINTERN) to amplify the gene in two fragments of between 300 and 440 bp from which contiguous sequences were generated to give the full 704 bp sequence. Additionally we amplified and sequenced a 506 bp fragment of the D-loop mtDNA (Stacy *et al.*, 1997) using primers (M15997/H16401) and 550 bp of Intron 7 of the nuclear gene Beta-Fibrinogen (*bfibr*) in the same localities (Table 1) using primers (BFIBR1/BFIBR2) previously used in closely related species (Seddon *et al.*, 2001).

Amplification was performed in 20-µl PCR reactions, containing <10ng of lyophilised DNA, 0.2 µM of each primer, 10 µl Bioline 2x PCR biomix (Bioline,UK) and Bovine serum albumin 0.1µg/µl. PCR amplification was performed using a G-Storm GS1 Thermal Cycler, with the following program: 95°C for 15 minutes; followed by 35 cycles of 95°C for 30 seconds, 48°C (Cyt*b*),60 °C (*bfibr*) or 58°C (D-loop) for 30 seconds, and 72°C for 45 seconds; and a final elongation at 72°C for 10 minutes. Amplified products were cleaned using ZR DNA sequencing clean-up kit (Zymo Research). DNA sequencing was then performed using BigDye v3.1 terminator and run at Manchester University DNA Sequencing Facility on an ABI 3730 48-well capillary DNA Analyser (Applied Biosystems, California, USA).

Phylogenetic analysis

Sequences were quality checked and aligned using BIOEDIT 7.2.5 (Hall, 1999) and further analyses were undertaken in MEGA 6.0 (Tamura *et al.*, 2013). The mutational model that best fit the data was identified using FINDMODEL (Posada and Crandall, 1998). The Maximum-likelihood (ML) trees were constructed using MEGA 6.0 (Tamura *et al.*, 2013). The robustness of the trees was assessed by 1000 bootstrap replications (Felsenstein, 1985). A Bayesian phylogeny was produced with MRBAYES v3.2.1 (Huelsenbeck and Ronquist, 2001). It was run for 100 000 000 generations and Bayesian posterior probabilities were set at a 50% majority rule consensus of trees sampled every 1000 generations and the consensus tree was generated in FIGTREE v1.3.1 (Rambaut, 2009). We consider nodes to be well supported with bootstrap values >70 (Hillis and Bull, 1993). A minimum spanning haplotype network was constructed using NETWORK 4.6.1.2. (Bandelt, Forster and Röhl, 1999) to examine genetic structure and geographical distribution of the mtDNA haplotypes for both Cyt*b* and D-loop. Haplotype (h) and nucleotide (π) diversity was estimated using DnaSP 5.10.01 (Librado and Rozas, 2009). Two neutrality test statistics, Tajima's D and Fu's Fs, were also estimated with DnaSP 5.10.01 (Librado and Rozas, 2009). The net distance between groups and average distances within groups were calculated using MEGA 6.0 (Tamura *et al.*, 2013).

To estimate how genetic variation was distributed within and among populations across geographical regions, an analysis of molecular variance (AMOVA) was performed based on pairwise differences using ARLEQUIN 3.0 (Excoffier and Lischer, 2010). AMOVA was conducted at three hierarchical levels of population subdivisions: among groups (UK and CNE (central northern Europe) groups); among populations (sub lineages within the UK); and within each population. F-statistics was estimated ARLEQUIN 3.0 (Excoffier and Lischer, 2010) based on mtDNA sequences (Φ ST) The significance of these parameters was estimated by 10,000 permutations of the distance matrix.

Divergence time estimation

The divergence time of UK populations was calculated using Bayesian sampling in BEAST 2.1.2 (Bouckaert *et al.*, 2014). For this analysis we used DNA sequences only for Cyt*b*, due to sequences being available on GenBank for continental European dormice (Mouton et al., 2012). This also allowed us to estimate the time of divergence for other genetic clades found in continental Europe (central northern Europe, Turkey, Balkans and Western Europe, Belgium). Analyses were performed under the GTR+G substitution model parameter (estimated by FINDMODEL), simulated with a substitution rate of 1% per million years, applying a relaxed log-normal molecular clock in BEAST 2.1.2 (Bouckaert *et al.*, 2014) The substitution rate was selected based on previous studies in the common dormouse (Mouton et al., 2012) and previous studies conducted in the dormouse family, Gliridae (Montgelard, Matthee and Robinson, 2003). The molecular clock was calibrated using prior distributions on the time to most recent common ancestor (TMRCA) of the UK clade. The UK clade was given a normal distribution truncated at lower and upper limits of 7.5 kya and 18 kya respectively, to coincide with the presence of the land bridge and the beginning of the Devensian time period when most temperate species were thought to disperse into the UK (Montgomery *et al.*, 2014). This time period spans both the EMH and LMH hypotheses and was chosen in order to allow us to resolve them. The analyses were repeated without the prior on the UK clade to test the effect of the priors on the posterior distributions. All other settings were defaults provided by BEAST. Two independent runs were performed with 100 000 000 Markov Chain Monte Carlo (MCMC) samples every 1000th generation. Convergence of chains was visualized using TRACER 1.6 (Rambaut and Drummond, 2013) with a burn-in of the first 10 million generations.

Demographic histories

Demographic history was analysed using mismatch analysis conducted in ARLEQUIN 3.5 (Excoffier and Lischer, 2010) and DNASP 5 (Librado and Rozas, 2009) for the UK and compared to the Central northern European clade (CNE) (i.e. Mouton et al. (2012). Multimodal distributions are considered to correspond to a condition of demographic stability or multiple colonization, whereas recent sudden population expansions would be observed by unimodal patterns (Slatkin and Hudson, 1991).

2.4 Results

2.4.1 Phylogenetic analysis and genetic diversity

A total of 704 nucleotide positions of the target Cyt*b* were resolved in all 125 individuals from around the UK. We found a total of five haplotypes, four of which are unique to known European haplotypes (all sequences have been deposited in GenBank; Table 1 & Fig 2a), of which 76 positions were parsimony-informative. We found nucleotide frequencies of 31% T, 26% C, 28% A and 15% G. Among samples from England and Wales nucleotide diversity (π) was 0.00275 ± 0.00023 per site (Table 2), marginally lower than the CNE lineage (0.00337± 0.00707). Haplotype diversity we found was similar to that reported in continental Europe (UK 0.727±0.052, EU 0.786±0.096).

ML analyses were performed using the GTR + Gamma model suggested for the data by the Akaike Information Criterion (AIC) in FINDMODEL. ML (Fig 3) and Bayesian inference tree showed identical topologies aligning UK dormice as a lineage close to CNE. The median joining network (Fig 2a) showed geographical partitioning of UK and CNE populations. In the cluster of UK haplotypes, only one mutational step was observed between each population forming geographical groupings of a SE England, N England and Wales (hap 39), Isle of Wight (hap 40), SW England (hap 41) and Suffolk (hap 40) (Fig 1&2a). In total, UK dormice show a 0.3% genetic difference with mainland EU. The Cumbria sample (the most northerly extant population in England) forms a cluster with this central haplotype group. Haplotype 39 (Table 1) was found in 11 populations and is the most frequent group sampled in this study that also form a grouping with CNE clade (Fig 2a).

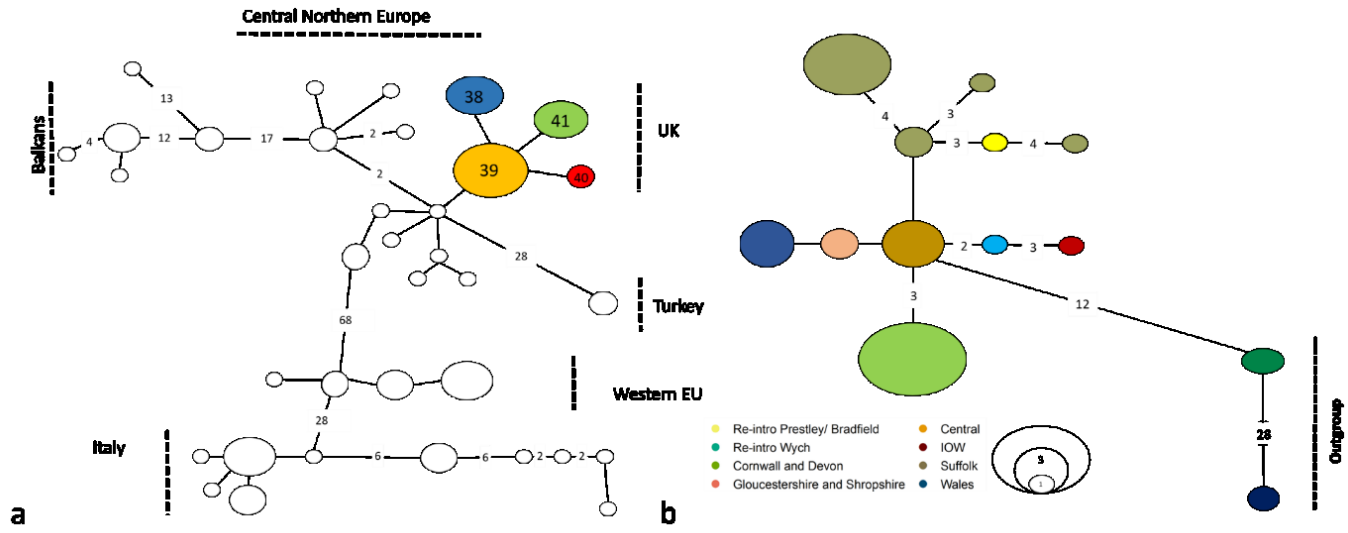

Figure 2. Haplotype map minimum spanning network. Numbers along the branches correspond to the number of mutational steps observed between haplotypes, no number is one mutational step, size of the circles correspond to the number of populations for each haplotype. (A) Number for UK haplotypes given in the circles correspond to Table 1. (B) D-loop haplotypes are coloured as found in key. Reintroduction sites in the key relate to * in Table 1 and outgroups are sequences from Central Northern Europe and Western European haplotypes found in Mouton et al. (2012).

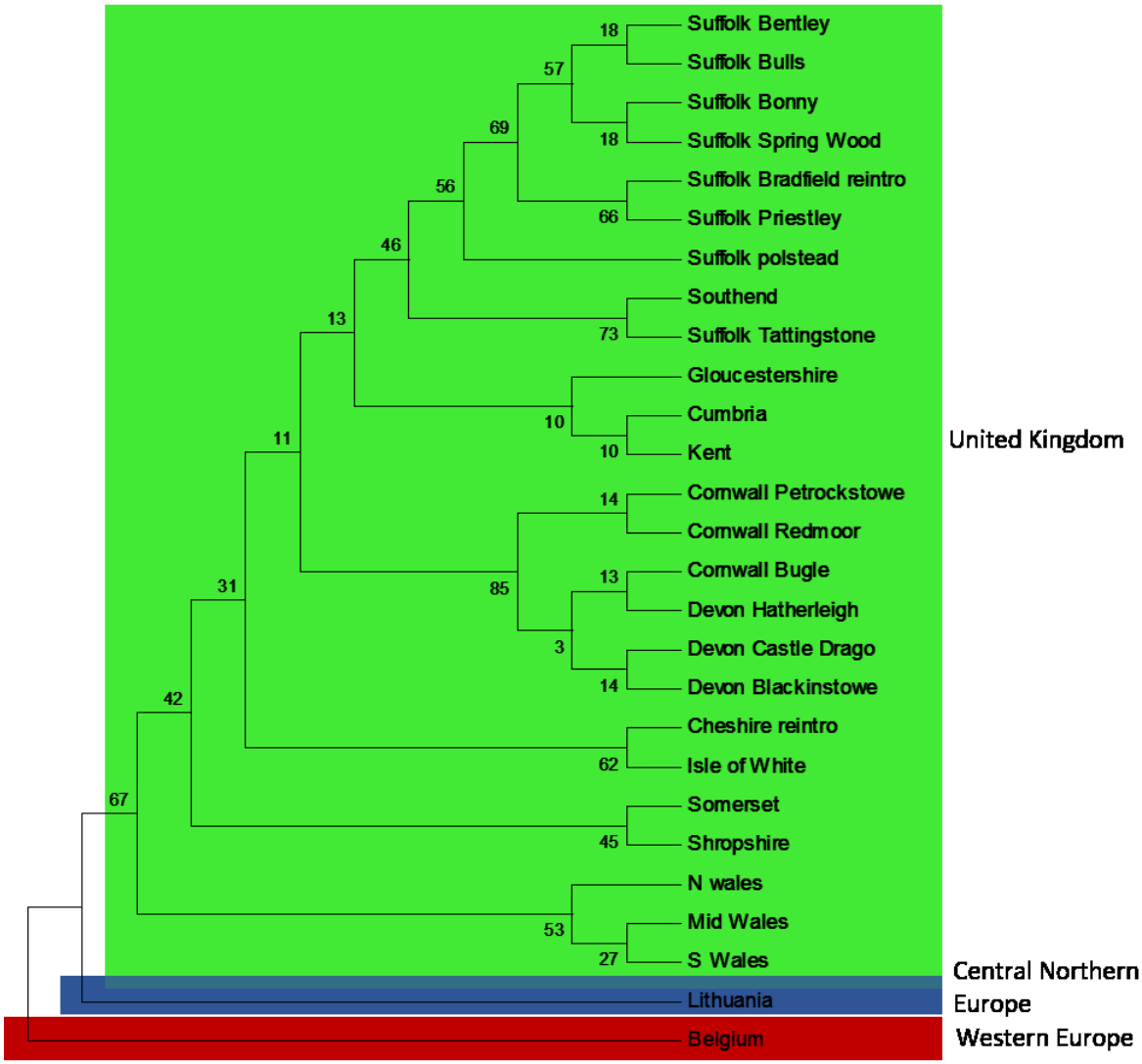

Figure 3. Maximum-likelihood (ML) topology for concatenated sequences (Cyt*b*, D-loop and *bfibr*). The numbers on the branches indicate the bootstrap values.

Table 1. Geographic location of sampling sites, n = number of individuals, haplotype ID for Cyt*b* (see fig 2a) and corresponding GenBank accession numbers for each gene. Reintroduction locations are indicated (*)

ID	**Location UK**	**n**	**Haplotype ID** Cyt*b*	**GenBank accession** Cyt*b*	**GenBank accession D-loop**	**GenBank accession *bfibr***
Polstead	Suffolk	5	Hap38	KP257560	KU312940	KU312944
Bonny	Suffolk	5	Hap38	KP257560	KU312940	KU312944
Bradfield*	Suffolk	5	Hap39	KP257563	KU312942	KU312944
Priestley*	Suffolk	5	Hap39	KP257563	KU312942	KU312944
Bentley	Suffolk	5	Hap38	KP257560	KU312939	KU312944
Bulls	Suffolk	4	Hap38	KP257563	KU312939	KU312944
Tattingstone	Suffolk	5	Hap38	KP257563	KU312941	KU312944
Spring wood	Suffolk	4	Hap38	KP257563	KY614305	KU312944
Southend	Southend	5	Hap39	KP257560	KU312933	KU312944
Briddlesford	Isle of Wight	5	Hap 40	KP257562	KU312935	KU312944
Hatherleigh	Devon	1	Hap 41	KP257561	KU312934	KU312944
Castle Drago	Devon	5	Hap 41	KP257561	KU312934	KU312944
Blackinstowe	Devon	5	Hap 41	KP257561	KU312934	KU312944
Petrockstowe	Cornwall	5	Hap 41	KP257561	KU312934	KU312944
Redmoor	Cornwall	5	Hap 41	KP257561	KU312934	KU312944
Bugle	Cornwall	5	Hap 41	KP257561	KU312934	KU312944
Callow	Somerset	5	Hap39	KP257560	KU312938	KU312944
Brooks	Shropshire	1	Hap39	KP257560	KU312938	KU312944
Roudsea	Cumbria	4	Hap39	KP257560	KU312933	KU312944
Bontuchel	North Wales	5	Hap39	KP257560	KU312937	KU312944
Clocaenog	North Wales	6	Hap39	KP257560	KU312937	KU312944
Brecknock	Mid-Wales	4	Hap39	KP257560	KU312937	KU312944
Siccaridge	South Wales	2	Hap39	KP257560	KU312937	KU312944
Bear Foot	Gloucestershire	4	Hap39	KP257560	KU312938	KU312944
Wych*	Cheshire	5	Hap40	KP257560	KU312933	KU312944
Lamberhurst	Kent	5	Hap39	KP257560	KU312933	KU312944
-	Lithuania	7	-	-	KU312936	KU312945
-	Belgium	10	-	-	KU312932	KU312943

A 506 bp fragment of D-loop was sequenced for 125 individuals (Table 1). Haplotype diversity (0.8738 ±0.0035) and nucleotide diversity (0.0616 ± 0.0071) were higher than we found in Cyt*b* (Table 2). Tests for departure from neutrality on both Cyt*b* and D-loop (Tajima's D and Fu's Fs) were significant, (both $P < 0.05$), consistent with a recent demographic expansion. The *bfibr* (726 bp) nuclear marker was sequenced for all individuals around the UK, Lithuania and France (no other sequences are available on GenBank for common dormice). This gene was found to be monomorphic across all populations in the UK, however there was divergence between the UK and continental European populations. As such *bfibr* and D-loop sequences were only further analysed as concatenated sequences along with Cyt*b*.

Table 2. Genetic diversity of Cyt*b* for both the UK and Europe and D-loop for the UK. Number of haplotypes (H), nucleotide diversity (π), Haplotype diversity (Hd) and the associated standard errors. Neutrality test statistics (Tajima's D and Fu's Fs) with significance derived from 10,000 simulations* $P<0.05$.

Lineage	**n**	**H**	π	**se**$_{\pi)}$	**Hd**	**se**$_{Hd}$	**Taj** D	**Fu** F_s
Cytochrome *b*								
UK	125	5	0.00275	0.00023	0.728	0.052	-0.11692	-0.821*
Central Northern Europe	21	13	0.00337	0.00707	0.786	0.096	-0.507	-6.318*
D-loop								
UK	125	8	0.0616	0.0071	0.874	0.035	-0.5758	-0.959*

Concatenated sequence analysis (1760 bp) showed a total of 7 unique haplotypes, and had higher resolution to reveal regional genetic variation than that of Cyt*b* alone (Fig 2b). This consists of additional genetic groups in Wales, North England and South East England, Central England, East England (Suffolk region), South West England (Devon and Cornwall) and the Isle of Wight. Phylogenetic analysis indicates a divergence between the clade containing the UK and CNE samples (Fig 3) with those from Western Europe. Due to a clustering of CNE and UK populations, AMOVA analyses only included these two genetic groups. The AMOVA analysis for Cyt*b* shows most of the variation is within populations (70.72%, $p < 0.001$) however, relatively high (24.62%, $p < 0.001$) variation between populations within groups and less variation between groups (4.66%, $p < 0.001$) was also observed (Table 3). The AMOVA for D-loop (Table 3) shows most variation was shown between populations within populations (75.40, $P<0.001$). This indicates population structuring between sampling locations within the UK but no significant variation between UK and CNE.

Table 3. Hierarchical distribution of mtDNA using Analysis of Molecular Variance (AMOVA) of Cyt*b* and Dloop (d.f. degrees of freedom, Φ Phist, P= significance value).

Source of Variation	**d.f.**	**Sum of Squares**	**Variance components**	**% variation**	**Φ st**	**P**
Cyt*b*						
Among groups	1	2.265	0.03610	4.59	0.67263	<0.001
Among populations Within groups	2	2.685	0.16259	20.62		
Within populations	21	12.329	0.58711	74.72		
Total	**24**	**17.280**	**0.78580**			
Dloop						
Among groups	1	15.431	0.05131	10.64	0.76039	<0.001
Among populations Within groups	2	5.227	0.36350	75.40		
Within populations	21	2.625	0.0631	13.96		
Total	**24**	**23.283**	**0.47791**			

In Cyt*b* and D-loop the reintroduced population of Wych in North England shows a close grouping with the Isle of Wight haplotype (Table 1). For Cyt*b*, only one mutation is observed between sequences as seen in both the ML tree and haplotype network (Fig 2&3), whilst for D-loop there are four mutational steps present (Fig 2b). Samples sequenced from Suffolk in East England show a close genetic grouping with each other, especially in the D-loop haplotype network (Fig 2b). Two populations sampled in Suffolk were part of a national re-introduction programme and clustered separately from natural populations in Suffolk (by a single mutation). Based on our Cyt*b* analysis, these reintroduced populations group with the South East and North England clade (Fig 2a), likely due to the genetic source of these populations from Southern England. Based on our D-loop analysis, one reintroduced Suffolk population (Bradfield) falls into the CE clade and the second reintroduced population groups with another Suffolk population (Bonny; Table.1 & Fig 2b). The last population is geographically close (less than 1km) with available connective habitat to the population in which it forms a haplotype connection with (see Fig 2b), but to assess whether there is evidence of gene flow or whether this is consistent with ancestral polymorphism, additional data and analysis would be required.

2.4.2 Divergence Time calculation and demographics

Molecular clock analysis was based on the mutation rate for Cytb previously estimated in the Gliridae (1% per Myr) and new calibration points for known periods of time when a land bridge was present between UK and EU between 7,5 and 25 kya at the end of the LGM (Shennan *et al.*, 2000). Based on these priors, TMRCA estimates show the existing UK dormice populations originated from a CNE clade around 10.8 kya (median 95% higher posterior density, HPD), at the end of the LGM (Fig 4). The 95% HPD estimates range from 8.7 to 14.9 kya and all sampled parameters achieved a minimum effective samples size (ESS) average of 314. Test runs with alternative prior distributions did not influence posterior estimates of this parameter. There is some variation in the estimates in the time of origin for this split (Fig 4), however the 95% HPD values fall within the timings of the land bridge being present after the end of the Younger Dryas period. Our TMRCA estimates for the divergence Turkey and CNE clades are 22.7 to 34 kya (median 95% HPD, 27.9 kya), a time at the end of the LGM as glacial caps were retreating from northern Europe. Estimates also place a Balkan and Turkey clade divergence at 35.6 kya and a Western European (Belgium) clade divergence at 65.5 kya. Mismatch distribution analysis (Fig 5) showed a unimodal distribution for the UK and CNE indicating a recent, rapid population expansion, which is consistent with an expansion at the end of the LGM when a land bridge facilitated dispersal of dormice to the UK.

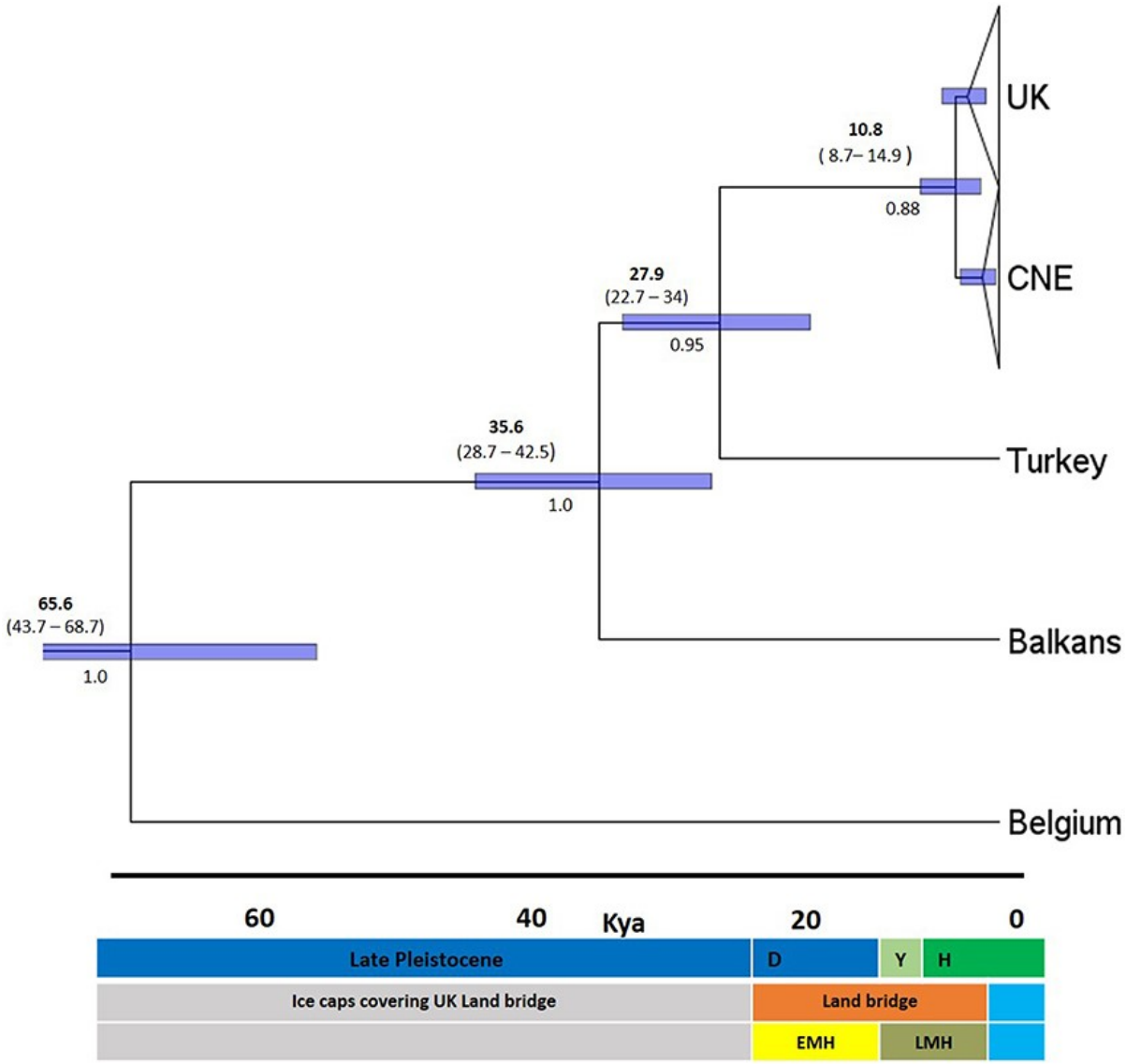

Figure 4. Maximum clade credibility chronogram of the Cyt*b* dataset. Derived from an analysis in BEAST using the GTR+I+G model and a relaxed clock. Median 95% higher posterior density (HPD) values in bold and age ranges below, Node bars also indicate 95% HPD age ranges. Dates in kya, definitions in middle key D- Devensian period, Y- Younger Dryas, H- Holocene period. Blue highlights period when UK was isolated from continental Europe by rising sea levels. The bottom bar indicates the approximate timings of the two colonization hypotheses early migration hypothesis (EMH) and late migration hypothesis (LMH).

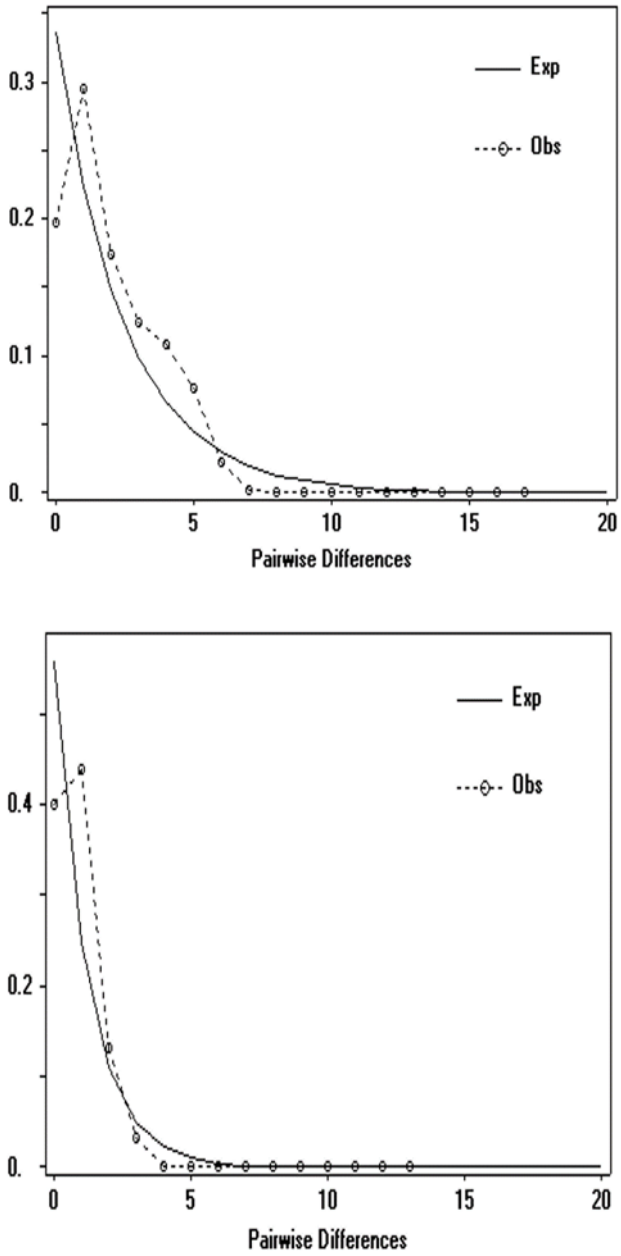

Figure 5. Mismatch analysis of Cyt*b* sequences (704 bp) top, central northern European population (n= 25), bottom UK populations (n=125).

2.5 Discussion

2.5.1 Genetic structure between UK and continental Europe

Here, we present the first evidence of genetic divergence between UK common dormouse populations and those in continental Europe. Our sequence analysis of Cyt*b*, in the context of published data from continental Europe (Mouton, *et al*. 2012), suggests significant geographic partitioning of sub-lineages within the UK. Further, it is shown that these UK lineages are most closely related to populations located in CNE (Fig 2) and the within-population variation detected here is similar to that identified within regional subgroups in continental Europe by Mouton *et al*. (2012). The nuclear gene *bfibr,* although monomorphic in the UK, shows genetic subdivision from continental Europe with a similar mutational difference to that of Cyt*b* and D-loop studied here. Comparisons of genetic diversity for mitochondrial genes observed in the UK are comparable to that found in the genetic clades of continental Europe which may be a result of recent genetic divergence in the order of magnitude in thousands of years. Because the entire mitochondrial genome is inherited as a unit, sequencing more than one mitochondrial gene, as we have done, whilst offering more resolution on the history of the maternal lineage, still represents a single lineage and thus has some inherent limitations. For this reason the addition of a nuclear gene in our analysis helps to confirm the identification of divergence between continental Europe and the UK. While there is evidence for reciprocal monophyly between continental European and UK dormice, further study of adaptive genetic variation in UK dormice is perhaps needed to inform management of the UK common dormouse as distinct.

2.5.2 Genetic structure within the UK

We describe the genetic structure of the common dormouse within the UK for the first time. Here, we present evidence of regional genetic clustering of populations around the UK based on mtDNA variation clustering (Fig 3). This regional variation is possibly explained by gross geographical features; the UK has several major rivers and uplands in the North, West and South of the country which may be important geographical boundaries leading to the further genetic clustering seen in this study. Although the nuclear gene *bfibr* was monomorphic in our samples, the allelic sequence variant we report is unique to the UK. Mitochondrial DNA evolves at a much faster rate (5-10 fold) and as such mtDNA is more suitable for resolving contemporary events and defining evolutionary significant units (Wan *et al.*, 2004). While the extent of regional genetic variation we describe is modest, it suggests the possibility of local adaptation and genetic differentiation which warrants further study at a finer geographical scale.

The phylogeographic structure we describe contrasts with that of previous small mammal studies. Other species of small mammal such as water voles, bank voles, field voles and pygmy shrews show distinct population clusters in Scotland, Wales and Cornwall, or a so-called "Celtic fringe" within the UK (Searle *et al.*, 2009). This is possibly explained by a two-stage colonization where the initial colonists were largely replaced by the second wave, leaving peripheral populations in northern and western areas of the UK. A pattern of single colonization was observed in dormice. This is possibly explained by the relatively low dispersal capability of the common dormouse.

Haplotype network analysis shows a clustering between the Isle of Wight and the reintroduced Wych population in North England. Based on the geographical distance between the Isle of Wight and Wych (over 130 km), the relatively close genetic relatedness of these populations is surprising. The Isle of Wight island population is relatively isolated off the south coast of England. However, the Wych population was reintroduced in 2001, with founding individuals originating from the Isle of White (pers. comm. Nida al Faluja, PTES). In Suffolk, the reintroduced populations we studied revealed one population grouping with natural Suffolk and South East England populations, suggesting genetic exchange between native and introduced stock. Thus, our analysis reveals the ability to pick up the genetic signatures of reintroduced populations to identify source populations when records are lacking. The consequences of admixture between lineages must be considered in genetic management to reduce the risk of losing adaptive potential of native populations and potential outbreeding depression in contrast with increasing genetic variation to reduce inbreeding depression (Frankham *et al.*, 2011; Houde *et al.*, 2011; Weeks *et al.*, 2011). Further research integrating ecological and demographic data and utilising data from polymorphic functional genetic markers will aid the investigation of the potential consequences and to assess present-day gene flow.

2.5.3 Colonization of mainland UK- Land bridge hypothesis

Single post-glacial colonization into the UK through a Central north western European clade is robustly supported in the haplotype network, Bayesian and ML trees. Molecular clock analyses timed this around the beginning of the Holocene period, supporting a late migration of dormice (LMH) along with several collateral lines of evidence. First, the land bridge 'Doggerland' connecting the UK and mainland Europe was present around the beginning of the Holocene period. It is thought that Doggerland landmass had suitable vegetation for dormice dispersal during the early Holocene period (Mix, Bard and Schneider, 2001; Spinney, 2008; Steffensen *et*

al., 2008) until the formation of the English Channel approximately 8000 kya (Weninger *et al.*, 2008). A key factor on colonization events would have been a cold period at the very end of the last glaciation, the Younger Dryas (11700-12900 kya) upon which a more temperate period followed (Hewitt, 2004; Steffensen *et al.*, 2008). Thus the TMRCA estimate after the Younger Dryas period is expected. In addition, the earliest dated dormice fossil remains in the UK from the post ice age period are from 9000 years ago using radio carbon dating (Montgomery *et al.*, 2014) concordant with the estimated ranges in this study. Britain also has a congruent mammal species composition as found in Belgium and the Netherlands (Montgomery *et al.*, 2014). Similarly, the Isle of Wight haplotype in southern UK, was isolated from the mainland around the same period. In total, these lines of evidence are consistent with the suggestion that dormice expanded through this land bridge to what is now the UK after the Younger Dryas period. This study suggests that the younger Dryas period may be more important than once thought in the shaping of the current phylogeographic structure in UK mammalian fauna.

2.5.4 Conservation implications of genetic structure

The pattern of regional genetic variation of the common dormouse described here has a direct relevance to conservation management in the UK. Habitat enhancements can be directly related to improving connectivity between populations which were once historically connected. Alternatively, this information can be used to inform the genetic captive management, reintroduction or augmentation of species. The program of reintroduction of common dormice in the UK, in conjunction with the national Biodiversity Action Plan for the species, has a goal of both bolstering the quality and size of extant populations but also restoring additional populations to sites which were once formerly occupied but have gone locally extinct. There have been some important successes in these reintroductions; however the captive born founders for these reintroduced populations come from stock of heterogeneous origin. This is evidenced in the results presented here, where the northerly reintroduced Wych population appears to be genetically discontinuous with geographically close populations and a similar situation can be seen in South East England (Suffolk populations). We further suggest that, because there is no critical overall extinction risk for the common dormouse, preserving the natural pattern of genetic variation observed in natural populations could, and perhaps should, be considered when reintroducing animals back into the wild. A second consideration is to explicitly consider the genetic biodiversity, represented by regional genetic structure, to understand the possible impacts of gene flow between these populations managing the risk of losing adaptive genetic variation via reintroduction itself.

Rising sea levels caused flooding of Doggerland that historically connected the UK and continental Europe around 7.5 kya, dormice have been isolated and under environmental change. Thus, the future persistence of the UK dormouse will be reliant on the phenotypic plasticity or the adaptive genetic variation available. Dormice in the UK have seen population declines of around 50% in the last 25 years (Bright et al., 2006), and have become extinct from many northern areas of the UK where they were historically present, a pattern not observed in the rest of northern Europe. What is particularly concerning for threatened species, such as dormice, is climate change and expanding human populations will lead to increased fragmentation and hence further isolation of genetic variants, such as those presented in this study. Variable annual climate fluctuations are known to affect hibernating mammal species, due to food availability upon emergence (Inouye *et al.*, 2000), and given the relatively low dispersal potential of dormice, genetic consequences of these climatic changes may be more severe. As such, if there are further population declines to the UK dormice, due to the relatively close genetic relationship of a CNE genetic clade, this may be a suitable source for future reintroductions.

There has been a considerable improvement in our current knowledge and understanding of the post-glacial expansion of species and the resulting genetic diversity of species on their range boundaries. This knowledge should play an important role in informing policy decisions both at local and national scales with regards to genetic conservation of species. Another consideration is that these peripheral populations on the range boundaries are often referred to as leading-edge populations and considered to easily become extinct (Hampe and Petit, 2005), it is considered that conservation efforts should ensure the survival of these populations as they play a critical role in the future response to climate change, due to their role in historical range movements expanding into new habitats and harbour evolutionary potential to respond to climatic changes (Lesica and Allendorf, 1995). Therefore, we propose that further research is required in common species such as the dormouse which are at risk or susceptible to declines. Finally, we recommend that monitoring programmes take into account not just population demographics but genetic make-up and predict the evolutionary potential of species to firstly, diagnose declines faster and secondly, to prevent further declines in the future.

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3 Chapter 3: Optimizing occupancy and detection probability in conservation monitoring: empirical case study the hazel dormouse

3.1 Abstract

In order to effectively conserve biodiversity ecologists need to conduct reliable and rigorously designed surveys in order to detect species and estimate local abundances. However, detection issues and ability to determine the survey effort required to reliably detect species presence or abundances can be challenging, especially for rare and elusive species. We use a forest dwelling small mammal, the hazel dormouse, a European protected species as a model system to explore a cost-effective sampling design that optimises the probability of detecting species and testing temporal covariates influence on detection probability. We collected detection/non-detection data via nest tube surveys in 144 sites from areas of South West England and South East England. We fitted single-season occupancy models to our data, the results of which showed that detection probability of dormice are highly temporally dependent with early months of the year having a low detection probability (0.21-053; April-June). Whilst detection probability peaked during breeding months (0.89; September). Survey intensity had a significant impact on detection probability, as such, it is recommended a minimum of 50 nest tubes are used in dormice surveys. For species such as dormice that are rare and low detectability, occupancy can be reliably estimated with comparatively lower number of surveys dependant on the starting month. Variation in detection probability can be complex and influenced by effects of both temporal and spatial scales, however here we show that temporal covariates can be controlled with comparatively low number of surveys with optimal starting periods and intensity of nest tubes used. Our findings provide empirical evidence to support robust ecological surveys, a legal requirement in the UK, for the presence of dormice and support conservation of this species in identifying the presence in sites previously unknown. Conservation practitioners may have limited knowledge of the relative abundance and likelihood of detection of a particular species and as such can apply these guidelines in different geographical locations.

3.2 Introduction

Understanding change in the abundance or distribution of species across heterogeneous and fragmented habitats is vital in order to effectively protect species at risk from anthropogenic activities and environmental change (Fischer and Lindenmayer, 2007). This requires

monitoring effort sufficient to identify trends in the presence or abundance of a species at variable temporal and spatial scales. While many aspects of such conservation monitoring are influenced by the biological characteristics of focal species, others are influenced purely by the sampling design being suited to answer a specific question (e.g., is the species present?; is the population changing in size?). While this appears straightforward, there is evidence that much conservation monitoring suffers from sampling design limitations, despite imperative for success, perhaps arising from limitations in time or human and economic resources (Legg and Nagy, 2006; Lindenmayer, Piggott and Wintle, 2013). Aside from ethical and conservation reasons, monitoring is often motivated by legal or practical imperatives. One example of this is the mitigation of sensitive species to development, where populations that would be impacted by human activities are identified and translocated as a method of preservation. There is evidence that mitigation translocation practices have increased rapidly recently (e.g., Ewen et al. 2012). However, the practice of mitigation is prone to the same limitations and imperatives as conservation monitoring and, unfortunately, has a similarly equivocal record of success (Germano *et al.*, 2015). Thus, there is appears to be a general and widespread need to improve the relationship between sampling design methods and conservation monitoring.

The concept of site occupancy is increasingly being applied in ecological monitoring to detect the presence of species and to identify population change (Bailey, Mackenzie and Nichols, 2014). Detection probability for rare or elusive species can be low, which can lead to site occupancy underestimation or a false conclusion that a species is absent (Storfer, 2003). However, methods exist to account for imperfect detection and, using presence and absence data, occupancy modelling can be used to detect and monitor species of conservation concern (MacKenzie *et al.*, 2003; Mackenzie and Royle, 2005). Many studies have shown how detectability varies among species due to survey methodology, observer experience, habitat and temporally over the survey season (Bailey, Simons and Pollock, 2004; Pellet and Schmidt, 2005; Petitot *et al.*, 2014). There is also a substantial literature on the theoretical sampling designs for occupancy modelling (e.g., MacKenzie et al. 2002; Mackenzie & Royle 2005; Bailey et al. 2007). There are several empirical examples of the required survey effort or intensity to reliably infer absence or to detect the precision or accuracy of estimates of presence however, these tend to focus on species-specific problems (Barata, Griffiths and Ridout, 2017). On the other hand, variance in detectability, for example during the breeding season or over a year, might be of special importance for species that are found in a variety of different habitats and are active in different seasons, especially if the pattern of activity may influence detectability. However, there are few studies that have investigated how occupancy estimates may be influenced by survey intensity or duration.

Our study investigates how sampling effort and temporal variance affects occupancy estimation in a small, arboreal mammal that naturally occurs at low density, the hazel dormouse (Bright et al., 2006; Bright & Morris, 1996). The hazel dormouse is legally protected across its European range (Wildlife and Countryside Act 1981; Habitat Directive Annex IV, Bern Convention Annex III) and is considered a bio indicator of woodland health (Bright et al., 2006)). Because of their conservation status, monitoring presence is mandated to determine if mitigation action is required. This is challenging both because of their low density and elusive nature, but also because they are inactive for up to six months each year. Habitat degradation has resulted in a reduction of suitable habitat for dormice across their range and, despite legal protection, dormouse range has become smaller and fragmented (by more than half since the 19th century (Goodwin *et al.*, 2017). Methodology to detect dormouse presence is laborious and there is little information available about how survey effort and timing relate to successful detection. Nest tubes are accepted as a standard method for dormouse surveys (Bright et al. 2006), perhaps because they are relatively inexpensive and easy to deploy, and are mainly used for short term surveys.

Here, we aimed to evaluate the impact of survey effort and duration on the detection of dormouse presence that would allow better allocation of survey effort and resources using nest tubes. We used a hierarchical occupancy modelling approach on surveys across multiple sites in two regions of the UK were dormice are known to be present in order to estimate variation in detection and occupancy. Specifically, we aimed to (i) Identify the most important determinants of detection and occupancy; (ii) Compare detection sensitivity relative to survey intensity, and (iii) Estimate the effect of start time and duration for the detection of presence/absence in this species. We discuss our results in the context of survey sampling design in a general framework, where there is a conservation imperative to detect presence but where time and resources may be limited.

3.3 Materials and Methods

3.3.1 Study system and sites

We collected presence/absence data during monitoring surveys using nest tubes (6 x 6 x 20 cm). This work was conducted in two regions of the UK, South-West England (Cornwall, Devon and Somerset) and East England (Suffolk). In the South West region, 16 nest tubes were placed at 20m intervals at 117 sites within the yearly period of 2002. In the area of Essex and Suffolk 50 nest tubes were placed at 20m intervals at 27 sites in the months of April to December in 2016. At both study sites nest tubes were placed on horizontal branches and put out in the

month of April, left until November of the same year, and were checked for presence or absence on a regular monthly period. For both studies, it is assumed that there are no systematic spatial or temporal effects between the surveys that hinder direct comparison. Within each study region the sites represented a range of typical dormouse habitat, including woodland, hedge and scrub. The presence of dormice was recorded on each visit based on the presence of dormouse nests or dormice by observers. Dormouse presence was identified as dormouse nests or individual dormice detected by trained observers. A matrix of presence and absence data was coded from the surveys from each site for each month surveyed.

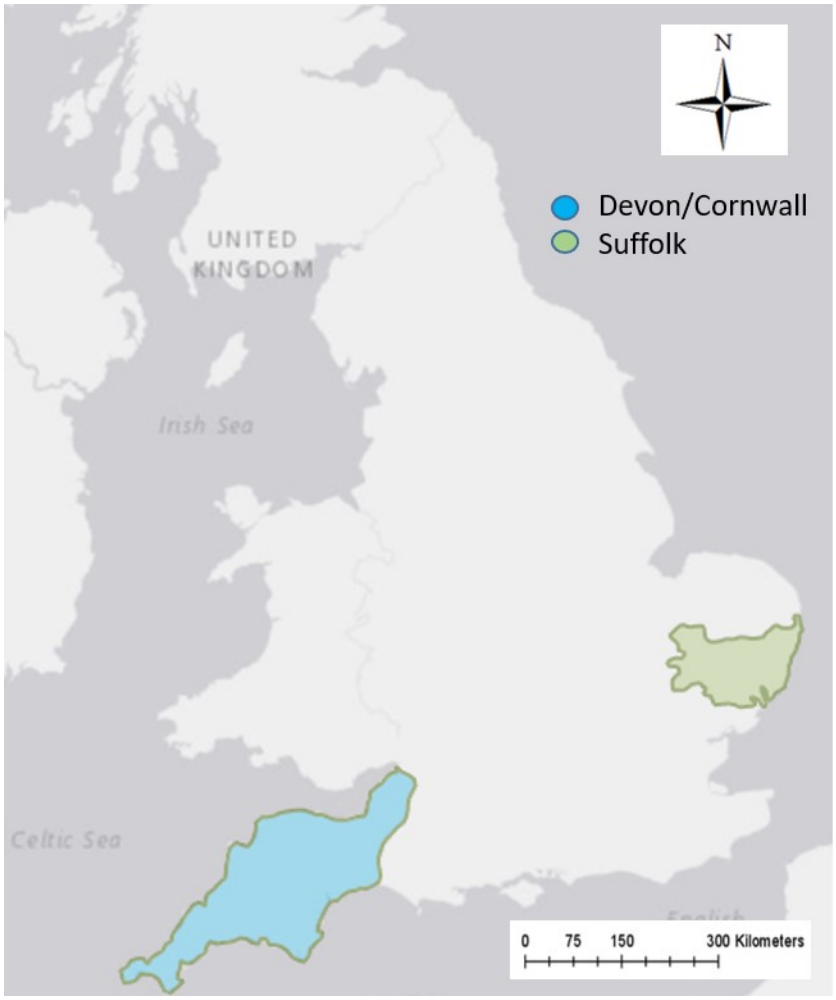


Figure 1. Geographical distribution of nest tube sampling sites within the UK, Blue, Devon and Cornwall region and green, Suffolk region.

3.3.2 Modelling species occupancy

Hierarchical occupancy modelling was used to estimate the proportion of occupied sites (ψ)and detection probability (p) for varying surveys consisting of 16 or 50 nest tubes using established methods (MacKenzie et al. 2002, 2003; Royle & Nichols, 2003; Royle & Dorazio, 2009). This model assumes the population is closed, i.e. site occupancy is constant throughout the survey season in order to ascertain the probability of detection (MacKenzie *et al.*, 2017). In addition to the assumption of closure, the model also requires the assumption that all sites are independent from each other during the closed season to reduce the likelihood of overestimation of occupancy (Rota *et al.*, 2009). Dormice are known to have small home ranges and are unable to disperse distances greater than 500m in a singles season (Büchner, 2008). In this study

distances between each site is greater than 1km, thus we can assume independence between each site.

The detection probability (p) refers to the chance of an observer detecting the presence of a species and takes into account the error of false identification . To identify whether temporal variation influenced detection probability we compared models with and without time (month) as a covariate. In each model, we used a complete matrix of presence/absence data from each site, occupancy was fixed to 1.0 and the logit link was used to model detection probability against the two models. We used corrected Akaike's Information Criterion (AICc) to rank candidate models (the model with the lowest AIC value having the optimal fit) and calculate their Akaike weights (competitive if delta AIC>2; Burnham & Anderson 2002). We used R v3.4.3 (R Core Team, 2017) and the package Unmarked (Version 0.10-6; Fiske & Chandler 2011) for all analyses.

To explore the effect of sampling effort and duration, we calculated occupancy and detection probability as above for each month for differing survey durations, from 1-8 months, by systematically analysing data starting in each possible month (N=36 models).

We extrapolated the number of nest tubes required (T_r) in order to achieve, 0.8, 0.9 or 0.95 detection probability (p_t) for each month, respectively. We did this by calculating the difference in detection probability (D) between the surveys of different intensity (i.e., 16 or 50 nest tubes). For this we assumed a linear relationship between survey intensity and detection probability for which there is a precedent (Ransom, 2012; Petitot *et al.*, 2014).

$$T_r = p_t\ (p\ /\ D)$$

Using our estimates of monthly detection probabilities, we evaluated competing survey designs according to nest tube number (50 nest tubes), survey starting month, and duration.

3.4 Results

In the South West region dormice were recorded in 32 sites out of the 117 (27.4%) and in Essex and Suffolk they were recorded in 19 out of 27 sites (70.3%). Data were analysed to compare models with and without time (the sequence of monthly visits) as a covariate to detection. For both surveys, including time as a covariate was a superior model (Figure 2; Table 1). Time-specific detection (detection refers to the conditional probability that given dormouse presence, presence is recorded) probability estimates are shown in Table 2. Occupancy (occupancy refers

to the predicted proportion of sites occupied, given the time-specific probability of detection) for 16 nest tubes was estimated as 32.6% (SE =/- 5.5%); occupancy for 50 nest tubes was estimated at 70.4% (SE =/- 8.8%). Time-dependent detection probability was estimated for both studies (Table 2; Figure 2), with mean detection for 16 nest tubes estimated at 17.9% (SE +/- 2.9%) and for 50 nest tubes, 55.8% (SE +- 4.1%). While the pattern of detection trough time is similar for both studies, detection probability was much significantly higher for 50 nest tubes at all time points, peaking at 89% (SE +/- 7%) in September ($t=7.05$, $df=7$, $P<0.01$). The lowest detection probability was estimated in the month of April (16 tubes, 8% SE +/- 4%, 50 tubes, 21% SE +/- 9%).

Table 1. Model fit for 16 and 50 nest tubes with and without including time (the sequence of monthly visits) as a covariate to detection.

	model	**N parameters**	**AIC**	**ΔAIC**
50 Nest tubes				
With time	**p_t_psi_dot**	**9**	**233.56**	**0**
Without time	p_dot_psi_dot	2	245.34	11.78
16 Nest tubes				
With time	**p_t_psi_dot**	**9**	**389.90**	**0**
Without time	p_dot_psi_dot	2	393.38	3.48

Table 2. Detection probability mean and standard error (SE) estimates by month for each of the two locations using 16 or 50 nest tubes per site.

month	mean 50	mean 16	SE 50	SE 16
Apr	0.21	0.08	0.09	0.04
May	0.53	0.26	0.11	0.08
Jun	0.42	0.13	0.11	0.06
Jul	0.42	0.08	0.11	0.04
Aug	0.68	0.31	0.11	0.08
Sep	0.89	0.31	0.07	0.08
Oct	0.68	0.16	0.11	0.06
Nov	0.63	0.13	0.11	0.06

3.4.1 Survey effort required to achieve a baseline of 95% detection

Based on the monthly probability of detection for 16 and 50 nest tubes, we extrapolated the sampling effort (number of nest tubes at each site) required to reach 80, 90 and 95% detection probability. A model of sampling effort and predicted probability of detection (per sampling occasion) can be seen in Figure 3. In the month of April due to low detection probability (Figure 2) in order to achieve a 95% baseline 248 nest tubes are required, the preceding months this falls below 128 nest tubes in February with the lowest number of tubes required in September, 60.

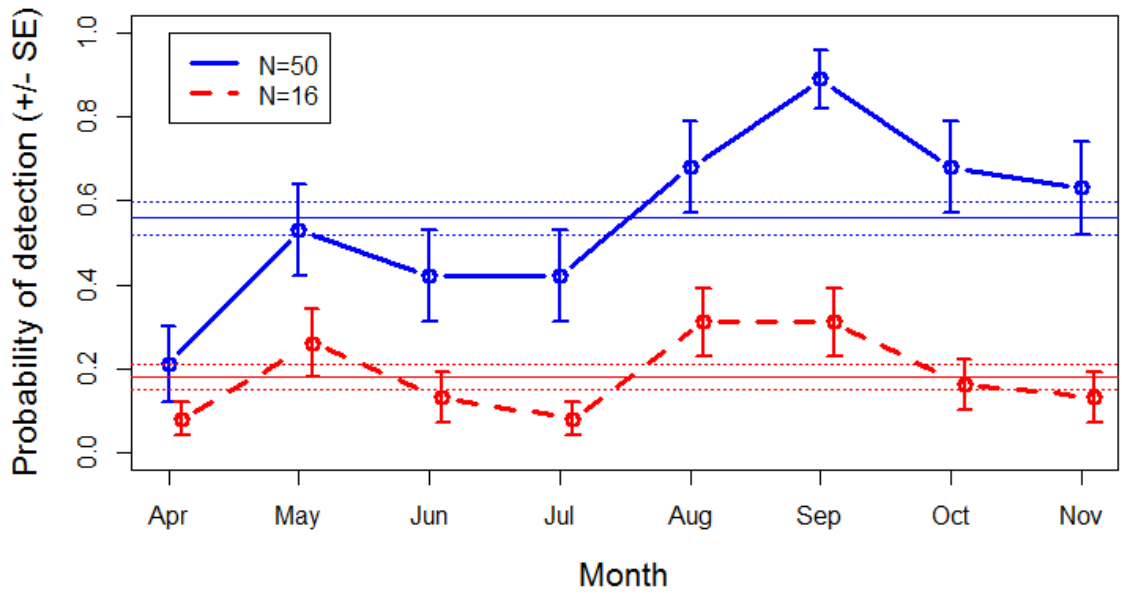
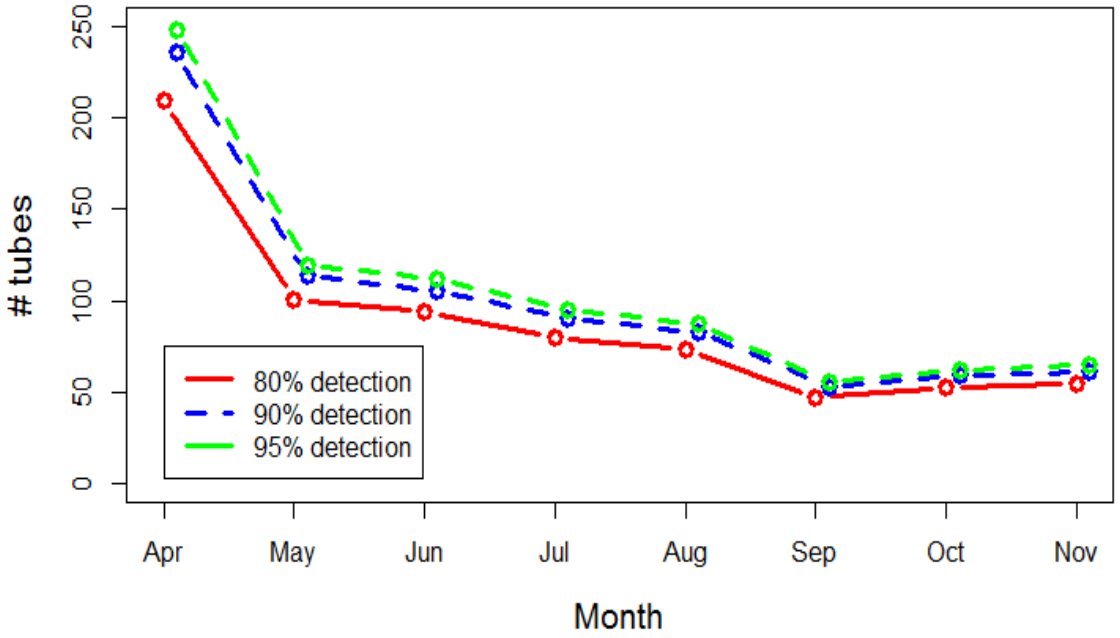


Figure 2. Detection probability as a function of time for all monthly surveys (per sampling date). Points are means, error bars are SE, horizontal solid lines are means flanked by dotted SE of overall detection ignoring time.

Figure 3. Predicted sampling effort, number of nest tubes required to achieve 80%, 90% or 95% detection (per sampling date).



3.4.2 Optimal survey start date and observation period number

Detection probability models were calculated for all possible combinations of starting month and number of sampling occasions (n=36 separate models) (Table 3). A graph showing the detection probability for surveys starting in every month (April to November) consisting of different numbers of sampling occasions (from 1 to 7) is shown in Figure 3. The highest detection probability (86.7%) is achieved by sampling 2 times in September and October. The only other combination achieving 80% detection is sampling 2 times starting in August (80.0%), although sampling once in September (79.3%), 2 times starting in May (77.8%), or 3 times starting in August or September (78.9%, 76.8%) are similar in efficiency and detection probability. The reduced average detection for increased sampling effort is a result of lower detection probability in early, late or middle months.

Table 3 Predicted cumulative probability as a function of i) sample start date and ii) number of sample occasions based on 50 nest tubes and the number of tubes required to achieve 95% baseline detection probability. Each number in the table is an independent model estimate of detection probability (n=36 models in total). Bold indicates survey methodology that reached 95% threshold for 50 nest tubes or the lowest number of nest tubes required to achieve a 95% probability.

		STARTING MONTH							
# months		Apr	May	Jun	Jul	Aug	Sep	Oct	Nov
1	p50 =	0.21	0.53	0.42	0.68	0.89	0.68	0.63	0.68
	p# = 95	124	79	88	78	58	78	88	78
2	p50 =	0.57	0.74	0.75	0.82	**0.95**	**0.99**	0.92	
	p# = 95	84	76	75	63	**50**	**50**	50	
3	p50 =	0.68	0.78	0.87	**0.99**	**0.99**	**0.99**		
	p# = 95	78	68	56	50	50	50		
4	p50 =	0.76	0.84	0.81	**0.99**	**0.99**			
	p# = 95	75	69	70	50	50			
5	p50 =	0.72	0.90	0.92	**0.99**				
	p# = 95	78	55	55	50				
6	p50 =	0.81	**0.99**	**0.99**					
	p# = 95	70	50	50					
7	p50 =	**0.99**	**0.99**						
	p# = 95	50	50						
8	p50 =	**0.99**							
	p# = 95	50							

3.5 Discussion

Our results suggest that detection probability is hugely impacted temporally through the year, whilst detection probability is significantly impacted by survey intensity and duration. We found that survey detection varies from 10% to 90% across our study. Relatively few sampling visits were required to estimate occupancy and detectability with a good precision above 80% power. The months of August and September have the highest detection probabilities so regular surveys that include these months greatly increase the probability of successfully detecting dormice if they are present. This correlates with the post breeding period in dormice were sub-adults and mature juveniles are dispersing and the population abundances are at their peak (Chanin & Woods 2003; Bright et al. 2006). Based on these findings, we suggest that specifically accounting for life history and behavioural variation in focal species of occupancy studies is perhaps the most important aspect of study design.

The detection probability is likely to vary between species, habitat, and other factors, and adjusting sampling design in response to these variables is vital in order to detect the presence of species with precision. In our study, the variation in detection probability we observed during the survey season indicates the importance of temporal aspects of sampling design, whilst survey effort, in terms of the number of nest tubes placed, was also important. In spring, dormice are emerging from hibernation and beginning to forage in order to meet the energy requirements for breeding and our results suggest that dormice may be slow to colonise nest tubes. The highest probability of detection we observed was during the post-breeding months of August and September, consistent with previous findings indicating the preference for natural nesting sites during periods when nest tube usage is low, but also reflects the differences in dormouse activity throughout the year (Chanin and Woods, 2003). There is some evidence to suggest that Hazel dormice generally use nest tubes as day shelters and rarely use them as breeding nest location (Juškaitis, 2014), which is related to the lack of breeding adults being found in this study.

Detectability has been found to be influenced by observer expertise; however, observers in order to conduct surveys for dormice must have gone through a rigorous licencing process that should ensure competencies to identify dormice nests and species. Thus, we can rule out observer bias in this study. However species misidentifications by different observers based on this this recommended protocol can lead to increase in false positives introducing bias in to occupancy estimates in the future (Royle and Link, 2006; Fitzpatrick *et al.*, 2009). However, we

suggest that these limitations should be explicitly considered in practical applications of monitoring study design for other species.

A major role in ecological monitoring is determining the presence or absence of species such as hazel dormice from sites that proposed development could contribute to significant adverse effects on dormouse populations. This includes surveying sites where development works, habitat removal, clearing woodland or removing connective habitat such as hedgerows may be conducted. When designing surveys due to limited budgets there is a trade-off between the number of sampling occasions, the possible duration and the number of sites that must be surveyed. As such the ability to conduct statistical analyses to better utilise wildlife monitoring programs can ensure resources are allocated sufficiently whilst retaining statistical confidence in data collected.

When conducting surveys there is a trade-off between the number of sampling sites and the number of sampling units (i.e. nest tubes), however, guidelines must be stipulated in order to ensure minimum statistical detectability is obtained. Nest boxes are the standard method of choice for monitoring dormice (Chanin and Woods, 2003); however, the financial and logistical costs of using nest boxes are not always feasible for detecting the presence of dormice in sites where presence is unknown. In the UK, guidelines for dormouse mitigation surveys state that a minimum of 50 nest tubes spaced 20 metres apart should be in place during the whole active season from April or May and checked regularly at least once per month (Chanin & Woods 2003; Bright et al. 2006; Chanin & Gubert 2011). An index of probability is then used, proportioning a score for each month April to November, with surveyors required to reach a minimum score of 20 out of 25 to judge the presence or likely absence of dormice (see Bright et al. 2006). Seasonal variation has been observed in nest box usage (Sanderson, 2004) and nest tubes (Chanin and Gubert, 2011), with higher use during May-June and September-October. Such variation during the season is believed to indicate the preference for natural nesting sites during periods of low box usage (Morris, Bright and Woods, 1990), however this may be due to differences in dormouse activity during these months (Chanin and Gubert, 2011) impacting on the detectability of dormice during the season. Whilst dormice foraging tends to be greater in the canopy during the months of June and August with the breeding season beginning in July/August and peak number of juveniles being observed in September (Bright et al. 2006). The current index of detection probability is based on nesting activity with the proportion of new nests in each month directing the monthly score. However, in this study comparisons of nesting activity between the two study areas fails to show congruence in the index scores for each month based solely on new nests. This is most likely due to differences observed in detection probability between locations due to habitat and number of nest tubes used.

Here we used an occupancy modelling framework to analyse presence/absence data to outline how survey design and effort varies depending upon the survey start time and the duration, importantly surveyors must consider this variability in detection probability throughout a season. Precision in detectability is increased by including the pre-breeding/post-breeding season in this species, august and September. However, there is little improvement in detectability by surveying after 3-4 visits. In our case, relatively few visits were sufficient to estimate detectability with good precision, whilst surveying in April has very poor detection probabilities so this monthly period is recommended to be avoided. Survey intensity is also important in order to increase detection probability the minimum number of nest tubes should be 50, as previously recommended by Chanin et al. (2003) whilst increasing the number of tubes used, if sites have sufficient space can increase this precision. As such, we have designed a statistically guided optimal survey framework based on the starting month of surveys (see Table 4) achieving a 95% detection probability to meet framework based on both time and intensity. For each month that surveys are started the optimal duration is given in order to achieve the detection threshold, with the condition that nest tubes are put out at each site the previous month.

Table 4. Proposed survey duration for each survey start month based on cumulative detection probability

Survey start Date	Minimum Duration
April	7months
May	6months
June	5months
July	3months
Aug	2months
Sep	2months

Conservation monitoring requires clear, data-informed guidelines in order to ensure economy. However, this presents a range of financial and logistical challenges to ecological practitioners. Here we used an occupancy-modelling framework to analyse presence and absence data to outline how survey design and effort varies depending upon the survey start time and the duration. The method of identifying optimal survey periods determination we used in this study provide a useful, yet simple tool for designing presence-absence surveys aimed at maximizing efficiency to detect species. This can result in reduced logistical costs and survey

effort for practitioners and higher success. Our results suggest that failing to understand the impact of survey effort on detection could lead to underestimation of occupancy. Thus, while the identification of optimal survey periods is a general solution to problem in the study of wild populations, it is particularly important in rare or elusive species, we conclude it is of special usefulness for species of conservation concern.

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4 Chapter 4: Landscape barriers influence forest connectivity and fine scale population structure in a small mammal *Muscardinus avellanarius*

4.1 Abstract

Anthropogenic alteration to landscape composition and structure can severely impact biodiversity, ecosystem functioning and services. Such changes can affect functional connectivity and gene flow (dispersal) thus influencing the genetic structure of populations. The hazel dormouse, *Muscardinus avellanarius* is a species that suffers the effects of habitat fragmentation. However, the degree to which landscape features impact dispersal or genetic structure lack is not well understood for most species. We use a landscape genetics approach to understand the influence of human-made landscape features (roads, railways and hedgerows) and natural features (slope, aspect and land cover) on functional connectivity in the hazel dormouse, a woodland species of conservation concern. Our results suggest that roads are associated with increase genetic differentiation and that this effect is greater for wide roads or motorways. However, we detected only a minor effect of railways on genetic differentiation. We show evidence that hedgerows and vegetation cover is associated with low genetic distance, possibly acting as corridors in the landscape. Our results are consistent with the assertion that conservation management to increase connectivity have been effective however, isolated populations may require active management and additional measures to mitigate habitat fragmentation and increase functional connectivity.

4.2 Introduction

Copious evidence implicates habitat loss and fragmentation as the leading causes of global biodiversity reduction, resulting in severe impairment of ecosystem functioning (Hooper *et al.*, 2012; Tilman, Reich and Isbell, 2012). Habitat fragmentation increases spatial isolation due to reduction in population connectivity (Leidner and Haddad, 2011), negatively impacting population resilience and disrupting population processes (Wiegand, Revilla and Moloney, 2005). One mechanism for this is the reduction of gene flow between and genetic diversity of within populations (Frankham, 2005; Allendorf and Luikart, 2007) which impacts the population persistence,, and is exacerbated by especially in the face of climate change (Willi, Van Buskirk and Hoffmann, 2006) and landscape use change (Fischer and Lindenmayer, 2007). This loss of population connectivity and subsequent reduction in genetic diversity can also

reduce adaptive potential of specie, i.e, their capacity to adaptively respond to these changes (Opdam and Wascher, 2004). Consequently, restoring connectivity and gene flow between fragmented habitat patches is often seen as an important mitigation strategy to address biodiversity decline. Thus, the identification and quantification of barriers and facilitators of gene flow, provides key information to inform the management of spatial connectivity between biological communities.

The field of landscape genetics aims to understand the relationship between landscape features and population genetic structure (Manel *et al.*, 2003; Storfer *et al.*, 2007a; Balkenhol *et al.*, 2009). Landscape features may influence landscape connectivity by acting as barriers to or promotors of dispersal that can directly influence migration and gene flow, and thus impacting the spatial genetic structure of species (DeSalle and Amato, 2004). (Spear *et al.*, 2010). Typically, the classical isolation-by-distance (IBD) model describes the relationship between genetic similarity and geographic distance for species considered to exhibit moderate dispersal (Wright, 1943). However, this relationship may be complex in terms of ecological or landscape determinants impacting on gene flow in species. Empirical studies have shown that gene flow may be influenced by landscape or ecological configuration, impacting dispersal capability or behaviour (e.g. mountain vizcacha (Walker, Novaro and Branch, 2007); roe deer (Coulon *et al.*, 2006); small mammals (Russo *et al.*, 2016) and fire salamanders (Kershenbaum *et al.*, 2014). Such physical barriers or features may result in fine-scale genetic structure within populations resulting in multiple factors shaping the pattern of genetic variation. Landscape genetics studies allow can inform targeted management approaches for species of conservation concern, this advances on the use of habitat preference data alone (Segelbacher *et al.*, 2010). Thus, it is vital to understand the combined role of landscape features on dispersal to quantify the effect on gene flow by promoting or hindering individual movement (Cushman *et al.*, 2006) and incorporate landscape genetics into conservation planning.

Least-cost pathway modelling has become important tool to model dispersal and gene flow to explicitly study the influence of landscape features independent of isolation by distance (IBD). Here, dispersal between focal populations is conceptualized as “resistance” within the landscape. Such isolation-by-resistance (IBR) models evaluate the relationship between the landscape composition and the genetic differentiation that exists (McRae, 2006). Use of both IBD and IBR methods are of particular importance for populations of threatened species, which suffer from the effects of small population sizes (demographic and genetic stochasticity), further exacerbated by fragmentation that can interfere with connectivity between populations or limit dispersal to new areas. This allows reliable conclusions to be inferred on the spatial genetic variation and key landscape determinants on habitat connectivity and restrictors to gene flow.

The hazel dormouse, *Muscardinus avellanarius*, is a small woodland mammal associated with deciduous woodland, characterized as having a small home range, low fecundity and occurring at low population densities in comparison to other small mammals (Juškaitis, 2014). There is evidence that hazel dormice presence is associated with features that contribute to woodland habitat complexity, such as mature shrub, hedgerow and hazel coppice stands, and with food plants such as hazel, honeysuckle, and bramble (Bright *et al.*, 2006). It has been suggested that isolated woods of less than 20 ha cannot sustain a viable population (Bright, Mitchell and Morris, 1994) and that habitat fragmentation is a major factor causing local extinction of dormice in the UK (Bright et al. 2006). Understanding the factors influencing population connectivity is important in this species, however, despite significant ecological research over several decades, the evidence for functional aspects of dispersal is equivocal. Whilst experimental data on the effect of landscape connectivity is particularly lacking for small mammal species such as dormice with relatively short generation times in order to increase our knowledge of landscape processes shaping genetic variability (Balkenhol et al. 2009). Dormice are considered to avoid crossing open ground or even small gaps in hedgerows connecting woodlands using radio telemetry (Bright et al. 2006; Chanin & Gubert 2011). However, there is some evidence of dormice dispersing over open ground (Büchner, 2008) or even crossing roads (Chanin and Gubert, 2012). Because the hazel dormouse is associated with productive, healthy woodland, it is a bioindicator of animal and plant diversity and is a compelling species to test the role of landscape complexity and features on population genetic structure and gene flow in order to inform practical conservation management for woodland species.

Our main aim of this study was to assess the influence of landscape features on the spatial genetic structure of the hazel dormouse. We use genetic data alongside landscape data to quantify the influence of landscape features on the effective dispersal and connectivity of the threatened hazel dormouse. We assessed the genetic variability in dormouse populations across its entire UK range, investigating: 1) the population genetic structure of populations; 2) the relative contribution of management activity, hedgerows and anthropogenic features (such as roads and railways) on dispersal capability; and 3) the role of landscape connectivity using resistance modelling across a spatially varying environment to identify habitat characteristics that are important to dispersal. We discuss the effect of conservation management on the future persistence of woodland mammals and dormice.

4.3 Materials and Methods

4.3.1 Study sites

We collected genetic samples from 29 hazel dormice populations in 7 regions of the UK that are part of the National Dormouse Monitoring Program (NDMP) (Table 1). Sites were selected due to the presence of key landscape features associated with dormice movement (roads, railways and hedgerows) between pairs of populations. Non-invasive genetic sampling of hair was conducted during nest-box surveys between April and November between the years 2013 and 2018 inclusive. A total of 706 samples were collected and stored in sterile tubes at −20°C after collection. All animals were handled under local and national guidelines from Natural England and Natural Resource Wales. Sampling was carried out over a two-day period at the end of every month over the active season of dormice during routine surveys of nest boxes placed 50m apart.

4.3.2 DNA Extraction and Genotyping

Total genomic DNA was extracted from hair roots using a Quick DNA extraction kit (Zymo research, USA) following the manufacturer's protocol with the addition of 20 µl of 1 M dithiothreitol during lysis. Eighteen microsatellite markers were selected from Mills et al. (2013) and PCR amplification was performed in 5-µl PCR reactions, containing < 10 ng of lyophilised DNA, 0.2 µM of each primer and 2.5 µl Qiagen Type-IT. PCR amplification was performed using a G-Storm GS1 Thermal Cycler with the following touch-down conditions; 95°C for 15 minutes; followed by 13 cycles of 95°C for 30 seconds, primer annealing for 30 seconds (decreasing by 1°C every cycle from 67°C to 55°) and 72°C for 45 seconds; then 25 cycles of 95°C for 30 seconds, 55°C for 30 seconds and, 72°C for 45 seconds, and a final elongation at 60°C for 10 minutes. Multiplexing up to 6 loci was conducted with combinations selected based on fragment size, T_m and fluorescent dye (FAM,TET and HEX). PCR products were processed at University of Manchester, Genomic Technologies Facility on an ABI 3730 48-well capillary DNA Analyser (Applied Biosystems, California, USA) and GENEMAPPER v3.7 (Applied Biosystems, California, USA) was used to assign allele sizes.

4.3.3 Statistical analyses

The presence of null alleles (non-amplifying alleles) was examined with MICROCHECKER (Van Oosterhout *et al.*, 2004). Observed (H_O), expected heterozygosity (H_e), and estimated null allele frequencies were calculated using CERVUS v3.0.2. The rarefaction procedure implemented in FSTAT v 2.9.3.2 (Goudet, 1995) was used to estimate the expected number of alleles, calculate inbreeding coefficient and compare allelic richness (r) for each population . Hardy-Weinberg

equilibrium (HWE) was analysed for each locus using FSTAT v 2.9.3.2 (Goudet, 1995). Linkage disequilibrium (LD) between all pairs of loci and F-statistics between clusters were estimated using GENEPOP v 4.1 (Rousset, 2008). Analysis of Molecular Variance (AMOVAs) was used to calculate the level of genetic differentiation among different populations, accounting for minimum sample sizesin FSTAT v. 2.9.3 software (Goudet, 2013). This method used microsatellite data collected to determine how much of the differentiation is due to differences between populations, between samples within population and/ or within samples.

4.3.4 Population structure

To examine the genetic population structure od dormice across the UK, we analysed data in STRUCTURE v 2.3.4 (Falush, Stephens and Pritchard, 2007). This was used to infer the most likely number of population clusters (*K*) and test our hypothesis of genetic differentiation between subpopulations. Bayesian clustering methods implemented in programs such as STRUCTURE are powerful for detecting barriers to gene flow within just 20 generations or less (Blair *et al.*, 2012). The analysis was implemented for 450 000 iterations following a burn-in of 50 000 iterations with no *a priori* locality data. Both the posterior probability of the data for the given value of *K* (LnPr(*X*|*K*)) and its rate of change (Δ*K*) (Evanno, Regnaut and Goudet, 2005) were used to evaluate population structure. Twenty independent runs were carried out for *K* values from K=1 to K=15 dependent on the number of populations sampled in each sampling region (i.e, Suffolk region contained highest number of populations N=12) and an admixture model with correlated allele frequencies was assumed (Falush, Stephens and Pritchard, 2003). F_{ST} is a standardised measure frequently used in landscape genetic studies (Storfer *et al.*, 2010) that allows for the direct comparison between populations correcting for within-population variation (Meirmans and Hedrick, 2011). For evaluation of genetic connectivity among all populations, we calculated the pairwise fixation index F_{ST} (Weir and Cockerham, 1984) among all 29 populations in the 'DiveRsity' package (Keenan *et al.*, 2013) in R v. 3.43 (R Core Team, 2017). Finally, we used Discriminant Analysis of Principle Components (DAPC) implemented in the R package 'adegenet' 2.1.0 (Jombart, 2008), to assign individual membership to genetic clusters, and to estimate the relationship between these clusters.

4.3.5 Landscape resistance

To examine the relationship between pairwise genetic distance (F_{ST}) and forest fragmentation, we conducted landscape resistance analyses within the region of Suffolk. We used a causal

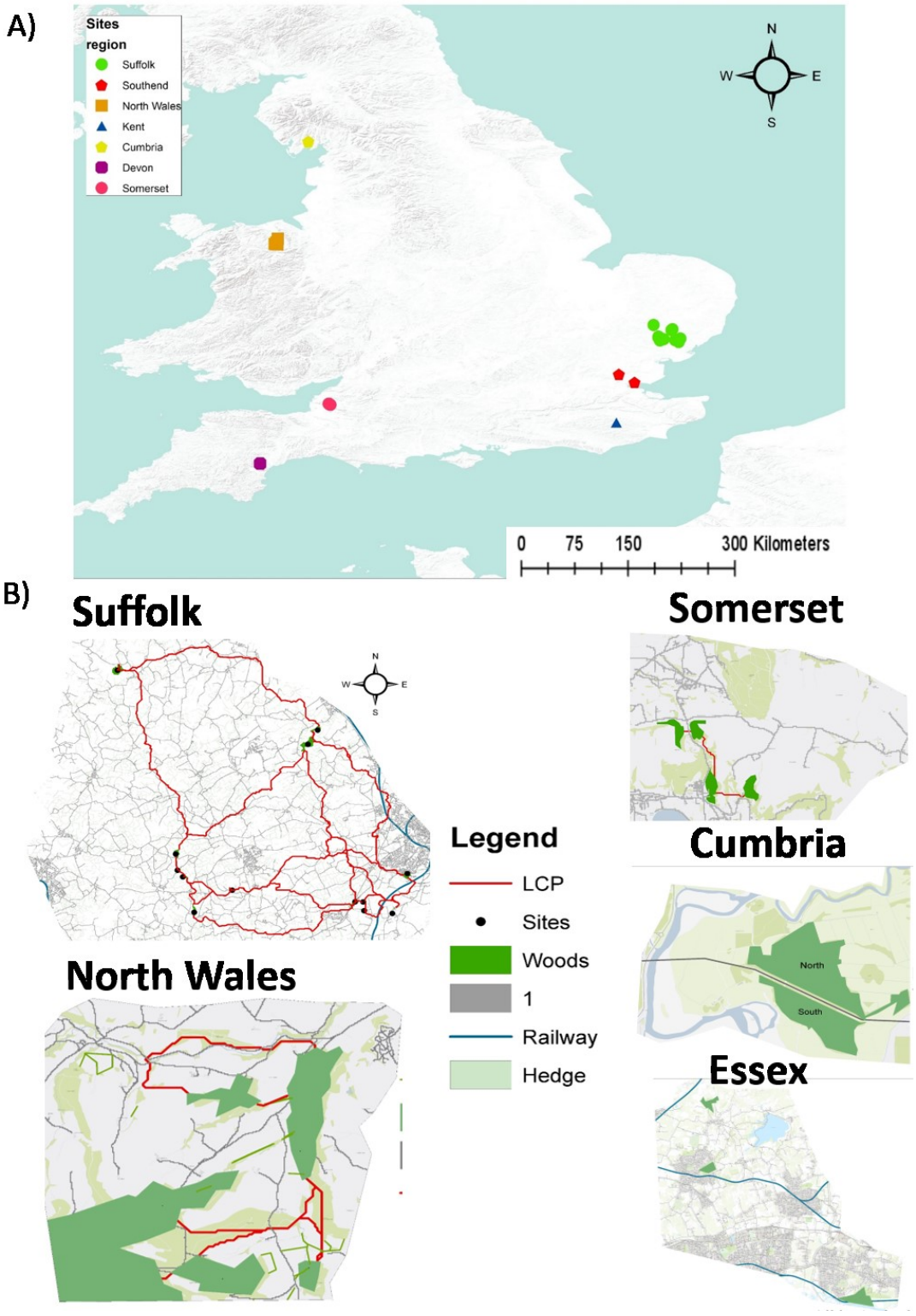
modelling framework (Legendre, 1993; Cushman *et al.*, 2006, 2013) to distinguish between isolation by distance (IBD) and isolation by resistance (IBR) as drivers of the empirical pattern of genetic differentiation. Genetic, geographic (Euclidean) and resistance distances were calculated. Geographic distance was obtained in ARCGIS v. 10.4.1 software (ESRI, Redlands, CA, USA) with the Euclidean distance calculation function and was used for comparing genetic differentiation against the isolation by distance model (Wright, 1943). To obtain resistance distances, we developed resistance surfaces from different landscape data sources in Gnarly Landscape v2.0 for ARCGIS 10.4 (ESRI). We used three land-cover classes (Land cover, aspect, slope) and three linear structure (roads, railways and hedgerows) features known to impact dormice movement and habitat selection were considered and raster's were in a 25 x 25m resolution. Land cover (LCM2015) data based on a 16-category scheme was downloaded from the Centre for Ecology and Hydrology (CEH, eip.ceh.ac.uk). We combined the three forest categories into one and the four grassland categories into one, all other categories were collapsed into one category of 'unsuitable habitat' for dormice i.e water bodies, urban buildings, coastal and rock structures. Slope and Aspect were derived from the digital elevation model (DEM) available from open source DigiMap (digimap.edina.ac.uk), calculated in Spatial Analyst toolbox v2.0 in ARCGIS 10.4 (ESRI, Redlands, CA) . . A hedgerow raster, anthropogenic linear roads and railways were extracted from the open data website DigiMap (digimap.edina.ac.uk). A 25m buffer was applied to linear landscape features and woodland boundaries to avoid breaks in raster features; to avoid bias in resistance values (Koen *et al.*, 2010), we applied a 2km buffer around all locations to define the study limits (Fig 1A).

Resistance values between 1 and 100 were assigned to each landscape variable. Values were chosen to illustrate were the landscape feature was not resistance (1), weak or moderately resistance (25 and 50 respectively) and high and total resistance (75 and 100 respectively). For each landscape variable we created resistance models to calculate the resistance distances between each population using two algorithms, least-cost path (LCP; Adriaensen et al. (2003) and circuit theory (CT;McRae 2006). The main difference between these algorithms is that LCP represents a single optimal pathway, whereas CT accounts for multiple pathways for dispersal; however both methods are typically employed for exploratory comparison in landscape models. Resistance distance by LCP was determined in Linkage Mapper v. 2.0 (Carroll, Mcrae and Brookes, 2012), implemented in ARCMAP v10.4.1(ESRI) and calculated the single least cost route within the landscape. CT in Circuitscape v.4.0 (McRae, Shah and Mohapatra, 2013) was used to allow for multiple paths to exist between each pair of populations. We used the pairwise mode option with focal points (i.e. populations), to calculate average resistance, based on a

network connecting cells to their eight immediate neighbours. Cumulative current maps and resistance matrices were produced for every pair of populations and visualized in ARCMAP 10.4 (ESRI). A null model of IBD using a resistance surface with all cells set to a value of 1 was used.

A two-step causal modelling approach was used to test significant effect of landscape resistance values that were calculated from Circuitscape. First, a partial mantel test of genetic distance to each of the resistance models, was conducted, given the spatial distance between samples (Partial 1). When there was a significant correlation of the first partial Mantel test, we calculated the effect of the IBD model on genetic distance, controlling for the landscape resistance variables (Partial 2) Where Partial 1 was significant and Partial 2 was non-significant, we inferred significant effects of the resistance model on genetic distance (Cushman *et al.*, 2006) . We assessed significance with a two-tailed *P*-value for partial Mantel tests in the R package 'Vegan' (Keenan *et al.*, 2013) based on 5000 permutations. Specifically, we expect that genetic distances be more highly correlated and show a better model fit with resistance distances than with geographic distances, if forest fragmentation reduces gene flow (e.g. McRae 2006). Here we use our Mantel tests when comparing resistance surfaces in highly fragmented landscapes, a major component in our study species, similar to their application in other studies (e.g. Balkenhol et al. 2009b; Zeller et al. 2016).

Figure 1. A) Map of regional sampling within the UK B-H) Regional maps indicating woodland sites and landscape features. Least cost paths are indicated in red for regions with > 2 populations.



4.4 Results

4.4.1 Genetic Diversity and Hardy-Weinberg equilibrium

A total of 706 individuals were collected from 29 locations (Table 1) within 7 regions of the UK and genotyped at eighteen microsatellite loci. In order to avoid spurious inferences caused by uneven sample sizes (e.g. underestimation of genetic clusters or identification of structure) we randomly selected between 20-35 individuals from each populations (Puechmaille, 2016) . These sample sizes achieve the minimum standards recommended for population genetic studies (Hale, Burg and Steeves, 2012). Five out of the twenty nine populations fell short of this sample size but were retained for analyses. Two loci were removed due to unsuccessful amplification across samples (Mav3) and monomorphic alleles in the other (Mav20).

The amplification success average over the 16 loci was 99.1% with all loci being polymorphic for all populations. There was no evidence of high incidence of null alleles (Table 1) or linkage disequilibrium (after B-Y FDR correction) between pairs of loci. Pairwise loci Fisher probability tests of deviation from genotyping equilibrium were significant <*0.*05 for all populations. Significant departures from Hardy-Weinberg equilibrium were found in a number of loci and populations however it is not attributable to specific loci (Appendix 2), however all populations showed expected levels of heterozygosity (Table 1) found in other studies (Naim et al. 2012; Mills et al. 2013). The mean expected heterozygosity ranged from 0.53 in Devon to 0.71 in Bradfield. Allelic richness and number of alleles were consistent among all sites (Table 1). F_{IS} values were close to 0 in all sites apart from Alton Water due to low observed heterozygosity in Suffolk indicating low genetic variability or inbreeding due to non-random mating, however sample sizes were smaller than all other populations at this site. Scotney Castle in Kent, considered to be an isolated population, shows the highest levels of allelic diversity and moderate levels of expected heterozygosity, whilst a slightly high empirical estimate of inbreeding (F_{is}= 0.19) possibly indicates the incidence of non-random mating..

Table 1. summary statistics for all populations within the seven regions samples; number of dormice samples (N), observed heterozygosity (Ho), expected heterozygosity (He), mena number of alleles (A), Allelic richness (Ar), inbreeding coefficient (Fis) and Hardy Weinberg test result (HWE)

Region (UK)	Wood	East	West	N	Ho	He	A	Ar	Fis	HWE
Suffolk	Polstead Wood	0.899296	52.00962	29	0.55	0.64	5.69	4.250	0.14	0
	Bonny Wood	1.027686	52.12509	26	0.59	0.68	6.19	4.647	0.12	0
	Priestly Wood	1.038665	52.13492	24	0.54	0.65	5.19	3.889	0.16	0
	Bentley Long wood	1.070074	52.01272	22	0.53	0.63	5.13	3.947	0.17	0
	Bentley	1.078588	52.01238	13	0.55	0.60	4.11	3.889	0.12	0
	Bradfield wood	0.828986	52.18248	23	0.68	0.71	6.56	4.934	0.05	0
	Tattingstone	1.108946	51.99124	12	0.49	0.64	4.88	4.360	0.23	0
	Spring wood	1.126782	52.03124	37	0.58	0.63	5.63	3.974	0.07	0
	Stoneydown	0.888908	52.03493	14	0.49	0.59	4.31	3.706	0.15	0
	Blessum	0.883635	52.03967	21	0.55	0.62	5.75	4.305	0.11	0
	Groton wood	0.882509	52.05124	21	0.57	0.61	5.13	3.757	0.05	0
	Layham	0.940536	52.02423	25	0.63	0.68	6.50	4.806	0.07	0
Southend	Hadleigh Wood	0.615153	51.54106	33	0.71	0.57	4.81	3.554	-0.23	0
	Norsey Wood	0.439975	51.63162	22	0.51	0.62	4.81	4.043	0.16	0
North Wales	Bontuchel	-3.36843	53.14443	34	0.64	0.67	6.00	4.366	0.04	0.06
	Coed Y Pennant	-3.38500	53.08300	34	0.59	0.66	6.00	4.464	0.01	0
	Coed Cooper	-3.36965	53.08256	17	0.63	0.66	5.13	4.232	0.03	0.04
	Coed Tre	-3.38205	53.10169	14	0.61	0.68	5.22	4.114	0.01	0
	North Clocaecoy	-3.40116	53.08670	16	0.62	0.64	5.31	4.339	0.038	0
Kent	Scotney Castle	0..40824	51.09188	32	0.55	0.67	7.06	6.718	0.19	0
Cumbria	Roudseawood North	-3.02586	54.23117	33	0.41	0.54	5.00	3.549	0.23	0
	Roudseawood South	-3.02723	54.22696	33	0.43	0.57	5.31	3.770	0.24	0
Devon	Haldon East	-3.56391	50.63461	15	0.68	0.58	3.31	3.848	-0.22	0
	Haldon West	-3.56355	50.63552	8	0.7	0.53	3.06	3.066	-0.35	0
	Haldon Central	-3.56533	50.63495	24	0.6	0.59	5.13	2.952	-0.02	0
Somerset	Roundhouse Hill	-2.78707	51.29333	18	0.54	0.66	5.25	4.987	0.17	0
	Batts Combe	-2.77923	51.29163	30	0.57	0.7	7.25	6.421	0.18	0
	Callow Rock	-2.79925	51.30204	27	0.58	0.7	7.13	6.365	0.17	0
	The Perch	-2.79299	51.30262	20	0.59	0.63	5.50	5.234	0.06	0.02

Table 2. Pairwise F_{ST} (below diagonal) and Euclidian distance (km) values between population pairs. Values are given for population pairs less than 90 km distance.

	Polstead	Bonny	Priestley	Bentley wood	Bentley	Brad	AW	SW	Stoney down	Blessum	G	Layham
Polstead		16	17	12	12	20	14	16	3	4	5	3
Bonny	**0.112**		1	13	13	15	15	12	14	14	13	13
Priestley	**0.118**	**0.050**		14	14	15	15	13	15	15	14	14
Bentley wood	**0.114**	**0.141**	**0.167**		1	25	3	4	13	13	14	9
Bentley	**0.118**	**0.199**	**0.184**	**0.024**		26	2	4	13	14	14	10
Bradfield	**0.148**	**0.113**	**0.112**	**0.112**	**0.125**		28	26	17	16	15	19
AW	**0.141**	**0.122**	**0.120**	**0.057**	**0.127**	**0.255**		3	16	16	16	12
SW	**0.180**	**0.180**	**0.183**	**0.189**	**0.189**	**0.218**	**0.108**		16	17	17	13
Stoneydown	**0.099**	**0.155**	**0.136**	**0.191**	**0.196**	**0.148**	**0.125**	**0.184**		1	2	4
Blessum	**0.069**	**0.159**	**0.164**	**0.153**	**0.139**	**0.129**	**0.158**	**0.215**	**0.111**		1	4
G	**0.115**	**0.122**	**0.148**	**0.157**	**0.191**	**0.124**	0.165	0.206	0.153	0.117		5
LayhamGrove	**0.074**	**0.090**	**0.108**	**0.095**	**0.150**	**0.098**	0.086	0.157	0.094	0.096	**0.111**	

Significant (following FDR correction) pairwise comparisons in bold ($p < 0.05$)

	Southend	Norsey	Cumbria N	Cumbria S	Coe dC	CoedYP	NorthC	Bont	Coed Tre	Perch	RH	Callo w	Batts Combe	Devon C	Devon E	Devon W
Southend		16														
NorseyWood	**0.2133**															
cumbria N				0.1												
Cumbria S			**0.003**													
CoedCooper						2	1	7	3							
CoedYPennant					**0.054**		1	7	4							
NorthClocaecoy					**0.158**	**0.092**		7	5							
Bontuchel					**0.188**	**0.139**	**0.088**		0.5							
Coed Tre					**0.084**	**0.101**	**0.125**	**0.051**								
Perch											1	0.2	2			
RoundhouseH										**0.132**		2	1			
CallowRock										**0.088**	**0.093**		2			
BattsCombe										**0.076**	**0.083**	**0.056**				
DevonC															0.2	0.2
DevonE														**0.040**		0.2
Devonw														**0.026**	**0.014**	

Significant (following FDR correction) pairwise comparisons in bold ($p < 0.05$)

4.4.2 Population Structure

Mean heterozygosity across all sites was 0.77 with allelic variation being relatively similar among populations (mean 4.21) (Table 1). Non-random mating F_{IS} was generally positive across population indicating increased mating between closely related relatives (Table 1). Global F_{ST} was 0.114 (G_{ST} =0.168) suggesting overall moderate population structure, whilepairwise F_{ST} ranged from 0.003 (no differentiation amongst populations) in Cumbria to 0.283 (great differentiation) in Essex (Table 2). Overall, AMOVA across all populations indicated that most genetic variation at these microsatellite loci was attributable differences within individuals (75%) and 17% among populations (Table 3). IBD over all sites within the UK was significant but weak (r=0.239, p = 0.024).

Table 3. Analysis of Molecular Variance (AMOVA) results for M. avellanarius performed for all sites

Source	df	SS	MS	Est. Var.	%
Among Pops	27	1526.167	56.525	1.039	17%
Among Individuals	659	3834.467	5.819	0.627	10%
Within Individuals	687	3136.500	4.566	4.566	73%
Total	1373	8497.135		6.231	100%

F-Statistics	Value	P
Fst	0.114	0.001
Fis	0.121	0.001
Fit	0.267	0.001

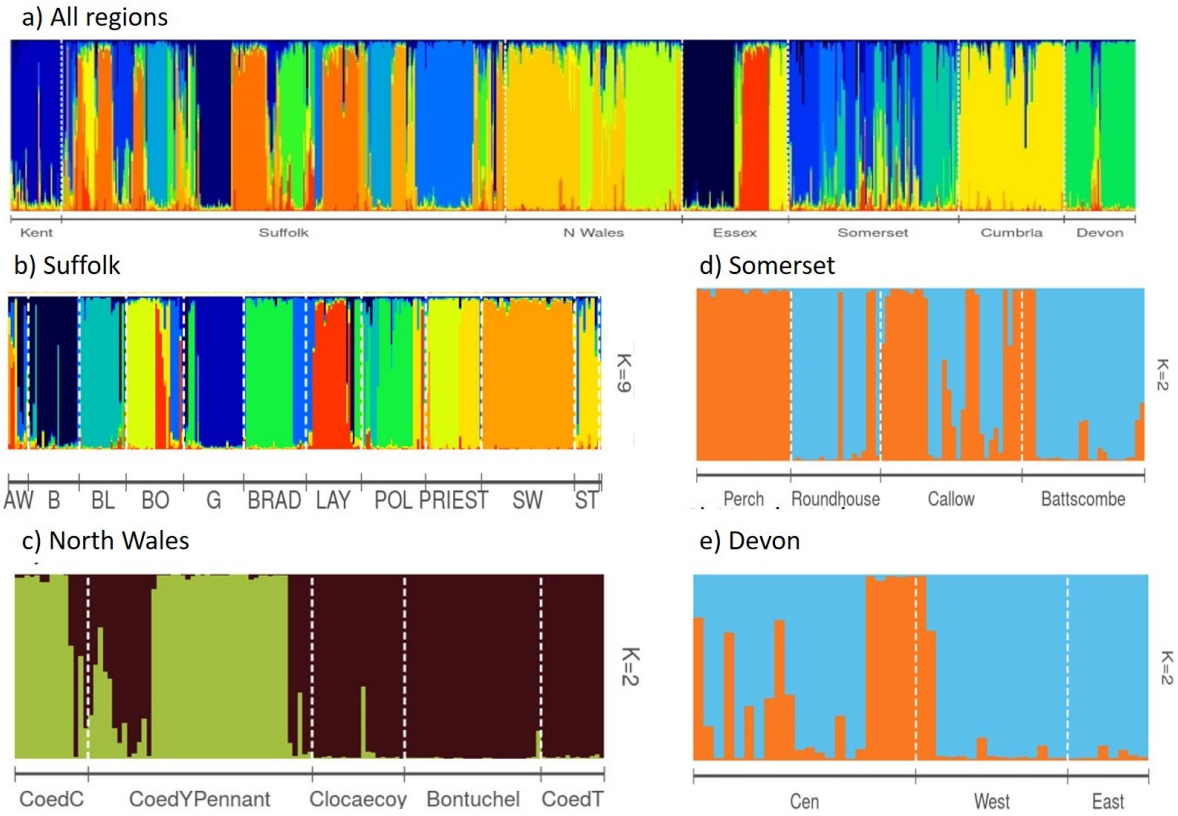


Figure 2. STRUCTURE Bar plot of estimated membership coefficient (Q) for each individual from a) all regions b) Suffolk region; c) Somerset region d) North Wales and e) Devon Region. Each vertical bar corresponds to an individual and bars are divided into proportions based upon the probability of assignment, K value indicates the predicted number of population clusters from STRUCTURE analyses using the Evanno et al. (2005) method.

Suffolk Region

STRUCTURE analyses indicated that the most likely value of LnPr(X/K) was K=8, however the method of Evanno et al. (2005) indicated K=7 with a clear tendency to asymptote (Fig.2a). Out of the 13 populations, three of the clusters (1. Bradfield; 2. Priestley and Bonny; 3. Spring Wood) indicating high genetic differentiation and isolation, based on pairwise F_{ST} from the other clusters (Table 2). Bradfield (the furthest Euclidian distance from other populations) and Springwood, isolated by a double carriage road, show high and significant levels of genetic differentiation (Bradfield 0.113 – 0218; Springwood (0.122-0.258) and show further separation on the PCoA (Fig 3.) Bonny and Priestley populations are connected by a 10 year old hedgerow and show high levels of gene flow (F_{ST}=0.05) but show greater levels of differentiation (F_{ST} >0.15) between all other populations indicating isolation. A railway present between the populations of Alton Water and Bentley area indicate that railways are not a barrier to dormice movement with low F_{ST}=0.062. AMOVA analysis suggested that 87% of genetic variation exists amongst individuals and only 6% among the 13 populations. IBD analysis was moderate and significant between Suffolk populations (r=0.526, adj.p = 0.002;Fig.4). Spatial autocorrelation analyses adjusting the P-value for multiple comparisons (p<0.005), a Mantel correlogram showed significant and positive fine scale genetic structure within in the first (<3.7km) and second (3.7-7.5km) distance values and non-significant in the other distance classes indicating short distance structuring of genetic variability (Fig.5).

Somerset and Devon Regions

Somerset and Devon are geographically (92km Euclidian distance) and genetically isolated (Table 2 and Fig.3) and as such the genetic STRUCTURE was analysed separately. Somerset populations show significant levels of gene flow between populations (F_{ST} 0.05-0.13) with the highest levels of genetic differentiation being observed between Roundhouse Hill population and the other three populations. Callow Rock is separated from the three other populations by a single carriageway road but low and significant levels of genetic differentiation are observed (Table 2). Structure analysis indicated K= 2 population clusters by both methods (Fig.2b). Devon sites separated by a dual carriage road but showed very low and significant level of genetic differentiation (average pairwise F_{ST} = 0.034). STRUCTURE indicated K=1 or 2. PCoA analyses identified one single cluster for all three populations (Fig.3).

Cumbria and North Wales Regions

Roudsea wood is made up of a North and South wood separated by a 10 m cleared track under an electrical line extending through the whole wood. STRUCTURE analysis identified the North and South Cumbrian woodlands as a panmictic population (K = 1) and a significant pairwise F_{ST} value of 0.003 (Table 2) indicating high levels of gene flow between these populations in both directions (Fig 1). North Wales populations show high to moderate levels of gene flow (F_{ST} 0.05-0.18) with the greatest level of gene flow occurring between the northern located populations of Bontuchel and Coed Tre and the southern populations of Coed Y Pennant and Coed Cooper populations (Table 2, Fig.1). STRUCTURE analyses indicated the most likely number of clusters as K=2 using the Evanno et al. (2015) method (Fig. 2c).

Essex Region

Essex populations show high and significant genetic differentiation between populations (F_{ST}>0.28). This differentiation is higher than populations in the region of Suffolk separated by similar distances that show more moderate levels of genetic differentiation than that seen, indicating higher level of genetic isolation. Although low levels of inbreeding are observed (F_{IS} -0.23) in the Southend woodland.

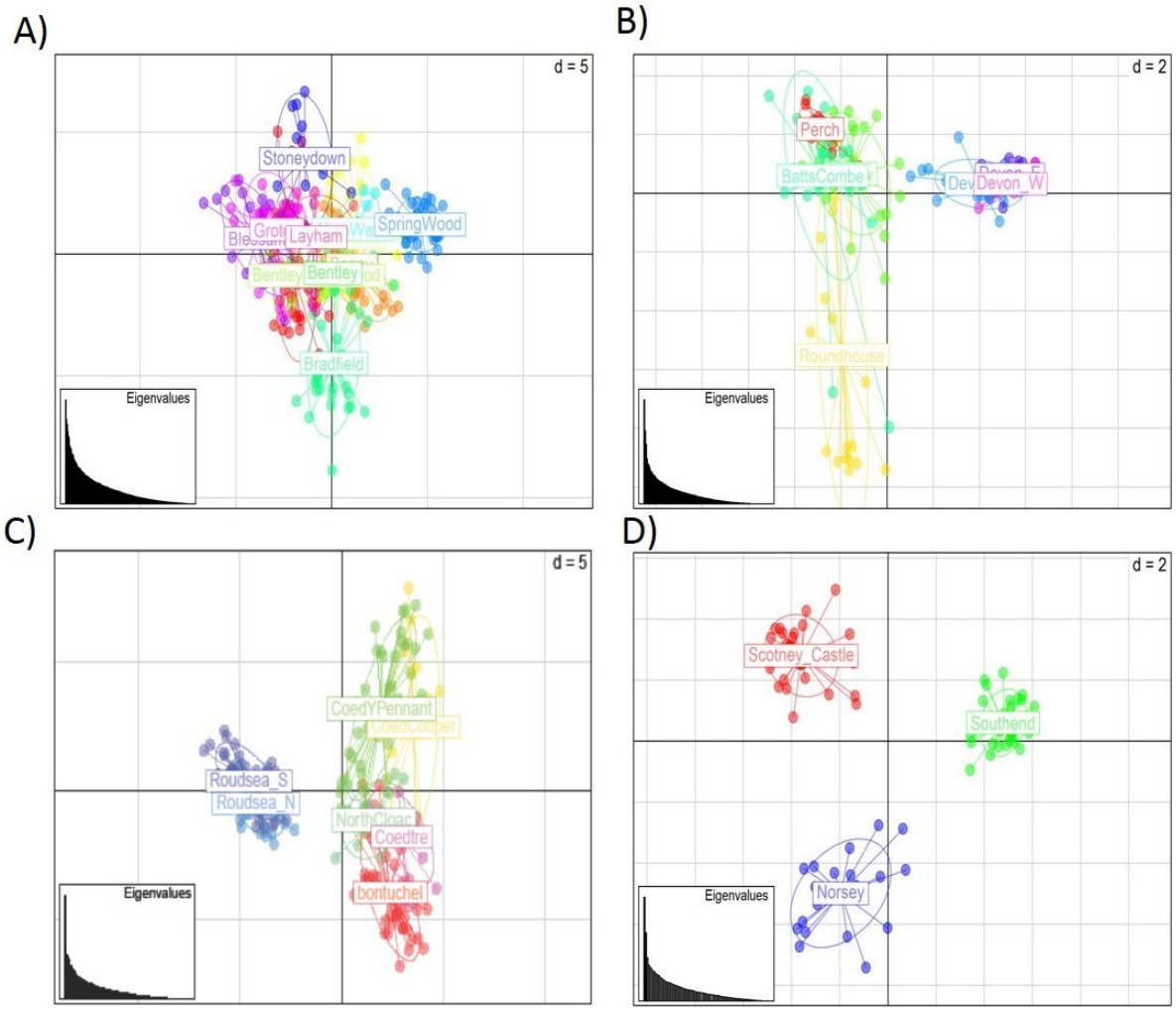


Figure 3. Principle coordinate analysis using corrected pairwise F_{ST} estimates for *Muscardinus avellanarius* at sampling locations of A) Suffolk ; B) Somerset and Devon; C) Cumbria and North Wales and D) Essex and Kent populations. Individuals are represented by dots, and sites are represented by inertia ellipses. The proportion of variance captured by the principle components and the discriminant analysis eigenvalues are displayed graphically (inset). Populations are grouped by colour and name of woodland.

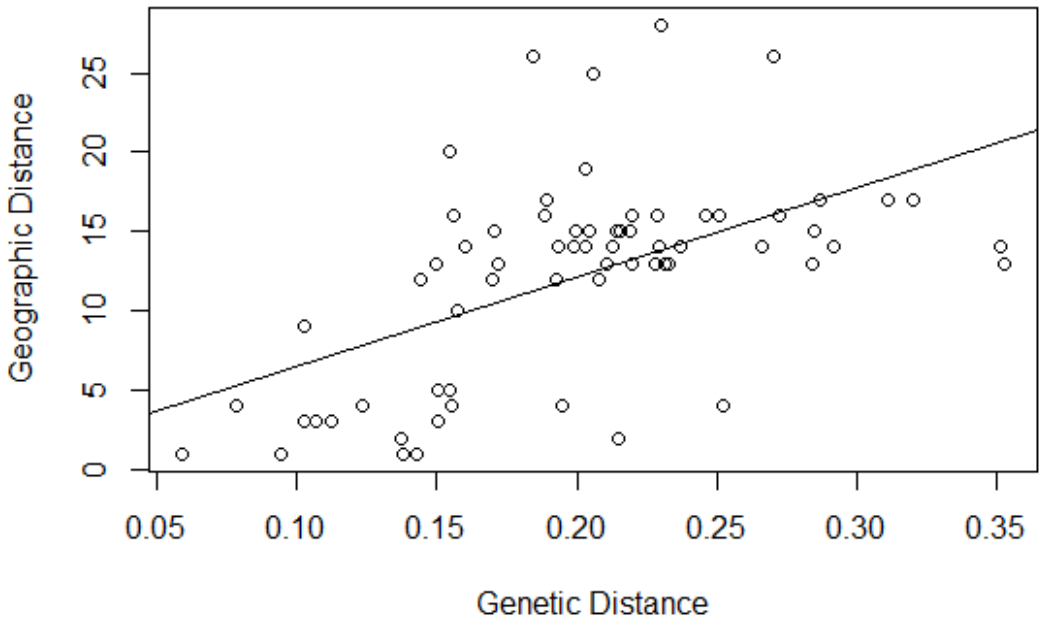


Figure 4. Genetic isolation by distance (IBD) in *Muscardinus avellanarius* within the region of Suffolk Region using pairwise calculation of linearised F_{ST} (F_{ST}/[1-F_{ST}]) and geographical distance (km).

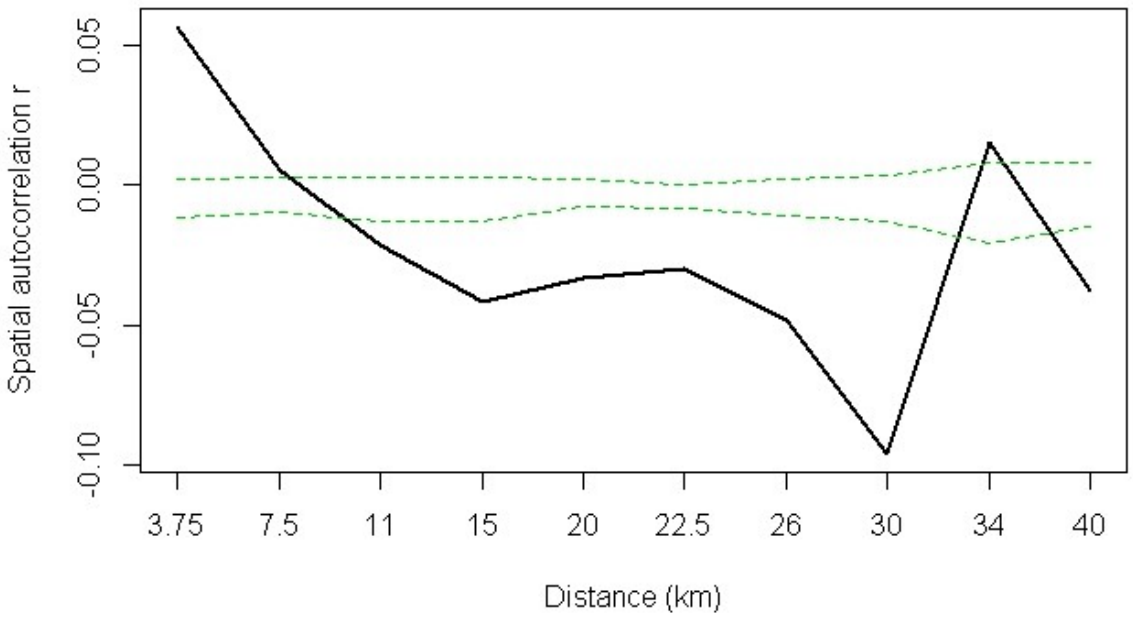


Figure 5. A correlogram showing positive correlation betweeen genetic distance (proportion of shared alleles) and euclidian distance (km) in the first two distance classes.

4.4.3 Effects of landscape features on genetic distance

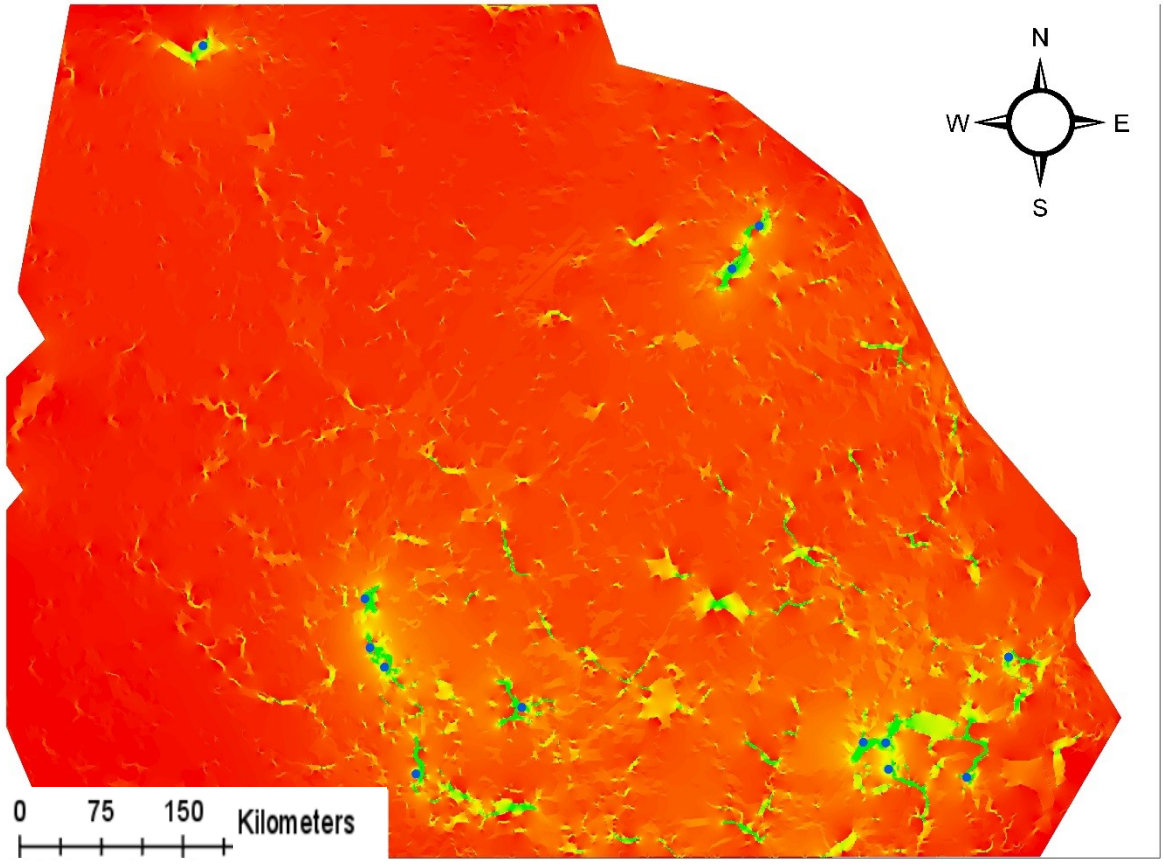
Landscape resistance effects on genetic distance were tested in the region of Suffolk due to the sampling effort of 13 populations in this study area. We found a significant effect of land cover and aspect on genetic distance (Land cover; $r_M = 0.154$, $P = 0.024$, Aspect; $r_M = 0.148$, $P = 0.012$) (Table 4). Hedgerows showed a significant negative correlation i.e. as connectivity increases genetic distance decreases ($r_M = -0.131$, $P = 0.012$), however it failed the causal modelling framework (did not pass) resulting in not explaining genetic distances observed. Roads similarly showed a statistically significant but weak correlation, failing the causal framework. Slope variables were not found to be significant in explaining genetic distance in any testand were excluded from further analyses. Roads and hedgerows were significant for Partial test 1 indicating possible support so were therefore included in the multivariate analyses (Table 4). Multivariate models were significant for models including land cover and aspect ($r_M = 0.128$, $P = 0.009$) (Table 4). This model with the inclusion of hedges had the greatest correlation ($r_M = .365$, $P = 0.059$) (Table 4). A further model with all variables included in the resistance calculations indicated a significant relationship ($r_M = 0.281$, $P = 0.0219$). All three models passed the causal modelling test. Mantel tests with the variables Land cover, roads and railways, were not significant.

LCP's generated from resistance maps highlight the least-cost connectivity routes between woodland patches (Figure 1b) taking into account Euclidian distance. Areas in red highlight restricted connectivity between populations, however corridors of predicted high connectivity (Green) highlight high levels of gene flow (Fig. 6). Most connective current flow is located in the southern regions of Suffolk, most likely due to increased sampling intensity and closer distances between woodland sites.

Table 4. Summary of landscape variables (LV) and causal modelling results after removing the effect of isolation by distance on genetic distance. Partial Mantel (r) tests for, Partial 1 (GD~LV|IBR) – partial mantel test between genetic distance and landscape variable and partial 2 (GD~IBR|LV's) – partial Mantel test between genetic distance and the landscape variable, partialling out the effect of IBR. If Partial 1 is significant partial 2 is run. If partial 2 is non-significant model is supported. Models run as univariate model or multivariate model (multiple variables as a combined resistance map). The hypotheses of using each variable are indicated. Significant effects are shown in bold and supported models, Yes or No.

Landscape Variable	Hypothesis	Partial Mantel 1	P-Value	Partial Mantel 2	P-Value	Supported
Univariate models						
IBD	Genetic distance increase with geographic distance	**(Standard Mantel) 0.517**	**0.001**			
Land Cover	Positive land cover, sources of food and water and promote gene flow	**0.1542**	**0.024**	**0.564**	**0.241**	**YES**
Roads and railways	Physical barrier to gene flow and dispersal	**0.081**	**0.048**	0.501	0.001	NO
Hedgerows	Connective feature for dispersal between woodlands	**-0.131**	**0.032**	0.542	0.004	NO
Aspect	Optimal aspects (less resistance) associated with availability of food and water	**0.148**	**0.012**	**-0.080**	**0.255**	**YES**
Slope	Resistance to gene flow increases with steeper slopes due to reduction in tree growth	0.012	0.342			**NO**
Multivariate models						
Land cover + Roads+ railways +Aspect +Hedges		**0.281**	**0.0219**	**0.224**	**0.451**	**YES**
Land cover + Roads + railways		0.122	**0.040**	0.0240	0366	**NO**
Land cover +Aspect + Hedges		**0.365**	**0.0059**	**0.0857**	**0.257**	**YES**
Land cover + Hedges + Roads+ Railways		**0.352**	**0.004**	**-0.1596**	**0.877**	**YES**
Land cover + Roads+ railways +Aspect +Hedges		**0.281**	**0.0219**	**0.224**	**0.451**	**YES**

Figure 6. Cumulative resistance map of top CIRCUITSCAPE model for hazel dormice (*Muscardinus avellanarius*) in Suffolk, UK. Colours indicate the predicted areas of conductance (green) and resistance (red) among the sampling locations (blue circles).



4.5 Discussion

Here, we investigate the genetic structure of woodland small mammals in multiple regions identifying landscape features that as genetic barriers to dispersal. Our results suggest the build-up of genetic structure amongst islands of fragmented habitat that is explained in part due to isolation-by-distance, and in part due to specific landscape features between different regions. The isolation-by-resistance analysis allowed us to identify specific landscape features, such as ground vegetation, hedgerows that facilitate gene flow and roads that inhibit gene flow. Our results suggest that dispersal in hazel dormice is strongly influenced by barriers in the landscape, with our main findings being that urban areas and roads are associated with restriction in dispersal and gene flow while habitat features such as hedgerows and forest cover are associated with increased gene flow.

Incorporating the influence of landscape heterogeneity and barriers on species movement is vital in order to understand the anthropogenic impacts that affect functional connectivity (Segelbacher *et al.*, 2010; Storfer *et al.*, 2010). Landscape genetic studies have provided case studies for the role of land use change (Khimoun *et al.*, 2017; Draheim *et al.*, 2018), road infrastructure (Kuehn *et al.*, 2007; Zhang *et al.*, 2013) and aspect (Castillo *et al.*, 2014) influencing gene flow and functional connectivity. However, the factors that drive these changes in population genetic parameters can be species specific especially for habitat specialists. Small mammals are also considered to be particularly vulnerable to fragmentation (van der Ree and McCarthy, 2005; Radford and Bennett, 2007), due to loss of habitat connectivity from housing, road or other human made modifications. However, studies of the effect of landscape structure on small mammals is lacking especially in species that are difficult to detect such as dormice in this study.

Genetic diversity is a key measure of population fitness, underlying the evolutionary potential of a population (Spielman, Brook and Frankham, 2004). Our results suggest that dormice populations are subject to significant loss of genetic variation due to genetic structuring amongst all populations. Previous studies in dormice found similar levels of genetic structure in the hazel dormouse at a local scale (Naim et al. 2012; Mills et al. 2015). However, these studies focused on isolation-by-distance patterns and levels of genetic diversity and noted that genetic differentiation could also be attributable to landscape barriers. This study, describes a significant but weak isolation-by-distance relationship with genetic distance, but also explains genetic structure is also attributable to isolation-by-resistance caused by barriers in the landscape and land use changes. This is especially important to consider for species that are

thought to naturally exhibit low reproductive potential and, being habitat specialists (Bright and Morris, 1996; Bright *et al.*, 2006; Juškaitis, 2014).

Dispersal in small mammals is considered to be due to factors, such as inbreeding avoidance, dispersal of young (mate searching), variation in habitat quality and local competition (Rémy *et al.*, 2011; Nichols, 2017). However, despite its importance, information regarding dispersal patterns for many species, especially for small mammals, is often limited. In this study, we find genetic differentiation due to roads. However, this genetic differentiation is not as clear as a road being present, but rather the width of the road and or habitat edge at the roadside. Dormice are a forest dependent species (Juškaitis, 2014) known to cross gaps (Bright, Mitchell and Morris, 1994; Bright *et al.*, 2006) whilst, long distance field crossings have been observed in dormice (Mortelliti *et al.*, 2013). However, this was based on artificial translocation of dormice to local isolated patches and may not represent natural dispersal. On the other hand dormice have been found present on central reservation of roads (Chanin and Gubert, 2012) whilst radio tracking studies has observed dormice voluntarily crossing roads (Kelm *et al.*, 2015). We observe a reduction in genetic diversity of populations affected by roads that may be a consequence of reduced population size and genetic drift (Holderegger and Di Giulio, 2010). As such the ever-expanding effects of roads may cause profound changes in animal populations, this study improves the knowledge of the impacts of roads on dispersal in small mammals. Further long-term comprehensive studies are there for necessary to improve our understanding of the genetic impacts of roads as barriers to gene flow.

The results of Landscape resistance IBR, controlling for IBD, showed that land cover or habitat type is strongly correlated with genetic distance. It is generally understood that natural populations can be impacted by IBD resulting in sub structuring and graduations in allele frequencies (Segelbacher *et al.*, 2010). However, very few studies use analysis methods to elucidate the effect of IBD and IBR and their role on genetic diversity and differentiation (Ruiz-Gonzalez *et al.*, 2015). This study indicates that spatial genetic structuring and diversity is related to both IBD and habitat characteristics and barriers. Dormice are a species considered to be significantly impacted by barriers in the landscape; however this study reveals that IBD still has a significant effect on genetic sub structuring. Thus, future landscape genetic approaches for species such as dormice require analysis models to still take into account the concept of IBD in order to understand population subdivision.

Dormice are found in a variety of different habitat types such as conifer woodlands, broadleaf woodlands and scrub habitats (Bright and Morris, 1996; Sanderson, Bright and Trout, 2004). Given that they utilize these habitats throughout its range, it is likely that they provide sufficient food and appropriate shelter for nesting and thus allows successful dispersal between these habitats. Accounting for roads and railways increases the strength of this correlation. Hedgerows are also found to reduce genetic distance. That is, hedgerows increase dispersal within a landscape, underscoring their use as a management tool in the UK to mitigate habitat fragmentation for Hazel dormice. . Although further work need to ascertain how quality of hedgerow influences gene flow and dispersal as denser hedges may better support dispersal and provide shelter as found in other species (Maudsley, 2000).

Spring wood in the most East population in Suffolk exhibited high genetic differentiation from dormice populations only 2-3 km apart. This population is surrounded by housing development on one side and a two-carriage motorway on the opposite side, indicating that such landscape features have profound effects on dispersal and isolation. There are bridges and underpasses present to create connective routs across the highway, however these contain no suitable habitat for movement (personal communication, Simone Bullion). The other genetic clusters see population separation by roads however low genetic differentiation is observed between these populations. However, the genetic subdivision increased with the cumulative effect of roads between population impacting on gene flow and dispersal. Based on this study roads do act as barriers to animal movement, migration and gene flow but the effects are subtle and not necessarily complete barriers. Roads can decrease functional connectivity, increasing the genetic differentiation of populations or the genetic distance between populations (Holderegger and Di Giulio, 2010). Moreover, this effect is amplified with the increase in width of roads. Double carriage roadways have a significant negative impact on movement in dormice across roads. However the effects of roads can be buffered for by ensuring edge habitat creating shorter crossing gaps across roads as found in populations of Somerset, North Wales and Devon that show significant and low genetic differentiation when roads are the key linear features causing separation of dormice habitat.

In the region of Suffolk, seven genetic clusters were identified with four isolated populations in terms of geographic distance, however these populations did show positive non-random mating (F_{IS}) a concern for future management efforts. The high level of inbreeding and low genetic variability observed are of particular concern as there may no longer be sufficient genetic variability for subpopulations to survive, for example the introduction of new diseases, or

climatic variability (Storfer *et al.*, 2007b). Bradfield, bonny and Priestley, the most northern woodlands in this study are reintroduced populations that have been established for over 10 years and are regarded as large stable populations. Resistance and genetic analyses indicate this population is severely isolated from counterpart within Suffolk. Dormice were introduced into Priestley (2001) and a connective hedgerow constructed in 2002/2003 to the Bonny population. Previous studies indicated that a historical population existed in Bonny woods pre-introduction to this wood (Combe et al. 2016). This study shows the success of this hedgerow to act as a facilitator to dormice dispersal with both populations showing high levels of admixture and relative migration in both directions. A similar pattern is observed in North Wales with least cost path analyses indicating low resistance along hedgerows and identify hedgerows as key connectivity routes between populations. Such features increase gene flow, reducing the negative impacts of genetic drift or inbreeding on small populations (Slatkin, 1987; Frankham, 2005).

There was relatively lower population differentiation amongst the other regions excluding Essex compared to those in Suffolk. The presence of power lines in Cumbria and a 10m wide clear-felled area showed no detectable impact on dormice dispersal, suggesting that dormice will cross land-cover gaps. This has implications on the management of the for the effects of structures such as electrical pylons and lines in species such as the Hazel dormouse.. While our study suggests that short tracks or gaps between populations do not have a detrimental impact on species dispersal, dense scrub along woodland margins may mitigate behavioural reluctance of the Hazel dormouse to cross gaps, which our study could not account for.

4.5.1 Implications for landscape connectivity and species conservation

Understanding the roles of landscape features on gene flow is known to be important for conservation management of species (Segelbacher *et al.*, 2010; Keller *et al.*, 2015), however the details of this are still relatively unknown for the majority of species. Hazel dormice are distributed throughout the UK, however there is evidence their its range area is in decline (Goodwin *et al.*, 2017), due to land use change and habitat fragmentation. . The observed effects of fragmentation on natural dormouse populations provides baseline data that may be useful for landscape genetic studies in the future, such as evaluating the role of wildlife bridges and connective routes.

Our results show that although some features such as roads and railway lines may increase dispersal resistance, they do not act as complete barriers to wildlife movements with corresponding populations one either side of single carriage roads indicating gene flow. Nevertheless, double carriage roadways have a significant negative barrier effect. As such transportation infrastructure measures that target connectivity such as underpasses and wildlife bridges will aid the re-establishment of landscape connectivity and gene flow. Least cost paths for the optimal resistance model were within the areas of high gene flow identified by the IBR modelling (Fig. 6) and these are areas that are considered important for the maintenance of gene flow and maintaining genetic structure. As expected, they correlated with areas of forest cover and avoids urban areas reinforcing the importance of these areas, even when woodland size may not be suitable to support large populations of dormice these sites may still be important to act as corridors for movement of dormice. Agricultural land plays an important role in restricting gene flow due to open spaces restricting movement as found in this study. However, agricultural land management typically facilitates hedgerows an important feature in promoting dispersal in this study. As such, although woodland habitat is a prioritization for conservation of dormice to sustain large populations, conservation managers should also ensure corridors are created through hedgerow regeneration. Further work on the suitability effectof hedgerow characteristics as habitat or facilitators of dispersal would improve our understanding of dispersal via these corridors to ensure re-establishment of locally extinct populations.

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5 Chapter 5: Density and climate effects on age specific survival and population growth: consequences for hibernating mammals

5.1 Abstract

The impact of factors such as density dependence and food availability are known to be important for predicting population change in a wide range of species, whilst, climate variation is also known to impact on the dynamics of populations. However, a challenge in ecology is understanding the contributory and interactive role of these drivers on population fluctuations in order to design effective conservation and management strategies. We analysed the effect of these processes using data from a long-term study of five marked hazel dormouse populations in Europe (four in the UK and one in Lithuania). We tested the relationship between population density and climate variation on demographic vital rates using an integrated population modelling approach, estimating age-specific overwinter survival, population growth, and fecundity. We applied model-averaging techniques using linear mixed effect models to test for the relative effects of local climate on these vital rates, and investigated the synergistic role of environmental stochasticity and ecological density-dependence. We found a strong negative effect of density dependence, rain, warm and more variable winter temperatures on population growth rates. Whilst, we also identified an interaction effect between climatic conditions and density on age-specific survival resulting in reduction of the hibernation season and decreasing the length and frequency of hibernation bouts. Although colder winters favor an increased mean population size, density-dependent feedback can cause the local population to be less buffered against occasional poor environmental conditions (warmer, wet winters). We suggest that management of woodland resources, such as food availability, could be used to mitigate stressors experienced by hibernating woodland species. We discuss our results in the context of the profound implications for understanding the impact of climate change on hibernating mammals and highlight the importance of regional-specific management of woodland resources.

5.2 Introduction

Understanding mechanisms underlying animal abundance is fundamental to ecology and important to inform species conservation management (Krebs, 2002; Hastings, 2010). This is especially true in fragmented landscapes where populations vary in size, demographic composition and facility for individuals to disperse, factors which are of key importance for

population persistence (Fahrig and Paloheimo, 1988; Boyce and McDonald, 1999; Mackey and Lindenmayer, 2001). In order to inform population management decisions, information must be attained on trends in abundance, population growth rate, reproductive success and dispersal. Population abundance is expected to show temporal stochasticity due to the combined effects of extrinsic factors, such as resource availability, predation or local climatic conditions, and intrinsic factors such as population density or dispersal (Bjørnstad and Grenfell, 2001; Melbourne and Hastings, 2008). These factors impact on populations via demographic parameters such as fecundity, survival, immigration and emigration, in turn driving population fluctuation. Thus, acquiring demographic data through monitoring can play a pivotal role in understanding the relationship between demography and the environment to predict change in population abundance or density.

Conservation monitoring of populations is a challenge for many species owing to logistic constraints due to monitoring effort and limited resource availability. This is exacerbated for rare or elusive species which may be relatively difficult to detect or identify, or which occur at low density. Further, some species exhibit variation in biology or conservation status over a wide geographical range, leading to further challenges in the collection of basic data, or, perhaps, spurious extrapolation. As a result of these issues, the basic population abundance for many species of conservation concern is uncertain even when monitoring is conducted. Because populations are expected to respond to conservation management, it is critical to monitor this response to measure the impact of management (Buckland *et al.*, 2007). Thus, a basic requirement to measure impact in conservation is the availability of monitoring data and a knowledge of the processes underpinning population change.

Demographic information, for example data collected by mark-recapture studies or long-term census data, is essential in order to investigate the drivers of population growth trends, for example to assess extinction risk in small or declining populations (Bonebrake *et al.*, 2010). The rate of population change depends on multiple vital rates such as survival, recruitment and fecundity. There is a history of debate surrounding the contribution of environmental and density-dependent processes which act on a populations vital rates (Andrewartha and Birch, 1954; Leirs *et al.*, 1997; Nowicki *et al.*, 2009; Ohlberger, Rogers and Stenseth, 2014), with current ecological theory recognising density dependence as an integral process that often has a role in regulating population abundance. Negative density dependent processes restrict population growth rates at higher population densities, which can help to stabilize populations and communities. As such when population abundance decreases, such restrictions tend to be relaxed resulting in an increase in per capita growth rate. Virtually all habitats are subject to environmental stochasticity, due to weather patterns and geographic situation, resulting in, for

example, changes in temperature and precipitation at a local scale (Walther *et al.*, 2002; Wilson *et al.*, 2005), or extreme weather events (Barker, 2007) . Climatic variation may directly impact survivorship or fecundity due to changes in the availability of food, shelter, or water (Vasseur and Fox, 2007; Loreau and de Mazancourt, 2013). What is less well understood is the degree to which environmental stochasticity influences population dynamics; there is some evidence that environmental stochasticity can alter the relationship between density and population parameters such as survival, fecundity and immigration and that this may be age specific (Miller, 2007; Richard *et al.*, 2014; Manning, Medill and Mcloughlin, 2015). However, no broad consensus exists over a wide range of species on the relative importance of climatic factors or density dependence in regulating populations or whether there is an interaction between these factors driving vital rates which may impact the risk of local extinction.

It has been suggested that environmental fluctuation may have a relatively high impact on hibernating species (Inouye *et al.*, 2000; Nowack, Stawski and Geiser, 2017), altering emergence date and consequent changes in phenology, increasing the impact on vital rates such as survival and fecundity. Temperature change has been noted as an important factor with a direct effect on a species energy and water reserves during hibernation, and on thermoregulation (Seebacher, 2009; Boyles *et al.*, 2011). Thus, in species adapted to undergo torpor or hibernation, it is particularly important to consider the role of environmental variation in maintaining homeostasis. Spatial variation in population demographics as a consequence of environmental stochasticity is poorly understood, and thus there is a need for the study of demographic variation in replicated subpopulations that vary in population density and environmental stochasticity.

Our main aim in this study was to investigate how important density-dependence and climate variation are for regulating population dynamics in a hibernating small mammal, the hazel dormouse (*Muscardinus avellanarius*). We performed the first comprehensive assessment of density dependence on growth and the role of climatic variables and their relative importance, using long term capture mark recapture (CMR) from five populations that vary in size, management practice and habitat composition. Specifically, we: 1) Compare variation in age-specific population growth and survivorship amongst our study populations; 2) investigate whether density dependence and climate variation is associated with population growth. We discuss our findings in the context of conservation management for hibernating mammals.

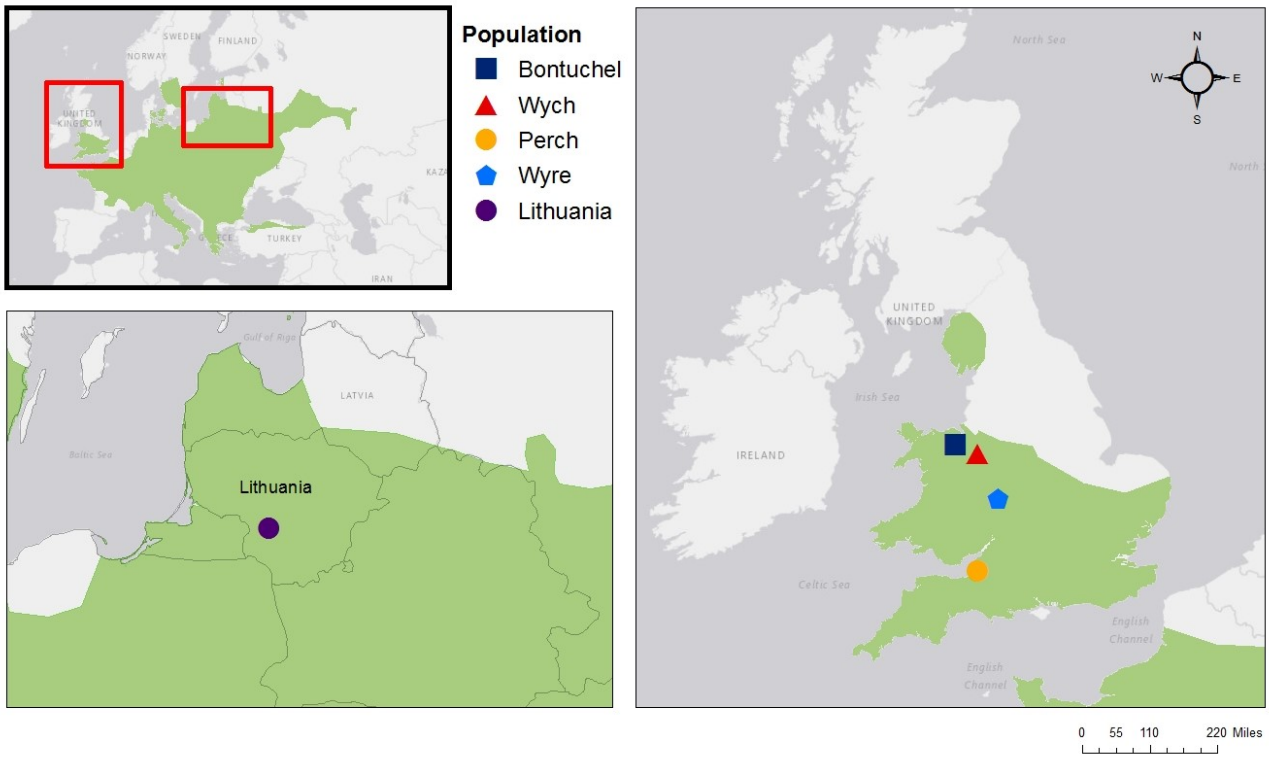
5.3 Materials and Methods

5.3.1 Model species

Hazel dormice are a small, semi-arboreal mammal associated with deciduous woodland, which exhibit a long hibernation period (~6 months) across its range (Bright, Mitchell and Morris, 1994). The hazel dormouse is characterised as having a small home range with low dispersal, low fecundity and occurring at low population density in comparison to other small mammals (Bright *et al.*, 2006). Because of a decline in its northern range, the species is strictly protected in Europe (Habitat Directive Annex IV, Bern Convention Annex III) and a species of conservation concern. For these reasons there are long term monitoring programs in place that occur over a wide range of local climatic conditions making this species a model species in ecology to test the role of climatic variation.

5.3.2 Study sites and data sources

Figure 1. Geographical location of CMR sites in the UK and Lithuania. The distributional range of the hazel dormouse is indicated in green.



CMR data were collected in five hazel dormouse populations: The Perch (Somerset, UK), Wyre forest (Worcestershire, UK), Bontuchel (North Wales, UK), Wych (Shropshire, UK) and Šakiai (South West Lithuania, Šakiai district; see Fig.1). In the UK populations, dormice were marked with 8mm passive implanted transponder (PIT) tags. In Lithuania, dormice were marked with aluminium rings (inner diameter – 2.5mm, height - 3.5mm). During each survey period the age-class (Adult or Juvenile young-of-the-year), sex of adults, litter size and weight (g) were recorded. All sites were monitored within each year between May and October. Wyre data were available for the period 2002-2016, Perch and Lithuania data were available for 2007-2016 inclusive. For Bontuchel and Wych, data were available for 2005-2016 inclusive. We analysed capture records for Wyre and Perch from monthly records taken for all 6 months between May and October, inclusive. For Bontuchel and Wych, monthly records were available for the months May, June, September and October. For Lithuania, records were available for the months July, August, September and October. To facilitate direct comparison of the populations, and because the expected lifespan of dormice in the wild is thought to reach up to six years (e.g., (Juškaitis, 2014)), capture histories were collapsed to yearly bins. Each of these four UK populations contain approximately 250 nest boxes placed at 20-40m intervals, the Lithuanian site includes 272 nest boxes spaced in a grid system at 50m intervals.

5.3.3 Bayesian integrated population model

Integrated population modelling (IPM) allows the simultaneous analysis of data from CMR and survey data consisting of annual counts of dormice, juveniles and total number of litters to account for spatio-temporal variation and uncertainty in parameter estimates (Zipkin and Saunders, 2018). Yearly bins of CMR data in a reduced m-array (Conroy *et al.*, 1989)were created for juveniles (<6months old) and adults (>6months old). Count data, annual counts of adult and juvenile dormice and productivity data, total number of litter were collated from survey counts during routine monitoring (Fig.2). Within the IPM framework (Fig.2), the temporal dynamics of dormice survey counts and CMR data were modelled using a state-space model in order to estimate the parameters; age-specific annual survival, population growth rate (λ), fecundity and population sizes. A separate IPM was developed for each population following methods described in (Abadi *et al.*, 2010) and (Harris, Combe & Bird 2015)) using Markov chain Monte Carlo (MCMC) simulations for parameter estimation. Goodness-of-fit tests on our CMR models suggested a good fit to these data (Cooch & White 2016). The analyses were implemented using JAGS version 3.4.0 (Plummer, 2003) called from R version 3.4.2 (R Core

Team, 2017) with package R2jags (Su and Yajima, 2012) and parameters were estimated using vague priors. To assess convergence, we ran four independent chains with different starting values of 100,000 MCMC iterations, with a burn-in of 50,000 iterations thinning every 100th observation resulting in 2,000 posterior samples. We confirmed model convergence using the Gelman-Rubin statistic (Gelman and Rubin, 1992). The hazel dormouse is relatively long-lived in comparison to other small mammals and, because we cannot exclude the possibility of immigration and emigration in our populations, we assumed that the populations are open to unobserved movement of individuals to and from the monitored populations. The IPM allowed the estimation for each year *t* the posterior means and the 95% credible intervals (CRI's) of the apparent survival rate (S) between each age class *i* and *i*+1.

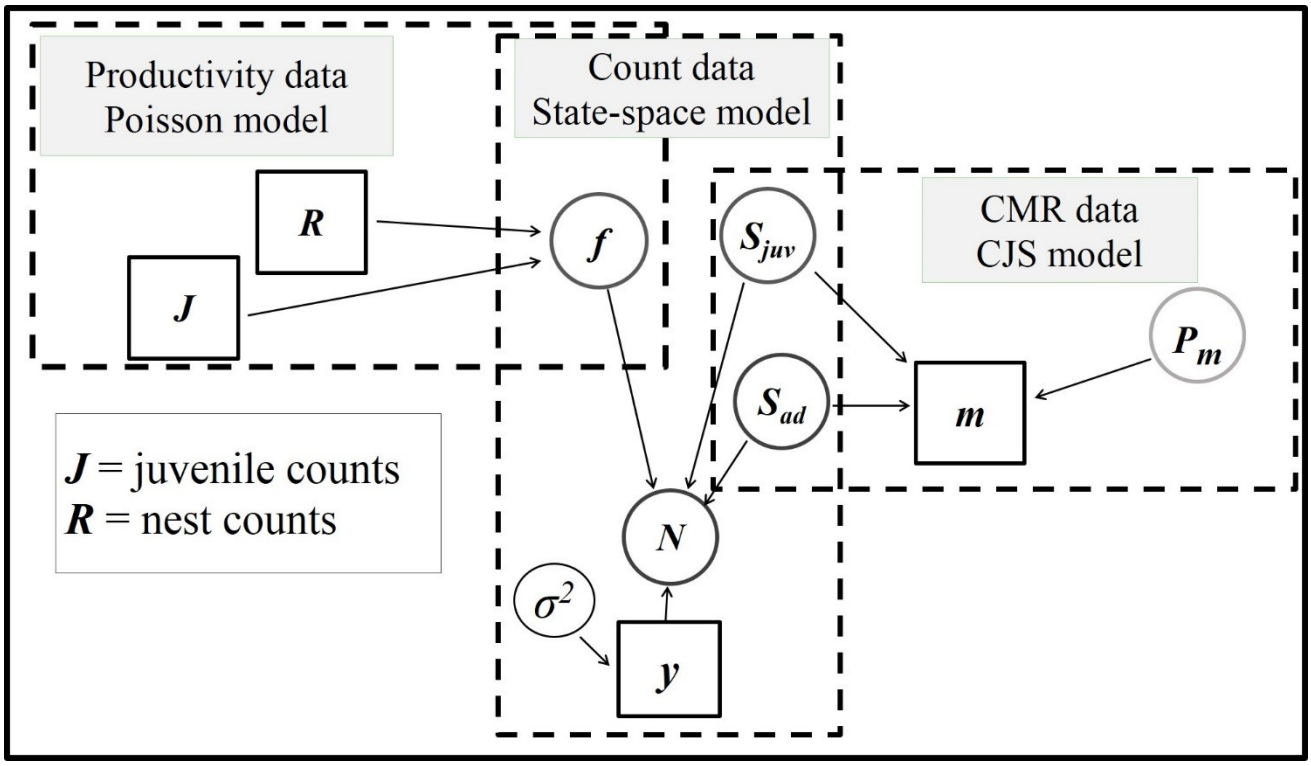


Figure 2. Graphical representation of an Integrated Population Model for the hazel dormouse, adapted from Abadi et al. 2010. Arrows demonstrate dependency between nodes and sub-models are represented by dotted rectangles. Node notations: R = number of nest counts, J = Juvenile counts each capture occasion, f= fecundity – number of young produced per

adult, S_{juv} = Juvenile survival probability S_{ad} = adult survival probability, m = CMR data, P_{m} = capture probability of marked individuals, y = population count data σ2 = observation error on count data, N = true population abundance.

5.3.4 Climate data

Dormice experience a hibernation-like torpor for up to six months, however the duration of hibernation varies geographically, being longer in more northern populations (Juškaitis, 2014). We collated weather variables from spring (March-May), summer (June-August), autumn (September-November) and winter (December-February). We obtained annual average temperature (°C) and precipitation (mm) data from local weather stations (within 10km of each site) using the R package weatherData. Average daily temperatures and precipitation variables were collated into seasonal and annual groupings (January – December of each annual period). Additional weather variables were collated for winter months and included the daily range in temperature, number of days above 10 °C, and daily maximum temperature. We tested for multicollinearity among explanatory variables, which can lead to ambiguous interpretation of results obtained from regression methods. None of the weather variables used were significantly correlated by this criterion, thus they were used in our analysis.

5.3.5 Density dependence

To test the effect of density on productivity in the following year, we modelled the population growth rate for each time period *t*, lambda (λ), as a function of population density estimates calculated from our IPM population (divided by woodland size in ha) for the previous year (time t – 1). The slope line describes the strength of density dependence. If the slope equals zero, there is no impact of density on population growth. A negative slope indicates negative feedback density dependence characterised by a decrease in population growth rate lambda (λ) as population abundance increases.

5.3.6 Climatic data analysis

We assessed the relative importance of different environmental variables to density, survival for adults and juveniles separately (*t* to *t* + 1), fecundity and population growth rate (λ) using Linear mixed-effect models (LME) and model averaging using AICc weights (Burnham and Anderson, 2002b). Using this approach, we generated subsets of the top models. We included

"year" and "population" as a random-effect in our models and all other explanatory variables were treated as fixed effects. Wych and Perch were excluded from analysis of juvenile survival due to low incidence of juvenile PIT tagging at these sites that differed methodologically from the other sites. Model averaging and LMM's were fitted using using "MuMin" and "nlme" packages in R version 3.4 (R Core Team, 2017).

5.4 Results

5.4.1 Demographic estimates using IPM

Bontuchel and Lithuania had the largest number of capture events with 2,017 (Unique = 1216) and 3,265 (Unique = 2,065) total captures respectively. The other three populations Wyre, Perch and Wych had 850 (Unique = 464), 314 (Unique = 192) and 298 (Unique = 273) capture events, respectively. Mean population growth rate varied amongst sites and during the study period. Wyre (λ=1.116, 95% CI = $\pm$ 0.365) and Bontuchel (λ=1.05, 95% CI = $\pm$ 0.108) showed variable population growth rates. The Lithuania population mean growth rate (λ=0.910, 95% CI = $\pm$ 0.311) also fluctuated however, the main cause for this was a rapid decline in population in the last 3 years. Perch (λ=0.949, 95% CI = $\pm$ 0.131) and Wych (λ=0.840, 95% CI = $\pm$ 0.76) both showed a rapidly increasing population in the first two years of study as seen in the growth rates, but declined steadily thereafter, with declines in population sizes.

Annual adult survival estimates were relatively similar between all five populations (Fig.3) (Bontuchel = 0.645, 95% CI = $\pm$ 0.054, Wych = 0.567, 95% CI = $\pm$ 0.076, Lithuania = 0.632, 95% CI = $\pm$ 0.045, Wyre = 0.651, 95% CI $\pm$ = 0.043, Perch = 0.627, 95% CI = $\pm$ 0.111). However, juvenile survival varied between populations (Bontuchel = 0.625, 95% CI = $\pm$ 0.154, Wych = 0.061, 95% CI = $\pm$ 0.060, Lithuania = 0.422 95% CI = $\pm$ 0.105, Wyre = 0.221, 95% CI = $\pm$ 0.133, Perch = 0.098, 95% CI = $\pm$ 0.074) Fig.3). The annual estimated number of young per adult was highest in Bontuchel (4.33 +1.22) followed by Lithuania 3.21 + 1.33, Wyre 2.80 + 0.81, Perch (1.83+ 0.8) and Wych (0.84 + 0.34).

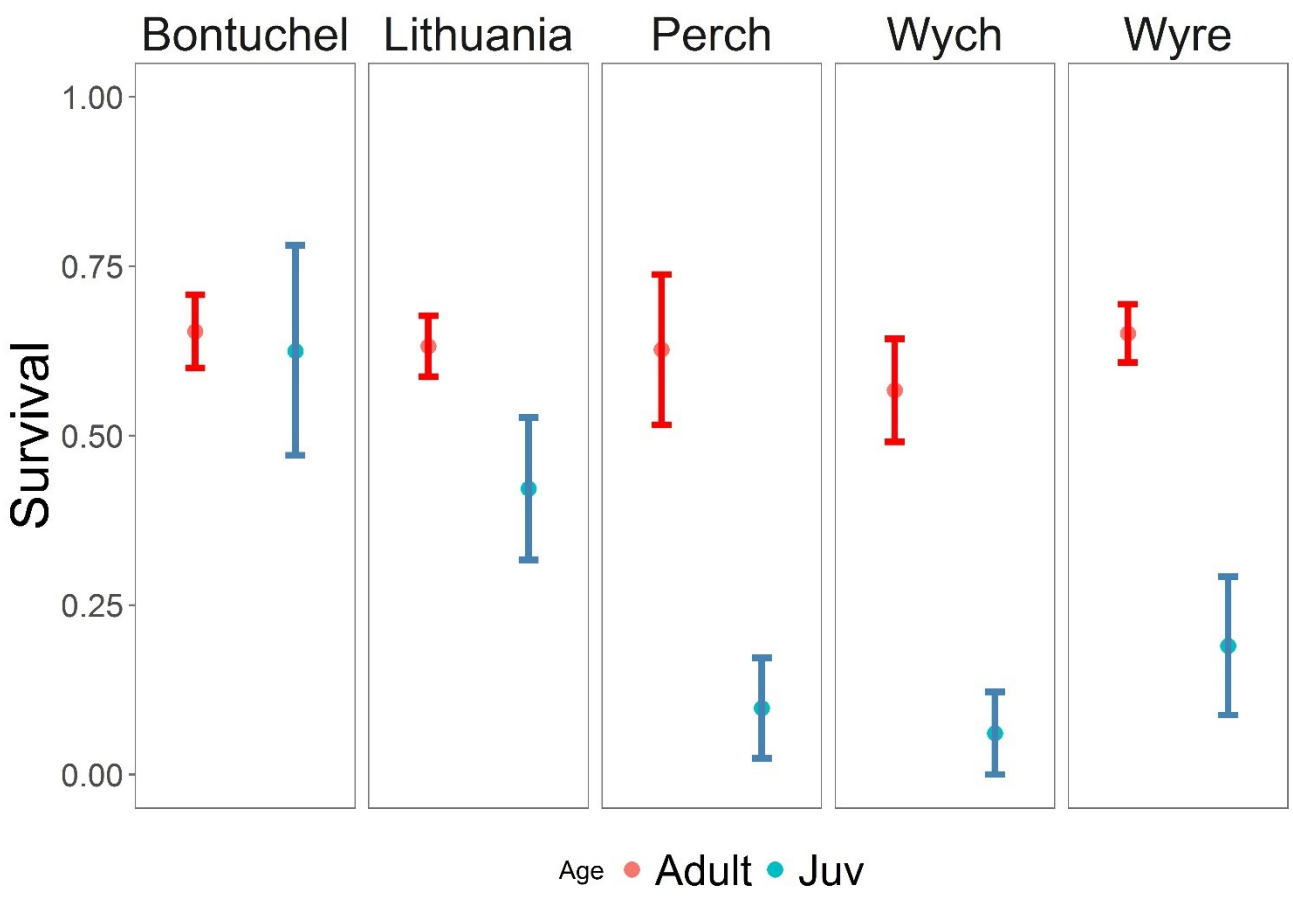


Figure 3. Survival rate estimates for each population (Solid circles; mean and 95% confidence interval) computed from the IPM, indicating differences for adults (red) and juveniles (Blue).

5.4.2 Density Dependence

Annual population growth estimates are shown in Fig.4. Four out of the five populations showed a significant negative correlation between population growth rate and density. The largest populations, Bontuchel and Lithuania, showed a strongly negative slope (Bontuchel, adj. $R^2 = 0.540$, Slope = -2.373, P = 0.023; Lithuania, adj. $R^2 = 0.672$, Slope = -2.013, P = 0.004), while Perch (adj.$R^2 = 0.540$, Slope = -0.456, P = 0.024) and Wyre (adj. $R^2 = 0.356$, Slope = -1.376, P = 0.014) both exhibited a less pronounced but significant negative relationship between population growth and density. However, the Wych population did not show a significant relationship between population growth and density (adj. $R^2 = 0.540$, Slope = 0.781, P = 0.329).

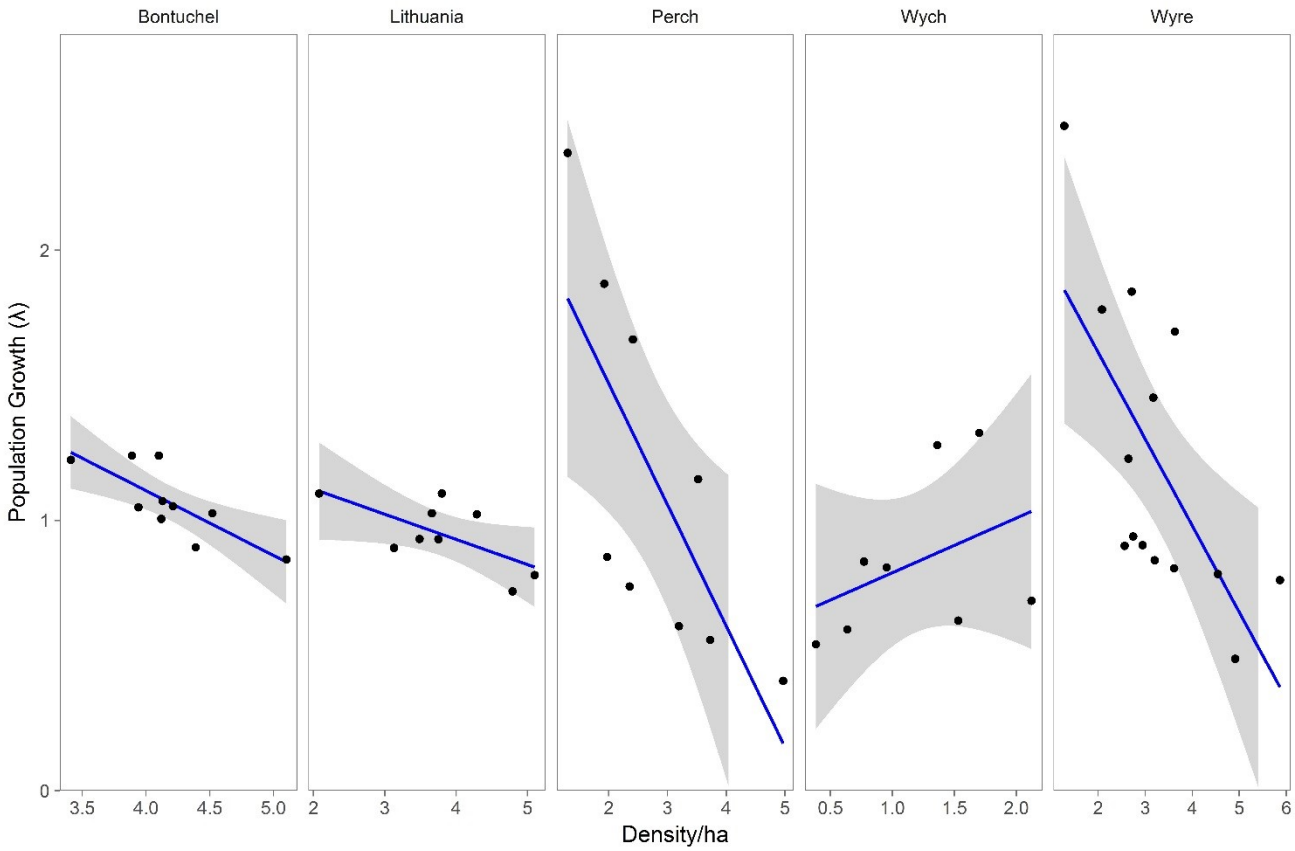


Figure 4 Rate of annual population growth (Lambda, λ) as a function of density per ha. Each circle is the annual estimate, the blue line represents the linear fit and shaded area is the standard error.

5.4.3 Climate variation

During the study periods 2002-2016, yearly average temperatures in the UK were between 9.2-11.1 ^{o}C, Lithuania average temperature was cooler, ranging between 6.3-8.2 ^{o}C. In the winter season, average temperatures in the UK were 5.3 ^{o}C whilst Lithuania was much cooler, -2.3 ^{o}C. Winter precipitation increased over the study period in the UK ranging from 176 to 556 mm during this season. Lithuania was dryer, ranging between 98 and 146 mm of precipitation during the study period. We also calculated the mean winter temperature range ranging from 3.7 ^{o}C observed in Bontuchel population and maximum of 8.5 ^{o}C in Perch. The average winter range for all populations was 5.2 ^{o}C during the 15 years. Annual precipitation also varied widely amongst the study sites with the UK populations experiencing from 820 to 1410 mm over the study period while the population in Lithuania experienced 425 to 820 mm. During the study period, there was a general increasing trend in annual temperatures, winter temperature and winter precipitation, observed in all populations.

5.4.4 Climate effects

Linear mixed-effect models (LME) with model averaging suggested that density was the single variable of highest importance in predicting population growth rate for dormice populations (Table 2), followed by mean winter temperature range, average yearly temperature, winter precipitation and average winter temperature. The top model results showed that, for population growth rate, the best model included density and mean winter temperature range (Table 2). When running only this LME model, density had a significant effect on population growth (β = -0.236, SE, 0.0551, $P<0.01$). Mean winter temperature range ranked high in explanatory power in the model averaging analysis but was not significant in our linear model (β = -0.124, SE = 0.063). Density and average annual temperature were the most important predictors for fecundity in our models (Table 2) with density having a significant negative effect on fecundity (β = -0.277, SE = 0.097) whilst annual temperature had a positive, but not significant, effect on fecundity (β = 0.208, SE = 0.113, P=0.071).

Model averaging indicated that explanatory variables in the highest ranking models (Table 1) all had a negative effect on adult survival (t+1), density (β = -0.073, SE = 0.0.097), mean range of winter temperature (β = -0.030, SE = 0.013), number of days above 10 ^{o}C (β = -0.0.003, SE = 0.001) and mean temperature (Winter) (β = -0.052, SE = 0.013) (Table 2). Whilst there was an interaction between density and the average winter temperature negatively impacting adult survival (β = -0.018, SE = 0.006) (Table 2). For juvenile survival density (β = -0.033, SE = 0.013),

annual temperature (β = -0.012, SE = 0.010), and annual precipitation (β = -0.0004, SE = 0.0002) all had a negative effect. There was a negative interaction effect on juvenile survival between density and average annual temperatures (β = -0.0128 SE = 0.006) (Table 2; Fig.5).

Table 1. Top ranked model results from LME's for all populations investigating population growth rate, fecundity and survival of Adults and Juveniles. The explanatory variables include density/ha (Density), mean temperature range winter (MTWinter), average annual temperature (Annual temp), mean winter temperature (Winter temp), mean winter precipitation (Winter PRCP), Number of days above 10 °C (Ndays10 °C) and annual precipitation (Annual PRCP). R^2 = proportion of variation explained for each model, ΔAICc = difference between AICc between this and the preceding model and weight is the Akaike weight for the given model.

Population growth rate (λ lambda)				
Model rank	**R^2**	**AICc**	**ΔAICc**	**Weight**
Density + MTWinter	0.79	61.27	0.00	0.34
Density + MTWinter + Annual temp	0.77	62.62	1.35	0.17
Density	0.75	62.64	1.37	0.17
Density + MTWinter + Winter PRCP	0.67	63.14	1.86	0.13
Fecundity				
Model rank	**R^2**	**AICc**	**ΔAICc**	**Weight**
Density + Annual temp	0.77	127.17	0.00	0.31
Density	0.76	128.12	0.95	0.20
Density + Annual temp + Annual PRCP	0.76	128.50	1.33	0.16
Density + Annual temp + MTWinter	0.74	129.80	2.62	0.08
Adult Survival				
Model rank	**R^2**	**AICc**	**ΔAICc**	**Weight**
Density + MRW + Ndays10 °C + MTWinter + Density:MTWinter	0.91	127.17	0.00	0.31
Density + MRW + Ndays10 °C + Winter + Density:MTWinter + AUT_PRCP	0.81	107.98	0.30	0.21
Density + MRW + Ndays10 °C + Winter + Density:MTWinter	0.71	107.54	0.75	0.17
Density + MRW + Ndays10 °C + Winter + Density:MTWinter + AUT_PRCP + Annual PRCP	0.70	106.87	1.41	0.12
Juvenile Survival				
Model rank	**R^2**	**AICc**	**ΔAICc**	**Weight**
Density + Annual temp + Annual PRCP + Density: Annual temp	0.92	77.53	0.00	0.24
Density +Annual PRCP + Annual temp + Density: Annual temp	0.81	77.33	0.20	0.22
Density +Annual temp + Density: Annual temp	0.78	76.85	0.68	0.17
Density +Annual PRCP + Annual temp +Winter PRCP + Density: Annual temp	0.74	75.90	1.63	0.11

Table 2. Results from model averaging procedure (using variables from the top ranked model; see table 1), parameter estimates (B) and standard error (SE), PRCP = Precipitation (mm).

Variable	*B*	SE
Population Growth		
Density	-0.236	0.055
MWtemp	-0.124	0.063
Fecundity		
Density	-0.277	0.097
Annual Temp	-0.208	0.113
Adult Survival		
Density	-0.073	0.098
MRW	-0.052	0.013
Ndays10 ^{o}C	-0.003	0.001
Winter temp	-0.030	0.013
Density:Winter temp	-0.018	0.006
Juvenile Survival		
Density	-0.033	0.013
Annual Temp	-0.012	0.010
Annual PRCP	-0.0004	0.0002
Density: Annual temp	-0.018	0.006

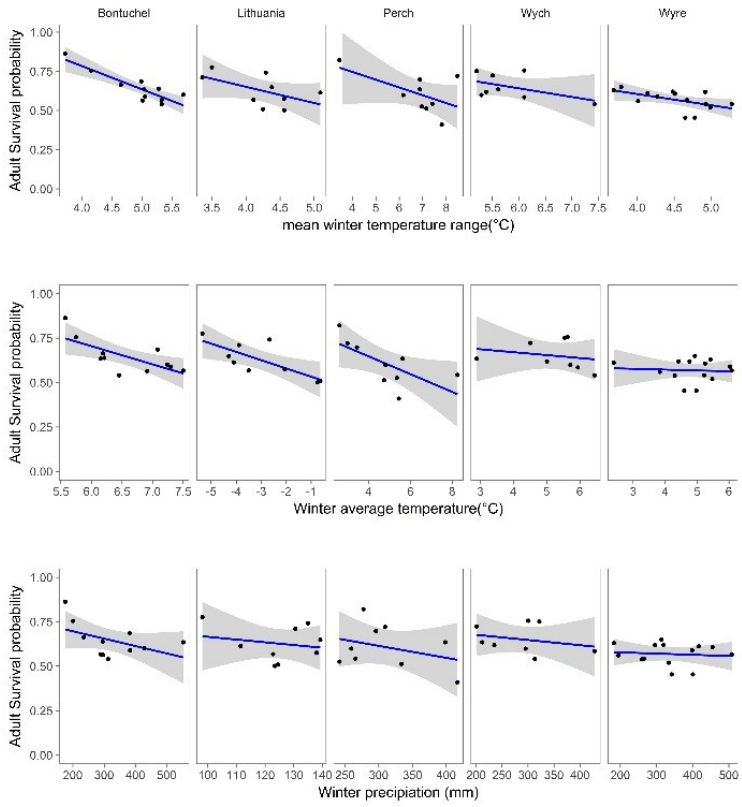


Figure 5. Adult survival plotted against the significant environmental variables in LME models, mean winter range of temperatures (°C), winter average temperature (°C) and winter precipitation (mm). Blue line indicates linear trend and grey shaded area indicates the standard error.

5.5 Discussion

Our main finding is that climatic factors and population density significantly interact to impact population growth and survival in dormice, showing an age-specific effect on over winter survival in adults and juveniles. Fundamental in the field of ecology is understanding what drives change in abundance of individuals over time, and how these specifically influence population vital rates (such as survival, growth, fecundity etc.). However, it has been cautioned drawing broad conclusions on the effect of climate changes on single demographic rates, rather it is crucial to observe the strength of the effect across processes (Jenouvrier, 2013). We show that early-life survival is impacted but the effect is greater for adult survival over winter, supporting previous studies that changes in population density have variable sensitivities resulting in reduced reproductive rate of adult females (Eberhardt, 2002).

There is a breadth of research focusing on our understanding of density dependence (Turchin, 2003; Churcher, Filipe and Basáñez, 2006; Morris and Maceachern, 2010). However, it has been noted that density-dependent interactions and the cumulative influence of variable local environments on the demographic parameters that drive population abundance are less well understood (Griffith *et al.*, 2016). Here we show a negative interaction effect of population density and temperature affecting survival in both adults and juveniles. In adults, increasing winter temperature (including range) and precipitation have a significant negative effect on over-winter survival. However, in juveniles increasing annual temperature and annual precipitation reduces survival rates but with a lower effect. Different aspects of weather variation impacting adults and juveniles has been noted in other species e.g. mammals, (Gaillard *et al.*, 2013); birds (Payo-Payo *et al.*, 2016).

5.5.1 Implications of climatic variability

Our results suggest that variance in climate variation *per se* can have an additive negative effect, along with density dependence, on driving population change. While climate variation has been implicated in negative impacts the vital rates, the mechanisms underlying climate-induced population change are poorly understood (McLaughlin *et al.*, 2002). Here we provide evidence climatic conditions negatively affect dormouse survival for both adults and juveniles. Winter climatic conditions comprising increased average temperatures and rainfall had a negative impact on adult dormouse survival. Importantly, we also found a relationship with increase in the mean winter temperature range negatively influencing survival rates. Global warming in the northern latitudes during winter months is thought to have severe consequences for species, and the trend is predicted to continue of increased mean winter temperatures, up to 3-4

°C by 2050 (Notaro *et al.*, 2011). As a result of warm winter temperatures, we observe changes in the timing of food resources, lengthening growing periods and earlier reproduction. This is a concern in hibernating species that may emerge earlier in spring and observe unequal shifts in phenology (Inouye *et al.*, 2000; Koppmann-Rumpf, Heberer and Schmidt, 2003; Adamík and Král, 2008). Thus, while climate change has been widely shown to affect species range limitations (e.g. Parmesan & Yohe, 2003), our results suggest that climate variation can also have direct negative consequences for hibernating species, such as the hazel dormice (Pretzlaff & Dausmann 2012).

Overwinter survival rates have been documented to be relatively high in hibernating small mammal species, (Turbill, Bieber and Ruf, 2011) such as the garden dormouse (Schaub and Vaterlaus-Schlegel, 2001) and edible dormice (Lebl *et al.*, 2011). Our results that rainfall and temperature during the winter months are negatively correlated with adult survival may suggest an indirect increase in mortality by decreasing the length and continuity of dormouse hibernation bouts. Here, increased energetic expenditure and lack of food resources may cause dormice to lose significant fat reserves and increase the likelihood of starvation (Pretzlaff, Rau & Dausmann 2014). Thus, in the spring and summer months, this increased energy usage over winter may negatively impact survivorship during the early active season, additionally impacting fecundity in females. There is evidence that some female dormice breed later in the season (e.g. September-October) in Lithuania (Rimvydas Juškaitis, 2003), possibly to account for low energetic resources to devote to reproduction in the spring. However, this may only be important in years with favorable environmental conditions (e.g., dry, warm and good food availability).

In juveniles, we observed a significant negative association on survival between density, yearly average temperatures, annual precipitation and a negative impact for the interaction between density and overall annual temperature. This may have additive or cancelling effects on top of density-dependent regulation, causing increased mortality or reduction in recruitment in times when density dependence is limiting or reducing growth in periods of increasing density. We observe that the negative impact of increasing temperatures on the subsequent survival of juveniles over their first winter coincides with a general trend in increased annual temperature at all sites across the study period. This could lead to reduction in the torpor period during the active season when food resources may be limited. Dormice have been found to go into torpor during spring and summer seasons when temperatures reach less than 14-15 °C and partly torpor below 19-20 °C (Juškaitis, 2005). Torpor reduces energy expenditure allowing individuals to shorten foraging times and possible exposure to predators (Liow *et al.*, 2009).

Thus, as a consequence of climatic changes species such as dormice may need to further intensify foraging in order to meet their energy requirements.

There is evidence that onset of the breeding season is correlated to environmental conditions as well as food resource availability (Bright & Morris, 1996; Juskaitis, 2014). In this study, we found a negative correlation between density and fecundity where in years with high density the fecundity decreased. Whilst we found no significant relationship between fecundity and weather, density and average annual temperatures tended to have high explanatory power for fecundity (Table 2). This supports previous findings on the importance of environmental factors in timing of breeding and torpor events (Juskaitis, 2014) however, our results suggest that density and food resources are important driving factors.

5.5.2 Density dependence

While population density as a regulatory process of populations has been previously noted as an important factor regulating population growth in small mammal species (Erb, Boyce and Stenseth, 2001), it has not been demonstrated before in dormice. Our evidence is consistent with the idea that dormouse populations are intrinsically regulated by density-dependent mechanisms. While we did not detect negative density dependence in the Wych population, this population has undergone a rapid decline in the last five years due to very low juvenile survival. This is possibly related to poor quality habitat and we note that the range of observed density in this population was much lower than that which we observed in all other populations during the study period.

Forest management practices can impact animal abundance by affecting habitat complexity and the availability of nest sites or food resources (Chaudhary *et al.*, 2016). Such practices can have a major, even if temporary, role in shaping the pattern of population demographics of the species which inhabit these forests (Lindenmayer, Franklin and Fischer, 2006; Lacerda and Nimmo, 2010). Forest management is also considered to have important effects on the population parameters of hazel dormice populations (Juškaitis and Šiožinyte, 2008; Sozio *et al.*, 2016) and across their range they are considered to be a species associated with early, successional woodland (Juskaitis, 2014). It can be seen during the time-period of this study that the Wyre population exhibited a trend in increasing abundance, survival and fecundity. This former conifer plantation is being managed to restore deciduous woodland (Trout et al. 2018). Thus, it must be considered that increasingly favorable conditions may have caused increased density and carrying capacity of the woodland. Likewise, at Bontuchel, coppicing

management has been implemented that could increase habitat structural complexity and food availability for hazel dormice. Periodic hazel coppicing is a forest management practice that considered to benefit dormice. Coppice understory with no canopy trees or coppice with a low density of canopy trees results in an unshaded and productive shrub layer. The practice maintains a successional stasis that is ideal for the species (Bright et al. 2006). Woodland ride and edge management is also undertaken, to allow light to penetrate further into the woodland and promote successional growth (Ramakers, Dorenbosch and Foppen, 2014). However, there is a lack of good evidence about the effectiveness of these practices for dormice or for other woodland species. Thus, while we identify some components of population regulation here, we still lack understanding on the role of forest management practices on dormice or other species.

Winter is a key season for hibernating species and fluctuations in temperature during this time can have serious consequences for individual fitness and survival to the following year. Because of increased stochasticity in temperature and rainfall negatively influencing population vital rates in this species, we suggest that climate change may have unpredictable conservation impacts on hibernating species that are also sensitive to habitat quality and fragmentation. To counter this, conservation management of resources should aim to buffer stressors specific to local populations in order to effectively protect species and reduce the probability of extinction in such species. In populations that are continually monitored, this could involve a scenario-based approach where management responds to both population change and environmental conditions. In dormice this includes management of felling and coppicing regimes in specific seasons of the year to take into account the vulnerability of species such as dormice or to increase ecological diversity improving woodland resilience to environmental stochasticity.

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6 Chapter 6: General Discussion

6.1 Introduction

The research presented in this thesis provides a comprehensive overview of the population genetics and ecological processes of *Muscardinus avellanarius* within the UK. This chapter summarises the findings of the research presented and considers the implications for conservation of the hazel dormice in the context of the current threat of habitat fragmentation and global climate change. This concluding chapter will outline the importance of the work, and identify further research motivated by the key findings. Wjilst single species conservation ma

6.2 Chapter key findings

Chapter two: Phylogeography of the hazel dormouse in the UK and Europe – Conservation units and management

The contemporary geographic distribution, demographic history and patterns or genetic diversity of species is considered to be shaped by the climatic conditions after the end of the last ice age. There has been a considerable improvement in our current knowledge and understanding of the post-glacial expansion of species and the resulting genetic diversity of species on their range boundaries. Chapter two investigated the phylogeographic structure of dormice within the UK using comparative analysis of two mitochondrial genes and one nuclear gene. This allowed the reconstruction of recent demographic history within and between continental Europe. Molecular clock analyses allowed the timing of dispersal into the UK to be calculated (7.5-11 Kya). This single post-glacial colonization into the UK through a Central North Western European clade supports a late migration hypothesis at the beginning of the Holocene period. This is concordant with the presence of a land bridge "Doggerland" connecting the UK and mainland Europe at this time (Spinney, 2008; Weninger *et al.*, 2008). Whilst the earliest

dated fossil of dormice in the UK using radio carbon dating is around 9000 years ago (Montgomery *et al.*, 2014).

The pattern of regional genetic variation identified has direct relevance to conservation monitoring within the UK. The information can be used to inform captive management, reintroductions or augmentation practices of this species. The reintroduction program currently source from a heterogeneous origin, however it is recommended that because there is no critical overall extinction risk in dormice, the natural pattern observed here should be preserved. This may help manage the risk of losing adaptive genetic variation and potential outbreeding depression via reintroductions or augmentations (Frankham *et al.*, 2011; Houde *et al.*, 2011; Weeks *et al.*, 2011).

Key Findings:

1. Dormice are genetically isolated from their continental European counterparts and should be managed as such.
2. Molecular dating calculates the time of divergence are coincident with the start of the Holocene period, 7.5-11 Kya, a time when woodland habitat was prevalent and a land bridge was present.
3. Evidence that dormice colonised the UK in a single expansion event.
4. Regional genetic variation into eight genetic clusters within the UK exists.
5. Population augmentation and reintroduction efforts should take into account this regional genetic variance, conservation management efforts should explicitly consider the genetic biodiversity that exists within UK dormice.

Chapter 3: Occupancy modelling for the detection of species

Chapter three used a large, comprehensive data set from nest tube surveys within the regions of Suffolk and Devon to investigate how temporal variation in detection probability within dormice can affect the ability to reliably estimate the presence or absence of a species. We fitted single-season occupancy models to our data, the results of which showed that detection probability of dormice are highly temporally dependent with early months of the year having a low detection probability (0.21-053; April-June). Whilst detection probability peaked during breeding months (0.89; September). Survey intensity had a significant impact on detection probability. Our findings provide empirical evidence to support robust ecological surveys, a legal requirement in the UK,

for the presence of dormice and support conservation of this species in identifying the presence in sites previously unknown. Conservation practitioners may have limited knowledge of the relative abundance and likelihood of detection of a particular species and as such can apply these guidelines in different geographical locations.

Key Findings

1. Detection probability was significantly temporally dependant throughout the season with early months having a low detection probability.
2. August and September are the months with highest detection probability, a period when dormice abundance is at a peak.
3. Survey intensity has a significant impact on detection probability, it is recommended a minimum of 50 nest tubes for surveys
4. Optimal survey periods and start dates can control for comparatively low number of surveys and variation in temporal covariates.
5. These guidelines can provide conservation managers and ecological monitors with a simple tool with sufficient power to detect species presence.

Chapter 4: The influence of landscape connectivity and barriers on genetic variation and gene flow

In Chapter four, population genetic analyses and landscape resistance analyses were conducted to evaluate the role of landscape features on shaping patterns of diversity within seven regions of the UK. It used classic population structure analyses to test isolation-by-distance (IBD) and isolation-by-resistance models (IBR) to predict landscape features that influence functional connectivity. Anthropogenic alteration to landscape composition and structure can severely impact biodiversity, ecosystem functioning and services. Such changes can affect functional connectivity and gene flow (dispersal) thus influencing the genetic structure of populations. Circuit theory analyses were conducted to calculate resistance matrixes in order to test the effect of landscape variables on genetic distance taking into account IBD.

Key Findings:

1. Our results suggest the build-up of genetic structure amongst islands of fragmented habitat that is explained in part due to isolation-by-distance, and in part due to specific landscape features between different regions.
2. The isolation-by-resistance analysis allows us to identify the landscape features, such as land cover, hedgerows and roads that facilitate or inhibit gene flow.
3. Land use in combination with non-natural landscape features have a significant effect on genetic diversity
4. Dispersal in hazel dormice is strongly influenced by barriers in the landscape, with our main findings being that urban areas and roads are associated with decreased gene flow while habitat features such as hedgerows and forest cover are associated with increased gene flow.
5. Results indicate that conservation management efforts to increase connectivity have had positive influences on dormice persistence, whereas more isolated populations require active management and additional measures to curtail habitat fragmentation and increase functional connectivity

Chapter 5 : Density and climate effects on age specific survival and population growth: consequences for hibernating mammals

Chapter five investigates the effect of density dependence and climatic factors on the population demography of five marked hazel dormice populations in Europe (four in the UK and one in Lithuania). An integrated population modelling approach was used to estimate age-specific overwinter survival, population growth and fecundity. The aim of this chapter was to determine the impact of density dependence and climatic variables collected from local weather stations on these population vital rates and whether adults and juveniles were impacted in similar patterns. There is a breadth of research focussing on our understanding of density dependence (Turchin, 2003; Churcher, Filipe and Basáñez, 2006; Morris and Maceachern, 2010). However the cumulative influence of variable local environmental conditions on key demographic parameters are less well understood (Griffith *et al.*, 2016). The results from the chapter have identified the

environmental drivers of these population parameters on hibernating mammals whilst providing evidence that density dependence has the greatest effect on population dynamics in this species. The results provide information that variability in winter conditions can have serious consequences for individual fitness, decreasing the dormancy season and leading to an increased extinction risk.

Key Findings

1. We found a strong negative effect of density dependence, rain, warm and more variable winter temperatures on population growth rates.
2. Identified an interaction effect between climatic conditions and density on age-specific survival resulting in reduction of the hibernating season and decreasing length and frequency of hibernation bouts.
3. In juveniles, a significant negative association on survival is found between density, yearly average temperatures and annual precipitation. Whilst ddult survival was negatively associated with, more variable winters and increased mean temperatures during this season.
4. Although colder winters favour an increased mean population size, density-dependant feedback can cause the local population to be less buffered against occasional poor weather (warmer, wet winters).
5. Management of woodland resources that can mitigate for the negative impacts of extreme weather conditions will greatly improve the future persistence of dormice populations.

6.3 Conclusions and implications for conservation

6.3.1 Conservation genetics and management

The phylogeographic and population genetics approach taken in Chapters 2 and Chapter 5 gives valuable insights to aid dormice restoration within the UK. A goal of landscape genetics is the identification of landscape features that may act as barriers or promotors to species movement through the use of genetic and landscape composition

data (Storfer *et al.*, 2007a; Bolliger, Lander and Balkenhol, 2014). This study adds analysis to the currently scarce availability of studies that test the effect of IBD and IBR (Ruiz-Gonzalez *et al.*, 2015). The identification of fine scale genetic structure not just by IBD but habitat characteristics and intraspecific barriers provides evidence for integration of analysis methods and conservation management efforts to consider the effect of mitigation methods that incorporate the effects of IBD and IBR. IBR studies, both empirical and simulation show that resistance values outperform these simple geographic distances (IBD) in explaining the genetic differentiation observed in wild populations (McRae, 2006; McRae and Beier, 2007; Segelbacher *et al.*, 2010), a similar case in this study. These findings are of interests to a wide array of scientists interested in landscape and evolutionary genetics, incorporating the role of IBD and IBR in determining spatial population genetic structure and gene flow. However, a major flaw in landscape connectivity studies is the lack of implementation of key findings to ensure future viability of populations (Correa Ayram *et al.*, 2016). Thus future work to consider the limiting factors of landscape barriers and the influence of mitigation methods for promoting dispersal on genetic structure should be incorporated into species conservation plans in order to aid the success of management efforts in species threatened by increasing urbanisation and habitat fragmentation.

The identification of both natural and anthropogenic barriers and corridors to movement and gene flow in this study provide conservation managers with important information for the prioritizations of improving landscape connectivity. The potential negative effects of structures such as roads, railways and urban structures would be consistent with the known negative decline of dormice populations in the UK associated with the loss of their primary woodland habitat (Bright *et al.*, 2006). Reduced dispersal due to these factors and declining population sizes being observed throughout its range (Goodwin *et al.*, 2017) can lead to a reduction in gene flow, thus altering genetic connectivity across dormice landscapes. This can lead to genetic challenges of inbreeding, which could create further challenges for the implementation of dormice conservation measures to mitigate these population declines. As a consequence, genetic assessment of dormice populations are vital, however the continued long-term monitoring of populations are required in order to ensure the viability of populations

and mitigate the declines being observed. A limitation of this genetic study is the fact that only six landscape variables were tested. The addition of environmental data and other data types such n this as radio telemetry or mark-recapture would greatly add to the knowledge of differences in animal movements. Whilst the effect of age or sex was not tested in this study, a causal factor observed in other species (Smith *et al.*, 2016). Landscape genomics provides the ability to further inform conservation management on the functional connectivity and effectiveness of connectivity measures (Segelbacher *et al.*, 2010; Aaron BA A Shafer *et al.*, 2015), whilst allow priority of selecting populations when conserving adaptive genetic variation (Allendorf, Hohenlohe and Luikart, 2010).

Hedgerows are of key importance for conservation efforts for habitat and dispersal routes between patches, especially in agricultural landscapes (Bright *et al.*, 2006) as tested here. As such, maintenance of hedgerow connections are vital to be increase dispersal between population whilst new hedgerow habitat can allow genetic exchange to reduce the effect of inbreeding and non-random mating. However, further creation of new hedgerow connections between isolated and fragmented populations will require future monitoring of genetic integrity in order to ensure inbreeding depression or outbreeding depressions are not negatively impacting the populations. Further research is also required to test the effect of variable hedgerow quality to quantify gene flow between habitat patches to further aid management recommendations.

Roads are known to exert various effects of conservation concern, due to mortality, alteration in dispersal behaviour etc that can further exacerbate fragmentation and restrict animal movements. Roads are generally considered to affect populations through a reduction in genetic diversity and gene flow, decreasing functional connectivity (Holderegger and Di Giulio, 2010; Zhang *et al.*, 2013). This study provides further evidence of the negative effects of roads on dispersal in small mammals. However such effects is based on size and width of road having significant impacts on increasing genetic differentiation. Whilst populations with woodland edge along single carriage roadways showed low genetic differentiation indicating dispersal and gene flow is relatively high and act as buffers to increase dispersal between populations.

Thus management efforts to increase woodland edges along barriers may have a positive effect on movement. Whilst the creation of habitat bridges and underpasses may be more suitable for wider roads with higher traffic levels.

The results of the phylogeographic study can be directly applied to restoration management efforts for species that similarly exhibit regional genetic variation. The current hope is to capture most available genetic diversity in the wild population to release the progeny back into the wild (Williams and Hoffman, 2009). Currently founders are generally selected based on a random strategy. However, current IUCN guidelines recommend the selection of founders based on source populations geographically close or from similar habitats (IUCN, 2012). The program of reintroduction of hazel dormice in the UK, in conjunction with the national Biodiversity Action Plan for the species, has a goal of both bolstering the quality and size of extant populations but also restoring additional populations to sites which were once formerly occupied but have gone locally extinct. There have been some important successes in these reintroductions (White, 2012); however the captive born founders for these reintroduced populations come from stock of heterogeneous origin. This is evidenced in the results presented here, where the northerly reintroduced Wych population appears to be genetically discontinuous with geographically close populations and a similar situation can be seen in South East England (Suffolk populations). We suggest that, because there is no critical overall extinction risk for the dormouse, preserving the natural pattern of genetic variation observed in natural populations could, and perhaps should be, considered when reintroducing animals back into the wild. A second consideration is to explicitly consider the genetic biodiversity, represented by regional genetic structure, to understand the possible impacts of gene flow between these populations managing the risk of losing adaptive genetic variation via reintroduction itself (Williams and Hoffman, 2009).

6.3.2 Species monitoring

A basic requirement to measure the impact of conservation is the availability of monitoring data and a knowledge of the processes underpinning population change and

abundances (Buckland *et al.*, 2007) and assess the extinction risk in small or declining populations (Bonebrake *et al.*, 2010). Fundamental to the field of ecology is understanding the mechanisms underlying animal abundances and fluctuations in order to inform practical conservation management (Krebs, 2002; Hastings, 2010) (Krebs 2002 and Hastings 2010). There is a breadth of research focusing on our understanding of density dependence (Turchin, 2003; Churcher, Filipe and Basáñez, 2006; Morris and Maceachern, 2010). However, it has been noted that density-dependent interactions and the cumulative influence of variable local environments on the demographic parameters that drive population abundance are less well understood (Griffith *et al.*, 2016). This research is relevant to the literature assessing the ecological effects of climate change and other intrinsic factors on species demographics. We demonstrate that some interaction with climatic variation have subtle and strong effects that might be hard to predict, which have important practical conservation management implications. As a consequence management of habitat resources for hibernators can buffer against the stresses of climate change effects on demography, aiding the future persistence of species vulnerable to changes in local climatic conditions.

Global warming in the northern latitudes during winter months is thought to have severe consequences for species, and the trend is predicted to continue of increased mean winter temperatures, up to 3-4 ^{o}C by 2050 (Notaro *et al.*, 2011). As a result of warm winter temperatures, we observe changes in the timing of food resources, lengthening growing periods and earlier reproduction. This is a concern in hibernating species that may emerge earlier in spring and observe unequal shifts in phenology (Inouye *et al.*, 2000; Koppmann-Rumpf, Heberer and Schmidt, 2003; Adamík and Král, 2008). Thus, while climate change has been widely shown to affect species range limitations (e.g. Parmesan & Yohe, 2003), our results suggest that climate variation can also have direct negative consequences for hibernating species, such as the hazel dormice (Pretzlaff & Dausmann 2012).

In dormice periodic hazel coppicing is a forest management practice that considered to benefit dormice. Coppice understory with no canopy trees or coppice with a low density of canopy trees results in an unshaded and productive shrub layer. The practice maintains a successional stasis that is ideal for the species (Bright et al. 2006). Woodland ride and edge management is also undertaken (Trout et al. 2018), to allow

light to penetrate further into the woodland and promote successional growth (Ramakers, Dorenbosch and Foppen, 2014). However, there is a lack of good evidence about the effectiveness of these practices for dormice or for other woodland species. Thus, while we identify some components of population regulation here, we still lack understanding on the role of forest management practices on dormice or other species.

6.4 Future work

In the face of anthropogenic challenges such as human development and global climate change there is still a lack of information on how a wide range of species will be affected in a range of habitats. The identification of adaptive genetic variation using genomic approaches will be key in future conservation studies in order to understand response of species or conservation management effort (Allendorf and Luikart, 2007; Allendorf *et al.*, 2010). High-through put sequencing tools promise to revolutionize many aspects of genetic research e.g. by allowing the identification of functional adaptive genetic variation. However a so-called 'conservation genomics gap' exist (Aaron B A Shafer *et al.*, 2015) due to the expense and expertise required to apply these tools to basic conservation questions. Although genomic technologies are increasing in usage through single nucleotide polymorphisms (SNPs), restriction-associated DNA sequencing (such as RADSEQ) the use of neutral markers is still relevant in conservation applications that have a timely conservation imperative. Further the use of Next-generation sequencing has a potential to further our knowledge of landscape genomics. A relatively new discipline that can further investigate the relationship between genomes and environmental heterogeneity within natural populations or the investigation of ex situ conservation such as captive breeding (Funk *et al.*, 2015). Such methods can be a powerful tool to conduct selective sweeps of candidate genes responsible for complex adaptive evolution of species.

Conservation genetic data can provide essential information for many aspects of managing threatened populations that are suffering declines by integrating genetic, ecological and demographic data. This data can aid monitoring of biodiversity (Thomsen and Willerslev, 2015), wildlife forensics and delineating units of conservation

concern (Palsbøll, Bérubé and Allendorf, 2007). Whilst such information can be used to implement management practices and policies or to direct government law for the protection of species (Haig *et al.*, 2016). However, the expense and expertise required to apply these tools to basic conservation questions is a challenge for applications outside academia, resulting in a so-called 'conservation genetics gap' (Aaron BA A Shafer *et al.*, 2015; Taylor, Dussex and van Heezik, 2017). The conservation genetics paradigm is that, basic information about genetic relatedness, inbreeding and gene flow are often critical to inform conservation management of small populations (Allendorf *et al.*, 2010). This information is often needed quickly and ideally should be accessible to workers without special expertise in genetics or genomics (DeSalle and Amato, 2004). However it is vital that information similar to that found in this study is made understandable by scientists, in order for wildlife practitioners to respect the usefulness of such information for both *in-situ* and *ex-situ* conservation programs.

The role of climatic variation on hibernating species within this study is a concern for the future survival of species that exhibit this behaviour. With global temperatures predicted to increase (Barker, 2007) it is of fundamental importance that future investigations explore this research framework particularly with other factors than temperature and investigate how in species such as dormice how woodland management efforts can buffer for the stressors of climatic fluctuations.

The results of this study provide evidence for the threats to the future persistence of populations of dormice within the UK. The sites sampled make up a large part of the dormouse range in the country and are part of the UK Dormouse Monitoring Programme. As such, some of the genetic variation observed may be in part due to the effectiveness of conservation and mitigation efforts in this species that aim to increase dormice abundance or woodland biodiversity. However, evidence is presented for the presence of inbreeding in isolated populations that lack connectivity. Genetic data on gene flow and connectivity can contribute to the inference of how species move across landscapes, this is vital in order to understand how species adapt, understanding how diseases spread within environments or how we can use management efforts to ensure genetic diversity is maintained. However, more investigation on the impacts of landscape and climatic variables on species dispersal and survival is needed, using long-term data sets and improved monitoring techniques.

6.5 References

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