Conservation and population genetics of British hedgehogs (*Erinaceus europaeus*)

NOTTINGHAM TRENT UNIVERSITY

A thesis submitted in partial fulfilment of the requirements of Nottingham Trent University for the degree of Doctor of Philosophy. September 2024.

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Copyright Statement

Acknowledgements

I thank my supervisory team, Dr Richard Yarnell, Dr Louise Gentle, Dr Axel Barlow, and Dr Philip Baker. I thank Richard for believing in me and giving me the chance to do this PhD, for helping me on every step throughout the process, and for taking me around the UK for my study. I have learned a lot from Louise, more than academic skills. I have to thank Axel for countless hours of training, from the beginning when I did not know how massive the work was to be undertaken. Philip's guidance will inspire me for a long time.

I wish to thank my tutors Prof Carl Smith, Dr Carrie Ijichi, and Dr Helen Hicks, academic administer Dr Ruth Bromiley-Miller, and internal assessor Prof Chungui Lu, for their help with various parts of this study. I am grateful for the support from the technician team especially Simon Taylor for helping with sampling and providing equipment, the laboratory team for helping prepare sampling kits, and the security team for safeguarding me on my nightly wanderings searching for hedgehogs. Thanks to my PhD cohorts.

A huge thank you to all the people who kindly provided me with samples for genetic analyses: Lauren Moore, Becki Lawson and the team from Garden Wildlife Health Project, Richard Wilkinson, Sophie Rasmussen, and Jacquie Wilson. I am thankful to Dr Deborah Dawson, Dr Gavin Wilson, and the team at the University of Sheffield, NERC Biomolecular Facilities, for helping with laboratory and analysis work for Chapter 4. Thanks to Lucy Knowles, for your expertise and help. Thanks must be extended to Sourish Kuttalam, Ben Owens, and the team at Bangor University for helping with laboratory work for Chapter 5. I am grateful to the volunteers who helped collect the data for Chapters 2 and 3. Thanks to Johanna Paijmans and Robert Davis for helping with the analysis. To all my collaborators and co-authors whom I cannot list here, thank you for your help. I am grateful to the School of Animal, Rural and Environmental Sciences, Nottingham Trent University for funding the genome sequencing. I acknowledge the support of the Supercomputing Wales project, which is part-funded by the European Regional Development Fund (ERDF).

I have to thank my brother Xinglong Yu for finding me a hedgehog before my PhD. I thank my parents Shaoliang Yu and Zhirong Wang. I thank my grandparents. To all my friends and family I cannot list here, thank you and I look forward to seeing you very soon.

My sincere thanks to all the people who were involved with this research. I would like to thank the hedgehogs for finally turning up, and thank Hawk, Hamilton, Imac, and Mackook (computers) for your ultimate stillness - I have lost in you, joyfully.

Contribution statement

Chapter 1

I wrote the manuscript with assistance from my supervisory team.

Chapter 2, Chapter 3

I undertook the survey work in 2021 and 2022, with data provided by Dr Richard Yarnell for the previous nine years combined. Numerous undergraduate and postgraduate students helped collect the data over the time period. I conducted the analyses and wrote the manuscripts with assistance from supervisors and collaborators.

Chapter 4

Samples were collected by Dr Lauren Moore from Nottingham Trent University. I undertook the lab work at the NERC Environmental Omics Visitor Facility. I conducted the analysis. I wrote the manuscript with assistance from supervisors and collaborators.

Chapter 5

Of the 132 samples sequenced, 76 samples were supplied by Garden Wildlife Health Project, Zoological Society of London, two Danish samples were provided by Sophie Rasmussen at the University of Oxford, three Orkney samples were collected by PhD student Richard Wilkinson from Nottingham Trent University, one Isle of Wright sample was provided by Mrs Jacquie Wilson at Isle of Wight Hedgehog Rescue. I collected other samples with Dr Richard Yarnell, Dr Louise Gentle, field technician Simon Taylor, and Dr Axel Barlow, through road surveys across Great Britain. I undertook DNA extraction at Bangor University, with assistance from Dr Axel Barlow, PhD students Sourish Kuttalam, and Ben Owens at Bangor University. The whole-genome sequencing was carried out by an external commercial facility. Dr Axel Barlow and I did raw data trimming, merging, and mapping. I conducted other analyses with training and assistance from Dr Axel Barlow. I wrote the manuscript with assistance from my supervisory team.

Chapter 6

I wrote the manuscript with assistance from my supervisory team.

Abstract

Population declines of common species constitute an important part of ongoing biodiversity loss. The ability to detect and preserve declining wildlife populations is paramount, but this is challenging for species that exist over large geographic ranges. The Western European hedgehog (*Erinaceus europaeus*) (hereafter termed hedgehog) is declining throughout its range with multiple potential causes suggested. This thesis aims to identify hedgehog population structure in Great Britain; infer demography, genetic diversity, and gene flow; and investigate factors driving population change. A combination of field studies, laboratory experiments, modelling, landscape genetics, and population genomic analysis was employed in an attempt to identify population structure at different spatial scales.

An 11-year spatial capture-recapture (SCR) dataset involving 207 hedgehogs was used to analyse survival and density at a rural site (Brackenhurst) in Nottinghamshire (**Chapter 2, Chapter 3**). Annual survival was relatively stable over the 11 search years. Annual apparent survival rates were 0.530 (95 CI 0.423–0.635), and 0.426 (95 CI 0.308–0.552) for adult females, and adult males, respectively. Survival during winter hibernation period was suggested to be high compared to that in active seasons. Annual population density averaged 14 hedgehogs/km^2, with adult female: juvenile female: juvenile male: adult male density ratios being 1.6: 1.5: 1.1: 1, respectively. Density on amenity: pasture: arable approximates to 10: 4: 1. These results are comparable to previous results. Density was found to be significantly positively associated with soil permeability, edge density, proximity to the nearest building, and distance to the nearest badger (*Meles meles*; intra-guild predator of hedgehog) sett. A new badger sett was identified halfway through the study period, resulting in a shift in the hedgehog density-weighted population centre, and a decline in overall density which was then stabilised, suggesting spatial segregation on the field scale, due to the landscape of fear response of hedgehogs to badgers, and coexistence on the landscape scale of both species.

Using genetic data genotyped with 14 microsatellite loci, contemporary gene flow among four neighbouring suburban populations (Farnsfield, Halam, Kirklington, Southwell) in Nottinghamshire separated by an agricultural landscape was evidenced based on a lack of genetic structure differentiating the populations (n = 236 hedgehogs; **Chapter 4**). Higher

relatedness and lower allelic richness were found in smaller suburban patches, potentially indicating an early stage of the establishment of different population structures due to a current lack of resources in rural habitats and the distance between urban areas that represent population centres.

Whole-genome analyses of 123 hedgehogs evenly sampled across Great Britain were used to infer population history, population structure, and genetic diversity of the hedgehogs (**Chapter 5**). Natural post-glacial colonisation of the hedgehog in Britain was supported, and a generally continuous genomic pattern was found, suggesting all present-day hedgehogs descend from the same post-glacial ancestral population. Individual heterozygosity was high in the south and decreased with latitude following the historical expansion routes. No evident differentiation was found to correspond to the presumed barriers, e.g., rivers, mountains, agricultural lands, city centres, and roads, revealing continuous gene flow on large scales. Limited evidence of severe inbreeding due to fragmentation was found, other than in some island populations. The recent population decline was found to have started a few centuries ago, likely coinciding with agricultural intensification.

The results from this study provide a baseline for future research and conservation of the hedgehog. As a model species in agroecosystems for informing habitat connectivity and quality, the results provided for the hedgehog have wider conservation implications. The study highlights the importance of understanding broad-scale genetic structure for the interpretation of local population patterns for hedgehogs and other common species, and proposes using whole-genome sequencing, with even-geographic sampling.

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Chapter 1: Introduction

1.1 Biodiversity loss

Human activities have a profound and largely negative impact on biodiversity (Pimm et al., 2001). Human-induced biodiversity loss is occurring at different levels: habitat disruption, species extinction, population extirpation and population size decline, and loss of genetic diversity (Dirzo et al., 2014). Studies have predicted that such biodiversity decline can deteriorate ecosystem function, affecting many species and local peoples (Diaz et al., 2019; Albert et al., 2023). The time window left for effective conservation is suggested to be shrinking (Barnosky et al., 2013; Haddad et al., 2015; Ceballos et al., 2017), with a need for empirical evidence-based strategies and broad-scale conservation actions (Barnosky et al., 2013; Haddad et al., 2015; Ceballos et al., 2017).

1.1.1 Habitat disruption

Human-induced biodiversity loss can be attributed to a range of factors, including habitat loss and fragmentation, exploitation, invasive species, and climate change (Dirzo et al., 2014). These effects vary among species and populations. The greatest threat to biodiversity, however, is widely suggested to be habitat loss and fragmentation due to land-use and sea-use change (Diaz et al., 2019). Human actions have directly altered at least 70% of ice-free land surface (IPCC, 2019), of which 50% is for agricultural land use (Foley et al., 2007); 70% of the forest is within 1 km of the forest edge, subject to degrading effects of fragmentation (Haddad et al., 2015); 49% of grassland area has been degraded (Bardgett et al., 2021); 66% of ocean surface is experiencing cumulative impacts (Halpern et al., 2015); 21% of wetland has been lost since 1700 (Fluet-Chouinard et al., 2023); and 63% of the longest rivers no longer flow freely (Grill et al., 2019).

1.1.2 Species extinction and population decline

Most species on the planet are lacking conservation assessment (Dirzo et al., 2014). Among those species that have been described, only 6% have been evaluated for the International Union for Conservation of Nature (IUCN) Red List (IUCN, 2024). Conservatively almost 200 vertebrate species have gone extinct over the last 100 years, possibly primarily driven by human-induced habitat disruptions. Some researchers have made attempts to predict the magnitude of this, based on the background extinction rate over the past two million years, that these species would have taken 10,000 years to naturally disappear (Ceballos et al., 2017).

While species extinction due to human-induced habitat disruption is widely recognised, less so is the population extirpation and decline of common species, which comprise a significant part of ongoing biodiversity loss (Ceballos et al., 2017; Finn, Grattarola and Pincheira-Donoso, 2023; van Klink et al., 2024). Studies have argued that billions of animal populations and 50% of the number of individuals are estimated to have been lost (Seddon et al., 2014; Ceballos et al., 2017). Out of the 32,776 species with population trends available on IUCN, 48% show declines, and of these 50% are those classified as Least Concern or Not Threatened (Ceballos et al., 2017; Finn, Grattarola and Pincheira-Donoso, 2023). Such declines in common species (e.g., many Least Concern or Not Threatened species) can cascade into ecosystem functioning (Dirzo et al., 2014), with a good example being the effects of decline in rural animals on agroecosystems (van Klink et al., 2024).

1.1.3 Loss of genetic diversity

Population extirpation and population decline often result in the loss of genetic diversity, the raw material of evolution which is with its loss likely irreversible in the time frame of conservation (Leigh et al., 2019). Low diversity in general has commonly been associated with an increased likelihood of genetic disease and a decreased adaptive potential in a changing environment, both of which can lead to increased extinction risks (Keller et al., 2002; Frankham, 2005; Fletcher et al., 2018). Quantifying genetic diversity loss is difficult due to a lack of historical data for most populations, but a conservative 6% of the within-population genetic diversity is estimated to have been lost over the past 200 years, based on genetic data from 91 species (Leigh et al., 2019).

1.2 Effects of agricultural intensification on common animals

Agricultural land-use change poses the highest threat to terrestrial species (Tilman, 1999; Tilman et al., 2017; Hartfoot et al., 2021). Over the past a few centuries, agricultural intensification and the subsequent loss and fragmentation of rural habitats have had severe impacts on the distribution and abundance of many previously common species (Tilman et al., 2017; Hartfoot et al., 2021). This is particularly relevant in Western Europe, where such changes in farmland have led to substantial declines in many animals (Donald et al., 2001; Rigal et al., 2023; Băncilă et al., 2023; Habel et al., 2021).

In the UK, agricultural land takes up around 70% of the total land (National Statistics, 2023). Widespread declines are evident in many common animals, including invertebrates (Warren and Bourn, 2011), amphibians (Petrovan and Schmidt, 2016), farmland birds (Krebs et al., 1999; Cornulier et al., 2011), and mammals (Mathews et al., 2018). These declines are thought to be primarily driven by agricultural expansion and intensification, including the conversion of natural grasslands to arable lands and pasture since the 1700s (Pretty, 1991), and the use of heavy machines, fertilizers, and pesticides since the 1950s (Robinson and Sutherland, 2002; Amar et al., 2010). The impacts of these agricultural practices on wild populations are far-ranging. The clearance of natural habitats directly decreases food and nesting material availability. The less visible effects, however, are also profound. The application of heavy machines, tillage, and the use of fertilizers and pesticides lead to soil erosion, salinisation, acidification, compaction, nutrient depletion, loss of soil organic matter, and contamination of chemicals, resulting in long-term declines in productivity and its environment moderating capacity (Stoate et al., 2001; Paoletti, 1999; Irmler, 2003; Bradley et al., 2006; Weil and Brady, 2017; Sandermana, Henglb and Fiskea, 2017). The disrupted habitats, and the decreased food and nesting materials may also exacerbate predation and competition pressure from sympatric species (Polis, Myers and Holt, 1989; Manlick et al., 2017). Further, the loss and fragmentation of habitats often result in the disruption of gene flow among populations, promoting the formation of small and isolated populations which may be pushed to the brink of extinction by low genetic diversity, inbreeding depression, and loss of genetic adaptive potential (Keller et al., 2002; Frankham, 2005; Fletcher et al., 2018).

1.3 The hedgehog: an overview

The Western European hedgehog (*Erinaceus europaeus*) (hereafter termed hedgehog) is a solitary, cryptic, nocturnal mammal. They are habitat generalists, widely distributed in different habitat types and often in low numbers (Morris, 2018). Across its range, the species contain three genetic clades identified based on mtDNA (Seddon et al., 2001). The first is from Italy northwards through Austria, Switzerland, Germany, the Netherlands, Scandinavia, and Estonia. The second is only found in western Europe, from Spain northwards through France, the Netherlands, and into the UK and Ireland. The third clade is restricted to Sicily, Italy (Seddon et al., 2001).

Hedgehogs are also present in many islands, often thought to be a result of introductions by humans, e.g., in Pianosa, Italy, where the founders of the population are thought to be translocated from Elba, Italy in the 20th century (Iannucci et al., 2018). The British hedgehog was documented to have been translocated to Ireland in the 12th century (Montgomery et al., 2014), New Zealand in the 19th century (Bolfíková et al., 2013), at least some of the 31 Scottish islands (where hedgehogs are/were present) in the 19-20th century, North Ronaldsay (Orkney, Scotland) in the 1970s, and St Mary's (Isles of Scilly, England) in the 1980s. On some islands, they failed to establish long-term populations for unknown reasons, whereas on some, they are currently being controlled as an invasive species (Harris, Morris and Wray, 1995; Morris, 2018).

The cryptic and nocturnal nature of the hedgehog makes it difficult to study. Nevertheless, the hedgehog is probably one of the most extensively studied common mammals, with the research covering, but not limited to: population density estimates (Schaus et al., 2020), habitat preferences (Hof and Bright, 2010), nesting behaviour and hibernation (Bearman-Brown et al., 2020), reproduction (Jackson, 2006; Jackson, 2007), feeding behaviour (Scott et al., 2023), accumulation of chemicals (Lieberman, 2021), anthropogenic injuries (Berger, 2024), diseases (Dastjerdi et al., 2019), interspecies interactions (Lee, 2021), dispersal (Haigh, 2011), phylogenetics (Seddon et al., 2001), and population genetics (Rasmussen et al., 2020). Further, the hedgehog is also a model species in agroecosystems for informing rural habitat connectivity and evaluating agri-environment schemes (Hof, Snellenberg and Bright, 2012; Pettett et al., 2017), being traditionally associated with agricultural lands and feeding on macroinvertebrates (Yalden, 1976). Although it is still relatively common

throughout its extensive range (Amori, 2016), the populations are reported to be declining in Western Europe, especially in rural areas (Yarnell and Pettett, 2020). The species has recently been downlisted from Least Concern to Near Threatened by IUCN (2024). It is protected under Appendix III (Protected Fauna Species) of the Convention on the Conservation of European Wildlife and Natural Habitats (Bern Convention) (Council of Europe, 1979), and in the UK under the Wildlife and Countryside Act (1981), listed as Schedule 6. Due to the reported declines in Great Britain, the species has recently been classified as Vulnerable on Britain's Red List (Mathews and Harrower, 2020).

1.4 Cause of hedgehog decline and knowledge gaps

The cause of hedgehog decline is likely multifaceted, including habitat loss and fragmentation due to agricultural intensification (reviewed in Yarnell and Pettett, 2020), the effects of its intraguild predator the badger (*Meles meles*; Young et al., 2006; Van de Poel, Dekker and Langevelde, 2015; Pettett et al., 2018; Hof, Allen and Bright, 2019), the use of chemicals such as pesticides (Lieberman, 2021), habitat loss and fragmentation due to urbanisation (Taucher et al., 2020), road fragmentation and mortality (reviewed in Moore et al., 2020), ecological traps such as parks in urban centres potentially causing inbreeding (Barthel et al., 2020; Araguas et al., 2022), diseases (Dastjerdi, et al., 2019) and other anthropogenic effects such as injuries (Berger, 2024). These effects are often confounded together, for example, the impacts of the increased badger density (after long-term persecution; Judge et al., 2017) and rural habitat degradation are difficult to disentangle because historical population distribution and population size, and long-term robust population dynamics are generally lacking (but see Kristiansson, 1990). So far, the main causes driving the decline are uncertain. Here, I provide a summary of key knowledge gaps for us to infer the current status of the hedgehog, and the main causes of the observed decline:

- *Current population estimates on agricultural lands are scarce and historical data are lacking*

Agriculture is the primary land use in Western Europe, and agricultural intensification has been a major cause of biodiversity loss (Robinson and Sutherland, 2002; Amar *et al*., 2010). In the UK, agricultural land makes up around 70% of land area, of which half is used for arable crops (National Statistics, 2023). Over the last few centuries, the effects of intensive

farming, due to machinery, the wide use of chemicals, and the removal of natural habitats such as grassland and hedgerows (Robinson and Sutherland, 2002; Amar et al., 2010), have had a considerable impact on biodiversity within the UK (Warren and Bourn, 2011; Krebs et al., 1999). These changes in landscape are also thought to have had a negative impact on hedgehogs, which were once thought to be ubiquitous across mainland Great Britain, and more associated with agricultural land (Yarnell and Pettett, 2020). However, recent monitoring suggests hedgehogs are declining, especially in rural areas (Battersby and Greenwood, 2004; Roos, Johnston and Noble, 2012; Wembridge et al., 2016; Wilson and Wembridge, 2018), and the species has recently been classified as Vulnerable on Britain's Red List (Mathews and Harrower, 2020). So far, robust habitat-specific density estimates remain scarce for the species, especially on agricultural lands (Mathews et al., 2018). Only a few estimates exist, with most of these being from pasture and amenity land (e.g., Schaus et al., 2020), and only one from arable land (northeastern France; Hubert et al., 2011), which showed low densities on arable land (0.7/km^2) and pasture (2/km^2), compared to that on the adjacent amenity land (36/km^2). Nevertheless, previous studies have documented difficulty in even locating hedgehogs on arable land (Hof, 2010; Pettett, 2016), and radio- and GPS-tracking studies have generally suggested that hedgehogs select amenity grassland and build-up areas over agricultural land (but see Haigh, Butler and O'Riordan, 2012). Where they can be found on arable land, they mostly occur on field margins that are close to amenity grassland or in close proximity to buildings (Pettett, 2016; Hof, 2010), further suggesting that arable lands might be unsuitable for hedgehogs. So far, the underlying mechanisms for the little occurrence of hedgehogs on arable lands are unknown. Studies carried out in rural Norfolk (Hof and Bright, 2010) and Yorkshire (Hof and Bright, 2010; Pettett et al., 2017), UK, have shown that hedgehogs tend to avoid arable land and suggested that this was due to the effects of badgers. However, the effects of badgers might be limited to local areas, while other factors might be more important in shaping wider population patterns of hedgehogs (Yarnell and Pettett, 2020). Likewise, a few studies have shown that hedgehog occurrence is low on agricultural lands and proposed the effects of recent agricultural intensification (Williams et al., 2018), but a lack of empirical data regarding historical hedgehog abundance and distribution on these lands makes inferences on the magnitude of population change difficult.

- *Dispersal and gene flow are largely unknown*

Habitat loss and fragmentation have had severe impacts on the distribution and abundance of many previously common species (Tilman et al., 2017), which often result in the disruption of gene flow among populations, promoting the formation of small and isolated populations which may be pushed to the brink of extinction due to demographic and environmental stochasticity, and fitness depletion resulting from low genetic diversity and inbreeding (Keller et al. 2002; Frankham, 2005; Fletcher et al. 2018).

Understanding dispersal and current gene flow between local populations can provide insights into the scale and extent over which functional population connectivity can exist (Walton et al., 2021). The currently widely used methods to study dispersal, such as radio- or GPS-tracking, are often for short term, and with a small number of adults (due to ethical considerations; Bearman-Brown et al., 2020) which may not be able to represent the wider population, making lifetime and population-level dispersal difficult to obtain. For common or previously common species, using population genetics to detect current gene flow between local populations, i.e., to inform dispersal, is also challenging. Minimal variation in genetic structure would be detected when current gene flow between populations is still ongoing, or when the genetic structure is being masked by stronger historical gene flow even though gene flow has ceased (Milligan et al., 2018; Lucena-perez et al., 2020). Thus, methods used widely to infer contemporary gene flow, such as assigning individuals captured from distinct populations to their natal population, typically lack power (Proctor et al., 2020). Relatively large overall population sizes also limit the power of traditional individual pairwise genetic pedigree methods via detecting closely related pairs of individuals to inform ongoing gene flow, as such individuals are often difficult to capture or detect (Taylor, 2015). A potential method for species exhibiting sex-biased dispersal (Li and Kokko, 2019) is through inferring asymmetric autosomal genetic structure between sexes, which can detect current gene flow without the effects of historical gene flow (Prugnolle and de Meeus, 2002). However, the method remains largely untested (but see Solmsen, Johannesen and Schradin, 2011; Pernetta et al., 2011; Walton et al., 2021), especially for populations that are separated by unoccupied landscapes where the signal of sex-biased genetic structure might not be detectable due to insufficient statistical power, if inter-patch connectivity is limited and only a small number of animals move through the unoccupied landscapes (Prugnolle and de Meeus, 2002).

Recent studies suggest that hedgehogs have a patchy and discontinuous distribution, e.g., across rural England and Wales (Williams et al., 2018), and where present in rural environments, they tend to occur in small populations near residential buildings (Schaus et al., 2020) while avoiding agricultural lands (Young et al., 2006; Hubert et al., 2011; Pettett et al., 2017), although they may use surrounding agricultural land occasionally (Hof, Snellenberg and Bright, 2012; Parrott, Etherington and Dendy, 2014). Even in suburban and urban areas where they tend to be centred on, they do not appear to be continuously distributed, for example, they were only found present in 26% of amenity grasslands (Parrott, Etherington and Dendy, 2014). As hedgehog home ranges are relatively small (e.g., 0.12 km^2 for females and 0.22 km^2 for males; Pettett et al., 2017) and their dispersal ability is poorly understood, questions remain about their ability to move across infrastructures such as roads (Huijser and Bergers, 2000) or fences, or move between suburban centred populations separated by uninhabited agricultural lands. If hedgehogs are unable or unwilling to traverse these landscapes, the local populations may be isolated, and are likely to experience increased genetic drift and inbreeding, and a subsequent loss of genetic diversity and lowered population viability (Reed and Frankham, 2003).

So far, the dispersal patterns of hedgehogs are uncertain. While Doncaster et al. (2001) indicated that hedgehogs do not have a clearly defined period of dispersal during their life history, Reeve (1994) and Haigh (2011) described juvenile exploration movements in their studies, but Rasmussen et al. (2019) then proved that juvenile hedgehogs only have small home ranges. Morris (2018) suggested that hedgehogs usually do not move distances larger than 4 km. However, these tracking studies are often short-term, on a small number of hedgehogs, and restricted to adult hedgehogs due to ethical considerations (Glasby and Yarnell, 2013). Thus, the results for previous tracking studies might not reflect the hedgehog's life-history dispersal. What is clear is that hedgehogs tend to display male-biased dispersal, yet whether this can be used to infer current gene flow, through inferring asymmetric autosomal genetic structure between sexes (Prugnolle and de Meeus, 2002), has not been tested.

Previous hedgehog genetic studies have shown complicated population patterns (Becher and Griffiths, 1998; O'Reilly, 2016; Rasmussen et al., 2020; Barthel et al., 2020; Osaka et al., 2022; Araguas et al., 2022). For example, in the UK, Becher and Griffiths (1998) showed differentiation between eight populations within a 15 km radius in Oxfordshire but could not

identify if limited intrinsic dispersal, natural barriers, or human-induced barriers were the cause. In Zurich, Switzerland, three genetic clusters of hedgehog populations were identified as being separated by natural (rivers) and man-made (major roads) landscape features (Braaker et al., 2017). By contrast, in Berlin, Germany, no clear population genetic differentiation was observed across the city, which was explained by the high percentage of greenery in Berlin which may provide stepping-stone habitats for hedgehogs to maintain connectivity (Barthel et al., 2020). Interestingly, the authors also suggested that the observed lack of genetic differentiation may be due to the mixing of populations via translocated hedgehogs from rescue centres. They also detected a large proportion of individuals with similar genetic profiles that were excluded from the analysis. These may have represented close relatives or, alternatively, population isolation and inbreeding. In Denmark, genetic differentiation was found to correspond to the nation's island structure. However, no significant differentiation was found within the island populations, suggesting gene flow between local populations within islands still occurred or had done so until recently (Rasmussen et al., 2020). It is notable that previous results did not evidence any signals of contemporary gene flow, either connected or not, which is of direct conservation relevance, as they were likely confounded by other factors such as historical gene flow. However, despite many uncertainties, severe isolation has been proposed to explain the status of some local hedgehog populations, e.g., in Regent's Park, London (O'Reilly, 2016), and Barcelona Zoo (Araguas et al., 2022), with the former showing extremely low genetic diversity, and the latter being reported to had gone extinct in 2018 before a recent translocation.

- Broad-scale genetic structure patterns are lacking

Genetic structure is often complex and shaped by different demographic processes, such as expansion or contraction, fragmentation, or changes in density, so knowledge of the broad-scale structure would enable comparable inferences across these factors (Milligan et al., 2018). Discrete differences in structure, i.e., the presence of a systematic difference in allele frequencies between subpopulations, can have confounding effects when estimating other population parameters and processes, e.g., increase observed population sizes (Byrne et al., 2020), or create spurious bottleneck signals (Chikhi et al., 2010). Thus, understanding structure on a large geographic scale is arguably a first critical step in conservation genetics.

Nevertheless, detecting differences in population structure in a relatively continuous setting is challenging, i.e., where there may simply be no fragmentation and a continuous population is spread over a large area, when the fragmentation is too recent for population genetic differences to have accumulated, or where large population size limits the power of genetic drift to create differences between populations, and traditional genetic markers such as mitochondrial DNA and microsatellite often lack statistical power on this (Petkova, Novembre and Stephens, 2016; Janes and Batista, 2016; Bradburd, Coop and Ralph, 2018; Proctor et al., 2020). Recent genomic studies have highlighted that whole-genome sequencing combined with newly developed genomic statistics (e.g., combining allele frequency and allele composition information; using model-flexible approaches) can overcome most of the limitations of classical genetic markers and expand the range of questions that can be addressed (Lawson et al., 2012; Milligan et al., 2018). On a broad genomic scale, these methods remain underutilised in natural populations, representing an important opportunity in conservation genetics (Leitwein et al., 2020).

To date, only a small number of genetic studies have been conducted on hedgehogs, and none have used whole-genome sequencing to better understand the broad-scale structure and evolutionary processes affecting hedgehog populations. So far, broad-scale population structures of hedgehogs are lacking and, as many potential factors seem to affect them and these factors are often confounded, local population patterns are difficult to interpret. Consequently, the main factors regulating hedgehog populations are uncertain, which hinders conservation actions.

1.5 Thesis aims, objectives, and structure

Hedgehogs are suggested to be declining across much of their range with reasons largely uncertain. This PhD research is developed to infer population structure, gene flow, genetic diversity, and demography of hedgehogs in Great Britain, and to identify factors shaping population structure. The thesis consists of the following Chapters:

Chapter 2
Adult hedgehog density at an agricultural dominated study site in Nottinghamshire is modelled based on an 11-year spatial capture-recapture (SCR) dataset. A new application of integrating both spatially and temporally changed habitats into one SCR framework is

demonstrated. The analysis provides robust density estimates and direct comparisons of the habitat effects on the density.

Chapter 3

Building on the adult hedgehog density presented in Chapter 2, this Chapter provides population-level, age- and sex-specific density, by adding juvenile density, growth rate, and survival of hedgehogs, at an agricultural dominated study site in Nottinghamshire (next to the study site in Chapter 2 and Chapter 3), to provide an example of long-term rural hedgehog population dynamics.

Chapter 4

As very low hedgehog density was found on agricultural lands in Chapter 2 and Chapter 3, I investigate if local populations separated by agricultural lands are connected by current gene flow, using microsatellite data (n = 14 loci) from 236 hedgehogs from four suburban populations separated by an agricultural matrix in Nottinghamshire, to provide evidence of contemporary gene flow among local hedgehog populations.

Chapter 5

Whole-genome analyses of 123 hedgehogs evenly sampled across mainland Great Britain and some neighbouring islands, are used to infer national-scale population structures, gene flow, genetic diversity, and demography. The results have direct conservation implications and provide a baseline for future hedgehog conservation research.

Chapter 6

The Chapter concludes the thesis by synthesising the findings from the previous Chapters in the wider context of existing literature. By doing so, this Chapter makes several suggestions for hedgehog conservation, and recommendations for future research.

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Chapter 2: Population density of adult hedgehogs in a spatiotemporally changing agroecosystem

Abstract

Spatiotemporal variation in density is key information needed to inform conservation management. Spatial capture-recapture (SCR) methods have been widely used to estimate animal density across a broad range of species in different ecosystems. However, SCR studies rarely incorporate habitat and predator covariates into the density modelling process, and typically assume habitat homogeneity, limiting inference on density variation across the study area. Further complexity may arise from sex-biased movement rates and biotic interactions, such as predator avoidance. Here, we used an 11-year dataset from a typical mixed agroecosystem in England to estimate landscape-scale spatiotemporal densities of Western European hedgehogs (*Erinaceus europaeus*). We extended the SCR application by simultaneously integrating spatially varied habitat covariates, and the spatiotemporal variation in predator (Eurasian badger, *Meles meles*) den site into one SCR framework. Density was spatially structured (range 0.39–13.54 on a 1 km^2 grid), and was lower in arable fields and highest in amenity grasslands next to buildings. Density was also positively associated with soil permeability, density of edge habitats, proximity to the nearest building, and distance from the nearest badger sett. A new badger sett appeared halfway through the study period, resulting in a hedgehog density-weighted population center shift away from the badger sett and a significant decrease in annual hedgehog density estimates. Density estimates were also 43% lower after incorporating spatiotemporal covariate heterogeneity into the modelling process. This highlights the need to integrate habitat covariates into density modelling to provide more accurate density estimations and insights into spatially structured populations to better inform conservation management decisions. Finally, our findings demonstrate the importance of long-term monitoring for understanding population responses to changes in predator presence and provide clear empirical evidence for a prey species altering space use in relation to the presence of a predator, supporting the landscape of fear hypothesis.

Keywords: spatial capture-recapture; agroecosystem; spatiotemporal habitat heterogeneity; population ecology; landscapes of fear; *Meles meles*; *Erinaceus europaeus*

2.1 Introduction

Accurate estimates of population density are necessary for understanding population dynamics (Fryxell et al., 2014). However, this is particularly difficult for populations that are exhibiting spatiotemporal variation in density (Royle et al., 2015). Spatial capture-recapture (SCR) can incorporate the within-population density into the overall estimates of density during the modelling process (Royle et al., 2015). SCR studies collect individual encounter history data, i.e., when and where the individuals are detected and not detected, and estimate the number and distribution of potential individuals that have never been detected, based on the detection probability of the detected individuals (Efford, 2004). In this process, the density modelling can be based on either habitat uniformity or an assumption of heterogeneity, with the former assuming all individuals are randomly distributed, and the latter assuming the individuals' distribution is shaped by the effects of habitats (Efford and Fewster, 2013). Most SCR density estimates have been based on the uniformity assumption (Tourani, 2022). Although a small number of studies have integrated spatial heterogeneity into density modelling, only a few have reported to what extent this could affect estimates (e.g., Efford and Fewster, 2013; Gerber and Parmenter, 2015). Further, for both spatially and temporally changing covariates, how the SCR models would perform when both spatiotemporally changing covariates are integrated into one framework is untested with empirical data.

The hedgehog (*Erinaceus europaeus*) (hereafter termed hedgehog) is a model mammalian species for informing landscape connectivity and evaluating agri-environment schemes (e.g., Hof, Snellenberg and Bright, 2012; Pettett et al., 2017). The hedgehog was traditionally thought of as being a rural dwelling mammal (Yarnell and Pettett, 2020), that primarily feeds on macroinvertebrates (Yalden, 1976). Their recent decline in abundance and occupancy is well reported across their range, with agricultural intensification and habitat fragmentation thought to be principal drivers in the agroecosystem (Roos, Johnston, and Noble, 2012; Wilson and Wembridge, 2018). However, previous abundance and occurrence estimates are limited by potential bias through a failure to account for imperfect detection, and a lack of long-term robust density estimates hinders our understanding of the mechanisms responsible (Morris, 2018). Furthermore, despite traditionally being associated with rural and agricultural lands, the hedgehog is experiencing faster declines in rural areas than urban ones (Wilson and

Wembridge, 2018). Hedgehogs also appear absent from many rural areas (Williams et al., 2018) and where they do occur their densities are relatively low (Schaus et al., 2020). Estimating rural hedgehog density is therefore particularly difficult as the populations tend to be at low density and may show considerable variation in habitat use over short distances (Pettett et al., 2017) which is likely to affect density at small spatial scales (Hubert et al., 2011). Therefore, fine-scale sampling across different habitat types is likely to increase the accuracy of density estimation.

As well as agricultural intensification likely impacting hedgehog populations, their main intra-guild predator the badger is also implicated in hedgehog declines (Young et al., 2006; Trewby et al., 2014; Pettett et al., 2017). However, direct evidence of the population-level interaction between hedgehogs and badgers is often confounded by other factors such as variation in levels of artificial food (Pettett et al., 2017), or differences in hedgehog activity in different habitats. Hedgehogs may also have developed strategies to enable co-existence with badgers such as spatial (Young et al., 2006; Williams et al., 2018) and dietary niche-partitioning (Lee, 2021; Trewby et al., 2014).

In this study, we used an 11-year search-based spatial capture-recapture dataset of hedgehogs collected in a typical agroecosystem in England, to estimate hedgehog density and to evaluate the effects of the spatially and temporally changed habitats. We extended an application by incorporating custom specifications into an existing SCR model (Sutherland, Royle and Linden, 2019), which allows simultaneous integration of both spatially and temporally changed covariates into one SCR framework. With the ongoing habitat alteration, and with the development of landscape-scale sampling, the application of integrating within-population density can be widely used for many species.

2.2 Methods

2.2.1 Study site and hedgehog survey

The study took place at Nottingham Trent Univesity's Brackenhurst Campus, Nottinghamshire, UK (site centroid coordinates: 53.06 N, -0.96 W; Coordinate Reference System EPSG:4326), which consists of 2.07 km^2 of mixed agricultural land (55.8% arable, 25.6% pasture, 6.9% amenity), including university campus buildings (Figure 2.1). The total

length of all edge types was 18.54 km, with buildings covering an area of 0.03 km^2. Between 2009–2014, there were three active badger setts, two within the survey area and one beyond (*approx.* 500 m to the nearest search field of the hedgehog). An additional badger sett was found during annual surveys in 2015, resulting in a total of four active setts between 2015–2022.

Hedgehogs were located during nocturnal spotlight (1 million candlepower) surveys conducted annually between March and October, 2009–2022; data from 2016, 2019, and 2020 were not included in the analysis due to low survey effort (*sensu* Schaus et al., 2020). Searches were conducted by walking the perimeter of all fields (Figure 2.1; Figure 2.2). All captured animals were marked by attaching coloured heat shrink tubes to dorsal spines for individual identification (Glasby and Yarnell 2013). For each capture, the animal's ID, sex, age (juvenile or adult), weight (g), GPS coordinates, and time were recorded. Juveniles were classified as young born in that year, and adults as those that had survived their first winter (Yarnell et al., 2019). As juveniles and adults have different detection rates and habitat use, this study utilises data from adults only. All work was approved by Nottingham Trent University's ethical review committee (Ethics approval code: ARE10) and licensed by Natural England (20121788 and 2018-36011-SCI-SCI).

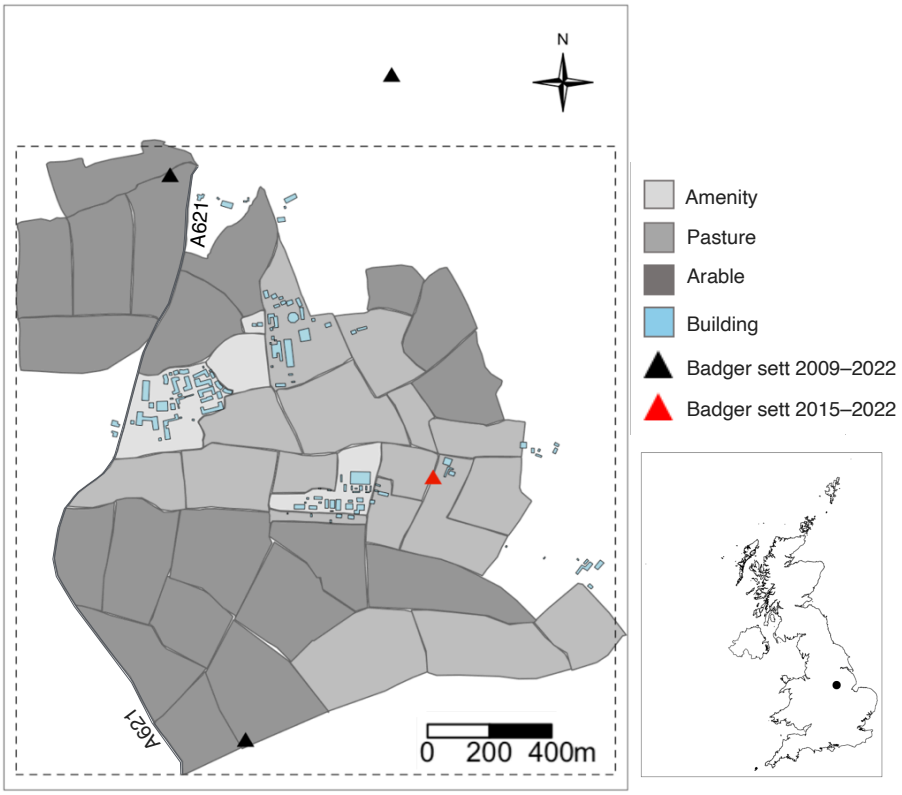

Figure 2.1. Habitat composition of the study site at Brackenhurst Campus, Southwell, England, showing badger sett locations, land use, and state space. Site centroid coordinates 53.06 N, -0.96 W (EPSG:4326). Amenity, pasture and arable fields are indicated in progressively darker shades of grey respectively. The study site is bisected by one major road (A621) towards the west. The state space for the hedgehog density modelling is shown within the dashed polygon. Triangles show the location of badger setts, with black triangles representing the setts that were active for the entire study period (2009–2022) and the red triangle representing the additional badger sett that appeared in 2015 and remained active beyond 2022.

Figure 2.2. Search fields. Site centroid coordinates: 53.06 N, -0.96 W (EPSG:4326). *Google Maps, Nottingham Trent University: Brackenhurst Campus, 1: 50,000.* https://www.google.co.uk/maps/place/Nottingham+Trent+University,+Brackenhurst+Campus/@53.0636857,-0.9666265,620m/data=!3m2!1e3!4b1!4m5!3m4!1s0x4879b7b6280396eb:0x8bb27bdbab0cd261!8m2!3d53.0636825!4d-0.9644325 [Accessed 05 October 2022]

Previous studies have indicated that hedgehog movement patterns are influenced by: the presence of buildings (Yarnell et al., 2014; Williams et al., 2018), likely driven by their attraction to anthropogenic supplementary feeding and/or a suggested lower risk of predation by badgers (Hubert et al., 2011; Pettett et al., 2017); edge habitats (Hof et al., 2012); and the presence of badgers (Hof and Bright 2010; Pettett et al., 2017). In addition, land use type and soil texture can influence the abundance of invertebrates such as earthworms, an important food resource, in turn potentially affecting the foraging behaviour of hedgehogs and/or badgers (Reeve 1994; Hof and Bright 2010; Pettett et al., 2017; Yarnell and Pettett 2020). To account for these relationships, the following variables were used as covariates in density modelling: BUILDING (euclidean distance from the centre of each grid cell to the nearest building); EDGE (the total length of all edge habitats [e.g. hedgerows, woodland edges, fences, etc.] in each grid cell); BADGER (euclidean distance from the centre of each grid cell to the nearest badger sett); SOIL (identified on site based on the permeability of topsoil and

subsoil; low permeability [type 0], slowly permeable reddish clay, mainly Worcester, passing to a blocky Mudstone; and high permeability [type 1], moderately permeable loams or silts overlaying slowly permeable reddish clay, mainly Whimple, Hopsford, and Mathon, passing to slightly permeable Dolomite [Ambrose et al., 2005]); and LANDUSE type (amenity, arable, or pasture categories) (Table 2.1). Data on buildings (all buildings with roofs) and edge habitats were extracted from OS MasterMap Topography Layers and high-resolution (25 m) Vertical Aerial Imagery (https://digimap.edina.ac.uk/; EDINA Digimap Ordnance Survey Service, 2022). Land use data were extracted from the 2021 UKCEH Land Cover Maps (Marston et al., 2022) and validated based on field observations. All habitat covariates were extracted based on 50 x 50 m grid cells across the state space (Figure 2.1; Figure 2.3). Spatial analyses were conducted using ArcGIS (ESRI 2015) and R (v4.2.2; R Core Team 2023) packages *sf* (Pebesma 2018; Pebesma and Bivand 2023), *tmap* (Tennekes, 2018) and *terra* (Hijmans et al*.,* 2023).

Table 2.1. Description of the spatial capture-recapture model parameters and covariates included in the modelling. Where p_0, is the baseline encounter probability; σ, sigma, is the movement parameter; and D, is hedgehog density.

Parameter	Notation	Description
p_0	SEX	Sex (categorical: female or male).
σ	SEX	Sex (categorical: female or male).
D	LANDUSE	Land use (categorical: 0, arable; 1, amenity; 2, pasture). The composition of land use types varied spatially but was constant across years.
	SOIL	Soil types (categorical: type 0, low permeability, slowly permeable reddish clay (mainly Worcester) overlaying a blocky Mudstone, and type 1, high permeability, moderately permeable loams or silts overlaying reddish clay (mainly Whimple, Hopsford, and Mathon), then passing to slightly permeable Dolomite. Soil composition varied spatially but was constant across years.
	BUILDING	Euclidean distance from the centre of the 50 x 50 m grid cell to the nearest building (continuous and standardised by z-score). This covariate was constant across years.
	BADGER	Euclidean distance from centre of the grid cell to the nearest badger sett (continuous and standardised by z-score). This covariate varied spatiotemporally as a new badger sett was created in 2015.
	EDGE	Edge density, refers to the total length of all types of edges within each grid cell (continuous and standardised by z-score). This covariate was constant across years.
	SESSION	Search year (categorical).

2.2.2 SCR modelling

Density was estimated using multi-session SCR models in the *oSCR* package (Sutherland, Royle and Linden, 2019) in R (R Core Team, 2023) with each hedgehog survey year defined as a SESSION (Table 2.1) and each search night (usually dusk to midnight) defined as an occasion. If an individual was captured more than once on a given occasion, only the first capture location was utilised. 50 x 50 m grid cells were regarded as effective traps (Schaus et al., 2020), such that survey effort was taken as the number of times each grid cell was surveyed in each session. Survey effort was incorporated in the density modelling process. The total state space was also based on a 50 x 50 m pixel grid (Figure 2.1; Figure 2.3) covering a total area of 3.70 km^2, which included the search area (2.07 km^2) (Figure 2.1; *sensu* Fuller et al., 2016; Morin et al., 2017). This is assumed to be large enough to contain probable animal locations (Efford et al., 2004) based on the approximate home range size of male hedgehogs (0.22 km^2: Pettett et al., 2017).

The baseline encounter probability (p_0) was the probability that an individual is detected at its activity centre (home-range centre). The movement parameter sigma (sig, σ) describes the distance over which the animal is likely to be detected. Both p_0 and σ were modelled as a function of sex (p_0~sex, σ~sex) as male hedgehogs often move larger distances than females (Reeve, 1994; Pettett et al., 2017). Before modelling the density, continuous covariates were standardized by converting to z-scores (Donovan and Hines, 2007). Correlation among predictor variables was checked with the cor() function in R, with the Pearson Correlation Index > 0.7 indicating considerable correlation; habitat covariates were not strongly correlated (< 0.4). Density was first modelled with the assumption of habitat uniformity (D~1) and then by incorportating the heterogeneity of the following covariates: land use type (LANDUSE: 0 [arable], 1 [amenity], 2 [pasture]); soil type (SOIL: type 0 [low permeability], type 1 [high permeability]); euclidean distance to the nearest building (BUILDING) and badger sett (BADGER); and density of edge habitats (EDGE). SESSION was also included to infer population trends across years.

To avoid having to test too many models, we conducted a hierarchical selection, based on Akaike information criterion values (AIC; Burnham and Anderson, 2002). We fitted the detection and movement models first and, using the most supported detection and movement

model, fitted the density models (as per Kervellec et al., 2023). We tested habitat effects on constant density models based on all combinations of covariates but limited the maximum number of covariates to three, as models with ≥ 4 covariates failed to converge. Temporal effects on density were modelled with SESSION as a covariate only and compared to the null density model. As the session-specific model outperformed the null density model, SESSION was added to the best-supported constant model that included other covariates for their additive effects (*sensu* Fondell, 2008). SESSION was initially run in chronological order (with 2009 as the intercept) for model fitting and for testing whether annual density estimates varied through time; density calculated based on the top-ranking model was presented unless otherwise specified. We then identified that the last SESSION (year 2022) had one of the lowest densities, and we therefore reordered SESSION so that the reference SESSION (intercept) for annual significance testing was year 2022.

We modelled both the realised density (indicating a single realisation of the number of individual activity centers per pixel) and estimated density (the mean number of activity centers in each pixel based on maximum likelihoods (Morin et al., 2017; Royle et al., 2017). As BADGER composition varied in relation to the presence of different numbers of badger setts in 2009–2014 and 2015–2022 (Figure 2.1; Figure 2.3), we simultaneously integrated two sets of BADGER composition in the SCR framework (Sutherland et al., 2019), generating two sets of estimated density in our results, for two time periods. To compare with other studies, the total number of hedgehogs was divided by 3.70 km^2 to produce a mean estimated density per annum. The density-weighted gravity centre was inferred using the wt.centroid() function in the *spatialEco* package (Evans and Murphy, 2021). To show the spatial variation in estimated density, we used a 50 m moving window to quantify the total number of hedgehogs on any of the 1 x 1 km grids, using the focal() function in the *terra* package (Hijmans et al., 2023), which takes into account a central cell and its neighbours for continuous space, and applying an aggregation function to all cells within the specified neighbourhood (Hijmans et al., 2023). The density was scaled up for land use types and fields by using a sum() function in R to sum the value in each pixel included (Royle et al., 2017). Comparisons of estimated densities between LANDUSE types were assessed using Wilcoxon rank sum tests (Haynes 2013) as the data failed to meet assumptions for normality for hedgehog density on any of the land use types. The relationship between hedgehog density and habitat covariates was plotted using the package *ggplot2* with a linear model method (Wickham, 2016).

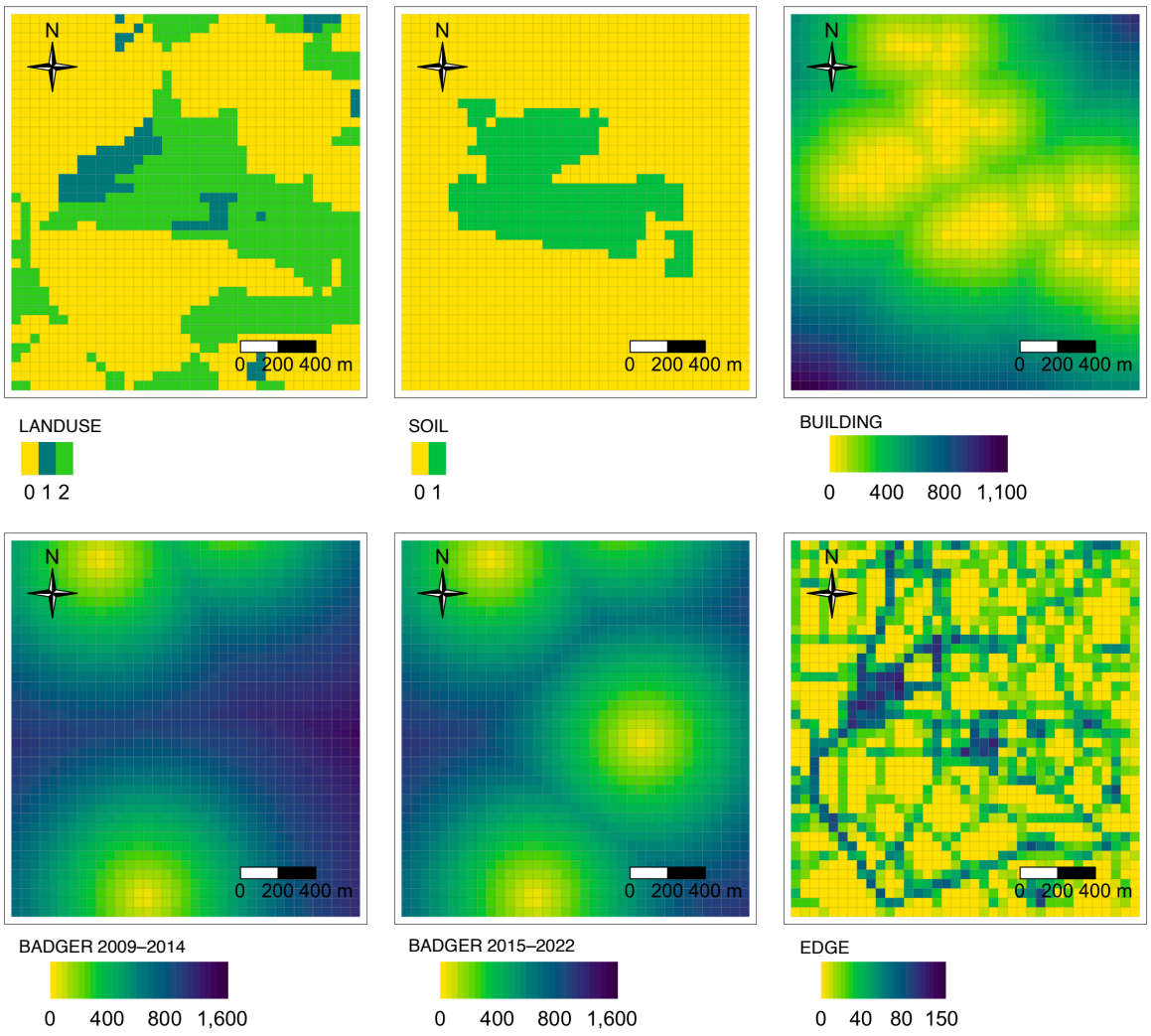

Figure 2.3. Map of habitat covariates used in hedgehog density modelling at Brackenhurst Campus, Southwell, England between 2009 and 2022. Site centroid coordinates 53.06 N, -0.96 W, EPSG:4326. Resolution: 50 x 50 m. Notations are summarised in Table 2.1. LANDUSE: arable (0), amenity (1), and pasture (2); SOIL: soil types, low permeability, slowly permeable reddish clay, mainly Worcester overlaying a blocky Mudstone (0); high permeability, moderately permeable loams or silts overlaying reddish clay, mainly Whimple, Hopsford, and Mathon, then passing to slightly permeable Dolomite (1). BUILDING: straight line distance (m) from the centre of each grid cell to the nearest building. BADGER: straight line distance (m) from the centre of each grid cell to the nearest badger sett for 2009–2014 and 2015–2022, respectively. EDGE: total length (m) of edge habitats within each grid cell.

2.3 Results

2.3.1 Summary of search effort and captures

Two spatial outlier captures were excluded as they rendered a high coefficient of variation in sigma which is known to affect density estimation (Kendall, 2019). Consequently, search effort consisted of 440 search nights over 11 years yielding 860 independent captures of 134 adults (77 female: 57 male), with mean number of captures per individual being 6 (95% CI 5–8). Detailed search effort and captures are summarised in Table 2.2.

Table 2.2. Summary of sampling effort for the spatial capture-recapture analysis of hedgehogs, Brackenhurst, UK, 2009–2022. Where, "No. traps" is the number of 50 x 50 m grid effective traps; "No. OS" is the number of occasions used in the Spatial Capture Recapture modelling; "No. trap OS" is the number of trap occasions; "No. unique ind (f:m)" is the number of unique individual hedgehogs in the session (female: male ratio); "No. cap" is the number of independent captures; "Average no. cap" is the average number of independent captures per captured hedgehog; and the "Average no. spatial cap" is the average number of independent spatial captures per captured hedgehog.

Session	State space (km^2)	Search area (km^2)	No. traps	No. OS	No. trap OS	No. unique ind (f:m)	No. cap	Average no. cap	Average no. spatial cap
2009	3.7	1.57	628	42	2582	16 (7:9)	48	3	2.69
2010	3.7	0.94	376	60	4806	21 (14:7)	89	4.24	3.81
2011	3.7	0.88	351	52	2569	21 (10:11)	79	3.76	3.62
2012	3.7	1.23	493	60	2591	31 (21:10)	167	5.39	4.77
2013	3.7	1.61	643	60	5221	26 (17:9)	181	6.96	5.46
2014	3.7	1.14	454	50	2705	22 (10:12)	127	5.77	5.18
2015	3.7	0.83	330	14	1639	16 (9:7)	33	2.19	2.12
2017	3.7	0.59	236	10	2206	6 (4:2)	15	2.5	2.17
2018	3.7	0.59	236	9	2284	8 (5:3)	17	2.12	2
2021	3.7	2.07	828	60	5280	11 (6:5)	67	6.09	4.91
2022	3.7	1.76	704	23	5974	8 (5:3)	35	4.38	3.62

2.3.2 Population density

For both baseline detection (p_0) and spatial scale parameter sigma (σ), models with SEX as a covariate were consistently supported over the null model, and all subsequent analyses were presented with SEX effects (p_0~SEX, σ~SEX). The sex-specific estimates of the movement scale parameter σ_{sex} were, σ_{female} = 118 m (95% CI 110–125), σ_{male} = 205 m (95% CI 183–227). Sex-specific estimates of the baseline encounter probability were, $p_{0female}$ = 0.014 (95% CI 0.012–0.016), p_{0male} = 0.005 (95% CI 0.004–0.006).

Among all constant density models (density assumed to be constant over time) that converged, the model with the lowest AIC value included an additive effect of SOIL type, distance to the nearest BADGER sett, and EDGE density (D~SOIL + BADGER + EDGE, p_0~SEX, σ~SEX). Estimated density based on the model was presented in Figure 2.4 and Table 2.3. There was a competing constant model (ΔAIC < 2) which included an additive effect of SOIL type, distance to the nearest BADGER sett, and distance to the nearest BUILDING (D~SOIL + BUILDING + BADGER , p_0~SEX, σ~SEX). Estimated hedgehog density per 50 x 50 m grid cell was significantly higher on SOIL type 1 (high permeability; β_{SOIL1} = 1.800 ± SE 0.437; p ≤ 0.001), with increasing distance from the nearest BADGER sett (β_{BADGER} = 0.479 ± SE 0.109; p ≤ 0.001), and with proximity to the nearest BUILDINGs (β_{BUILD} = -0.916 ± SE 0.462; p ≤ 0.05). LANDUSE did not appear in the top-ranking models, likely due to model convergence issues. However, when modelled as a single covariate (D~LANDUSE, p_0~SEX, σ~SEX), estimated density was significantly higher in amenity land, compared to pasture ($\beta_{Pasture}$ = -1.727 ± SE 0.294; p ≤ 0.001) and arable land (β_{Arable} = -2.242 ± SE 0.316; p ≤ 0.001).

Table 2.3. Mean estimated hedgehog density inferred from different density models over three time periods. Hedgehog density is presented as the number of hedgehogs per km^2 (95% confidence intervals) per year, across three time periods: 1) 2009 to 2014 which represents the time period before a new badger sett appeared (Density (2009–2014) Pre-Badger); 2) 2015 to 2022 which represents the time period after the new badger sett appeared (Density (2015–2022) Post-badger); and 3) the density estimate from the total time period 2009 to 2022 (Density 2009–2022). The densities from each time period were estimated from the four spatial capture-recapture models listed.

Density model	Density (2009–2014) Pre-Badger	Density (2015–2022) Post-badger	Density (2009–2022)
D~SESSION + SOIL + BADGER + EDGE, p_0~SEX, σ~SEX	8.52 (4.58–16.27)	3.76 (1.68–8.62)	6.36 (3.26–12.79)
D~SESSION, p_0~SEX, σ~SEX	14.95 (9.63–23.23)	6.658 (3.56–12.60)	11.18 (6.87–18.40)
D~SOIL + BADGER + EDGE, p_0~SEX, σ~SEX	6.08 (3.75–10.24)	5.90 (3.58–10.09)	5.99 (3.66–10.16)
D~1, p_0~SEX, σ~SEX	10.45 (8.43–12.96)	10.45 (8.43–12.96)	10.45 (8.43–12.96)

Based on the constant model with the lowest AIC value, the addition of SESSION further improved model fit. Thus, our top-ranking model was D~SESSION + SOIL + BADGER + EDGE, p_0~SEX, σ~SEX (Table 2.4). Across years, and in comparison to the intercept (SESSION 2022), all years between 2010 and 2015 had significantly higher hedgehog densities except 2009. There was no significant difference in hedgehog density in years 2017 to 2021 in comparison to 2022, which corresponds with years after which the additional badger sett appeared (Figure 2.4; Table 2.5). To illustrate the influence of an additional badger sett appearing in 2015, when the two BADGER time periods were summarised, the average annual estimated density decreased after the new badger sett appeared from a mean of 8.52 km^{-2} (95% CI 4.58–16.27) between 2009 to 2014 to 3.76 km^{-2} (95% CI 1.68–8.61) between 2015 to 2022 (Table 2.3).

Table 2.4. Overall summaries of the constant spatial capture-recapture models used for inferring trends in the hedgehog density, Brackenhurst, UK, 2009–2022. For each model, log-likelihoods (logL), number of parameters (K), delta AICc (ΔAICc), AICc weight (Weight), and cumulative AICc weights (CumWt) are presented. Density (D) was fixed (~1) or modelled as a function of soil condition (SOIL), distance to nearest badger sett (BADGER sett), total edge density on 50 x 50 m grid (EDGE), and distance to nearest building (BUILDING). Detection probability (p) and movement (sig) were modelled as a function of sex (~SEX). Covariate notations are included in Table 2.1. Only models that converged are included.

Model	logL	K	AICc	ΔAICc	Weight	CumWt
D(~SESSION + SOIL + BADGER + EDGE) p(~SEX) sig(~SEX)	5529.918	19	11097.84	0.000	1	1
D(~SOIL + BADGER + EDGE) p(~SEX) sig(~SEX)	5548.918	9	11115.84	18.001	0	1
D(~SOIL + BUILDING + BADGER) p(~SEX) sig(~SEX)	5549.178	9	11116.36	18.521	0	1
D(~SOIL + BADGER) p(~SEX) sig(~SEX)	5551.496	8	11118.99	21.156	0	1
D(~SOIL + EDGE) p(~SEX) sig(~SEX)	5558.39	8	11132.78	34.945	0	1
D(~SOIL + BUILDING) p(~SEX) sig(~SEX)	5559.934	8	11135.87	38.033	0	1
D(~SOIL) p(~SEX) sig(~SEX)	5561.239	7	11136.48	38.642	0	1
D(~EDGE + BADGER + BUILDING) p(~SEX) sig(~SEX)	5561.842	9	11141.68	43.849	0	1
D(~BADGER + BUILDING) p(~SEX) sig(~SEX)	5563.613	8	11143.23	45.390	0	1
D(~EDGE + BUILDING) p(~SEX) sig(~SEX)	5571.007	8	11158.01	60.179	0	1
D(~BUILDING) p(~SEX) sig(~SEX)	5574.175	7	11162.35	64.516	0	1
D(~EDGE + BADGER) p(~SEX) sig(~SEX)	5573.38	8	11162.76	64.925	0	1
D(~BADGER) p(~SEX) sig(~SEX)	5572.808	9	11163.62	65.781	0	1
D(~LANDUSE) p(~SEX) sig(~SEX)	5579.457	8	11174.91	77.079	0	1
D(~EDGE) p(~SEX) sig(~SEX)	5581.016	7	11176.03	78.196	0	1
D(~SESSION) p(~SEX) sig(~SEX)	5576.118	16	11184.24	86.401	0	1
D(~BADGER) p(~SEX) sig(~SEX)	5593.378	7	11200.76	102.921	0	1
D(~1) p(~SEX) sig(~SEX)	5603.192	6	11218.38	120.548	0	1
D(~1) p(~1) sig(~SEX)	5649.981	5	11309.96	212.128	0	1
D(~1) p(~1) sig(~1)	5655.590	4	11319.180	221.346	0	1

Table 2.5. Maximum likelihood estimates (MLE) and standard errors (SE) of the estimated parameters for model D∼SESSION + SOIL + BADGER + EDGE, p_0∼SEX, σ∼SEX, with SESSION 2022 as the reference year (intercept). In this model, D is the density of hedgehogs and considers an additive effect of the session, soil, distance to nearest badger sett, and edge density. The baseline detection probability p_0 and the scale parameter σ are both sex dependent. The sex ratio Ψ is for the probability of being a male. Values shown in bold indicate significant difference.

Parameter	Notation	Estimate	SE	*p*-value
p_0	Intercept: female	**-4.223**	**0.069**	**< 0.001**
	SEX: male	**-0.977**	**0.115**	**< 0.001**
σ	Intercept: female	**-2.142**	**0.032**	**< 0.001**
	SEX: male	**0.557**	**0.063**	**< 0.001**
D	Intercept: 2022	**-6.067**	**0.476**	**< 0.001**
	SESSION 2009	0.773	0.434	0.075
	SESSION 2010	**1.05**	**0.417**	**0.012**
	SESSION 2011	**1.062**	**0.418**	**0.011**
	SESSION 2012	**1.537**	**0.399**	**< 0.001**
	SESSION 2013	**1.136**	**0.405**	**0.005**
	SESSION 2014	**1.132**	**0.414**	**0.006**
	SESSION 2015	**0.972**	**0.435**	**0.025**
	SESSION 2017	-0.089	0.541	0.869
	SESSION 2018	0.22	0.501	0.661
	SESSION 2021	0.319	0.465	0.492
	SOIL1	**1.943**	**0.402**	**< 0.001**
	EDGE	**0.337**	**0.102**	**0.001**
	BADGER	**0.412**	**0.123**	**0.001**
Ψ	Male	**-0.396**	**0.152**	**0.009**

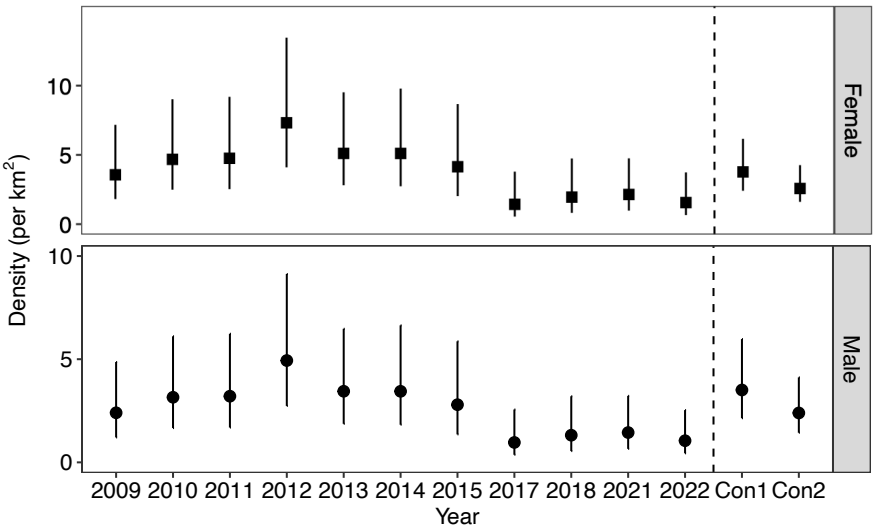

Figure 2.4. Estimated density (mean number of hedgehogs per km^2) of female (top) and male (bottom) adult hedgehogs at Brackenhurst Campus, England, between 2009 and 2022. Density estimates are derived from nocturnal spotlight surveys and spatial capture-recapture analysis. Estimates are provided for each year separately, with 2016, 2019, and 2020 excluded due to low sampling effort in these years. Average density estimates are also provided from two time periods relating to different BADGER sett locations during the study, with 'Con1' representing the presence of three BADGER setts between 2009 and 2014, and 'Con2' representing 2015 to 2022 when four BADGER setts were present. The annual session-specific densities (2009–2022) are derived from the top-ranking model: D~SESSION + SOIL + BADGER + EDGE, p_0~SEX, sig~SEX. For different BADGER sett location periods (Con1 and Con2), the estimates come from the model: D~SOIL + BADGER + EDGE, p_0~SEX, sig~SEX). Error bars correspond to 95% confidence intervals to the mean.

Across all years, mean estimated density from the top-ranking model (6.356 km^{-2} [95% CI 3.258–12.788]) was 43% lower than the model (11.180 km^{-2} [95% CI 6.874–18.396]) that assumed landscape uniformity and no predator covariates (D~SESSION, p_0~sex, sig~sex) (Table 2.3). The results show that when covariates were not included in the density models, the overall hedgehog density was inflated 1.5 times. Therefore, incorporating spatiotemporal covariate heterogeneity into the SCR modelling process demonstrated that hedgehog density is substantially spatially structured. Indeed, hedgehog density ranged 0.39–13.54 per km^2 based on 50 m moving windows, showing the high variation in spatial density.

Based on the top-ranking model, estimated hedgehog density per 50 x 50 m grid cell was significantly higher on land with SOIL type 1 (high permeability; β_{SOIL1} = 1.939 ± SE 0.4; 0.060 per 50 m grid cell: 95% CI 0.059–0.061) than that on SOIL type 0 (low permeability; 0.007 per 50 m grid cell: 95% CI 0.006–0.007), with the former being 9 times higher as the latter. Estimated hedgehog density per 50 x 50 m grid cell was also significantly higher with increasing distance from the nearest badger sett (β_{BADGER} = 0.412 ± SE 0.123; $p \leq 0.001$), and with increasing edge density (β_{EDGE} = 0.321 ± SE 0.102; $p \leq 0.05$) (Figure 2.5).

Estimated hedgehog density derived from the top-ranking model was summersied based on land use characteristics, showing estimated hedgehog density was significantly higher in amenity land (0.034 per 50 m grid cell: 95% CI 0.012–0.055), compared to pasture (0.012 per 50 m grid cell: 95% CI 0.004–0.019; Wilcoxon rank sum exact test, $p \leq 0.001$), which was significantly higher than arable land (0.004 per 50 m grid cell: 95% CI 0.001–0.007; Wilcoxon rank sum exact test, $p \leq 0.001$; Figure 2.6), with amenity: pasture: arable ratios approximating 9: 3: 1. The results highlight the low densities on the arable land use (Figure 2.8; Figure 2.9).

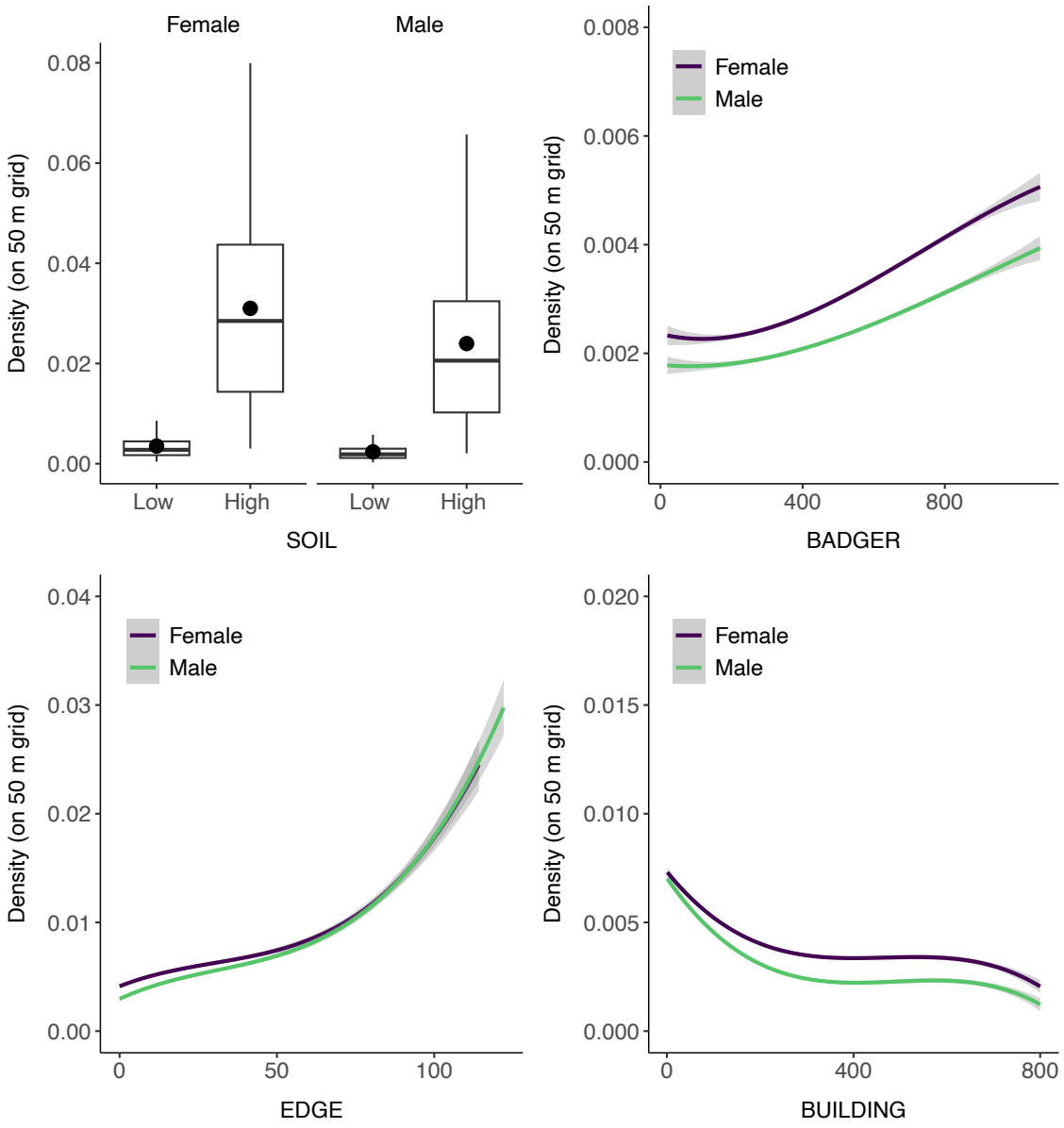

Figure 2.5. Estimated female and male adult hedgehog density at Brackenhurst Campus, England, in relation to SOIL type, BADGER sett location, proximity to BUILDINGs and EDGE density. Hedgehog density is presented as the number of hedgehogs per 50 x 50 m grid cell per year (females and males shown separately). Boxplots (median, 25% and 75% quartiles, 95% confidence interval, and mean [black point]) of estimated hedgehog density values on SOIL type (Low: low permeability; High: high permeability; Table 2.1) and plots of mean estimated hedgehog density values on distance (m) to the nearest BADGER sett, of EDGES (m) within each 50 x 50 m grid cell, and distance (m) to the nearest BUILDING (grey shaded areas indicate 95% confidence intervals to the mean). The output is derived from model: D~ SESSION + SOIL + BADGER + EDGE, p_0~SEX, σ~SEX.

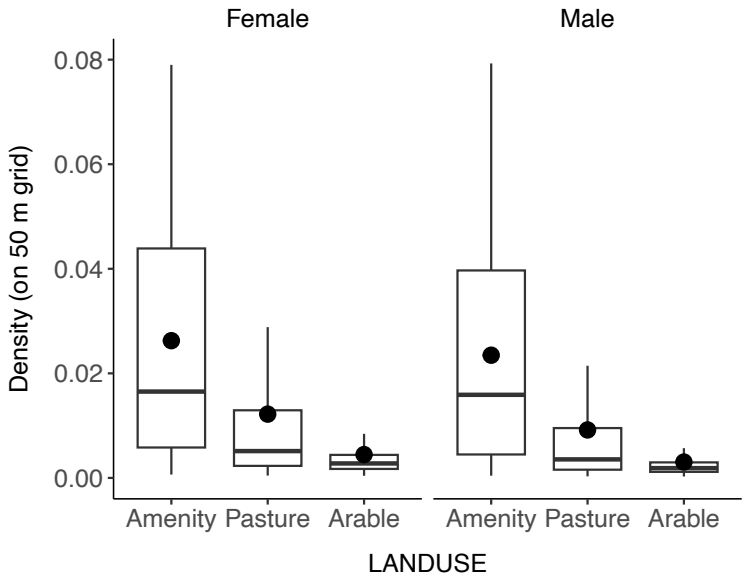

Figure 2.6. Boxplots (median, 25% and 75% quartiles, 95% confidence interval, and mean [black point]) of estimated hedgehog density in relation to land use characteristics at Brackenurst Campus, England, between 2009 and 2022. Hedgehog density is presented as the number of hedgehogs per 50 x 50 m grid cell per year (females and males shown separately). The output is derived from model: D∼SESSION + SOIL + BADGER + EDGE, p_0∼SEX, σ∼SEX.

To further illustrate the influence of spatiotemporal covariates on hedgehog density, the realised hedgehog density was plotted. The hedgehog population density-weighted gravity centre shifted *approx.* 300 m from where the new badger sett was located, to areas with more buildings to the northwest (Figure 2.7). For all the surrounding arable land, hedgehog densities were consistently low across all SESSIONS (Figure 2.8; Figure 2.9).

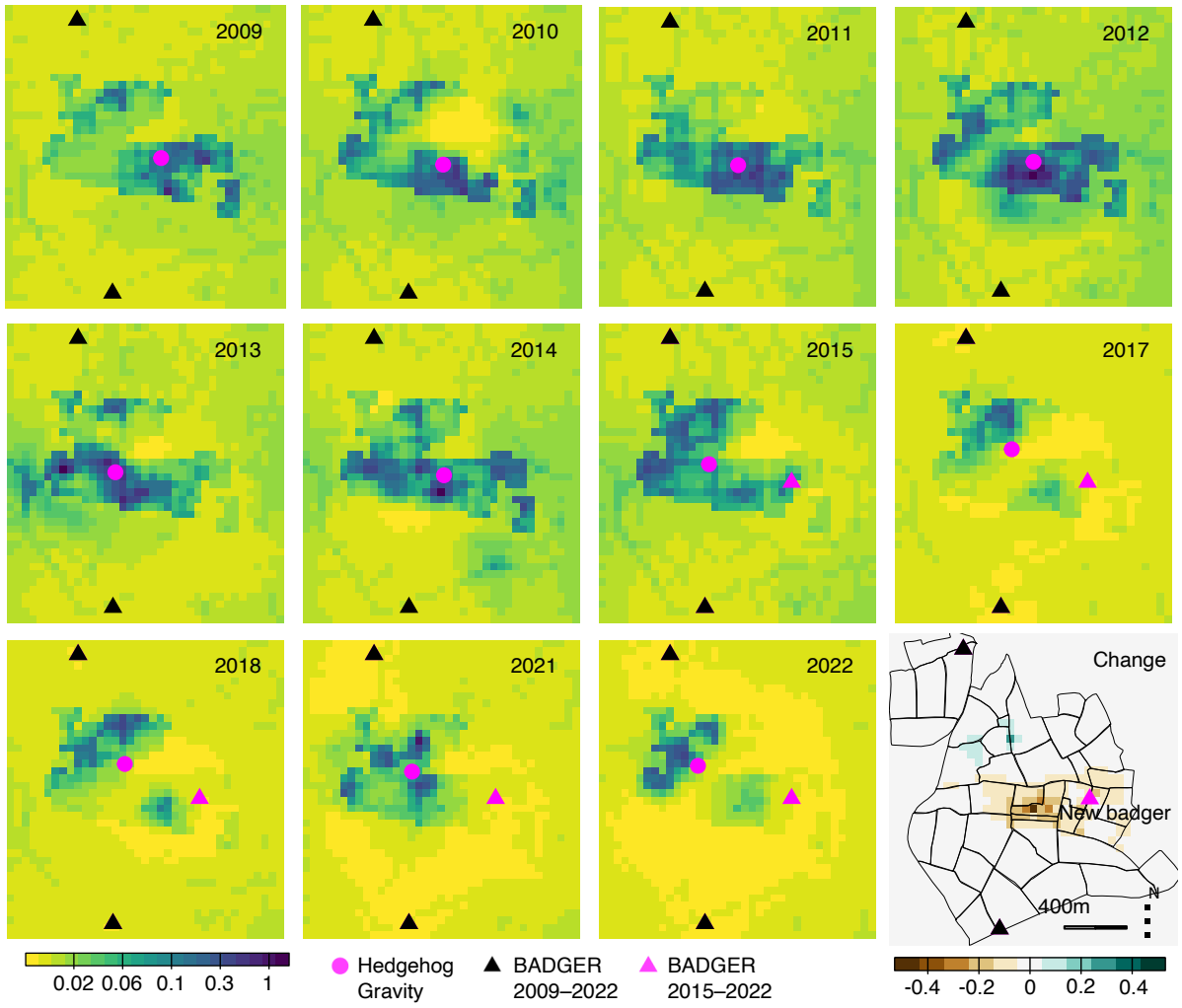

Figure 2.7. Realised density of adult hedgehogs at Brackenhurst Campus, England, between 2009 and 2022. Site centroid coordinates 53.06 N, -0.96 W, EPSG:4326. Density estimates are derived from nocturnal spotlight surveys and spatial capture-recapture analysis. Hedgehog density is presented as the number of hedgehogs per 50 x 50 m grid cell per year (females and males combined). Pink point represents the hedgehog density-weighted gravity centre (Hedgehog Gravity) of the year. Triangles show the location of badger setts, with black triangles representing the setts that were active for the entire study period (2009–2022) and the pink triangle representing the additional badger sett that appeared in 2015 and remained active beyond 2022. Spatial density estimates of hedgehogs are provided for each year separately, with yellow to dark purple indicating density from low to high (with 2016, 2019 and 2020 excluded due to low sampling effort in these years). The density-weighted gravity center shifted approximately 300 m from southeast (i.e., from where the new badger sett was located) to northwest. Spatial changes in density estimates between two time periods relating to different BADGER sett locations during the study are provided in the last panel

(Change), with orange indicating where hedgehog density delined, to white indicating no changes in density, and then to blue indicating hedgehog density increasing, showing spatial changes in hedgehog density in response to the additional badger sett. Estimates are derived from the model: D∼SESSION + SOIL + BADGER + EDGE, p_0∼SEX, σ∼SEX.

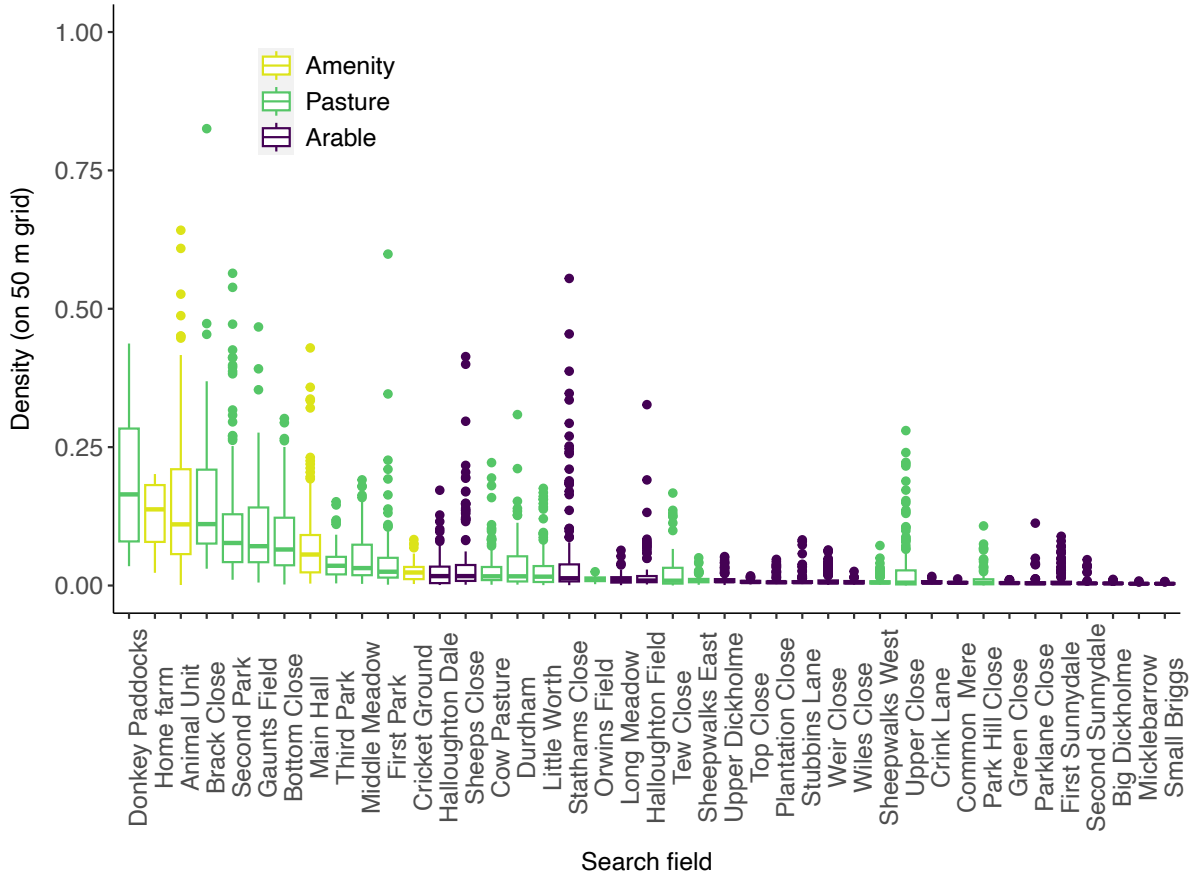

Figure 2.8. Boxplots (median, 25% and 75% quartiles, and 95% confidence interval) of estimated hedgehog density in relation to land use fields at Brackenhurst Campus, England, between 2009 and 2014, before the new badger sett appeared. Site centroid coordinates: 53.06 N, -0.96 W (EPSG:4326). Hedgehog density is presented as the number of hedgehogs per 50 x 50 m grid cell per year (females and males combined). The output is derived from model: D~SESSION + SOIL + BADGER + EDGE, p_0~SEX, σ~SEX.

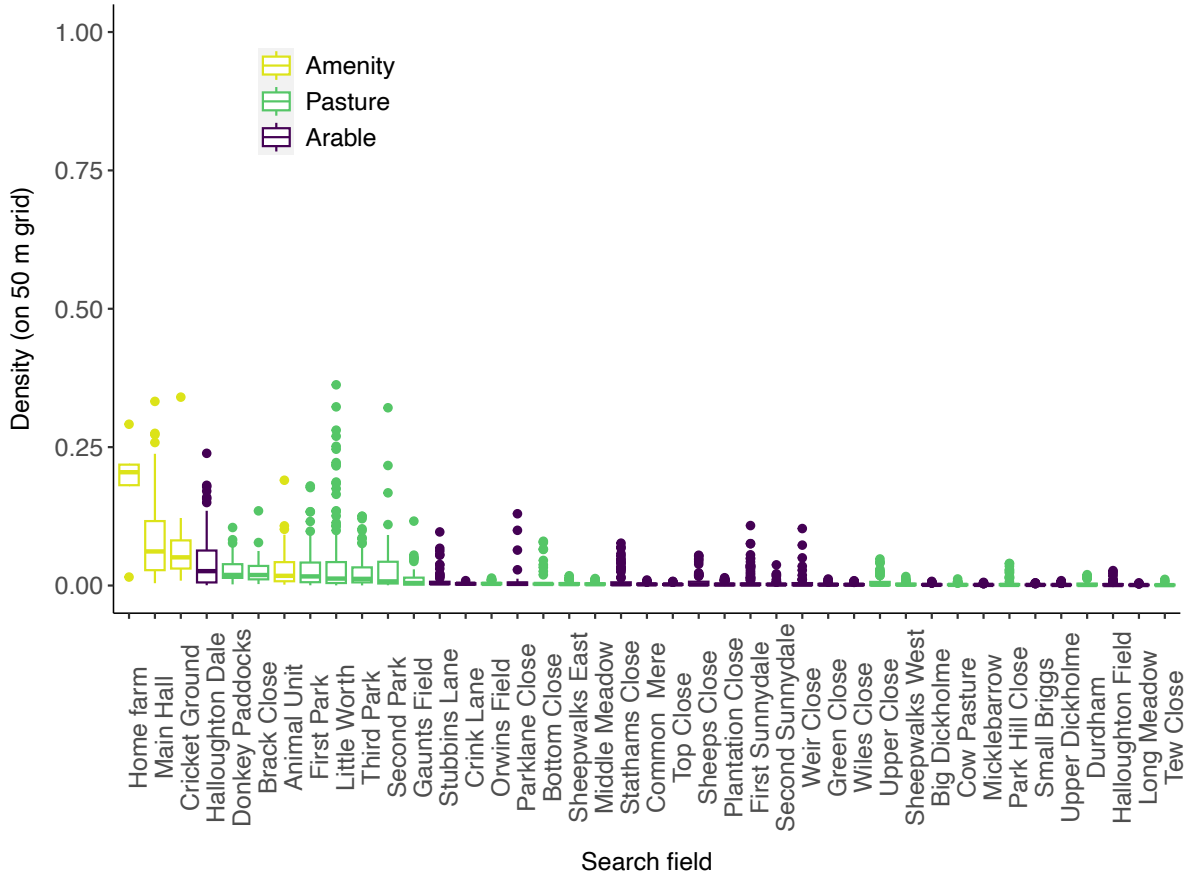

Figure 2.9. Boxplots (median, 25% and 75% quartiles, and 95% confidence interval) of estimated hedgehog density in relation to land use fields at Brackenhurst Campus, England, between 2015 and 2022, since the new badger sett appeared. Site centroid coordinates: 53.06 N, -0.96 W (EPSG:4326). Hedgehog density is presented as the number of hedgehogs per 50 x 50 m grid cell per year (females and males combined). Compared to Figure 2.8, densities in Brack Close, Bottom Close, Middle Meadow, Cow Pasture, and Second Park decreased from previously high values to near zero, potentially due to effects of increased badgers (indicated as a new badger sett appeared in 2015 on Bottom Close); densities in Donkey paddocks, Gaunts field, and Little worth were largely retained after the new badger sett appeared, likely due to the high-density edges and buildings (Figure 2.1; Figure 2.2) in these areas providing shelter for the hedgehogs. Hedgehog densities were consistently low across years in Upper Close, Park Hill Close, Tew Close, Orwins Field, Sheepwalks West, and Sheepwalks East, suggesting effects of other factors rather than badgers, e.g., soil quality and cropping. The output is derived from model: D~SESSION + SOIL + BADGER + EDGE, p_0~SEX, σ~SEX.

2.4 Discussion

Based on an 11-year SCR hedgehog dataset, our study represents the longest SCR density modelling of a mammal in an agroecosystem, and a rare example of long-term monitoring of a hedgehog population (but see Kristiansson 1990). By simultaneously integrating both spatially and temporally varied covariates into one SCR framework, we illustrate how spatially structured population densities can be greatly overestimated (by 43%) if habitat uniformity is assumed. The long-term monitoring also allowed identification of a decline in population density related to an increase in predator presence, while also being linked to habitat characteristics.

Previous applications of SCR have typically been based on habitat uniformity, with a small number of recent studies integrating spatial heterogeneity, which assumes the individual's distribution is shaped by habitat and site-specific characteristics (e.g., Fuller et al., 2016). Direct comparisons of SCR density values between those based on habitat uniformity versus assumptions of heterogeneity are scarce, and have contrasting findings. Efford and Fewster (2013) and Gerber and Parmenter (2014) showed no considerable difference between density estimates from both assumptions. By contrast, the density of American mink (*Neovison vison*) was found to be 1.9 times higher when distance to nearest city was integrated into density modelling (Fuller et al., 2016). Where habitats affect the within-population individuals' distribution, population density estimates can be dramatically biased if such effects are not accounted for, as demonstrated here. Therefore, the most appropriate assumption may depend on the spatial extent of the sampling and the range of habitats occupied by the target population. This will be especially important for species with specific habitat preferences at small spatial scales, and this might also explain why other studies did not find such differences (Efford and Fewster, 2013; Gerber and Parmenter, 2014).

As one of the few studies to tackle both spatially and temporally varying covariates in the density modelling framework, our study highlights the utilisation of SCR as a framework for population monitoring studies (Sutherland et al., 2019). This is particularly important for the assessment of population size trends in space and time. In our example, without taking into account habitat heterogeneity, we would have almost doubled our estimated number of animals occurring across the state space, and would have been unable to link certain habitats with hedgehog density, which would lead to misleading assumptions about the population

state of this declining species. We therefore recommend that future SCR studies incorporate habitat heterogeneity into the modelling process to provide greater inference into variation of density across landscapes, and provide more accurate densities from which management decisions can be based and evidenced.

Our findings confirmed predictions that hedgehog density is substantially spatially structured at a local scale and linked to previously reported habitat and land use associations (Yarnell et al., 2014; Williams et al., 2018; Lee et al., 2025). Hedgehog density was lowest in arable fields (Pettett et al., 2017), and highest in amenity, with pasture fields being intermediate (Parrott, Etherington and Dendy, 2014). The higher density on amenity grassland is likely associated with the close proximity of buildings which was also demonstrated here and supports previous studies showing hedgehog density is typically higher in urban environments (Hubert et al., 2011; Schaus et al., 2020). The low density estimates associated with arable fields add to a growing evidence base that suggests these are unsuitable for hedgehogs (Pettett et al., 2017; Lee et al., 2025). These habitat associations are likely driven by varying food and shelter resources in each habitat, with intensive arable farming leading to lower macroinvertebrate abundance caused by soil compaction, homogenisation of landscapes, and use of macroinvertebrate pesticides.

Indeed, our study demonstrates that overall hedgehog density is positively related to edge density. Edge habitats, such as hedgerows, have previously been identified as being important for hedgehogs (Rodriguez Recio et al., 2013) in facilitating movements (Hof et al., 2012), providing nest sites (Bearman-Brown et al., 2020), a refuge from predators, and food resources (Hof et al., 2012; Pettett et al., 2017). The edge effect supports the view that homogenisation of habitat is detrimental for hedgehog populations, and that habitat complexity and resulting high edge densities may help improve connectivity in the landscape and be beneficial. We recommend that areas of arable land should aim to maintain hedgerows and edge habitats to increase habitat suitability for hedgehogs at the local scale.

A novel finding from this study is the positive association of hedgehog density and the area with high soil permeability. The suggestion that soil permeability may influence the distribution of hedgehogs was raised by Jackson (2007), where the density of island translocated hedgehogs was twice as high on more permeable sandy-soiled machair versus peaty-soiled blacklands. Unfortunately, in both our study and Jackson's (2007), the location

of more permeable soil was confounded by overlapping amenity and pasture fields. By contrast, less permeable soils, where hedgehog densities were lower in the present study, were largely associated with arable fields, which are used infrequently by hedgehogs (Pettett et al., 2017). Disentangling and determining whether soil or land use influences hedgehog density is worthy of further research. However, the observed correlation between the hedgehog density and soil raises the possibility that the habitat effects on hedgehogs may be beyond the contemporary land cover or land use, and may also be related to historical land use or geology. Hedgehog density estimates are needed on more sites that quantify soil types, to better understand the mechanisms underlying the observed relationship between soil types and hedgehog density.

The estimated hedgehog density was negatively related to distance to the nearest buildings, as has been documented in previous studies (Yarnell et al., 2014; Williams et al., 2018). The higher hedgehog densities found in built-up areas or urban areas are often suggested to be driven by hedgehog's attraction to anthropogenic supplementary feeding (Hubert et al., 2011; Pettett et al., 2017) and lower risk of predation by badgers (Hubert et al., 2011; Pettett et al., 2017), but the two effects are often difficult to disentangle (Lee et al., 2025). Interestingly, our study area lacked intentional supplementary food, suggesting that the hedgehog's association with buildings could be due to the landscape of fear response of hedgehogs to badgers (Young et al., 2006). However, we were unable to quantify the variability of natural food availability across the state space, which may have helped explain some of the spatial patterns observed in this hedgehog population (Hof, Snellenberg and Bright 2012).

Our findings provide further evidence of spatial segregation between hedgehogs and badgers (e.g., Young et al., 2006; Pettett et al., 2017; Williams et al., 2018; Turner et al., 2022; Lee et al., 2025). Previous studies have shown the negative correlation between both species and typically explained the relationship as being a result of badgers exerting a negative influence via competition and/or predation. However, the alternative hypothesis of differential species-specific habitat selection has received little support or consideration (Lee et al., 2025). Furthermore, none of the correlation studies were able to demonstrate a population response of hedgehogs to badgers over time as they provided a snapshot in time of the spatial distribution of each species. Trewby et al. (2014) were the first to survey hedgehog populations over a period of six years in relation to reductions in badger abundance due to

culling for disease management. They showed that hedgehog indices of relative abundance increased in areas where badger abundance was expected to have decreased, and relative to control areas where badger abundance was assumed constant. However, there is some uncertainty in how the relative indices of abundance accurately reflect population density or varying activity patterns (see Hayward et al., 2015).

Our study is the first to show a hedgehog population response to increasing badger setts (a proxy for abundance [Judge et al., 2017]). Hedgehogs shifted their density-weighted centre, away from the badger sett after it appeared in the middle of the study area. Prior to that, the density-weigthed centre was similar across years. We were unable to determine the mechanism driving this observed pattern. Plausible non-exclusive explanations include: 1) direct predation of hedgehogs in the immediate vicinity of the new badger sett removing individuals, 2) reduced activity near the sett in response to a perception of increased predation risk (landscape of fear); and or 3) increased competition for shared food resources causing hedgehogs to shift their foraging to areas away from the competing badgers (Lee et al., 2025). Some hedgehog predation was recorded during the study, but only one hedgehog carcass was found after 2015 with the characteristic signs of badger predation, namely a hollowed out skin. These rates of identified predation in the study were similar before the appearance of the new badger sett (unpublished data). Therefore, although a potentially contributory factor, it is more likely that the shift was caused by a landscape of fear and/or due to competition for shared food resources. Furthermore, the addition of a badger sett did not lead to overall extinction of the population, but rather a drop and subsequent stabilisation of density in response to the new predator spatial configuration in the landscape. This suggests that if badgers increase in an area, and there are refuges in the landscape that provide the resources for hedgehogs to access, hedgehogs can shift local habitat selection and remain in the wider area. However, the ability of hedgehog populations to respond to increasing badger abundance is likely to be affected not only by the habitat and resource availability in the area, but also by the magnitude of and spatial extent of the badger increase. Our results suggest that the appearance of badgers in previously occupied hedgehog habitat led to on average lower hedgehog densities overall, suggesting that badgers may have had a negative influence on hedgehog abundance at this site. Badger densities at the study site are relatively high for England (7 per km^2; Lee et al., 2025), but our results do suggest that the potential for badgers to influence hedgehog densities at various scales is possible (Williams et al., 2018).

2.5 Conclusion

This study demonstrates the value of long-term population datasets combined with habitat and predator covariates. Using this approach, we have shown that population density varies over small spatial scales and that prey population centres can shift in response to the presence of predators, supporting the landscape of fear hypothesis. Understanding how declining populations use habitat differentially at varying spatial scales can inform wildlife management, providing greater insight into how species preferentially use and move through landscapes. Furthermore, incorporating spatial habitat heterogeneity into the SCR modelling framework produced lower density estimates than models that assumed habitat uniformity. This is important because there is a risk of overestimating abundance of species of conservation concern by taking a landscape uniformity approach, which could lead to the incorrect conservation assessment of endangered species.

2.6 References

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Chapter 3: Population dynamics of rural hedgehogs estimated using long-term spatial capture-recapture

Abstract

Population dynamics among demographic groups of mammals sets the foundation for our understanding of conservation requirements. The hedgehog (*Erinaceus europaeus*) is suggested to be declining across much of its range, especially in Western Europe. So far, their population-level spatiotemporal density, growth rate, and survival are largely unknown. Based on an 11-year spatial capture-recapture (SCR) dataset collected in a typical agroecosystem in England, building on the previously reported adult density (Chapter 2), here we estimated the overall population density by adding the contemporary juvenile density to the adult density, age- and sex-specific growth rate and apparent survival. Juvenile and adult hedgehogs had similarly population trends in modelled densities (declined after the appearance of a new badger sett and then stabilised) and were subject to similar effects of habitats, i.e., being significantly positively related to soil permeability, edge density, distance from the nearest badger sett, and proximity to the nearest building, although juveniles were more associated with buildings. Modelled annual population density was 13.3 hedgehogs/km^2, with juvenile female: adult female: juvenile male: adult male ratios being 1.7: 1.6: 1.3: 1, and on amenity: pasture: arable approximating to 14: 4: 1. Modelled annual apparent survival rate, with emigration at least partially accounted for, was 0.530 (95% CI 0.423–0.635) for adult females and 0.426 (95% CI 0.308–0.552) for adult males. 15.63% (95% CI 0–31.32%) of males were recaptured after their second year April, lower than that in females (28.21%, 95% CI 10.35–46.06%), likely supporting natal dispersal in males. A wide range of mortality causes were recorded, of which the majority (52%) were human-related. However, as 86% of the marked hedgehogs disappeared for unknown reasons and with death undetected, the main driver of hedgehog mortality may be unknown, illustrating the complexity of inferring mortality rates and causes of mortality to population dynamics and highlighting the need for conservation management to consider not mortality, but rather identification of drivers of population changes is needed through long-term studies.

Keywords: spatial capture-recapture, spatiotemporal density, survival, population growth rate, agroecosystem, *Erinaceus europaeus*

3.1 Introduction

Population dynamics among demographic groups of mammals sets the foundation for our understanding of conservation requirements (Keith et al., 2015). Population dynamics in mammals is often shaped by complex interactions between the animal's intrinsic factors, e.g., life stages, sexes, and density, and the changing environmental factors, e.g., seasonal resource availability, local weather conditions, and interspecific competition (Camaclang et al., 2015; Combe et al., 2022). For many species, accurate estimates of population parameters are often lacking and, when available, often derived from mixed groups or adults only, despite estimates of juveniles being integral for understanding population dynamics (Camaclang et al., 2015). Further, juveniles are considered to have different habitat preferences compared to adults due to their different tolerance to environmental pressures (Gaillard et al., 2000), and foraging and ranging behaviours (Nie, Liu and Chen, 2022; Gravel, Lai and Berteaux, 2023), thus an understanding of their population patterns is essential for identifying environmental factors limiting population growth and setting appropriate conservation strategies (Combe et al., 2022).

So far, the only empirical long-term study investigating hedgehog population dynamics (density, growth rate, and survival) was carried out in a village in Sweden, Northern Europe, based on capture-mark-recapture, in which the annual density was found relatively stable and the apparent survival fluctuated (Kristiansson, 1990). However, due to the different climates and land use histories between Northern and Western Europe, these results in Swedish hedgehogs might not represent population patterns in Western Europe where hedgehog is declining in both range and abundance (Roos, Johnston and Noble, 2012; Taucher et al., 2020).

Based on an 11-year spatial capture-recapture (SCR) dataset collected in a typical agroecosystem in England, building on the previously reported adult (those that had survived their first winter) density (Chapter 2), here we present contemporary juvenile (young born in the year) density and population-level overall density, sex and age structure, growth rates, and survival, to provide the first long-term SCR-based population dynamics of hedgehogs.

The results from this study can provide a baseline for future research and conservation of the hedgehog.

3.2 Methods

3.2.1 Study site and data collection

The study site and data collection methods in this study are the same as those in Chapter 2.

3.2.2 Juvenile density

The SCR density modelling methods (close SCR models in the oSCR package; Sutherland, Royle, and Linden, 2019) used in this study for juvenile hedgehogs are consistent with that used for the contemporary adult hedgehogs of the same population in Chapter 2, with the exception that, in this study, the sampling occasions for each year were truncated from the date when the first juvenile was captured in the year, which was mainly in June, so that the detection rates of juveniles would not be underestimated by including occasions before they emerged. Further, covariate "LANDUSE" (land use types including Arable, Amenity, and Pasture) was not included in juvenile density modelling, due to the related convergence issues found in adult density modelling in Chapter 2. The description of the SCR model parameters and covariates included in the juvenile density modelling is shown in Table 3.1. Models with convergence issues (e.g., CV (coefficient of variance, equals SE/estimate) is close or higher than the estimate; Schmidt et al., 2022) were not presented.

Table 3.1. Description of the SCR model parameters and covariates included in the modelling of juvenile hedgehog density. Where p_0, is the baseline encounter probability; σ, sigma, movement parameter; D, hedgehog density. (Details are included in Table 2.1, Chapter 2)

Parameter	Covariate	Meaning
p_0	SEX	Sex (female, male)
σ	SEX	Sex
Density, D	SESSION	Hedgehog search year
	SOIL	0: low permeability; 1: high permeability
	BADGER	Distance to the nearest badger sett
	EDGE	Edge density, total length of all types of edges
	BUILD	Distance to the nearest building

3.2.3 Non-spatial and spatial CJS modelling survival

We used the R package openCR (Efford and Schofield, 2020) to fit spatial Cormack-Jolly-Seber (CJS) models for estimating survival, based on maximum likelihood. CJS models do not model the first capture of each animal. They condition on the first capture and model subsequent recapture probabilities and apparent survival (Efford and Schofield, 2020). Each survey year was defined as a session, and mostly each search night (usually from dusk to midnight) was defined as an occasion. Searches were based on fields (Figure 2.1 and Figure 2.2 in Chapter 2) with survey effort being measured by constructing a 50 x 50 m grid cell across the searched area and by recording how many searches were traversed in each grid cell. The habitat mask was also based on a 50 x 50 m grid cell across the study area (same as the "state space" in Figure 2.1). Each individual was assigned to an activity centre in each session (home-range centre which describes the potential centre of an animal's movement activity in a session). Spatial CJS modelling assumed that individual activity centre was either static/fixed ("Spatial static"; dispersal not allowed) among sessions, or changed ("Spatial normal"; dispersal allowed). For the latter, the spatial scale parameter describing activity centre relocation was denoted as α, modelled as a random walk with bivariate normal distribution (BVN) of step length. Detection probability (λ_0) was modelled as a function of the distance between individual activity centre and trap. Within-session individual movement parameter was denoted as sigma (σ), indicating to which spatial extent the detection probability of the animal within the session is close to zero, conditional on its activity centre in the session. The spatial scale detection parameter σ differs from the activity centre movement scale parameter α, with the former related to home range within a session, and the latter related to distances that home-range centre relocated between sessions. We kept detection probability (λ_0) and scale parameter sigma (σ) constant across sessions which can boost sample sizes and generate more precise estimates as tested in our preliminary analysis. Survival was modelled either with covariate "Session" included (session-specific models, allowing survival rate to be changed by sessions) or not included (constant models, assuming survival rate to be constant across sessions). For years with missing data (due to insufficient survey effort), 2016, 2019–2020, survival rates were truncated for single-year inference by adding intervals between sessions in the modelling. The spatial CJS modelling was run separately for: female adults, male adults, female adults and juveniles combined, and male adults and juveniles combined. Models within each demographic group were then compared using an AIC-based model selection method (Burnham and Anderson, 2002) separately. In

addition to the spatial CJS models, we also fitted the non-spatial CJS models, using openCR (Efford and Schofield, 2020), based on similar methods but ignoring the spatial distribution of animals.

3.2.4 Known mortality

Dead hedgehogs were found and reported by anyone and were not restricted to being found during systematic surveys so that the data collection period could be extended. Cause-specific detection rates of dead hedgehogs were not accounted for, so that the patterns of detected mortality are not suitable for extrapolating. Dead hedgehogs collected were mapped using the R package sf (Pebesma, 2018; Pebesma and Bivand, 2023), with the presumed cause of mortality provided, mainly based on necropsy or direct inspections. For predation, for example, those individuals we found skinned were presumed to have been predated by badger, and those with punctures were presumed by fox, whereas those that were attacked on the head but not consumed might be by dog or other species (Doncaster et al., 1994; Reeve, 1994; Morris and Reeve, 2008).

3.3 Results

3.3.1 SCR modelling juvenile density

The dataset for SCR modelling of juvenile density includes 62 juvenile hedgehogs (38 female: 24 male) from 165 search occasions. The number of captures for each individual ranges from 1 to 10, including 20 individuals (15 female: 5 male) captured multiple times, 15 twice (6 female: 9 male), and 27 (17 female: 10 male) once (Table 3.2).

Table 3.2. Summary of sampling effort of the spatial capture-recapture of juvenile hedgehogs, Brackenhurst, UK, 2009–2022. Where, "No. traps" is the number of 50 x 50 m grid effective traps; "No. OS" is the number of occasions used in the Spatial Capture Recapture modelling; "No. trap OS" is the number of trap occasions; "No. unique ind (f:m)" is the number of unique individual hedgehogs in the session (female: male ratio); "No. cap" is the number of independent captures; "Average no. cap" is the average number of independent captures per captured hedgehog; and the "Average no. spatial cap" is the average number of independent spatial captures per captured hedgehog.

Session	State space (km^2)	Search area (km^2)	No. traps	No. OS	No. trap OS	No. unique ind (f:m)	No. cap	Average no. cap	Average no. spatial cap
2009	3.70	0.86	343	26	955	11 (6:5)	17	1.55	1.45
2010	3.70	0.47	187	21	856	6 (3:3)	21	3.50	3.00
2011	3.70	0.43	170	13	462	4 (4:0)	16	4.00	3.00
2012	3.70	0.57	227	19	964	6 (5:1)	10	1.67	1.67
2013	3.70	0.48	193	13	465	6 (2:4)	16	2.67	2.33
2014	3.70	0.47	186	13	482	3 (3:0)	8	2.67	1.67
2017	3.70	0.59	236	8	1735	3 (2:1)	7	2.33	2.00
2021	3.70	1.90	759	32	3057	9 (5:4)	16	1.78	1.78
2022	3.70	1.76	704	20	5584	14 (8:6)	33	2.36	2.21

Annual juvenile density

For both baseline detection (p_0) and within-session spatial scale parameter sigma (σ), models with "sex" as a covariate were consistently supported over the null model, and all subsequent analyses were presented with sex effects included (p_0~SEX, sig~SEX). The top-ranking constant density model included a significant negative effect of distance to the nearest building (D~BUILD, p_0~SEX, sig~SEX; Table 3.3; Table 3.4). Based on this model, sex-specific estimates of the baseline encounter probability are, $p_{0female}$ = 0.039 (95% CI 0.028–0.057), p_{0male} = 0.004 (95% CI 0.002–0.007). Sex-specific estimates of the movement scale parameter σ_{sex} are, σ_{female} = 54 m (95% CI 47–63 m), σ_{male} = 149 m (95% CI 117–189). Both p_0 and σ varied significantly between sexes (P < 0.001). When the covariate "Session" was added to the top-ranking constant model, D~SESSION + BUILD, p_0~SEX, sig~SEX, the model had a higher AIC value than the constant model and thus was less supported (the model was also with high CV values), suggesting a lack of significant changes in population trends, although a slight decline was observed in 2018, which likely coincides with the period after the appearance of the new badger sett (Figure 3.1).

Juvenile hedgehog density and habitat effects

Based on the top-ranking model (D~BUILD, p_0~SEX, sig~SEX), the estimated hedgehog density per 50 x 50 m grid cell was significantly negatively related to distance to the nearest building (β_{BUILD} = -5.286 ± SE 1.242; p < 0.001). The annual estimated number of juvenile hedgehogs across the 3.70 km^2 state space is 25 (23–27), including 14 (95% CI 13–15) females and 11 (95% CI 10–12) males, respectively. The density for juvenile females and males combined averaged 7 (95% CI 6–8) hedgehogs per km^2. The estimated sex ratio female: male was 1.3: 1. When using 50 m moving windows to quantify density on a continuous landscape, the scaled estimated number of juvenile hedgehogs on a 550 x 550 m grid ranged from 0 to 3, and on a 1 x 1 km grid ranged from 0 to 13 (females and males combined).

SOIL, BADGER, EDGE, and LANDUSE did not appear in the top-ranking model, likely due to model convergence issues when additive effects of covariates were considered, potentially due to the insufficient captures of juvenile hedgehogs for running complex models. However, when modelled as a single covariate seperately (D~SOIL; D~BADGER; D~EDGE; D~LANDUSE; with p_0~ SEX, σ~SEX for all), each covariate had significant effects on the estimated hedgehog density. Specifically, the estimated hedgehog density per 50 x 50 m grid cell was higher on land with soil of high permeability (type 1; β_{SOIL} = 2.946 ± SE 1.041; P < 0.05); increased with increasing distance to the nearest badger sett (β_{BADGER} = 0.383 ± SE 0.165; P < 0.05); increased with increasing edge density (β_{EDGE} = 0.908 ± SE 0.145; p < 0.001); lower in Pasture ($\beta_{Pasture}$ = -1.420 ± SE 0.395; p < 0.001) and Arable land (β_{Arable} = -3.331 ± SE 1.215; p < 0.05), compared to that in Amenity land. When calculated based on the top-ranking model (D~BUILD, p_0~SEX, sig~SEX), across search years, mean estimated density per 50 x 50 m grid on Amenity land was 0.088 (95% CI 0.077–0.099), Pasture 0.025 (95% CI 0.021–0.029), Arable 0.005 (95% CI 95% CI 0.004–0.006), with amenity: pasture: arable ratios approximating to 18: 5: 1, and female: male to 1.3: 1 (Figure 3.2).

Table 3.3. Overall summaries of the constant spatial capture-recapture models used for inferring trends in juvenile hedgehog density, Brackenhurst, UK, 2009–2022. Overall summaries of the constant spatial capture-recapture models used for inferring trends in the hedgehog density, Brackenhurst, UK, 2009–2022. For each model, log-likelihoods (logL), number of parameters (K), delta AICc (ΔAICc), AICc weight (Weight), and cumulative AICc weights (CumWt) are presented. Density (D) was fixed (~1) or modelled as a function of soil condition (SOIL), distance to nearest badger sett (BADGER sett), total edge density on 50 x 50 m grid (EDGE), and distance to nearest building (BUILDING). Detection probability (p) and movement (sig) were modelled as a function of sex (~SEX). Covariate notations are included in Table 3.1. Only models that converged are included.

model	logL	K	AICc	ΔAICc	Weight	CumWt
D(~BUILD) p(~SEX) sig(~SEX)	846	7	1705.13	0.00	1	1
D(~EDGE) p(~SEX) sig(~SEX)	854	7	1721.82	16.69	0	1
D(~SOIL) p(~SEX) sig(~SEX)	860	7	1733.21	28.08	0	1
D(~LANDUSE) p(~SEX) sig(~SEX)	861	8	1738.90	33.77	0	1
D(~BADGER) p(~SEX) sig(~SEX)	871	7	1755.42	50.29	0	1
D(~1) p(~SEX) sig(~SEX)	874	6	1759.08	53.96	0	1
D(~1) p(~SEX) sig(~1)	902	5	1814.62	109.49	0	1
D(~1) p(~1) sig(~1)	907	4	1822.17	117.05	0	1

Table 3.4. Maximum likelihood estimates (MLE) and standard errors (SE) of the estimated parameters for model D~BUILD, p_0~SEX, σ~SEX. In this model, D is the density of hedgehogs and considers an additive effect of the session, soil, distance to nearest badger sett, and edge density. The baseline detection probability p_0 and the scale parameter σ are both sex dependent. The sex ratio Ψ is for the probability of being a male. Values shown in bold indicate significant difference.

Parameter	Notation	Estimate	SE	P-value
p_0	Intercept: female	**-3.189**	**0.191**	**< 0.001**
	SEX: male	**-2.389**	**0.352**	**< 0.001**
σ	Intercept: female	**-2.911**	**0.077**	**< 0.001**
	SEX: male	**1.006**	**0.144**	**< 0.001**
D	Intercept	**-8.845**	**1.44**	**< 0.001**
	d.beta.BUILD	**-5.286**	**1.242**	**< 0.001**
Ψ	Pr(male)	-0.263	0.305	0.388

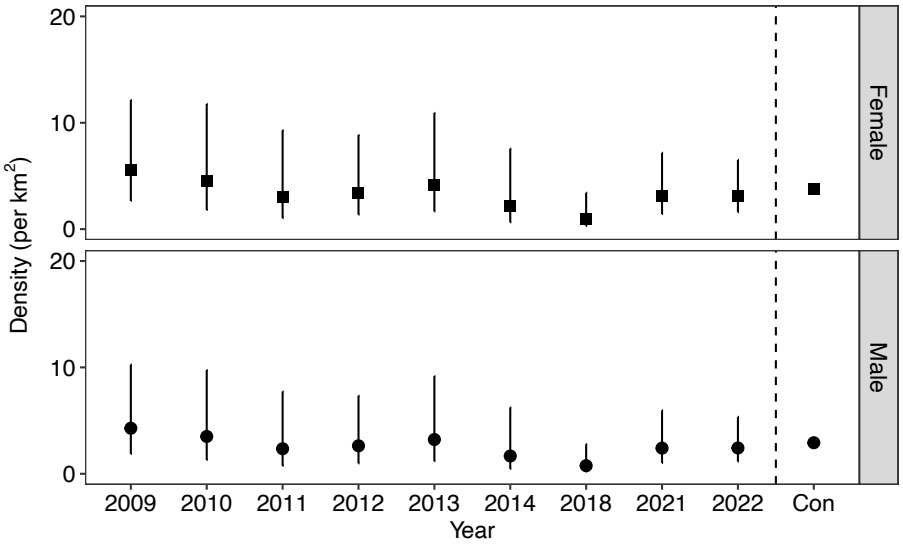

Figure 3.1. Annual estimated density of juvenile hedgehogs. Females and males are shown separately. Con: constant juvenile density, based on the constant density model: D~ BUILD, p_0~SEX, sig~SEX. Density for each year was based on the according session-specific density model: D~SESSION + BUILD, p_0~SEX, sig~SEX.

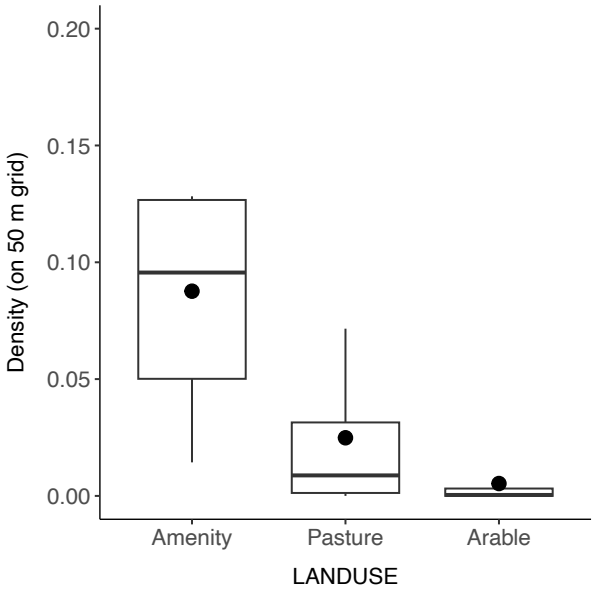

Figure 3.2. Boxplots (median, 25% and 75% quartiles, 95% confidence interval, and mean [black point]) of annual estimated density of juvenile hedgehogs based on land use characteristics. Hedgehog density is presented as the number of juvenile hedgehogs per 50 x 50 m grid cell per year (females and males were combined). (The output is derived from model: D~SESSION + BUILD, p_0~SEX, sig~SEX).

Annual realised juvenile density

To further illustrate the density-habitat relationships, the realised juvenile density for each year session and the mean across years are mapped (Figure 3.3) and plotted by fields (Figure 3.4), showing that juvenile hedgehogs were largely confined to amenity land, or other areas close to buildings, while the surrounding arable land and large areas of pasture fields consistently had low density across years. (Habitat composition is included in Figures 2.1–2.3 in Chapter 2).

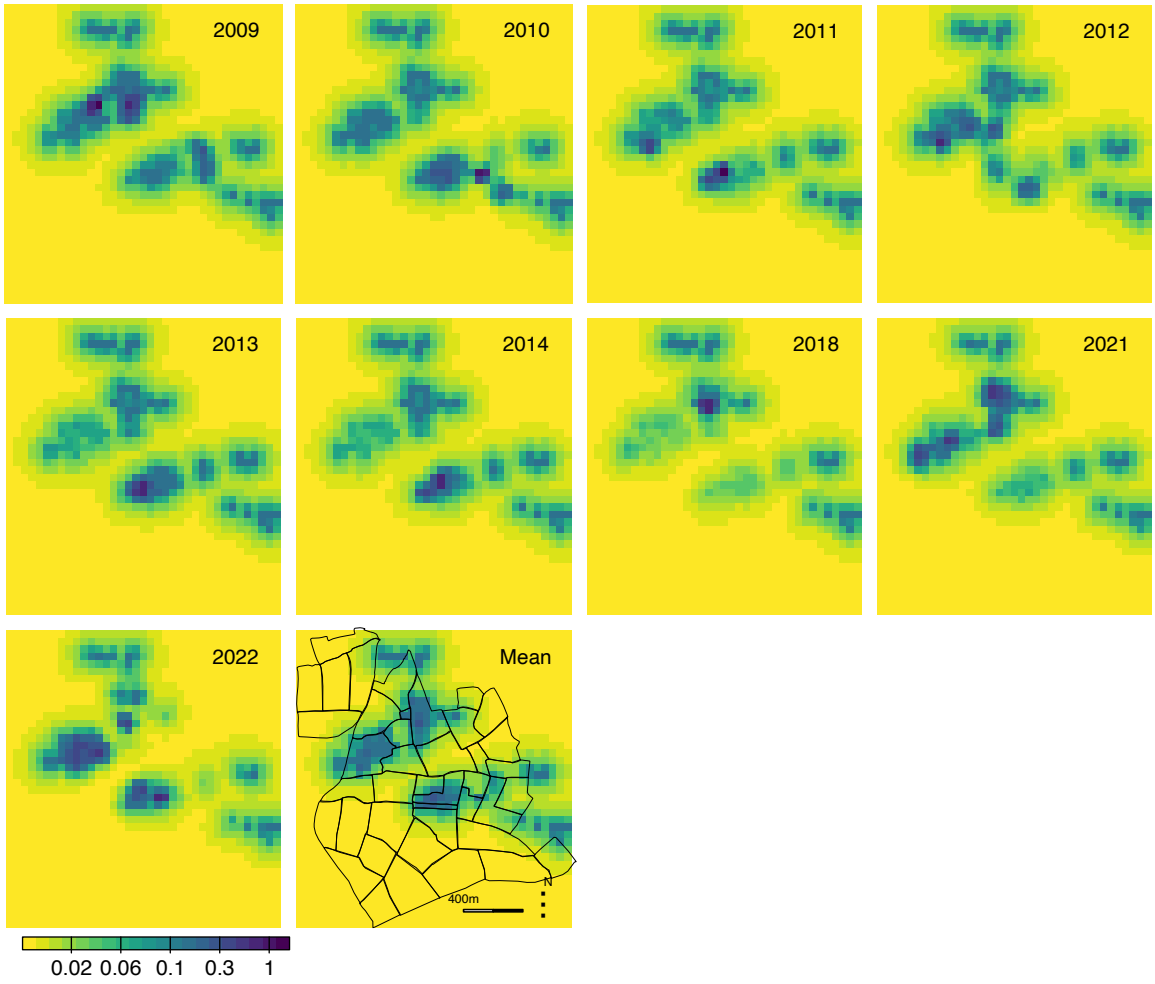

Figure 3.3. Realised density of juvenile hedgehogs. Annual realised density of juvenile hedgehogs from 2009 to 2022 and the mean across years (females and males were combined). Site centroid coordinates 53.06 N, -0.96W (EPSG:4326). Habitat composition is included in Figures 2.1–2.3 in Chapter 2. (The output is derived from model: D~SESSION + BUILD, p_0~SEX, sig~SEX).

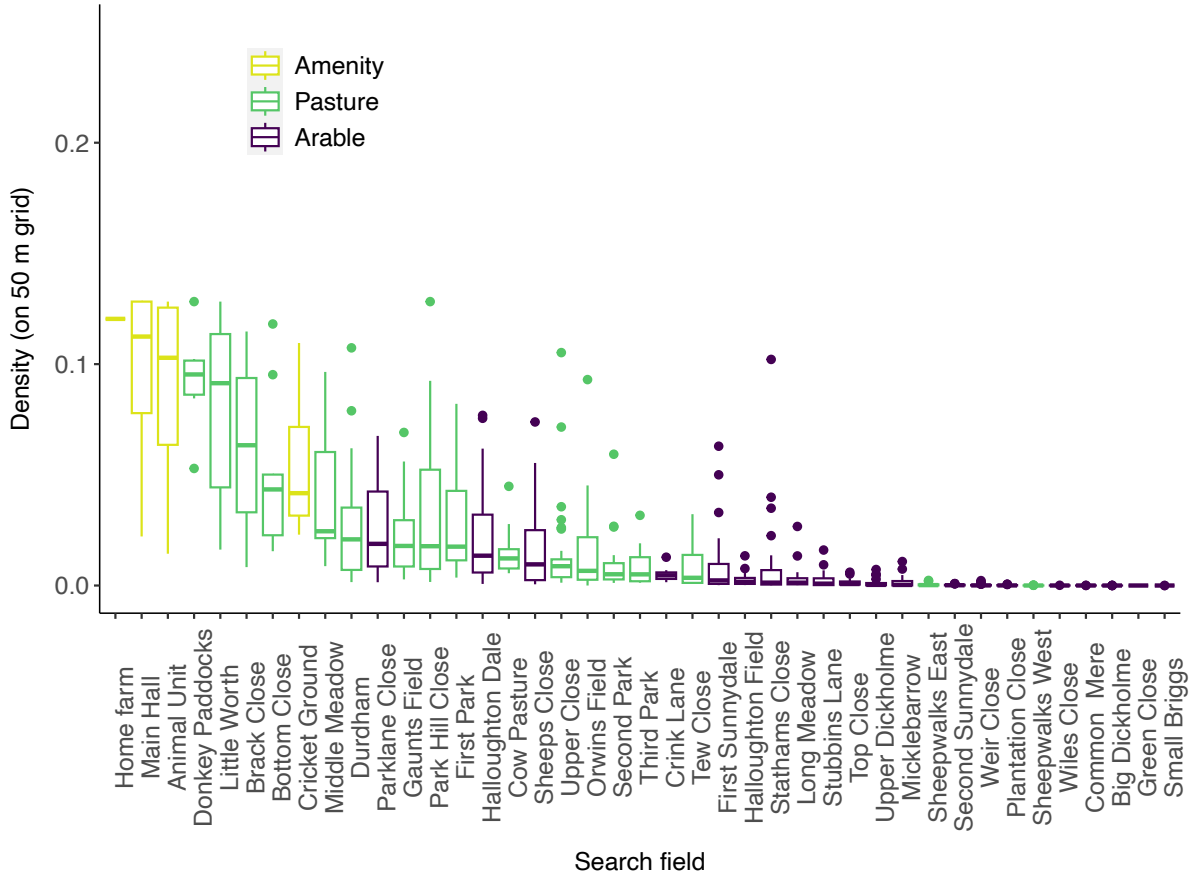

Figure 3.4. Boxplots (median, 25% and 75% quartiles, and 95% confidence interval) of annual realised density of juvenile hedgehogs based on fields. Mean 2009–2022. Females and males were combined. (The output is derived from model: D~SESSION + BUILD, p_0~SEX, sig~SEX).

3.3.2 Comparisons between juveniles and adults in detection and density

Based on the SCR density modelling, 28% of juveniles and 69% of adults were estimated to have ever been detected during the study period. In juveniles, annual estimated density per 50 x 50 m gird on amenity land was 0.089 (95% CI 0.077–0.099), pasture 0.025 (95% CI 0.021–0.029), and arable land 0.005 (95% CI 0.004–0.006), with that on amenity: pasture: arable approximating 18: 5 :1, and female: male to 1.3: 1. In adults, annual estimated density per 50 x 50 m gird on amenity land was 0.034 (95% CI 0.012–0.055), pasture 0.012 (95% CI 0.004–0.019), and arable land 0.004 (95% CI 0.001–0.007), with that on amenity: pasture: arable approximating to 9: 3: 1, and female: male to 1.6: 1. With juveniles and adults combined, the overall annual estimated population density was 13.3 hedgehogs/km^2, with juvenile female:

adult female: juvenile male: adult male ratios being 1.7: 1.6: 1.3: 1, and on amenity: pasture: arable approximating to 14: 4: 1. Juvenile and adult hedgehogs had similarly trends (decreased and then stabilised) in estimated densities, and were subject to similar effects of habitats, i.e., being significantly positively associated with land with high soil permeability, positively related to distance to the nearest badger sett and edge density, and negatively related to distance to the nearest building. The main observed differences were that juveniles were more associated with buildings than adults.

3.3.3 Spatial CJS modelling survival

The dataset used for nonspatial and spatial CJS modelling of survival includes 1068 captures (576 adult female: 348 adult male: 96 juvenile female: 48 juvenile male) from 174 unique hedgehogs (99 female: 75 male) collected from 440 systematic search occasions, with an average of 6 (95% CI 5–7) captures per individual. 112 individuals (61 female: 51 male) were classified as adults on their first captures; and 62 individuals (38 female: 24 male) as juveniles on their first captures, in which 21 (15 female: 6 male) were recaptured during adult stage (i.e., after the first winter of the individual), were also included in adult survival modelling (with captures during adult stage only), resulting in 133 unique adults (76 female: 57 male). Detailed search efforts and captures per year were included in Table 3.5.

Table 3.5. Summary of sampling effort of the spatial capture-recapture of the hedgehogs, Brackenhurst, UK, 2009–2022. Where, "No. traps" is number of 50 x 50 m grid effective traps; "No. search nights" is number of searched nights; "No. OS" is number of occasions; "No. unique ind" is number of unique individuals in the session; "No. spatial cap" is number of spatial captures. am: af: jm: jf indicates age and sex, with age based on the capture in the session (rather than based on the first capture during the study period).

Session	State space (km^2)	Search area (km^2)	No. traps	No. search nights	No. OC	No. unique ind (am:af:jm:jf)	No. spatial cap (am:af:jm:jf)	No. moves (am:af:jm:jf)
2009	3.70	1.57	628	42	42	27 (9:7:5:6)	39 (15:7:7:10)	36 (6:25:2:3)
2010	3.70	0.94	376	62	60	27 (7:14:3:3)	55 (21:14:4:16)	79 (12:54:1:12)
2011	3.70	0.88	351	52	52	25 (11:10:0:4)	60 (37:10:0:13)	65 (26:31:0:8)
2012	3.70	1.23	493	81	60	37 (10:21:1:5)	118 (91:21:1:5)	153 (80:73:0:0)
2013	3.70	1.61	643	67	60	21 (9:6:4:2)	90 (62:16:6:6)	156 (53:99:2:2)
2014	3.70	1.14	454	50	50	24 (11:10:0:3)	76 (58:10:0:8)	100 (45:53:0:2)
2015	3.70	0.83	330	14	14	16 (7:9:0:0)	22 (13:9:0:0)	16 (6:10:0:0)
2017	3.70	0.59	236	10	10	9 (2:4:1:2)	16 (5:4:1:6)	11 (2:6:0:3)
2018	3.70	0.59	236	9	9	8 (3:5:0:0)	13 (8:5:0:0)	8 (5:3:0:0)
2021	3.70	2.07	828	64	60	21 (5:7:4:5)	44 (21:7:7:9)	60 (16:37:3:4)
2022	3.70	1.76	704	23	23	22 (3:5:6:8)	47 (9:5:17:16)	42 (6:19:11:6)

Across demographic groups and modelling methods, constant survival rates were more supported than session-specific survival rates, supporting stable temporal trends in apparent survival (Figure 3.5; for simplicity, only survival rates of adult males and adult females based on the spatial normal movement models ("Spatial normal") were plotted). Across groups, the spatial normal movement models fitted better than the spatial static models ("Spatial static"), supporting home range shifts (dispersal) between sessions. Then, based on the constant survival model, and spatial normal movement model, the survival estimate (φ) for adult females was 0.530 (95% CI 0.423–0.635), for adult males was 0.426 (95% CI 0.308–0.552), with no strong differences between the two groups based on the overlapped 95% CI values (Table 3.6). The estimated spatial scale of between-session home-range relocation α was close for adult females and adult males, supporting similar levels of dispersal/emigration in the two groups. However, when juveniles were combined with adults of the same sex, α was substantially increased for males, but not for females, likely supporting natal dispersal in males (Table 3.6).

Table 3.6. Non-spatial and spatial modelling of hedgehog survival.

	Non-spatial	**Spatial static**	**Spatial normal**
(a) Apparent survival φ			
Female adult	0.426 (0.334–0.524)	0.530 (0.442–0.636)	0.530 (0.423–0.635)
Male adult	0.366 (0.263–0.483)	0.422 (0.305–0.548)	0.426 (0.308–0.552)
Female adult juvenile combined	0.462 (0.38–0.546)	0.572 (0.478–0.662)	0.571 (0.478–0.660)
Male adult juvenile combined	0.393 (0.299–0.495)	0.442 (0.337–0.553)	0.447 (0.343–0.556)
(b) Baseline detection λ0			
Female adult	0.113 (0.099–0.128)	0.016 (0.013–0.02)	0.017 (0.014–0.022)
Male adult	0.127 (0.109–0.148)	0.009 (0.007–0.011)	0.010 (0.008–0.012)
Female adult juvenile combined	0.117 (0.105–0.13)	0.017 (0.014–0.02)	0.019 (0.016–0.022)
Male adult juvenile combined	0.109 (0.095–0.124)	0.008 (0.006–0.01)	0.010 (0.008–0.012)
(c) Detection scale σ (m)			
Female adult		131 (118–146)	130 (117–145)
Male adult		260 (220–308)	242 (202–291)
Female adult juvenile combined		122 (112–132)	121 (111–132)
Male adult juvenile combined		267 (230–311)	234 (200–275)
(d) Movement kernel scale α (m)			
Female adult			158 (59–421)
Male adult			158 (78–318)
Female adult juvenile combined			180 (100–326)
Male adult juvenile combined			354 (193–648)

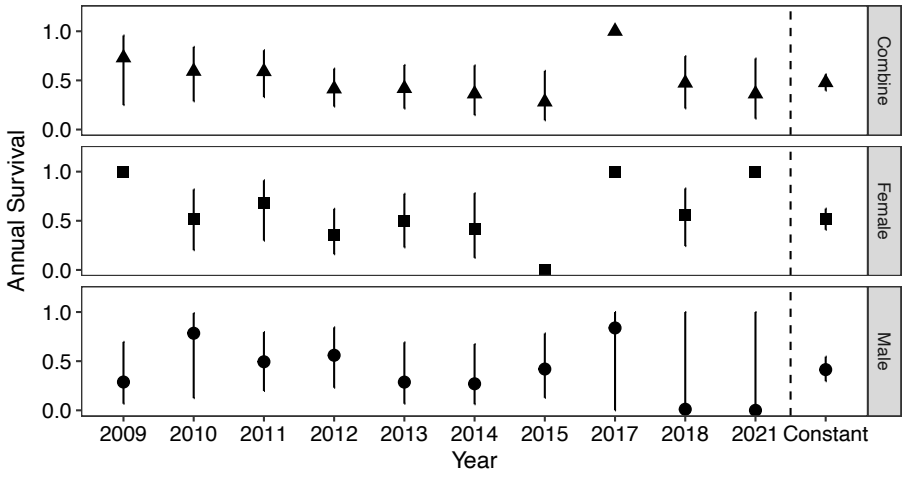

Figure 3.5. Apparent annual survival rates of adult hedgehogs. Based on session-specific and constant spatial models. 2009–2021. Males and females were run separately and combined.

3.3.4 Non-spatial CJS modelling survival

Apparent survival rates were also estimated using traditional non-spatial CJS models. Compared to the spatial apparent survival rates, the non-spatial survival rates were lower, being 0.426 (0.334–0.524) and 0.366 (0.263–0.483) for adult females and adult males, respectively, likely supporting dispersal/emigration (annually c. 10%) for both groups.

3.3.5 Known mortality

A total of 25 marked hedgehogs (14% of 174 marked; ID-identified) were found dead during the study, comprising 8 adult females, 15 adult males, and 2 juvenile females (age at the last capture; all were captured more than once), leaving 149 hedgehogs (86% of the 174) disappeared for unknown reasons. Of the 25 ID-identified dead hedgehogs, 20 were adults at first capture, surviving in the population for an average of 426 (range 10–1068) days since first capture (4 female hedgehogs, 357 (range 10–1068) days; 16 male hedgehogs, 444 (range 16–1015) days); five were juveniles at first capture, surviving in the population for an average of 651 (range 257–1050) days since first capture (4 female hedgehogs, 552 (range 257–816) days; 1 male hedgehog, 1050 days).

In addition to the 25 ID-identified dead hedgehogs, a further 21 dead hedgehogs with no known identity were found (ID-unidentified), resulting in a total of 46 dead hedgehogs found (Figure 3.6; Table 3.7). The ID-identified and ID-unidentified hedgehogs showed little difference in both causes and the spatiotemporal distributions of mortality, thus were combined for summarising cause-specific mortality. The 46 dead hedgehogs comprise 37 adults (13 female: 15 male: 9 unknown), and 9 juveniles (1 female: 1 male: 7 unknown), estimated based on the body size of collected dead hedgehogs.

Of the 46 dead hedgehogs, 24 were likely human-caused mortalities (52% of 46; roadkill, electrocution, drowning, livestock trampling, and movement of hay bales), 19 naturally died (41%; starvation in cold weather, and predation; predation presumed by domestic dogs were included due to uncertainty), and three with causes unknown (7%). Of those killed on roads, all but two were located on the main A621 road, with roadkill density being around 0.5 hedgehog per km of main road per year. A roadkill hot spot was identified at the west entrance of the Brackenhurst Campus, near the hedgehog core population centre. Nine (20%) were presumed to be predated by badgers, including one on amenity land and eight on pastures with little difference in the number predated before and after the new badger sett. Seven hedgehogs (15%) were likely predated by foxes, dogs, or other species but not badgers, as the carcasses were not skinned. Five were killed by poorly installed electric fencing (11%). Three were likely to have died of starvation in dry and cold weather (7%). Dead hedgehog density on/next to amenity: pasture: arable was 37: 55: 1.

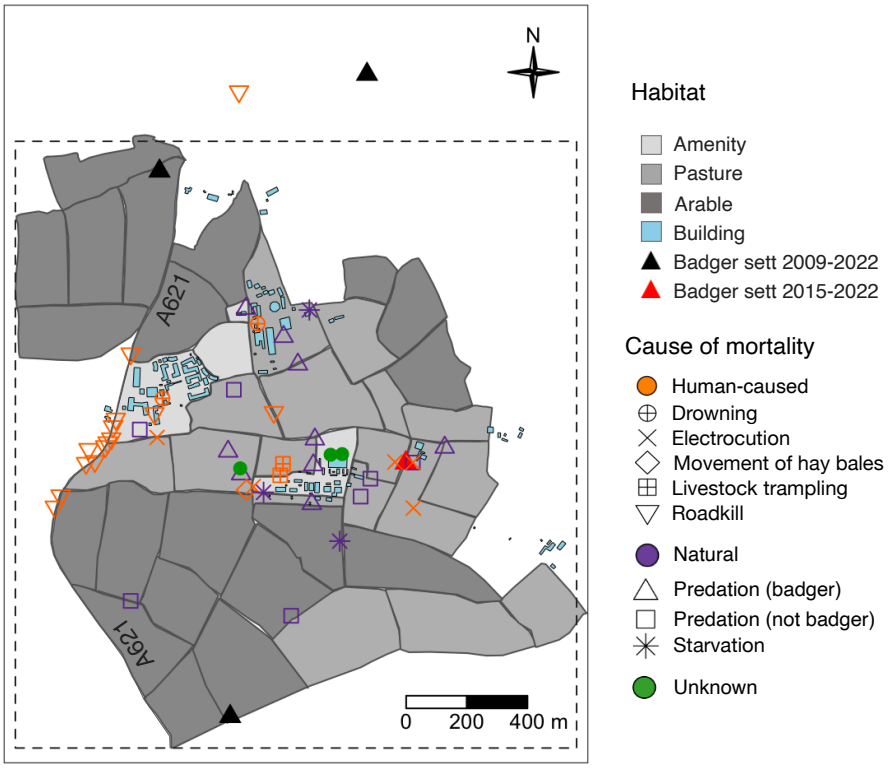

Figure 3.6. Map of dead hedgehogs collected (n = 46; 2009–2022). Legends of habitats were included in Figure 2.1. The presumed causes of mortality were provided. Details are included in Table 3.7. One marked hedgehog found dead outside of the systematic search area (shaded area) was included.

Table 3.7. Causes of known mortality. The presumed cause of known mortality (Cause of mortality), the number of hedgehogs to a certain cause (No. hedgehogs) and the according proportion to the overall known mortality (% known of the total 46 collected dead hedgehogs) were provided. * F: female, M, male, U: sex unknown, A: adult on collection, J: juvenile on collection; ID: ID-identified; No-ID: ID-unidentified.

Cause of mortality	No. hedgehogs	% known death (n= 46)	Notes*	Cause of mortality (detailed)	No. hedgehogs (ID-identified: ID-unidentified)	% known death (n= 46; detailed)	Notes (detailed)*
Human-induced	24	52.17	8F: 10M: 6U; 1J: 23A; 2 in Mar, 5 in May, 7 in Jun, 5 in Jul, 2 in Aug, 3 in Sep; 14ID: 10No-ID	Roadkill	14 (7: 7)	30.43	4F: 6M: 4U; 1J: 13A; 2 in Mar, 2 in May, 4 in Jun, 3 in Jul, 1 in Aug, 2 in Sep. Roadkill hotspot: near the west entrance of the Campus
				Electrocution	5 (4: 1)	10.87	1F, 3M, 1U; 5A; 1 in May, 2 in Jun, 1 in Jul, 1 in Sep
				Drowning	2 (1:1)	4.35	2F; 2A; 1 in May, 1 in June
				Livestock trampling	2 (1: 1)	4.35	1M, 1U; 2A; 1 in May, 1 in Aug
				Movement of hay bales	1 (1: 0)	2.17	1F; 1A; 1 in July
Natural	19	41.30	5F: 6M: 8U; 6J: 13A; 2 in Apr, 3 in May, 4 in Jun, 5 in Jul, 1 in Aug, 1 in Sep, 2 in Oct, 1 in Nov; 10ID: 9No-ID	Predation (badger)	9 (5: 4)	19.57	2F, 3M, 4U; 3J: 6A; 2 in Jun, 4 in Jul, 1 in Aug, 1 in Oct, 1 in Nov; 1 Amenity, 8 Pasture; all near sparse trees
				Predation (not badger)	7 (2: 5)	15.22	3F, 0M, 4U; 3J, 4A; 1 in Apr, 1 in May, 2 in Jun, 1 in Jul, 1 in Sep, 1 in Oct; 1 Amenity, 4 Pasture, 2 Arable; 1 likely by fox, 1 unknown, 5 likely by dog or other species
				Starvation (in cold weather)	3 (3: 0)	6.52	3M; 3A; 1 in Apr, 2 in May
Unknown	3	6.52	1F, 2U; 2J, 1A; 3 in July; 1ID: 2No-ID	Unknown	3 (1: 2)	6.52	1F, 2U; 2J, 1A; 3 in Jul

3.4 Discussion

The density of Brackenhurst hedgehogs decreased after the new badger sett appeared, mirroring reported population declines over wider spatial scales (Hof, Snellenberg and Bright, 2012; Van de Poel, Dekker and Van Langevelde, 2015; Pettett et al., 2017; Hof, Allen and Bright, 2019). The population was then stabilised at a lower density. The apparent survival (Chapter 3) trends were relatively stable across the study period. The results likely suggest the negative effects of badgers on hedgehogs on the local scale, but the potential coexistence of both species on the landscape scale. However, densities on all arable lands and some pastures on our study site were extremely low across the search years, with the underlying mechanisms warranting further research.

3.4.1 Detection rate and density

Based on the SCR density modelling, only 28% of juveniles and 69% of adults were estimated to have been detected, despite extensive searches being conducted, highlighting the importance of accounting for detection rates in density estimation of hedgehogs.

Juveniles and adults in our study had similarly density trends and were subject to similar effects of habitat types, being significantly positively associated with land with high soil permeability, edge density, distance from the nearest badger sett, and proximity to the nearest building. The main observed differences were that juveniles were more associated with buildings, and juveniles had slightly higher density than adults on amenity lands, but slightly lower density than adults on pasture and arable lands. Annual estimated density per 50 x 50 m gird on amenity: pasture: arable in juveniles was 18: 5 :1, compared to that in adults 9: 3: 1. With juveniles and adults combined, the overall annual estimated density was 13.3 hedgehogs/km^2 for the population, with juvenile female: adult female: juvenile male: adult male ratios being 1.7: 1.6: 1.3: 1, and on amenity: pasture: arable approximating to 14: 4: 1. The high density on amenity lands compared to that on pastures were consistent with previous studies (e.g., Parrott, Etherington and Dendy, 2014; but see Haigh, 2011). As we are aware, arable-specific density could only be retrieved from one previous study, which was carried out in northeastern France, based on direct counting of unique hedgehogs (Hubert et

al., 2011), in which density patterns based on land use characteristics were similar to that found in our study. These findings are in line with the results of previous radio- or GPS-tracking studies, showing hedgehogs tend to avoid arable lands and pastures and select amenity lands (Pettett et al., 2017). However, an exception is in a farmland-dominated area in Ireland, where the hedgehogs were found to frequently use pastures and arable land (Haigh, Butler and O'Riordan, 2012), suggesting the species may utilise these lands in some areas. Therefore, although currently the population is likely stabilised (2015–2022), we yet do not know if density on the arable lands and pastures had dropped before the study started. But if so, this might indicate that most of their previous habitats on site were severely degraded. (Habitat effects on hedgehog density was discussed in more details in Chapter 2).

3.4.2 Survival

Adult males and adult females had similar levels of between-year home-range relocation, indicating similar levels of dispersal/emigration (annually c. 10%) of both groups. However, when juveniles were added to adults of the same sex, the scale of between-year home-range relocation in males (354 m, 95% CI 193–648) became considerably higher than that in females (180 m, 95% CI 100–326), likely suggesting natal dispersal related to juvenile males. The findings that only 15.63% (95% CI 0–31.32%) of juvenile males were recaptured after their second year April, compared to 28.21% (95% CI 10.35–46.06%) in females also likely supports this (Appendix I , Table 3.9, and Figure 3.7 B).

In our study, the annual apparent survival rates of adult hedgehogs were found to be c. 0.5 based on spatial CJS modelling, and c. 0.4 based on traditional non-spatial CJS modelling. The difference between the spatial and non-spatial modelled survival rates is comparable to that in other studies (e.g., Schaub and Royle, 2014; Efford and Schofield, 2020), potentially because emigration is not taken into account in non-spatial models. However, even though the spatial models can account for emigration to some extent, these models are suggested to be only effective when data span the range of movement of the animals (Efford and Schofield, 2022). Given that long-range movements (at least > 3 km) in hedgehogs are likely (Chapter 4) and our study area was relatively small, even the spatial modelled survival rates in our study are likely to be underestimated.

The annual apparent survival of adult hedgehogs was found to be 0.54 in the Swedish population in Kristiansson (1990), estimated based on direct captures and with detection rate and emigration not being accounted for. Although different methods were used and thus direct comparisons of results might not be appropriate, the survival rates found in both studies appeared to be close, i.e., roughly around half of the marked adult individuals were not recaptured in the following years. Nevertheless, factors affecting the observed apparent survival rates were uncertain. In both studies, most of the marked hedgehogs disappeared for unknown reasons with death not detected. Kristiansson (1990) explained that roadkill caused most mortality during active seasons and the harsh weather during the winter hibernation period in Sweden caused most annual mortality, with the former based on the collected dead hedgehogs while the latter based on the disappearance of marked individuals, assuming individuals that were not found dead in a year but not recaptured in the following year died in winter. However, this assumption might lead to an overestimation on winter mortality, i.e., if detection of dead hedgehogs during active seasons were low. We yet do not know the detection rates of dead hedgehogs, but when based on monthly survival analysis (Appendix I, Table 3.8, Table 3.9, and Figure 3.7), mortality during winter hibernation period in our study was suggested to be low compared to that during active seasons, consistent with that was found based on radio-tracking in Bearman-Brown et al. (2020; England), and in line with that winter is generally associated with lower mortality in hibernation mammals (Turbill, Bieber and Ruf, 2011).

Based on the spatial CJS modelling, we found that when juveniles were combined with adults of same sex, the estimated spatial scale of between-session home-range relocation substantially increased for males, but not for females, likely supporting natal dispersal in males. Indeed, for those with first capture as juveniles, only 15.63% (95% CI 0–31.32%) of males were recaptured after their second year April, lower than that in females (28.21%, 95% CI 10.35–46.06%) (Appendix I, Table 3.9). Our results are in line with that found in Reeve (1994; England) and Haigh (2011; Ireland), supporting natal dispersal/exploration movement of hedgehogs during the early stage of their life history. However, this might be context-specific and not be true for all populations, e.g., little natal dispersal was found in the Danish hedgehogs in Rasmussen et al. (2019). Furthermore, juvenile survival varied substantially from different studies, e.g., 35% of juveniles survived their first winter in England (Morris,1969; see Kristiansen, 1990), and 66% survived to their second year in Sweden

(Kristiansen, 1990), presumably because of different environmental contexts or using different methods.

A wide range of causes of detected mortality was found and many are human-caused (52% of the 46 collected dead hedgehogs), such as roadkill, electrocution, drowning in garden pond, horse trampling, and by movement of hay bales. Roadkill (30% of 46) and badger predation (20% of 46) accounted for half of the detected mortality, but as this is likely the most obvious mortality to find, the main driver of hedgehog mortality may be unknown. Notably, the detected mortality only accounted for 14% of the population, with the cause of mortality for 86% of individuals being unknown, illustrating the complexity of inferring mortality rates and causes of mortality to population dynamics and highlighting the need for conservation management to consider not mortality, but population changes through long-term studies.

3.5 Conclusion

This study presents the first long-term SCR-based density and apparent survival estimates of the hedgehog. The results show that juvenile and adult hedgehogs had similarly trends in densities and were subject to similar effects of habitats, i.e., being significantly positively related to soil permeability, edge density, distance from the nearest badger sett, and proximity to the nearest building, with juveniles being more associated with buildings. Modelled annual apparent survival rate of adult hedgehogs was around 0.5, which seems to be enough for the population to maintain population size. Natal dispersal in male hedgehogs is likely supported. A wide range of mortality was detected. The detected mortality, however, only accounted for 14% of the population-level mortality. Of these, roadkill and badger predation accounted for the majority of detected mortality, but as this is likely the most obvious mortality to find, the main driver of hedgehog mortality may be unknown which hampers conservation management intervention. Our result illustrates the complexity of inferring mortality rates and causes of mortality to population dynamics, highlighting the need for conservation management to consider not mortality, but rather identification of drivers of population changes is needed through long-term studies.

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3.7 Appendix I: Minimum survival of Brackenhurst hedgehogs

Methods

Minimum survival was summarised based on direct captures, i.e., from the first capture to the last capture, assuming the animal died immediately after the last capture, using both systematic and opportunistic data. Yearly data were pooled. Three juveniles first caught before July were included and treated as being first caught on 1st July of the year due to limited datasets. Detection rates of dead animals were not accounted for, so the mortality patterns are not suitable for extrapolating. Only captures with individual ID, coordinates, age, month, sex, and body weight available were included in this analysis, and only hedgehogs captured more than once were retained.

The minimum survival for interval t+1 is conditioned on that of the last interval S_t, $S_{t+1} = S_t *((Nt_{+1} - D_{t+1})/N_{t+1})$, where N_{t+1} is the number of risks during the monthly age interval t+1, D_{t+1} is the number of those being last caught in interval t+1. Note with the newly caught individuals incorporated, N_{t+1} here is $N_t - D_t + J_t$, where Jt is the number of newly joined (those being first caught) in interval t. Minimum survival rates were compared between demographic groups with a log-rank test using the R package 'survival' (Therneau, 2024) with the function survdiff().

Age structure of the population and cumulative monthly survivorship were summarised based on the minimum survival. The exact ages were only known for individuals first caught as juveniles, for which the year the first capture occurred was the birth year and the age at the year was zero, and then for those that survived the next April (survived to the next May) was treated as reaching one year old. This is because juvenile hedgehogs in this population became independent since June or July, and thus they were likely to be born in May or June (Morris, 2018). For individuals first caught as adults, they were considered to be at least one year old at first capture, so their ages, with the year the first capture occurred being treated as one, were minimum estimates. The survivorship curves were plotted using the geom_line() function in the R package ggplot2 (Wickham, 2016). As no data were available during hibernation, survival from October to April was depicted as horizontal lines. Since the minimum survival here indicates the period from the first capture to the last capture, if an

individual is captured in October but never captured again after, it is treated as dead in that October although death could occur after, e.g., during the winter hibernation period (potentially November to March).

Results

A total 207 hedgehogs were included in our dataset, in which 153 were captured more than once and retained for calculating the minimum survival, comprising 106 individuals with first capture as adults (female: male = 54: 52), and 47 with first capture as juveniles (female: male = 28: 19). Apparent survival days, age structure, and monthly age-specific survival rates were summarised.

For individuals with first capture as adults, the average minimum survival was 351 (95 CI% 282–421) for females and males combined, 404 (95 CI% 302–507) days for females, and 296 (95 CI% 201–391) for males, and with no significant difference between females and males (chi-squared X^2= 2.4, d.f. = 1, p = 0.1). For individuals with first capture as juveniles, the average minimum survival was 220 (95 CI% 114–327) for females and males combined, 288 (95 CI% 129–448) days for females, and 120 (95 CI% 0–244) for males, and with that in males being significantly lower than in females (chi-squared X^2 = 3, d.f. = 1, p = 0.08). For females, no significant difference in minimum survival was found between those with first capture as adults and as juveniles (chi-squared X^2 = 1.9, d.f. = 1, p = 0.2), while for males, significantly lower minimum survival was found for those with first capture as juveniles than as adults (chi-squared X^2 = 9.1, d.f. = 1, p = 0.003). These results suggest low minimum survival in males than in females during the early stages of their life history. Indeed, for those with first capture as adults, 27.36% (95% 14.94–39.77%) of males were recaptured in the following year (after April), lower than that in females (50.67%, 95% 35.19–66.15%) (Table 3.8; Figure 3.7 A). Similarly, for those with first capture as juveniles, only 15.63% (95% CI 0–31.32%) of males were recaptured in the following year (after April), lower that in females (28.21%, 95% CI 10.35–46.06%) (Table 3.9; Figure 3.7 B).

With all age and sex groups combined, 29.74% (95% CI 21.21–38.26%) of the hedgehogs (all age and sex groups combined) survived to two years old, and 13.61 (95% CI 7.69–19.51%) to three years old, 4.39 (95% CI 1.45–7.53%) to four years old, and 0.49% (95% CI 0–1.24%) to five and the same continue to six years old, and none beyond.

The longest apparent survival was at least 6 years for females and 4 years for males. Monthly survival was lowest in July (64.70%, 95% CI 45.23–72.31%) and highest in October (87.89%, 95% CI 79.69–89.91%). Averaged monthly survival from April to July was 74.14% (95% CI 58.57–79.21%), from August to October was 74.36%, 95% CI 60.34–77.76%), with no significant difference between the two groups (Wilcoxon test, W = 936, P > 0.5). As winter mortality would be more likely to be added on August to October than April to July which was not observed in our data, the results potentially suggest low winter mortality.

Table 3.8. Age structure and cumulative minimum survival of hedgehogs with first capture as adults, for females (AF; n = 54) and males (AM; n = 52) separately and averaged (AFAM, n = 106). Age at the first capture was treated as one. Calculated from the April of the year in which the first capture occurred. Only individuals captured more than once were included.

Age (years)	Month	Survival %; AF	Survival %; AM	Survival %; AFAM
1	Apr	90 (76.85–100)	85.71 (70.75–100)	87.8 (78.4–97.2)
	Jul	63.12 (46.41–79.83)	47.67 (31.55–63.78)	54.9 (44.03–65.77)
	Oct	53.65 (37.82–69.48)	31.46 (18.08–44.84)	42.12 (32.31–51.94)
2	Apr	50.67 (35.19–66.15)	27.36 (14.94–39.77)	38.55 (29.12–47.98)
	Jul	29.81 (17.43–42.18)	20.52 (9.86–31.18)	24.99 (17.36–32.61)
	Oct	20.86 (10.33–31.39)	16.41 (6.93–25.9)	18.56 (11.93–25.19)
3	Apr	19.37 (9.2–29.55)	15.05 (5.98–24.11)	17.14 (10.75–23.52)
	Jul	10.43 (2.84–18.02)	8.21 (1.57–14.84)	9.28 (4.68–13.88)
	Oct	7.45 (1–13.9)	5.47 (0.07–10.87)	6.43 (2.59–10.26)
4	Apr	7.45 (1–13.9)	4.1 (0–8.77)	5.71 (2.08–9.34)
	Jul	2.98 (0–7.09)	1.37 (0–4.05)	1.43 (0–2.86)
	Oct	1.49 (0–4.4)	0 (0–0)	0.71 (0–1.79)
5	Apr	1.49 (0–4.4)		0.71 (0–1.79)
	Jul	1.49 (0–4.4)		0.71 (0–1.79)
	Oct	1.49 (0–4.4)		0.71 (0–1.79)
6	Apr	1.49 (0–4.4)		0.71 (0–1.79)
	Jul	0 (0–0)		0 (0–0)

Table 3.9. Age structure and cumulative minimum survival of hedgehogs with first capture as juveniles, for females (JF; n = 28) and males (JM; n = 19) separately and averaged (JFJM, n = 47). Age at the first capture was treated as zero. Calculated from the July of the birth year. Only individuals captured more than once were included.

Age (years)	Month	Survival %; JF	Survival %; JM	Survival %; JFJM
0 (year of birth)	Jul	66.67 (39.99–93.34)	75 (44.99–100)	70 (53.07–86.93)
	Oct	28.21 (10.35–46.06)	15.63 (0–31.32)	22.55 (12.96–32.14)
1	Apr	28.21 (10.35–46.06)	15.63 (0–31.32)	22.55 (12.96–32.14)
	Jul	18.8 (4.72–32.88)	7.81 (0–18.77)	14.09 (6.49–21.7)
	Oct	14.1 (2.13–26.07)	3.91 (0–11.61)	9.86 (3.39–16.34)
2	Apr	14.1 (2.13–26.07)	3.91 (0–11.61)	9.86 (3.39–16.34)
	Jul	11.75 (0.93–22.58)	3.91 (0–11.61)	8.46 (2.41–14.5)
	Oct	11.75 (0.93–22.58)	3.91 (0–11.61)	8.46 (2.41–14.5)
3	Apr	7.05 (0–15.28)	3.91 (0–11.61)	5.64 (0.8–10.47)
	Jul	2.35 (0–7.01)	0 (0–0)	1.41 (0.02–3.45)
	Oct	2.35 (0–7.01)		1.41 (0.02–3.45)
4	Apr	2.35 (0–7.01)		1.41 (0.02–3.45)
	Jul	2.35 (0–7.01)		1.41 (0.02–3.45)
	Oct	0 (0–0)		0 (0–0)

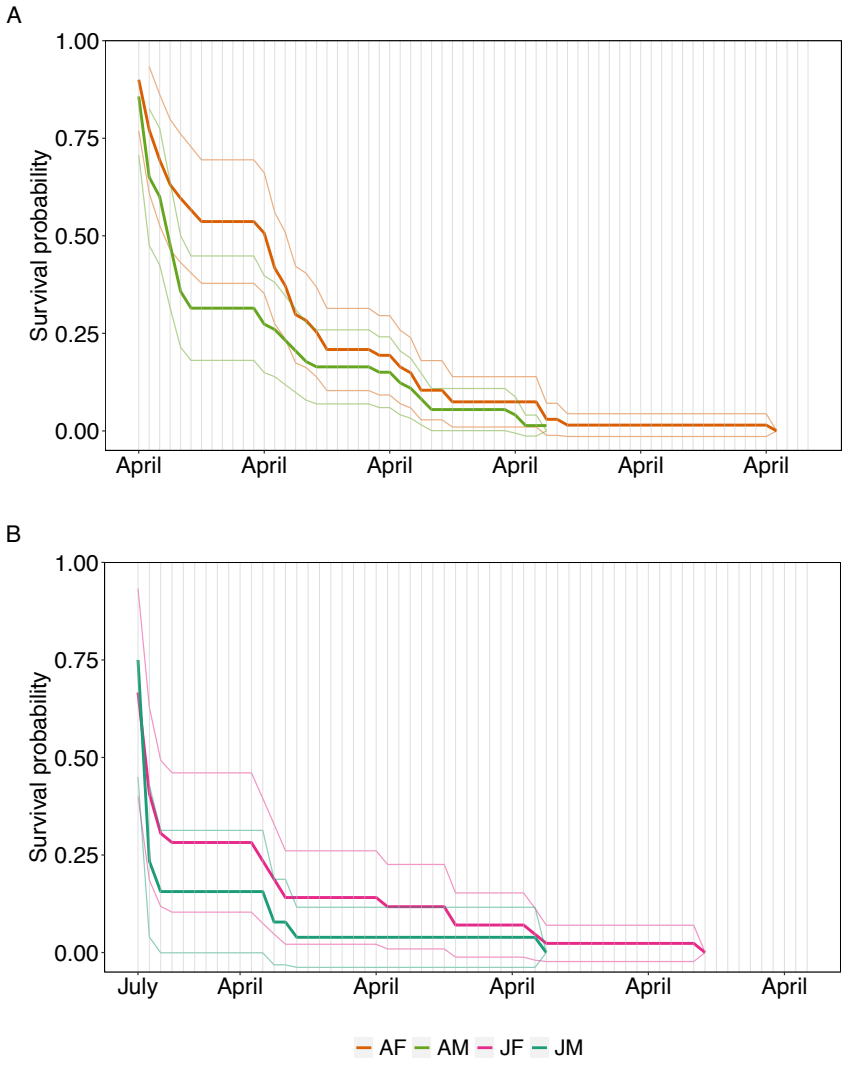

Figure 3.7 Survivorship of hedgehogs. Monthly age-specific survivorship of hedgehogs with first capture as adults (A; the April of the year in which the first capture occurred, and then the April of following years through the hedgehogs' capture history were labelled), and juveniles (B; the July of the birth year, and then the April of following years through the hedgehogs' capture history were labelled). Survival rates in panel A and panel B are independent. AF: female with first capture as an adult (survived for at least one winter); AM: male with first capture as an adult. JF: female with first capture as a juvenile (born in the year); JM: male with first capture as a juvenile.

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Chapter 4: Unexpected landscape-scale contemporary gene flow and fine-scale genetic diversity in rural hedgehogs

Abstract

Agricultural intensification is one of the major forces driving populations of many traditionally common native species into smaller, fragmented populations which are prone to isolation and loss of genetic diversity. Identifying the spatial extent and characteristics of rural systems that support gene flow and promote genetic diversity for these species is thus essential for their long-term conservation. Here we used asymmetric autosomal genetic structure between sexes to investigate current gene flow among four neighbouring suburban populations of hedgehogs (*Erinaceus europaeus*) in England, which are separated by agricultural land. Contrary to expectations, we found that individuals belonged to a single genetic population despite the populations being separated by unoccupied agricultural land. Spatial autocorrelation was significant in adult female hedgehogs, but non-significant in adult males, revealing male driven contemporary gene flow between local populations. The results suggest that male hedgehogs are capable of moving between population patches separated by at least 3 km across the agricultural matrix. This finding is crucial to aid the development of efficient conservation strategies for hedgehogs in similar agricultural landscapes as, for the first time, it shows the extent that previously assumed isolated populations across a perceived inhospitable landscape are connected by current gene flow. Higher within patch relatedness, and lower allelic richness were found from smaller suburban patches than larger patches (after accounting for potential effects of sample size), largely reflecting local population size, indicating an early stage of genetic diversity loss due to habitat loss and associated fragmentation. Our study illustrates that considering current gene flow and local genetic diversity together is important to better understand habitat effects on genetic variation and to inform future conservation management.

Keywords: contemporary gene flow, sex-biased, genetic diversity, habitat fragmentation, metapopulation dynamics

4.1 Introduction

In recent decades, agricultural intensification and the subsequent loss and fragmentation of rural habitats have had severe impacts on the distribution and abundance of many previously common species (Tilman et al., 2017). This is particularly relevant in Western Europe, where such changes in farmland have led to substantial declines and range fragmentation for many common species including birds (Donald et al., 2001; Rigal et al., 2023), amphibians (Petrovan and Schmidt, 2016; Băncilă et al., 2023), and butterflies (Habel et al., 2022). Understanding current gene flow between local populations can provide insights into the scale and extent over which functional population connectivity can exist (Walton et al., 2021). However, this is challenging as the genetic structure of the population is often difficult to detect, being masked by stronger historical gene flow that may have long since disappeared (Milligan et al., 2018; Lucena-perez et al., 2020). Thus, methods used widely to infer contemporary gene flow, such as assigning individuals captured from distinct populations to their natal population, typically lack power where there is minimal variation in genetic structure (McMahon, Teeling, and Höglund, 2014; Proctor et al., 2020). Also, relatively large overall population size limits the power of traditional individual pairwise genetic pedigree methods via the detection of closely-related pairs of individuals to inform ongoing gene flow, as such individuals are often difficult to capture or detect (Taylor, 2015).

A potential method for overcoming these issues that is applicable to species exhibiting sex-biased dispersal is through inferring asymmetric autosomal genetic structure between sexes (Li and Kokko, 2019). This can detect current gene flow, without the effects of historical gene flow (Prugnolle and Meeus, 2002). However, the method remains largely untested (but see Solmsen, Johannesen and Schradin, 2011; Pernetta et al., 2011; Walton et al., 2021), especially for populations that are separated by unoccupied landscapes where the signal of sex-biased genetic structure might be less visible if inter-patch movement is highly restricted (Prugnolle and de Meeus, 2002).

The Western European hedgehog (*Erinaceus europaeus*) is considered a model species in agroecosystems for informing rural habitat connectivity and evaluating agri-environment schemes (e.g., Hof et al., 2012; Pettett et al., 2017). The species has undergone significant population decline across its geographic range (e.g., Roos, Johnston and Noble, 2012; Hof and Bright, 2016; Taucher et al., 2020), with rural habitat loss and fragmentation thought to

be the principal drivers (Wilson and Wembridge, 2018). Recent studies also suggest that rural hedgehogs have a patchy and discontinuous distribution (Williams et al., 2018), and where present in rural environments, they tend to occur in small populations near residential buildings (Schaus et al., 2020) where they will occasionally use the surrounding agricultural matrix (Parrott et al., 2014; Hof et al., 2012). As hedgehog home ranges are relatively small (e.g., 0.12 km^2 for females and 0.22 km^2 for males; Pettett et al., 2017) and their dispersal ability is poorly understood, questions remain about their ability to move between suburban centred populations separated by a largely uninhabited agricultural matrix (Yarnell et al., 2014). If hedgehogs are unable or unwilling to traverse the agricultural matrix, and the suburban populations are indeed isolated, they are likely to experience increased genetic drift and a subsequent loss of genetic diversity and lowered population viability (Reed and Frankham, 2003; Spielman et al., 2004).

The isolation of populations and their likelihood of losing genetic diversity will depend on the distance between populations and the size of the populations. A lack of suitable habitat will also play a role in shaping the within-population genetic variation, leading to reduced genetic diversity at local scales. This is because smaller patches can only accommodate lower effective population sizes and, consequently, local populations will experience higher levels of genetic drift and retain lower levels of genetic diversity (Keyghobadi, 2007). However, very few studies have considered the effects of landscape structure on genetic diversity within populations (Dileo and Wagner, 2016). In hedgehogs, whilst much work has focused on hedgehog genetic structure, genetic diversity remains insufficiently understood (Rasmussen et al., 2020).

Interpretation of previous studies on hedgehog gene flow is also hampered by ascertainment bias in analysis, historical gene flow (Araguas et al., 2022), and the unknown wider genetic population structure across its geographical range. For example, Becher and Griffiths (1998) showed population differentiation between eight populations within a 15 km radius in Oxfordshire but could not identify if natural barriers to intrinsic dispersal or human induced habitat fragmentation were the cause. In Zurich, hedgehog population structure might be confounded by sampling biases caused by sampling closely related individuals (Braaker et al., 2017; Barthel et al., 2020). Furthermore, a population in central London was found to have low genetic diversity, but whether this was due to current isolation, historic or recent founder effects remains unknown (O'Reilly, 2016).

Here, we used genetic data from hedgehogs residing in four local suburban sites with varying local hedgehog population sizes and used asymmetric autosomal genetic structure between sexes, to provide the first evidence of contemporary gene flow across a perceived inhospitable agricultural matrix. We also inferred the genetic structure, relatedness patterns, and genetic diversity, both within and across sites, to evaluate the effects of habitat composition on the genetic variation in the hedgehogs.

4.2 Methods

4.2.1 Study area and sampling design

Hedgehogs were sampled from four suburban centres (Farnsfield, Halam, Kirklington, and Southwell) in rural Nottinghamshire, England (Figure 4.1). Site Southwell is next to site Brackenhurst in Chapter 2 and Chapter 3. The spatially-varying distribution of the hedgehogs within our study is representative of most rural hedgehog populations in England as: 1) sites were < 10 km apart from their nearest neighbour, and hedgehogs have been sighted in 91.4% of 10 x 10 km grids across England (Hof and Bright, 2016) and 2) sites were largely separated by agricultural land which takes up 69% of land cover in England (National Statistics, 2022). The sites had varying local hedgehog population sizes, and differing amounts of preferred suburban and grassland habitats (Figure 4.1; Table 4.1; Table 4.2).

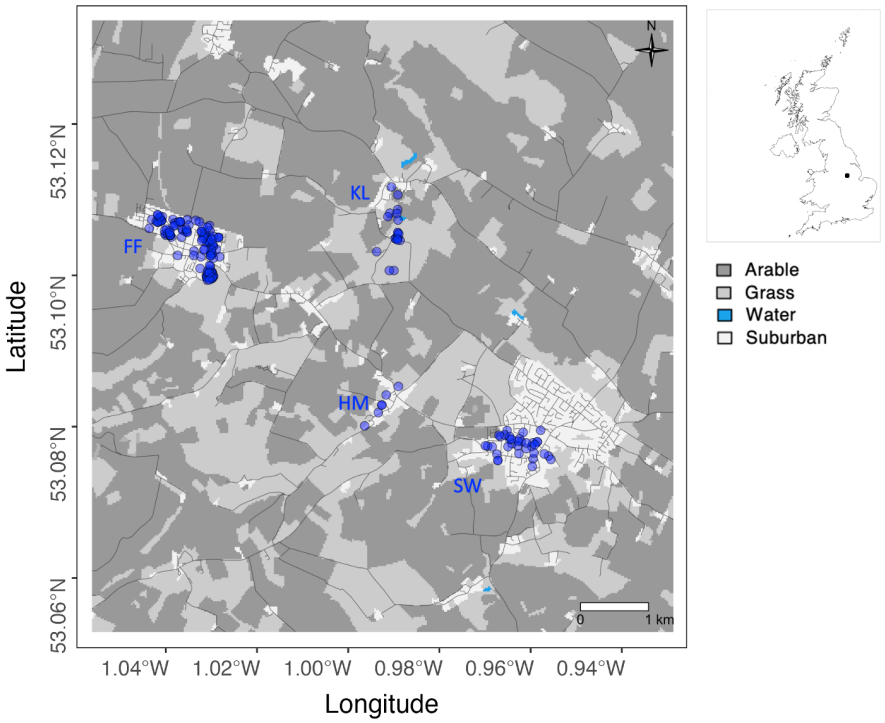

Figure 4.1. Sampling locations of hedgehogs from 4 sites in Nottinghamshire, UK. n = 183; only for those with coordinates available. Darker blue shaded points indicate geographically close or overlapped samples. Grey lines indicate roads. * Abbreviations: KL: Kirklington; FF: Farnsfield; HM: Halam; SW: Southwell. Base map: UKCECH Land Cover Map 2020 (Marston et al., 2022); projection: EPSG:4326.

Table 4.1. Sampling sites, samples used in the final analysis, and landscape composition. * Abbreviations: n: sample size; A: adult, J: juvenile, M: male, F: female, NA: not known. D: hedgehog density (individuals per km^2); Patch: suburban patch size in km^2. Suburban, Grass, Arable, Buildings, Roads: mean densities (proportions) of the habitat compositions within a 1 km radius of individual sampling location. Suburban includes Buildings and Roads in the area.

Site	Village	n (AM: AF: JM: JF: NA)	D	Patch	Suburban	Grass	Arable	Buildings	Roads
FF	Farnsfield	149 (47: 47: 20: 17: 18)	72	1	0.26	0.06	0.47	0.05	0.05
HM	Halam	6 (3: 1: 0: 0: 2)	5	0.15	0.08	0.08	0.54	0.01	0.03
KL	Kirklington	24 (5: 5: 5: 4: 5)	18	0.3	0.05	0.15	0.44	0.01	0.03
SW	Southwell	57 (11: 16: 0: 9: 21)	45	2.5	0.50	0.04	0.21	0.09	0.08
Overall		236 (66: 69: 25: 30: 46)							

Table 4.2. Link-based landscape composition. * Abbreviations: KL: Kirklington; FF: Farnsfield; HM: Halam; SW: Southwell. Geodist: geographic distance between pairwise site centroids in km. Suburban, Grass, Arable, Buildings, Roads: densities (proportions) of the factors calculated within a 1 km buffer around lines drawn between pairwise site centroids. Suburban includes Buildings and Roads in the area.

Pairwise sites	Geodist (km)	Suburban	Grass	Arable	Buildings	Roads
HM:KL	3.07	0.05	0.40	0.54	0.01	0.04
HM:SW	1.98	0.26	0.35	0.38	0.05	0.03
FF:HM	3.71	0.13	0.34	0.53	0.02	0.05
FF:KL	3.14	0.12	0.31	0.57	0.02	0.05
FF:SW	5.66	0.21	0.34	0.45	0.04	0.02
KL:SW	4.31	0.17	0.38	0.44	0.03	0.04

Between 2020 and 2021, 276 hedgehog samples were collected from a ~1 km^2 area in each suburban population. The majority of samples (n = 247) were hairs plucked from live hedgehogs during systematic spotlight transects. Additional soft tissue samples mainly from ears (n = 29) were collected from road killed hedgehogs within the suburban centres. Geographic coordinates, sex, and age information were recorded where possible. For hedgehogs with multiple captures, the midpoint between sampling coordinates was taken as the location of that hedgehog's sample. 'Juvenile' hedgehogs were defined as those born that calendar year. All tissue samples were stored in 50 ml of absolute ethanol in screw-topped

rubber-sealed falcon tubes and transferred to a -20 °C spark-proof freezer as soon as possible until DNA extraction.

Local hedgehog density (number of individuals per km^2) was estimated based on spatial-capture-recapture as part of a wider research project (Moore, 2023). Landscape composition was qualified using both node-based and link-based methods, with the former focusing on within-patch habitat availability and the latter inter-patch permeability (Dileo and Wagner, 2016). For node-based landscape composition, the density (proportion) of suburban, grass, and arable land within a 1 km radius of each sampling location was calculated based on UKCEH Land Cover Map 2021 (Marston et al., 2022), with all types of grasslands included as 'Grass'. In addition, densities (proportions) of buildings and roads were calculated for the same area based on Ordnance Survey Open Built Up Areas v.1.0, 2022, and Ordnance Survey Open roads v.2.4, 2023, respectively. As different base maps were used, some buildings and roads were included in the suburban category, thus densities (proportions) of these habitat compositions combined do not equal 1. The average landscape composition densities were calculated for each site to provide a within-site landscape composition (Table 4.1). For link-based landscape composition, densities (proportions) of the same variables were calculated within a 1 km buffer around lines drawn between pairwise site centroids (Table 4.2), using the same maps.

All hedgehog surveying and sampling were performed in accordance with ethical standards of the Animals (Scientific Procedures) Act, 1986, under a Natural England licence to capture and handle hedgehogs (2018-36011-SCI-SCI), and supported by Nottingham Trent University ethics committee (codes: ARE192014a and ARE192014b).

4.2.2 DNA extraction and genotyping procedures

DNA was extracted from all samples collected (n = 276) using an ammonium acetate precipitation method (Nicholls et al., 2000). Hair samples (n = 247) had 10 ml DDT (Dichlorodiphenyltrichloroethane) added to help digest the keratin protein in them.

Twenty-eight hedgehog-specific primer pairs (Becher and Griffiths, 1998; Henderson et al., 2000; Curto et al., 2019) were tested on the soft tissue samples (n = 29) using polymerase

chain reaction (PCR) methods to determine primer performance, optimise annealing temperatures, and to verify the expected amplicon length, using 5 replicates per sample. After optimisation, 12 primer pairs were removed due to difficulty in amplification or scoring, or non-polymorphism, resulting in 16 primer pairs remaining for amplification and informative analysis.

To genotype the hedgehog samples, the 16 primer pairs were combined into 3 multiplex PCR panels using Multiplex Manager (Holleley and Geerts, 2009) (Table 4.3), and amplified using the QIAGEN Multiplex PCR kit (Qiagen, Hilden, Germany), following the manufacturer's instructions. The genotyping PCR mixture (5 ul) consisted of 2 µl DNA template (approximately 5 ng of DNA; approximately 1 ng per ul PCR mixture), and 2 µl master mix supplied with the QIAGEN Multiplex PCR kit, and 1 µl primer mix (equally mixed). PCR amplifications were performed in an Applied Biosystems 2400 thermal cycler using an initial incubation step at 95 °C for 15 min to activate the QIAGEN HOTSTAR Taq DNA polymerase, followed by 44 cycles involving denaturation at 95 °C for 30 s, annealing at 58 or 59 °C (Table 4.3) for 60 s, extension at 72 °C for 60 s; followed by a final extension step at 60 °C for 30 min. On average 2.3 replicates (range 2–3) were performed for each PCR reaction. For genotyping, 1 µl of PCR product was diluted to a ratio of 1: 80 H_2O, and 1 µl of this solution was added to 9 µl formamide and 0.2 µl of GeneScan 500 LIZ Size Standard (Applied Biosystems, Warrington, UK).

An ABI 3730 48-well capillary DNA Analyser was used to separate the PCR products, and alleles were scored using GENEMAPPER v.3.7 (Applied Biosystems, California, USA). Only alleles with no mismatch in at least 2 replicates, and only individuals that amplified alleles for a minimum of 8 primer pairs, were retained (n = 239). Further, CERVUS v.3.0 (Kalinowski et al., 2007) was used to identify any identical genotypes that were potentially due to the recollection of road killed hedgehogs that were previously sampled. Three pairs of genotypes were detected as identical, so the profile with the fewer loci genotyped was removed. These resulted in a final analysis of genotype data from 236 unique individuals (Table 4.1). GenePop 4.0 (Raymond and Rousset, 1995) was used to test loci for deviation from Hardy-Weinberg equilibrium (HWE) and detect linkage disequilibrium between pairs of loci. Polymorphic information content (PIC), and null allele frequencies were estimated using the R package *hierfstat* (Goudet and Jombart, 2022).

Table 4.3 Multi-locus panels (Set) used for genotyping. Annealing temperature, allele size range for each primer pair. Each primer pair was assigned to a panel (MP Set, multipanel set: 1, 2, or 3) to minimise overlap, and labelled with a flurophore dye (Dye: 6-FAM, PET, VIC, or NED), as indicated. Source ref, Source reference: 1, Becher and Griffiths, 1997; 2, Henderson et al., 2000; 3, Curto et al., 2019. Forward and reverse primer sequence and motif are given. * EEU36H and W30 had low amplification success rates, and EEU36H was also not in Hardy-Weinberg equilibrium (HWE), possibly due to genotyping errors, thus were not included in the following analyses (although no obvious effects were found in the structure analyses including both loci).

Marker	Forward and reverse primer sequence (5'– 3')	MP Set	Dye	Annealing temp. (°C)	Range (base pairs)	Source ref.	Microsatellite motif	Chromosome
EEU1	ACCCACATCTTATGCCTTTCAGTGAT TAAATGTCAATGGCCATCTGTTATAACAA	2	FAM	59	132–149	1	(CA)15	14
EEU12H	CTGCATGTACCTCTCCTCTACCTC TTTTCTTTTTCCACCGGTGTTATC	2	PET	59	96–102	2	(CT)15	15
EEU2	GTAGGGACCGAGGGCTTGAACTG GACTGGCATTCACCCTAAAACACAT	3	FAM	58	257–266	1	(CA)18	19
EEU3	CAACAGAAGACAGGAGCAGATACAGG GAACTTCCACCAGAACATCAAGGCT	1	FAM	58	156–174	1	(CA)18	19
EEU36H*	GACTCTGGAACTCAAAACCAGG GGTAGACAGAGAGATCAAAAGGGA	2	VIC	59	149–151	2	(CT)21	2
EEU5	TGCATGAGGAACCAAATTCAA CAGCATGGATGTCCCACTACT	3	FAM	58	116–133	1	(CA)24	11
EEU6	CAGTGAAGTTAAGGGTGGCTTT TATGCTGGGTGGGTCTCTTCT	3	VIC	58	153–161	1	(GA)18	5
W10	ATAGCTGGATAGTGGTCTGG ACATCTTTTCTTCCTCACAGT	3	FAM	58	409–414	3	(AAAAC)7	12

W19	AGAGATCAGACTAACGTTTTT GGGGAGAATTTGGTACTGTA	1	NED	58	387–391	3	(TTCT)13	2
W29	CATTACCGTGCACACAGA GTTTGATCCCCACCACTTAA	2	NED	59	409–416	3	(CT)15	3
W30*	TCTCATTGGATAGTGCACTG TGCCTAATAGCAAATACACA	2	VIC	59	387–426	3	(CT)17	9
W32	CAGTCAATGCATTCCCAATC TGTGTGGTACAGGGAATAGA	1	VIC	58	414–416	3	(GT)13	20
W33	AGAAAAGACCTCAGGAGACT CCTGGAGAGTGGAAAAGTTA	1	PET	58	416–428	3	(CA)11	7
W7	TTAGCTTGGTTTTCACAGGT GAGTGGCAGTCTTCAAGTAG	1	FAM	58	394–419	3	(TCTTT)9	2
W8	ATAGGAGGACTGGCGATC AATGGAGGGAGTAGATGGG	2	FAM	59	357–397	3	(TTCCT)10	9
W9	TTCAATCTCAAGTACCACATT GATGCACCTGGTTGAGAG	3	PET	58	398–424	3	(TTTCT)10	10

4.2.3 Genetic structure and contemporary gene flow

4.2.3.1 Gene flow

Patterns of gene flow between sites were first investigated by analysing pairwise F_{ST} (Weir and Cockerham, 1984) using the package *hierfstat* (Goudet and Jombart, 2022).
We used boot.ppbetas (5000 iterations) for bootstrapping the F_{ST} (sensitivity analysis was done to determine the optimal number of bootstrap iterations). Patterns of gene flow were investigated further by undertaking an individual-based clustering analysis using the package LEA (Frichot and François 2015) as it is suggested to be more robust to Hardy-Weinberg equilibrium (HWE) assumptions (Frichot and François 2015) than commonly employed genetic clustering software like STRUCTURE (Pritchard, Stephens and Donnelly, 2000) or ADMIXTURE (Alexander and Lange, 2011). Ancestry coefficients were calculated for each individual, with K being set from 2 to 4 (the number of prior local populations), where K is the number of assumed ancestry populations, and coefficients were compared.

Gene flow was then explored using two genetic clustering methods: discriminant analysis of principal components (DAPC; Jombart et al., 2008), and spatial principal component analysis (sPCA; Montano and Jimbart, 2017), using the package adegenet (Jombart, 2008; Jombart, 2017). In DAPC, we used four sites as prior populations to show how the genetic distribution of the samples was related to their original sampled sites. And then in sPCA, allele frequencies and their spatial autocorrelation were analysed on an individual, rather than population, basis. sPCA allows tests of global and local spatial structure, with high global structure indicating that individuals are genetically similar to their geographic neighbours, and high local structure indicating genetic dissimilarity on local scales (Montano and Jombart, 2017). The genetic structure in sPCA was estimated from lagged scores summarizing genetic variability which also account for the geographic location of samples. The lagged scores of each component can be translated into a colour from the RGB colour channel such that the different shades of the red, green, and blue colour system give an indication of genetic differentiation with similar colours representing genetic similarity. In our analysis, the first two components were retained as suggested by the eigenvalues, and the results were plotted on 25 m land cover grids based on the UKCEH Land Cover Map 2021 (25 m rasterised land parcels, GB; Marston, et al., 2022), using the package terra (Hijmans et al., 2023). To detect any influence of sex on genetic structure, analyses were undertaken separately for all hedgehogs, adult males only, and adult females only.

4.2.3.2 Relatedness

Relatedness between groups of individuals was inferred using the package *related* (Pew et al., 2015), with group-based population simulations. As the accuracy of relatedness estimators is genetic-marker dependent and relies on the true relatedness being estimated on the population's relatedness structure, to determine which relatedness estimator is most appropriate for the dataset. The relatedness estimates of seven different estimators were compared, including two likelihood methods, dyadml and trioml, and five non-likelihood methods, lynchli, lynchrd, quellergt, ritland and wang, using simulated data from 100 individuals based on the existing genotype data and expected values of relatedness (e.g., 0.5 parent-offspring or full siblings, 0.25 half siblings, 0 unrelated). The Pearson's correlation coefficient between observed and expected relatedness for each estimator was calculated for

the simulated data, and the relatedness estimator with the highest correlation coefficient was selected to use in subsequent analyses.

To determine if hedgehogs within certain groupings were more related to each other than expected if randomly mixed with samples across the relative groups, further simulations were undertaken using the *grouprel* function. Group sizes were preserved, but individuals were shuffled randomly for 20–100 iterations based on sample sizes, providing a distribution of expected relatedness while assuming a random distribution of individuals across the relative groups. The expected relatedness was compared with the observed relatedness, and P-values were calculated based on the proportion of simulations that had average within-group relatedness greater than or equal to the observed value using the function *grouprel*. To assess relatedness, hedgehogs were grouped for 7 analyses: 1) 190 individuals of known age were grouped as 'juvenile' or 'adult'; 2) 135 adult individuals of known location were grouped into their four original sites (FF, HM, KL, SW); 3) 71 adult males of known location were grouped into their four original sites (FF, HM, KL, SW); 4) 64 adult females of known location were grouped into their four original sites (FF, HM, KL, SW); 5) 135 adult individuals were grouped by sex; 6) 93 adult individuals from site FF were grouped by sex; and 7) 27 adult individuals from site SW were grouped by sex. Separate analyses for adult hedgehogs from sites HM and KM were not undertaken due to insufficient sample sizes.

4.2.3.3 Genetic diversity

In order to characterize genetic diversity on different spatial scales, the following metrics were calculated across all sites overall, and for each site separately, using the package *hierfstat* (Goudet and Jombart, 2022): observed heterozygosity (H_O), expected heterozygosity (H_S = within sites; H_T = across sites), allelic richness (A_R), and inbreeding coefficient (F_{IS}), where high genetic diversity, indicative of increased gene flow, is associated with high heterozygosity and allelic richness, and a low inbreeding coefficient. We used boot.ppfis (5000 iterations) for bootstrapping the F_{IS} (sensitivity analysis was done to determine the optimal number of bootstrap iterations). Two methods were then used to investigate whether the observed genetic diversity within sites were impacted by sample size and habitat type: 1) heterozygosity values were calculated as an effect of increased sample size using the package *hierfstat* (Goudet and Jombart, 2022); and 2) 6–10 samples from a 100 m radius (except for the six samples from Halam, which were distant from more than 100 m but combined into

one group due to small sample size) around each sampling location were grouped, with locations with less than 6 samples excluded, and genetic diversity values calculated for each group using the package *sGD* (Shirk and Cushman, 2011), and visualised using the packages *sf* (Pebesma, 2018; Pebesma and Bivand, 2023), and *tmap* (Tennekes, 2018).

All data analysis was carried out in R Statistical Software (v.4.2.2; R Core Team, 2023).

4.3 Results

Data from 236 unique individual hedgehogs, genotyped using 16 markers, were included in the final analysis. Loci EEU36H and W30 had low amplification success rates, and EEU36H was also not in Hardy-Weinberg equilibrium (HWE), possibly due to genotyping errors, thus were not included in the following analyses (although no obvious effects were found in the structure analyses either including or excluding both loci). The remaining 14 loci were potentially from 12 different autosomal chromosomes. For loci located on the same chromosomes, no linkage-disequilibrium was detected between pairs of loci in more than 3 out of 4 sites. One locus (W10) showed a higher probability of the presence of null alleles but was retained as no obvious effects were found in the subsequent analyses (i.e., same genetic variation patterns were found either include or exclude the locus). No consistent linkage-disequilibrium was detected between pairs of loci in more than 3 out of 4 sites. The number of alleles per locus ranged from 2 to 7, the mean observed heterozygosity per locus was from 0.154 to 0.864 (Table 4.4).

Table 4.4. Indices of the Primer pairs across the 236 unique genotypes were included in the final analysis. * Abbreviations: Percentage of individuals genotyped (P_geno), number of alleles (*A*), mean observed heterozygosity (H_O), mean gene diversities within sites (H_S), overall gene diversities across sites with the whole population taken as panmictic (H_S), Inbreeding coefficient (F_{IS}) following Nei (1987) per primer pair, calculated using the package *hierfstat,* Polymorphic information content (PIC), average non-exclusion probability for the identity of: first parent (NE-1P), second parent (NE-2P), estimated probability of null-alleles F(null).

Marker	P_geno	*A*	H_O	H_S	H_S	F_{IS}	PIC	NE-1P	NE-2P	F(Null)
EEU1	0.822	2	0.255	0.254	0.251	-0.003	0.231	0.965	0.885	-0.017
EEU12H	0.966	3	0.558	0.457	0.458	-0.222	0.369	0.903	0.800	-0.075
EEU2	0.784	3	0.252	0.222	0.231	-0.135	0.301	0.946	0.834	-0.078
EEU3	0.881	5	0.684	0.695	0.717	0.015	0.714	0.649	0.471	-0.009
EEU5	0.860	3	0.668	0.603	0.598	-0.108	0.476	0.837	0.726	-0.065
EEU6	0.941	3	0.632	0.617	0.612	-0.024	0.478	0.838	0.723	-0.058
W10	0.839	2	0.182	0.211	0.210	0.138	0.204	0.973	0.898	0.108
W19	0.932	2	0.177	0.167	0.166	-0.063	0.141	0.988	0.929	-0.016
W29	0.856	3	0.463	0.505	0.507	0.083	0.449	0.865	0.739	0.075
W32	0.903	2	0.213	0.191	0.207	-0.120	0.190	0.977	0.905	-0.024
W33	0.894	3	0.154	0.143	0.145	-0.070	0.172	0.983	0.910	-0.046
W7	0.911	7	0.756	0.699	0.709	-0.082	0.678	0.689	0.512	-0.056
W8	0.847	6	0.864	0.778	0.785	-0.110	0.728	0.628	0.449	0.001
W9	0.881	6	0.681	0.597	0.605	-0.141	0.639	0.725	0.539	-0.052
Overall	0.880	3.571	0.467	0.439	0.443	-0.065				

4.3.1 Genetic structure and contemporary gene flow

F_{ST} values were low (95% CI values all cross zero) for all pairwise site comparisons, indicating that the local populations are not strongly genetically differentiated (Table 4.5).

Table 4.5. Pairwise F_{ST} (95% CI values based on bootstrapping for 5000 iterations) between sites. * Abbreviations: KL: Kirklington; FF: Farnsfield; HM: Halam; SW: Southwell.

Pairwise sites	F_{ST} (95% CI)
HM:KL	0.00 (-0.08–0.06)
HM:SW	0.01 (-0.06–0.07)
FF:HM	0.01 (-0.06–0.09)
FF:KL	0.01 (-0.04–0.08)
FF:SW	0.02 (-0.02–0.07)
KL:SW	0.02 (-0.04–0.10)

Individual-based clustering analyses failed to recover any discernible geographic population structure, with all sites containing a mixture of individuals assigned to each of the K population clusters, for each investigated value of K (Figure 4.2).

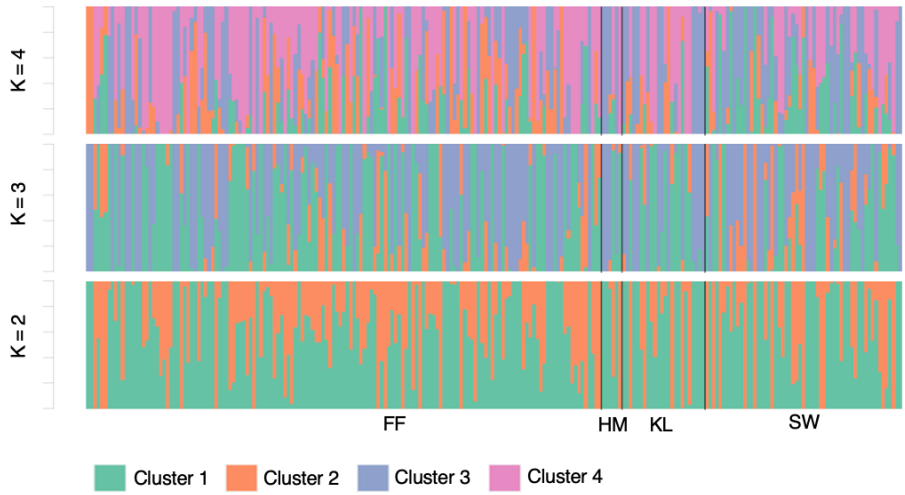

Figure 4.2. Genetic clustering for the 4 hedgehog populations. Estimated using the package LEA. Cluster proportions are showing in y-axis (range: 0–1), and each bar represents one hedgehog, for K = 2–4 . * Abbreviations: KL: Kirklington; FF: Farnsfield; HM: Halam; SW: Southwell.

Findings from the DAPC analysis with the four study sites as prior populations showed the genetic distribution of samples roughly mirrors geography, suggesting an effect of geographic distance on the genetic divergence. But notably, samples from each site were not discretely clustered, instead showing considerable overlap across sites, indicating an absence of discrete, well-structured populations (Figure 4.3).

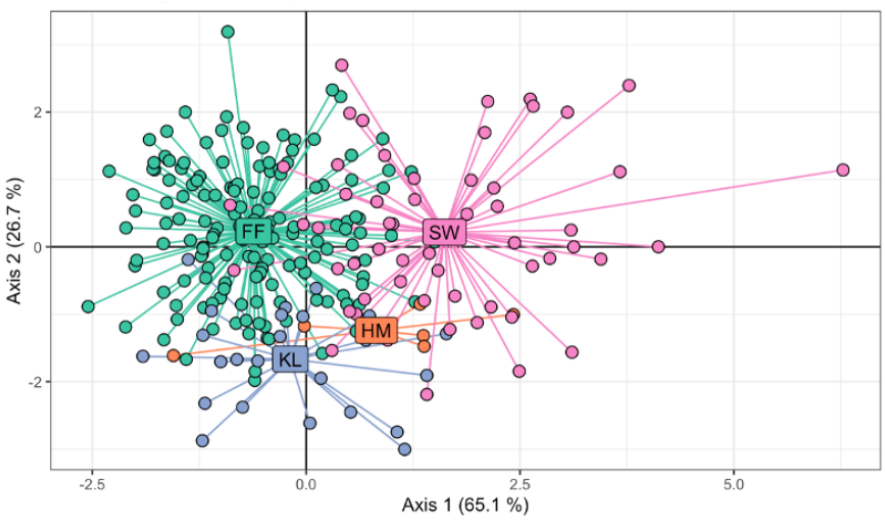

Figure 4.3. Cluster analysis of genetic variation using DAPC. n = 236, with the four study sites as the prior groups. Individual hedgehogs are represented by points and coloured by their sampled site (not genetic clusters). * Abbreviations: KL: Kirklington; FF: Farnsfield; HM: Halam; SW: Southwell.

Findings from the sPCA global structure analysis indicated that there was a significant positive spatial autocorrelation for individuals of all ages and sexes ($\lambda = 0.015$, n =183, $p < 0.001$; Figure 4.4 A), indicating that individuals were more genetically-similar to their close geographic neighbours. When analysing adult males only, the positive spatial autocorrelation becomes non-significant ($\lambda = 0.023$, n = 70, $p = 0.367$; Figure 4.4 B), whereas it is significant for adult females only ($\lambda = 0.032$, n = 64, $p = 0.013$; Figure 4.4 C). Tests for negative spatial autocorrelation (decreased genetic similarity between close geographic neighbours) found no significant correlation ($p > 0.05$) between genetic variation and geographic distance for any of the hedgehog groups (all: $\lambda = 0.009$, n =183; male only: $\lambda = 0.021$, n = 70; female: $\lambda = 0.024$, n = 64; Figure 4.4), showing no genetic dissimilarity on a local scale. The difference in positive spatial autocorrelation between the sexes indicates that gene flow is currently being maintained across the study area, and this is mainly driven by the males.

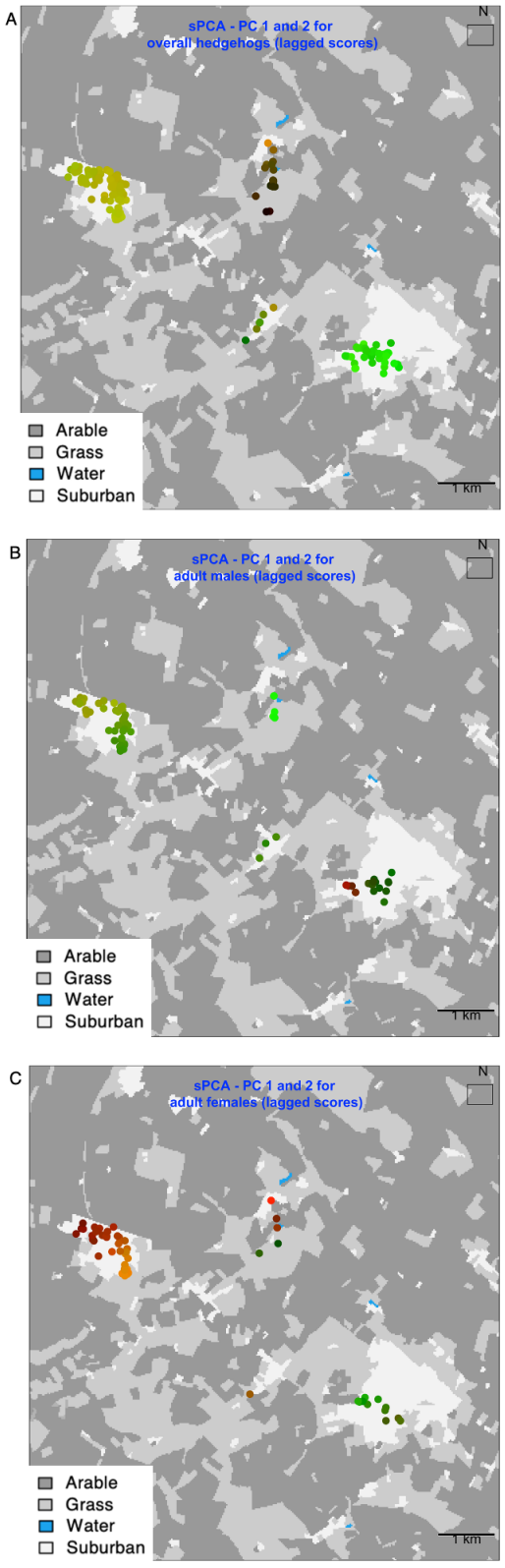

Figure 4.4. Spatial genetic variation of hedgehogs. Inferred from the spatial principal component analyses (sPCA), using the first two principal components. Points represented individual hedgehogs, with the similarity in the colours indicating genetic similarity. A: all hedgehogs, B: adult males, C: adult females.

4.3.2 Relatedness

The output from all of the seven estimators show similar results and all correlated to the expected values of relatedness (Figure 4.5). The Pearson's correlation coefficient from the lynchard estimator (0.8) was slightly higher than others and was, therefore, used for the relatedness analysis.

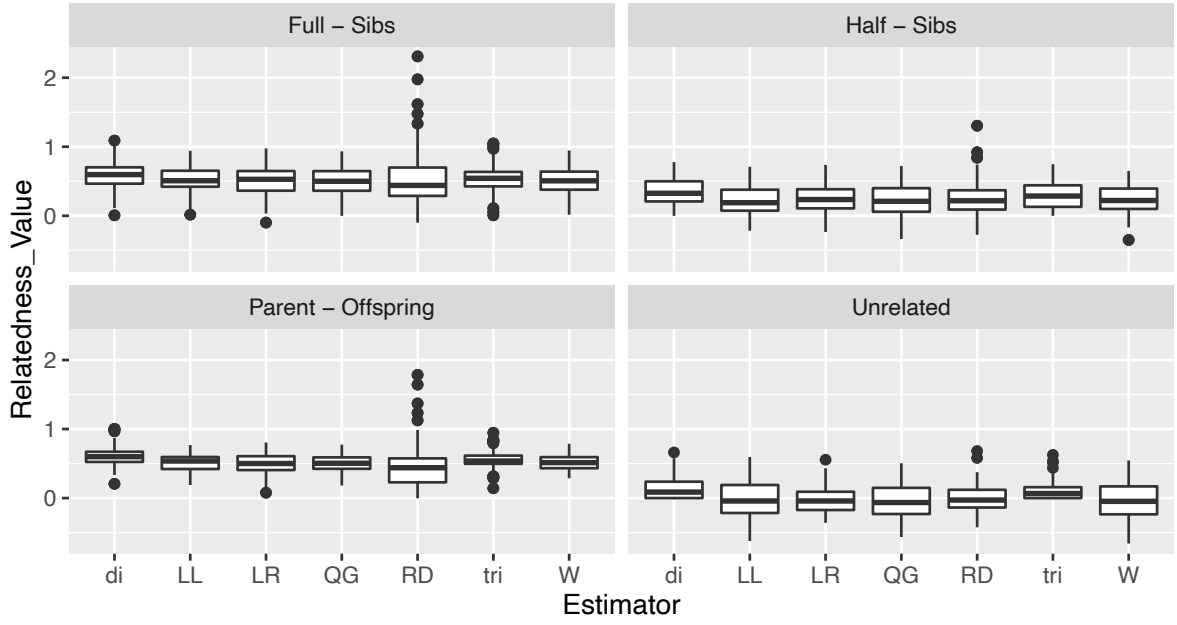

Figure 4.5. Performance of seven estimators of relatedness. * Abbreviations: di: dyadml; LL: lynchli; LR: lynchrd; QG: quellergt; RD: ritland; tri: trioml; W: wang.

A total of 190 hedgehogs of known age were grouped as 'juvenile' or 'adult' and were taken as input in one run using the package *related*: the juveniles showed significantly higher relatedness within-group (observed relatedness $r = 0.014$, $n = 55$, $p < 0.05$), and adults significantly lower relatedness within group ($r = -0.004$, $n = 135$, $p < 0.05$), than expected when being randomly mixed across age groups, indicating age effects on the relatedness potentially due to delayed natal dispersal and the lack of generation overlaps in juveniles. As our interest was to infer effects of potential dispersal (movement) on the relatedness patterns across the landscape, for the following analyses, juveniles were excluded and only adults were retained.

When the adult individuals of both sexes combined were grouped into their four original sites (FF, HM, KL, SW), individuals from the same site showed significantly higher relatedness than expected when being randomly shuffled with individuals across sites ($n = 135$, $p < 0.05$) for each site except for HM which had low sample size ($n = 4$), suggesting individuals from within-sites are more related than across sites. The two larger sites (SW and FF) had lower within-site relatedness than the two smaller sites (KL and HM), but relatedness values ($\leq$ 0.01; Table 4.6) were low for all sites (e.g., compared to the expected 0.125 between first cousins). Then, to infer sex effects on the relatedness distribution, this analysis was run for adult males and adult females separately. A significantly higher within-site relatedness than expected when being randomly shuffled with individuals of same sex across sites were only found for females only at site FF ($n = 47$, $p < 0.05$), potentially reflecting a reduction in statistical power compared to the combined-sex analysis.

4.3.3 Genetic diversity

Values for observed heterozygosity (H_O), expected heterozygosity (H_S), and allelic richness (A_R) were generally higher on larger suburban patches (e.g., SW) than smaller patches (e.g., HM) (Table 4.6; Figure 4.6 A–B), and this is not due to sample size effects (Figure 4.7), suggesting that the hedgehogs were unevenly distributed across the study area, indicating an early stage of varied genetic diversity in relation to habitat type. Mean A_R (2.45; values from the four sites averaged) was 30% lower than overall A_R (3.5; samples from the whole area were taken as panmictic; Table 4.6), showing reduced genetic diversity on local scales, which potentially reflects demographic change, whereas mean observed heterozygosity was close to overall heterozygosity, suggesting such demographic change happened in relatively recent times. Estimates of inbreeding coefficient F_{IS} values were low for all sites (95% CI values cross zero for FF, and HM, and being negative for KL and SW; Table 4.6), but some locations showed higher inbreeding (within 6–10 individuals per location) (Figure 4.6 C).

Table 4.6. Genetic diversity (n = 236) and relatedness (adults only, n = 135). Observed heterozygosity (*H*o), expected heterozygosity (within sites *H*s, across sites H_T), allelic richness (A_R), and inbreeding coefficient ($F_{IS;}$ bootstrapping for 5000 iterations). Mean: sum of each site/number of sites. Relatedness: lynchrd relatedness in adult hedgehogs. * Abbreviations: KL: Kirklington; FF: Farnsfield; HM: Halam; SW: Southwell.

Site	FF	KL	HM	SW	Mean	Overall
H_o	0.47	0.44	0.42	0.53	0.47	0.48
H_S , H_T	0.44 (H_S)	0.40 (H_S)	0.40 (H_S)	0.50 (H_S)	0.44	0.46 (H_T)
A_R	2.44	2.35	2.41	2.61	2.45	3.50
F_{IS} (95% CI)	-0.06 (-0.13–0.01)	-0.09 (-0.15– -0.03)	-0.04 (-0.19–0.11)	-0.06 (-0.11– -0.02)	-0.06	-0.05
Relatedness	0.01	0.07	0.04	0.03		

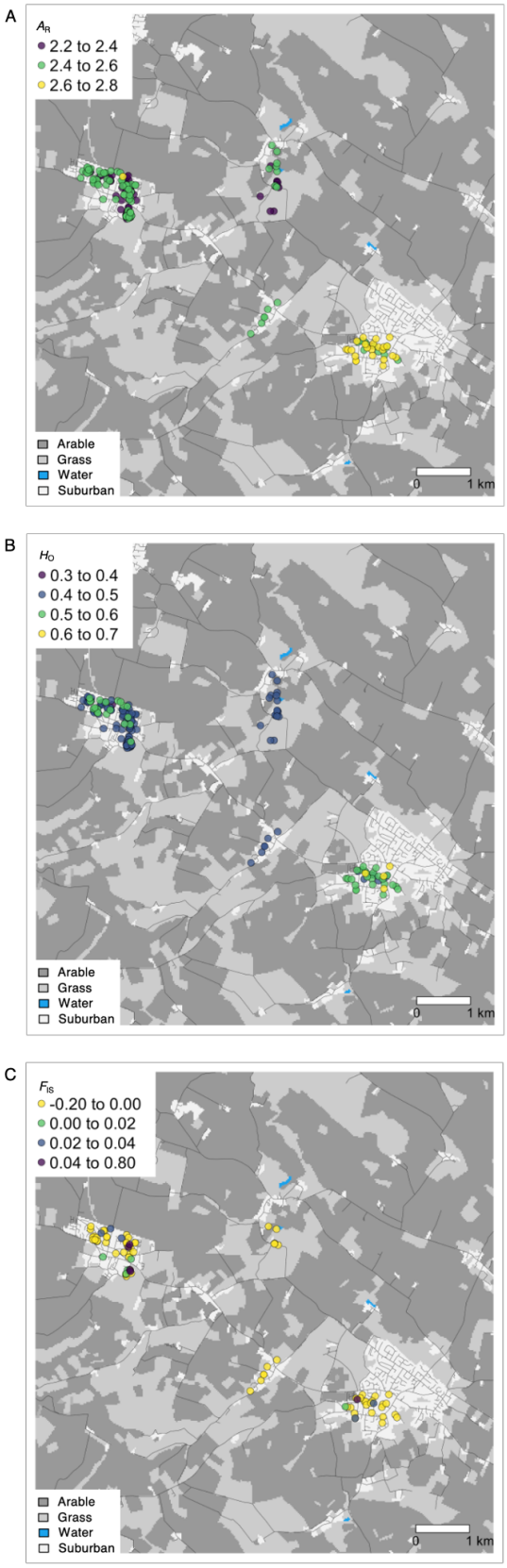

Figure 4.6. Distribution of genetic diversity. For groups of 6–10 samples within a 100m radius of each sampling location (n = 183). A: diversity allelic richness (A_R), B: observed heterozygosity (*Ho*), C: inbreeding coefficient (F_{IS}).

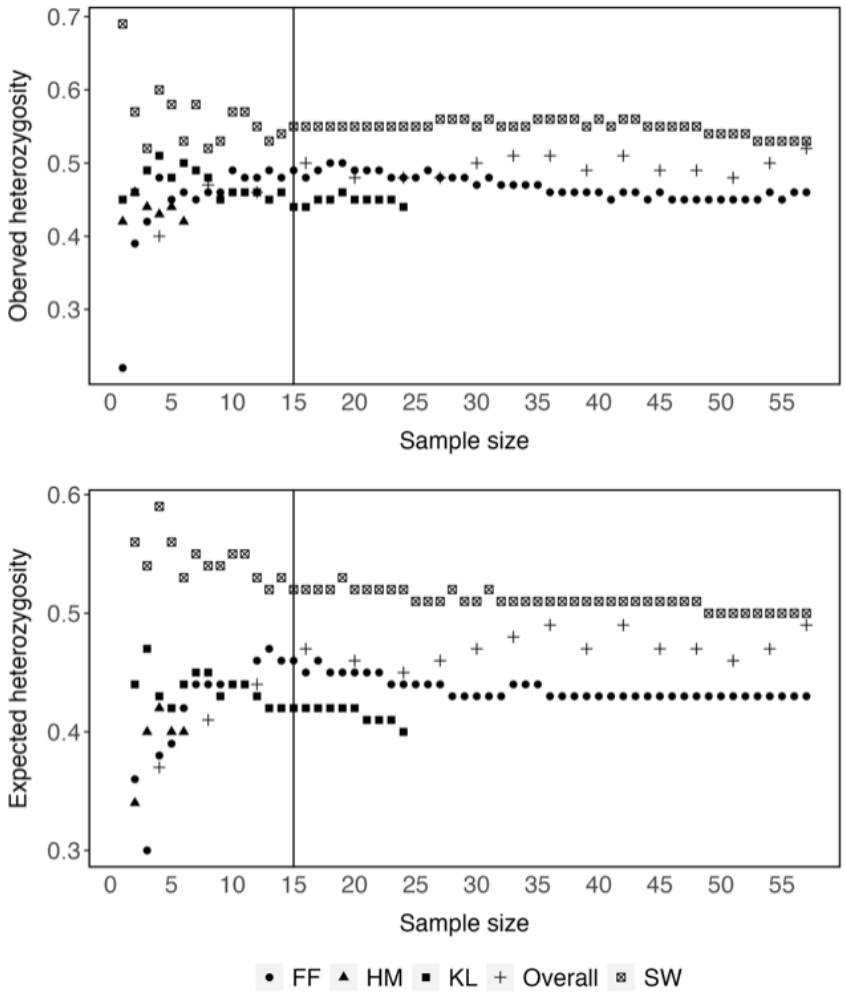

Figure 4.7. Observed (H_o) and expected heterozygosity (H_S) as a function of sample size. Samples were subsetted from single sites FF, HM, KL, SW, and across all sites (Overall); showing that heterozygosity values became less biased when the sample size reached around 6, and nearly stabilised around 15, and thus indicating that the varied genetic diversity values observed in this study were not due to sample size effects.

4.4 Discussion

In this study, we utilized the asymmetry in genetic variation between sexes as a powerful measure of current gene flow. This provided evidence of population-level, long-distance movement in a declining farmland mammal across a perceived inhospitable agricultural matrix. Smaller suburban patches had lower allelic richness and heterozygosity than larger patches (after accounting for potential effects of sample size), reflecting local population size,

suggesting an early stage of varied genetic diversity due to habitat loss and the associated fragmentation.

The low F_{ST} values between pairwise sites (95% CI values all cross zero), no private alleles in any sites, and little evidence of differentiation in structure across the sites, indicate that the hedgehogs across the study area still belong to one genetic population. The genetic similarity observed here is in accordance with studies in urban Berlin (Barthel et al., 2020), and urban Helsinki (Osaka et al., 2022), which also suggested that their hedgehogs were not genetically differentiated. This is, however, in contrast to studies in rural Oxfordshire (Becher and Griffiths, 1998), and urban Zurich (Braaker et al., 2017), in which distinct genetic differentiation patterns were observed. Nevertheless, the recent human-induced fragmentation, which is of direct conservation relevance, could not be concluded in these studies as influencing the observed genetic variation patterns as they were likely confounded by other factors, such as historical gene flow patterns. Such historical gene flow might obscure current fragmentation, leading to little to no genetic structure being detected. Conversely, where genetic structure is detected, it could be due to natural barriers and not necessarily due to recent landscape changes (Milligan et al., 2018; Lucena-perez et al., 2020).

To better understand contemporary gene flow, we used asymmetric genetic variation between sexes. For species exhibiting sex-biased dispersal (Li and Kokko, 2019), such as the hedgehogs, it is possible to detect contrasting genetic structure patterns between males and females using biparental inherited genetic markers. However, this is only possible where gene flow occurs between local populations, as the signal of this asymmetric sex-biased genetic structure is lost in just one generation if gene flow ceases (Prugnolle and de Meeus, 2002). Consequently, this method has the potential to reveal current gene flow without the confounding effects of historical gene flow (Solmsen, Johannesen and Schradin, 2011). We found a sex-biased difference in genetic variation, where the sPCA showed a significant positive spatial autocorrelation between allele frequency and geographical location in adult females ($\lambda = 0.032$, n = 64, p = 0.013; Figure 4.4 C), but not in adult males ($\lambda = 0.023$, n = 70, p = 0.367; Figure 4.4 B). This indicates that contemporary gene flow is occurring across the studied agricultural matrix, and is mainly driven by the movement of males.

This is the first evidence of the status of contemporary gene flow among local hedgehog populations on a landscape scale. Our results suggest that long-distance movement across the

agricultural matrix (at least 3 km here) in hedgehogs might be more extensive and frequent than often suggested by spatial-capture-recapture, and GPS tracking studies which are often short-term, small-scale, and restricted to adult hedgehogs due to ethical considerations (Glasby and Yarnell, 2013). For example, Pettett et al. (2017), estimated home ranges of adult females and males to be ~0.1, and ~0.2 km^2, respectively, and movements across agricultural matrix were rare. Our results suggest that hedgehogs require suitable corridors through which they can move between suburban population centres to maintain connectivity. Increasing the extent and quality of field margins (Yarnell and Pettett, 2020) and minimising road crossings that may act as barriers to hedgehogs (Moore et al., 2020; Moore et al., 2023) are likely to help facilitate such movement and reduce future risks of population fragmentation and isolation. Future studies should aim to use broad-scale population genetic structure to better understand matrix permeability, alongside the dispersal capabilities of hedgehogs, including what features and habitats they can navigate through so that they can be protected and maintained (Bowler and Benton, 2005).

Observed heterozygosity *H*o values were found higher than expected heterozygosity *H*e values, and inbreeding coefficient F_{IS} (95% CI values cross zero for FF, and HM, and being negative for KL and SW) were low for all sites. This pattern can arise due to multiple factors, including heterozygote advantage, gene flow (migration), or the Wahlund effect (population substructure). Given that contemporary gene flow was found to have been connected between populations in structure analyses (mainly mediated by movement of males), the most likely explanation for this pattern is gene flow, although heterozygote advantage cannot be entirely ruled out. As such, although some locations showed higher inbreeding than others (within 6-10 individuals per location; Figure 4.5 C), our results suggest a generally lack of inbreeding in all local populations, potentially indicating a large population size of the hedgehogs on a metapopulation level.

However, higher within site relatedness, and lower allelic richness were found on smaller suburban patches than larger patches (after accounting for potential effects of sample size), largely reflecting lower local population sizes, which suggests an early stage of genetic diversity loss in relation to small suitable habitat patches and associated fragmentation. Mean allelic richness across all four sites was lower than overall richness, while mean observed heterozygosity was close to overall heterozygosity, further suggesting the populations in this study were showing signs of recent habitat fragmentation. This is based on the theoretical

prediction that allelic richness for neutral loci generally respond more strongly and rapidly to demographic change than heterozygosity when the population is experiencing recent decline and restricted gene flow (Barrandeguy and García, 2021). Our results, thus highlight that for declining species which often show varied local density, variability in spatial patterns of genetic diversity can happen at a fine scale, even when some gene flow is still apparent and population differentiation is negligible. Similar results, i.e., reduced local genetic diversity despite large-scale gene flow, were also found in the declining common woodland birds in Australia (Harrison et al., 2012). This suggests that although population differentiation is generally suggested to proceed faster than loss of genetic variation following habitat disruptions (e.g., Keyghobadi 2005), this might not always hold true, especially for species that are still with large population sizes, or that are mobile enough to maintain gene flow, but suffering different levels of local declines. Our results thus illustrate that considering current gene flow and local genetic diversity together is important to better understand habitat effects on genetic variation and to inform conservation management.

4.5 Conclusion

We have demonstrated several unexpected findings for the genetic status of a declining common mammal species across an agricultural matrix. The most important finding was that, despite the small size and high temporal stability of adult hedgehog home ranges, there is clear evidence that long-distance movement (at least 3 km) in hedgehogs is more frequent than previously thought based on home ranges. This finding is crucial to aid the development of a conservation strategy for hedgehogs as, for the first time, it shows the extent that previously assumed isolated populations across a perceived inhospitable landscape are connected by current gene flow. Higher within population relatedness, and lower allelic richness were found on sites with lower suburban land cover, largely reflecting local population size, indicating an early stage of reduced genetic diversity in relation to habitat loss and associated fragmentation. We suggest hedgehog conservation can aim to prevent further declines by identifying what features are needed to facilitate hedgehog movement between population centres and facilitate improved connectivity and resultant gene flow.

4.6 References

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Chapter 5: Population genomics of British hedgehogs

Abstract

The hedgehog is reported to be declining in both abundance and distribution across Western Europe. So far, data regarding the genetic status of the species are scarce, and its large-scale population structure, genomic diversity, and demographic history are largely unknown. Factors shaping the structure of the population are contentious. Often, where differentiation in genetic structure is shown, effects of natural processes, anthropogenic barriers, the species' site fidelity, or ascertainment bias in sampling might be confounded, and where the differentiation in genetic structure is lacking, time lag effects, being masked by low genetic diversity, or admixture due to released rescued hedgehogs, are apparent. Here, we present the first whole-genome sequencing of the hedgehog, with 123 individuals geographically-evenly sampled across Great Britain. A generally continuous genetic variation distribution was observed, highlighting gene flow on large geographic scales. Moderate genetic diversity was retained, other than that in some island hedgehogs. Recently disrupted inbreeding coefficients and recent population decline were detected, likely at least partially due to the agricultural intensification. Our findings suggest that the previous concerns of isolation of populations and low genetic diversity are yet to be fully realised, but early signatures of such problems were detected meaning action maintain connectivity across large spatial scales to improve local habitat quality and maintain connectivity across large scales would help maintain hedgehog genetic diversity.

Keywords: population history, genetic structure, genetic diversity, demography, *Erinaceus europaeus*

5.1 Introduction

Declines in common species due to human-induced habitat disruption constitute an important part of biodiversity loss (Dirzo et al., 2014; Seddon et al., 2014; Ceballos et al., 2017; Leigh et al., 2019; Finn, Grattarola and Pincheira-Donoso, 2023; van Klink et al., 2024). Detecting and preserving the declining populations of these species is paramount, since population parameters such as demography, connectivity, and genetic diversity, often need to be evaluated on large geographic scales (Allendorf, 2017). While population genetics is useful in informing broad-scale population patterns (Allendorf, 2017), it can be difficult to attribute current genetic patterns to specific demographic events, e.g., either natural (Carroll et al., 2020) or human-induced (Lucena-Perez et al., 2020; Pozzi et al., 2023), which limits conservation actions. Traditional genetic markers such as microsatellite or mitochondrial DNA often lack spatial and temporal resolution to disentangle the effects of different demographic processes on the populations (McMahon, Teeling and Höglund, 2014). Recent studies have shown that whole-genome sequencing, combined with newly developed population genomic statistics, can help reconstruct population history by retrieving genomic signatures, enabling the effects of different processes to be evaluated better (Bradburd, Coop and Ralph, 2018; Milligan et al., 2018; Leitwein et al., 2020). However, this often requires an understanding of the distribution of genetic variation across habitats and geographic regions (Lucena-Perez et al., 2020; De Jong et al., 2023), which is lacking for many common species. We here present the first whole-genomic sequencing of the Western European hedgehog (*Erinaceus europaeus*; hereafter termed hedgehog), sampled across Great Britain, United Kingdom (UK), where the population is considered to be suffering a range of genetic and ecological pressures, and represents a suitable model for exploring the genetic structure and diversity patterns of a common species that faces anthropogenic habitat disruptions.

The hedgehog is widely distributed across mainland Great Britain and many of the surrounding islands (Morris, 2018). The colonisation history of the species in these areas is unclear, which hinders the interpretation of local genetic patterns. The only range-wide phylogenetic study for the species found that hedgehogs from Britain belong to the same genetic clade as those from France and Spain (Seddon et al., 2001). However, as only two mitochondrial haplotypes were found in the British hedgehogs, whether they colonised Britain naturally or not cannot be further explored with the data, e.g., through investigating the geographic patterns of the genetic variation. It is thus not clear whether hedgehogs spread

to Britain naturally during the short period of existence of land bridge between Great Britain and mainland Europe in the interglacial period (around 9000 kya; thousand years ago; Preece, 1995) or whether the population was recently introduced by humans (Seddon et al., 2001; Bolfikova et al., 2013).

To date, few genetic studies of hedgehogs have been conducted in the UK. For example, hedgehogs in Regent's Park, London have low genetic diversity (O'Reilly, 2016), whereas in Oxfordshire they have moderate genetic diversity, with distinctly differentiated populations (Becher and Griffiths, 1998). Although both findings are explained as a consequence of recent fragmentation, multiple explanations could apply in each case, which hampers interpretation and is less likely to inform conservation interventions. For instance, the low genetic diversity found in the London population could reflect high levels of inbreeding, which may be related to intense or long-term fragmentation, or recent or historical founder effects. In Oxfordshire, the moderate genetic diversity and high differentiation between local populations may reflect recent fragmentation, or other processes where increased genetic drift has occurred. By comparing genetic variation on a broad scale, it is possible to interpret whether the identified low diversity and high differentiation are local context-specific patterns, or general features of hedgehogs in the UK which are probably due fragmentation, founder effects, fragmentation, or intrinsic factors such as site fidelity.

In Europe, interpretation of previous genetic results is also hampered by the unknown large-scale structure patterns of the species. Studies have suggested various anthropogenic factors limiting hedgehog distribution and movement, e.g., agricultural lands (Hubert et al., 2011), highly urbanised city centres (Turner, Freeman, and Carbone, 2022), and roads (Huijser and Bergers, 2000). However, how these factors would affect the landscape-scale gene flow cannot be concluded from previous genetic studies, representing a long-standing conservation question. Often, where differentiation in the genetic structure was shown, the effects of natural barriers such as water bodies or mountains, the species' site fidelity, or ascertainment bias in the sampling or analysis, might have been confounded (Becher and Griffiths, 1998; Braaker et al., 2017; Araguas et al., 2022); and where differentiation in genetic structure was found lacking, the potential time lag effects, being masked by low genetic diversity, or admixture due to released hedgehogs from rescue centres, could all be causal (Barthel et al., 2020; Rasmussen et al., 2020; Osaka et al., 2022). Further, previous genetic studies in hedgehogs were mainly based on microsatellite genotyping (e.g., Braaker et al., 2017), with a

few based on or combined with mitochondrial DNA (e.g., Osaka et al., 2022), or Reduced representation sequencing approaches (e.g., Rasmussen et al., 2020). These results are important for us to understand the local genetic variation though, often lack of efficiency in facilitating comparisons among studies. So far, genome-wide genetic diversity and historical demography of the hedgehog have not been investigated, and whether the genetic consequence of natural or anthropogenic effects would represent a significant underlying driver of the current observed decline (Spielman, Brook and Frankham, 2004), and whether the current decline is part of a prolonged historical decline are yet unknown, which are concerning given that these potential effects have been found in even apparent large continuous populations (Hoelzel et al., 2012; Gousy-Leblanc et al., 2020).

The aim of this study was to assess genetic diversity and structure patterns for the British hedgehogs and to understand the contribution of historical and contemporary processes shaping their demographic history. We hypothesised that:

1. the hedgehog colonised Britain naturally. If it was a single colonisation, i.e., all present-day hedgehogs descend from one ancestral population post-LGM (Last Glacial Maximum), then we would expect to observe a continuous south-to-north cline in genetic variation, in accordance with serial founder effects along the northward expansion routes from the south. If it was two or multiple colonisations, then the south-to-north cline would be lacking. Instead, deep differentiation due to being isolated historically in different glacial refugia, might be observed, although more complex scenarios could be possible. Alternatively, if the population was established by human-induced hedgehog translocations, various genetic diversity centres and relatively low genomic diversity might be found.

2. the historical population was ubiquitous. If the individual heterozygosity is continuously distributed, then it is largely retained and it supports a ubiquitous distribution of the hedgehogs during historical times. Otherwise, if individual heterozygosity is patchily distributed, coinciding with the current varied population distribution, e.g., higher in urban areas and lower in rural areas, the hedgehogs might have always been patchily distributed since historical times, or have been through severe loss of genetic diversity during recent times.

3. local hedgehog populations are structured by landscape features, such as rivers, mountains, agricultural lands, city centres, and roads, assuming a low dispersal ability of the species. Alternatively, if at least some hedgehogs are capable of surmounting the potential barriers, little structure across large regions would be observed.

4. the population is suffering from recent habitat disruptions. We infer temporal effective population sizes and temporal inbreeding coefficients of the hedgehogs, and expect recent patterns to be different from historical patterns, reflecting recent anthropogenic effects on the hedgehogs. Alternatively, if recent patterns are similar to historical patterns, the effects of recent habitat disruptions might not be prevalent.

5.2 Materials and methods

We used 123 samples with approximately even sampling distribution across Great Britain to infer population genomic patterns (Lotterhos and Whitlock, 2015; Meirmans, 2015), and one sample from Denmark to serve as outgroup in structure analysis (Sedden et al., 2001). We additionally included published data from an individual from New Zealand (NCBI accession number: SAMN12110467) in heterozygosity and historical demography analyses. DNA was extracted from tissue samples and sequenced using Illumina technology to an average depth of 5.45x (95% CI 5.01–5.89x; Appendix II, Table 5.1).

5.2.1 Dataset, sampling, laboratory procedures, and data processing

Tissue samples from 123 British hedgehogs collected post mortem from 2011 to 2023 inclusive were utilised in this study. Seventy-six of the samples were selected from the frozen archive of the Garden Wildlife Health project, a general wildlife health surveillance scheme in Great Britain co-ordinated by the Zoological Society of London (www.gardenwildlifehealth.org), which conducts post-mortem examinations on wild animals, including hedgehogs. These samples were strategically supplemented mainly from roadkill collections to provide even spatial coverage. The average distance between sampled British hedgehogs was 37 km (95% CI 36–37 km). The sampling map was generated using the R package tmap (Tennekes, 2018) with the map of Great Britain (http://www.diva-gis.org/gdata to directory ./spatial, Accessed 10 October 2023) and Hadrian's Wall (https://data.ncl.ac.uk/articles/dataset/Hadrian_s_Wall_-_Frontier_system/11855592

to ./spatial, Accessed 16 December 2023; Hingley, 2012) as backgrounds. The Danish hedgehog was collected from Zealand, Denmark. Samples were stored in absolute ethanol or at -20 ºC prior to DNA extraction. DNA was extracted using a commercially available kit (DNeasy Blood & Tissue Kit, QIAGEN) following the manufacturer's protocol. Extracted DNA was quantified using a Qubit 3 fluorometer and dsDNA HS assay kit, following the manufacturer's instructions. Library preparation and whole-genome sequencing were carried out by an external commercial facility (Novogene UK Ltd., Cambridge), using an Illumina NovaSeq platform, producing paired-end 150bp sequencing reads, aiming for 6x mapped read depth.

Data processing was carried out within the BEARCAVE data analysis environment (adapted from https://github.com/nikolasbasler/BEARCAVE), involving trimming adaptor sequences and removing reads < 30 bp using Cutadapt v. 1.18 (Martin, 2011), and merging overlapping paired-end reads using FLASH v. 1.2.11 (Magoč and Salzberg, 2011). The processed reads were then mapped to the reference genome mEriEur2.1 (GenBank number: GCA_950295315.1) using the BWA v. 0.7.17 (Li and Durbin, 2009) mem algorithm and SAMTools v. 1.17 (Li and Durbin, 2009), filtering for mapping quality (-q 30) and potential PCR duplicates (mkdup). The final mapped read depth was calculated using ANGSD v. 0935 (Korneliussen et al., 2014) by setting a minimum base quality score of 30 (-minQ 30), and a minimum mapping quality score of 30 (-minMapQ 30).

5.2.2 Nuclear genome analyses

Nuclear genome analyses were restricted to autosomal chromosomes. The software ANGSD v. 935 (Korneliussen et al., 2014) was chosen for a series of analyses as it can overcome the biases that may arise due to differential coverage across the genome. Instead of other methods that rely on direct SNP/genotype calling from the data, ANGSD uses genotype likelihoods in downstream analyses. This allows the incorporation of statistical uncertainties into the analysis which, in turn, reduces biases caused by differential coverage across the genome. For analyses involving ANGSD, the following filtering options were applied: minimum base quality score 30 (-minQ 30), minimum mapping quality score 30 (-minMapQ 30). Population genomic analyses were performed requiring a minimum mapping depth of 3 reads (-setMinDepthInd 3), and requiring data for > 75% of individuals (-minInd). A minimum allele frequency of 25% is required (-minFreq 0.25), with the major/minor alleles

determined from genotype likelihoods. R version 4.3.1 (R Core Team, 2023) was used for all analyses unless otherwise specified.

5.2.3 Population structure

Patterns of population structure among British hedgehogs were first inferred using principal component analysis (PCA; Menozzi, Piazza, and Cavalli-Sforza, 1978). An allele covariance matrix was generated in ANGSD using majority-base sampling (-doIBS 2) requiring a minimum base quality score of 30 (-minQ 30) from reads with a minimum mapping quality score of 30 (-minMapQ 30), a minimum mapping depth of 3 reads (-setMinDepthInd 3), requiring data for > 75% individuals (-minInd 93), and a minimum allele frequency of 25% (-minFreq 0.25), with the major and minor alleles determined from genotype likelihoods (-doMajorMinor 1). This filtering resulted in 1,009,243 sampled variable sites. The allele covariance matrix was then used as input for PCA in R using the "eigen" function.

A Neighbour-joining (NJ) tree was calculated, rooted with the Danish hedgehog. ANGSD was used to calculate a distance matrix for the 124 hedgehogs (123 British, and one Danish) using the filters described above, which resulted in 1,033,603 sampled variable sites. The NJ tree was then generated in R using the APE v. 5.7-1 library (Paradis and Schliep, 2019).

To investigate individual ancestry proportions and gene flow we used NGSadmix v. 32 (Skotte et al., 2013) which is a maximum likelihood (ML) implementation of STRUCTURE (Pritchard, Stephens and Donnelly, 2000), that can use genotype likelihoods as input. First, ANGSD was used to calculate genotype likelihoods based on SAMtools model (-GL 1), using the filters described above with the addition of a maximum mapping depth of 12 (-setMaxDepthInd 12), and SNP p-values of $> 1 \times 10^{-6}$ (-snp_pval 1e-6). This filtering resulted in 1,192,263 sampled variable sites. The number of ancestral populations, K, was set from 2 to 4. The resulting estimated admixture proportion files were then processed in R and displayed as colour-coded bar plots. To investigate isolation by distance, the above BEAGLE file, containing the genotype likelihoods, was used as input in ngsDist v. 3 from ngsTools (Fumagalli et al., 2014) for generating individual pairwise genetic distances. These were then plotted against individual pairwise Euclidean geographic distances in R. The geographic distances were generated using the R packages sf (Pebesma, 2018; Pebesma and Bivand, 2023) and metagMisc (Mikryukov, 2017).

5.2.4 Effective migration and diversity surfaces

Effective migration between neighbouring demes and diversity within demes were investigated using Estimation Effective Migration Surfaces (EEMS; Petkova et al., 2015). EEMS uses the average genetic distance across markers to explicitly model the effective migration rate (m) between neighbouring demes and diversity within each deme (d) to approximate a continuous genetic pattern, while drawing attention to where variation is elevated or depressed relative to expectations from the geographic distance, thus it can help account for effects of geographic distance. For generating migration and diversity surfaces, EEMS analysis was carried out based on the above individual pairwise genetic distance calculated in ngsDist. The coordinates for the habitat boundaries were generated with an online Google Maps API tool (http://www.birdtheme.org/useful/v3tool.html). 1,000,000 burnin and 20,000,000 sampling iterations (thinning every 999 iterations) were used. The number of demes was set as 50, 100, and 200, based on our sampling density. The estimated migration and diversity surfaces were plotted using the R package rEEMSplots, which is included in the EEMS software.

5.2.5 Genome-wide heterozygosity estimates

Site allele frequencies were calculated for each sample in ANGSD, assuming the reference genome as the ancestral state, using the base and map quality filters described above, and requiring a maximum read depth less than two times the mapped read depth, and a minimum read depth higher than half the mapped read depth if > three, otherwise it was set as three. realSFS in ANGSD was then run using 100kb non-overlapping windows and genome-wide heterozygosity calculated by summing the total number of polymorphic sites and dividing by the total number of sampled sites across all windows. As the average mapping coverage varied among samples, we investigated the effect of coverage on our heterozygosity estimates. We down-sampled the high-coverage (28x) genome data from the New Zealand hedgehog to different levels of coverage and estimated heterozygosity. A steady and predictable increase in mean heterozygosity was found with increasing mapping depth (Figure 5.1; Appendix II, Table 5.2). We thus corrected the heterozygosity estimates calculated from medium-coverage British hedgehog datasets to that predicted for the high-coverage individual, based on the results of downsampling (Appendix II, Table 5.2).

Heterozygosity was mapped using the R package sf. To investigate any relationship between latitude and heterozygosity we performed linear regressions for heterozygosity and latitude, via the R package ggplot2 (Wickham, 2016).

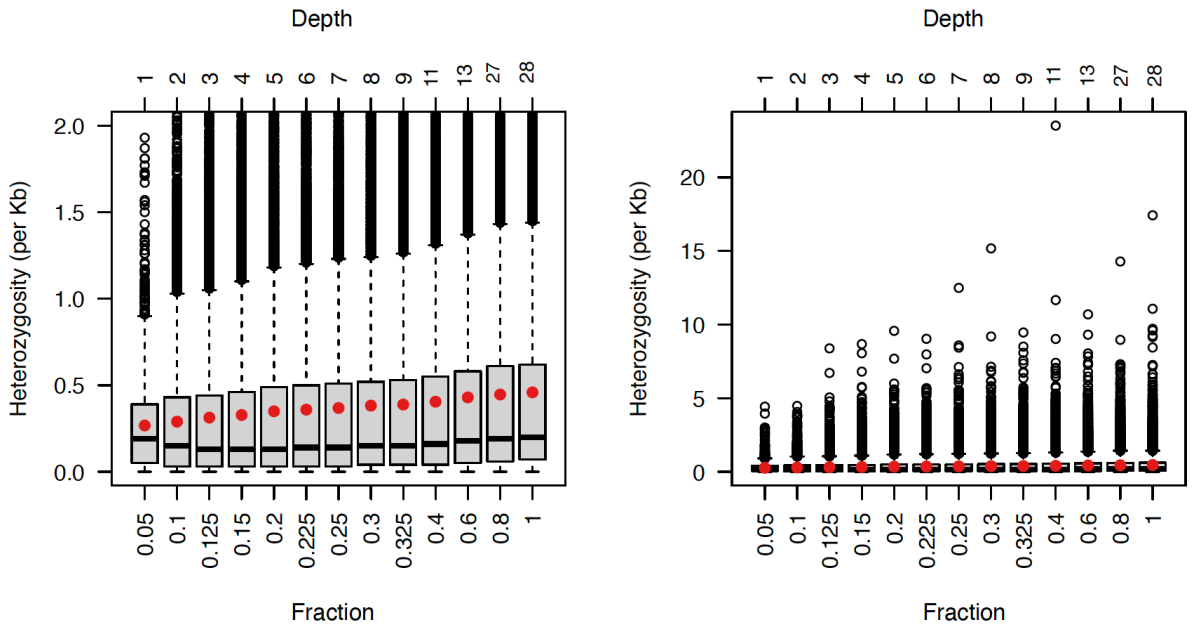

Figure 5.1. Relationship between heterozygosity and mapped read depth. Plotted with different y-axis scales. Fraction: the fraction of the downsampled dataset to the 28X dataset. Depth: mapped read depth of each downsampled dataset.

5.2.6 Summary of runs of homozygosity

A run of homozygosity (ROH) segment is a contiguous region of an individual's genome where only homozygous genotypes are observed. These homozygous genotypes are chromosome segments inherited identically by descent from a recent common ancestor through both maternal and paternal lineages (Thompson, 2013). A key feature of these segments is that filtering them by length provides a means to interrogate the approximate timescale of inbreeding. The longest segments reflect recent episodes of inbreeding whereas shorter segments reflect older episodes (Thompson, 2013). The fraction and number of ROH (FROH and NROH respectively) within individual genomes reflect the population's demographic history. Large populations generally have lower FROH and NROH. FROH and NROH can be increased following colonisation routes due to series founder effects and will

be exacerbated in small populations or populations that have experienced recent bottlenecks (Foote et al., 2021; De Jong et al., 2023).

Since homozygous tract length (L; Mb) declines as a function of recombination rate (r, cM/Mb) and time (g, the number of generations back to the most recent common ancestor for the two homologous sequence copies within an ROH), the expected length of ROH is L = 100/(2g*r) (Thompson, 2013). For example, 0.1 Mb ROH in hedgehogs would correspond to two IBD tracts coalescing in a parental common ancestor 330 generations ago (assuming a recombination rate, r, equivalent to that in mole rats, *Spalax galili*; 1.515 cM/Mb; Li et al., 2015), which equates to 660 years ago (assuming a generation time of 2 years of hedgehogs; Morris, 2018).

Heterozygosity was calculated for 50 kb non-overlapping windows as described above. We allowed up to 2 heterozygote sites per 50 kb window, and then a length of at least two consecutive such windows was required to call an ROH. The minimum length of the called ROHs was 100 kb and increased in steps to 50 kb. We summarised the following statistics: fraction of the mapped autosomal genome in ROH of all lengths (FROH) to represent the overall inbreeding coefficient, and the ROH fraction within the following length bins: 100–300 kb, 300–500 kb, 500 kb–1.5 Mb, and > 1.5 Mb, to approximate coalescence before 660–200, 200–130, 130–40, and within 40 years ago; number of ROH fragments (NROH) within the length bins. The individual FROH, and NROH > 1.5 Mb (representing the more recent episodes of inbreeding) were mapped based on geographic locations using the Great Britain map and Hadrian's wall as backgrounds, with both using the R package sf, and FROH and the average length of ROH (Mean length of ROHs (Kb)) were plotted in as scatterplot, via the R package ggplot2.

5.2.7 Demographic inference

The demographic history, i.e. temporal fluctuations in effective population size (Ne) was reconstructed using the pairwise sequentially Markovian coalescent PSMC (Li and Durbin, 2011) and Stairway Plot 2 (Liu and Fu, 2020), for the time periods > 8,000 years ago and 8,000 years ago to near present, respectively. This is because PSMC tends to perform best at inferring relatively ancient demographic histories as the density of coalescent events tends to

increase at deeper time scales (Terhorst, Kamm and Song, 2017) but suffers from imprecise estimates toward the present as a consequence of limited recent coalescent events (Liu and Fu, 2020; Terhorst, Kamm and Song, 2017). In contrast, Stairway plots 2 which depends on the SFS (site frequency spectrum) tends to perform best toward the present, as more ancient inferences are confounded by the impacts of recent demographic changes and saturation of sites present in the SFS (Liu and Fu, 2015). For both PSMC and Stairway Plot 2, plots were calibrated using a mutation rate of 3.03 x 10^-9 per site per generation (as calculated for mole rats, *Spalax ehrenbergi*; Li et al., 2020) and a generation time of 2 years of the hedgehog (Morris, 2018).

PSMC analysis (Li and Durbin, 2011) was carried out with the high-coverage (28x) dataset from the New Zealand hedgehog, which is a descendant of a population that was translocated from Great Britain around 150 years ago (Bolfíková et al., 2013) and thus can represent the historical demography of the British hedgehog. PSMC analysis was also performed for the New Zealand hedgehog, downsampled to 8x to show how the coverage would affect the demographic reconstruction, as well as for two samples (5x, 6x) from Scotland and three (5x, 6x, 8x) from England and Wales to compare historical demographic patterns of these hedgehogs. For each of the above dataset, heterozygous positions were recovered using bcftools (Li et al., 2009), filtering data for low mapping (< 30) or base quality (< 30). Minimum and maximum depths were set at half and double the coverage of each dataset, respectively. PSMC was run using maximum numbers of iterations (-N) 25, maximum 2N0 coalescent time (-t) 15, initial theta/rho ratio (-r) 5, and parameter pattern (-p) 4+25*2+4+6. Twenty bootstrap replicates were performed for each run, using random re-sampling with replacement. Stairway Plot 2 analyses were carried out for all hedgehogs from south of Hadrian's Wall, and all from north of Hadrian's Wall excluding individuals from the translocated Orkney population. We first generated unfolded population-based SFS in ANGSD using 100 kb moving windows, then carried out the Stairway Plot 2 analysis with default parameters: with 67% of sites for training, four different breakpoints (nseq/4, nseq/2, nseq/4*3, nseq-2 where nseq is 2*number of individuals), and 200 bootstrapping rounds. Plots were produced using the R package ggplot2.

5.3 Results

5.3.1 Data

We generated whole-genome sequencing data from 124 hedgehog samples, comprising 123 from Great Britain (Figure 5.2, with orders listed; Appendix II, Table 5.1), and one from Denmark. Together with one previously published individual from New Zealand (NCBI accession number: SAMN12110467), our dataset provides genome-level data from 125 hedgehogs (Appendix II, Table 5.1).

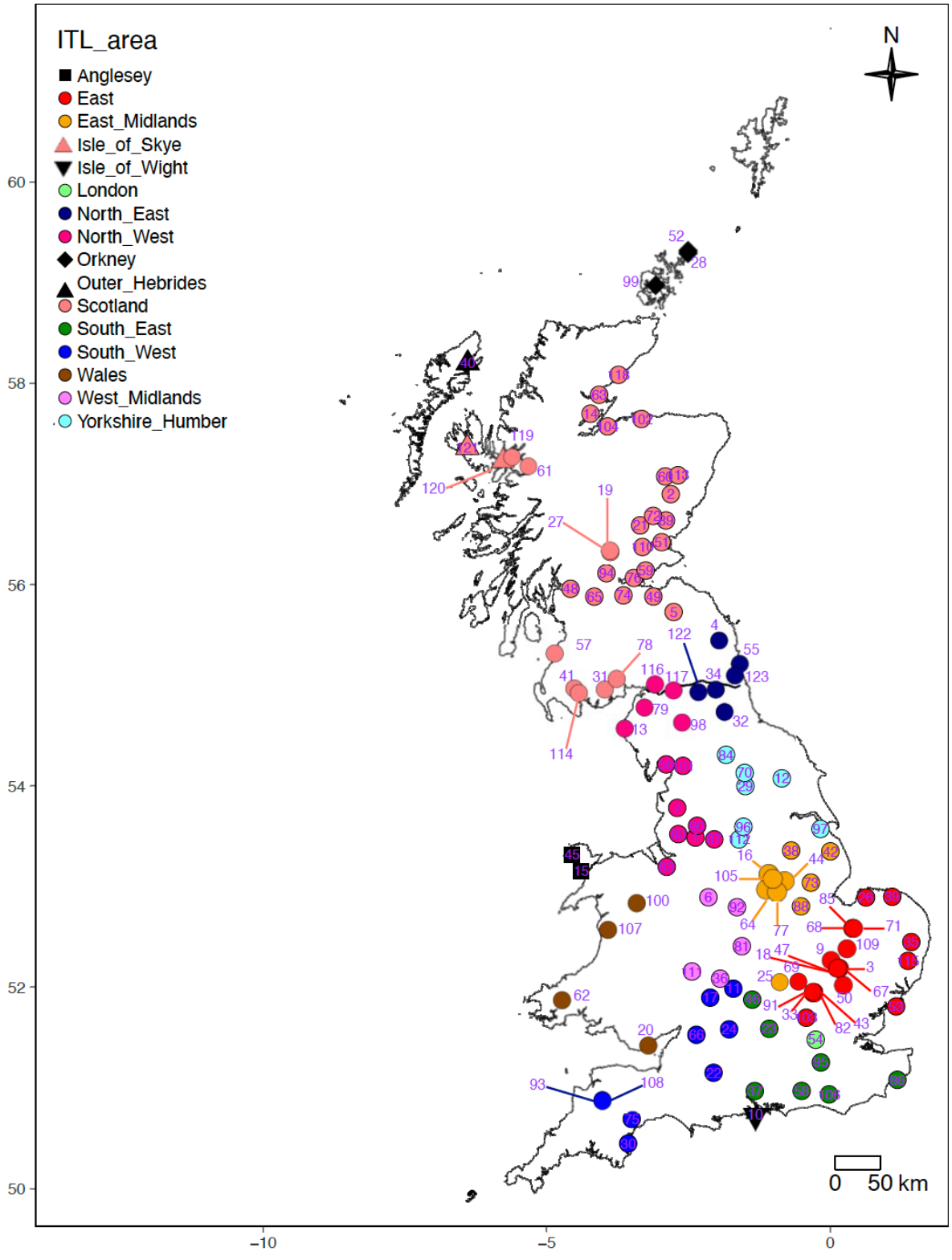

Figure 5.2. Sampling map. Individuals are coloured based on sampled ITL (the International Territorial Levels) areas and labelled by order in Appendix II, Table 5.1.

5.3.2 Population structure

We first explored the population structure of British hedgehogs by performing a PCA (Figure 5.3). The result reveals two distinct clusters, with the northern and southern clusters broadly corresponding to Scotland (northern), and England and Wales (southern), respectively. Within the southern group a continuous south-to-north cline is evident, suggesting a lack of long-term strong barriers within this region, except for the Orkney human-translocated hedgehogs (n = 3, orders 28, 52, 99) which were closer to hedgehogs from North England where their ancestors might be translocated from.

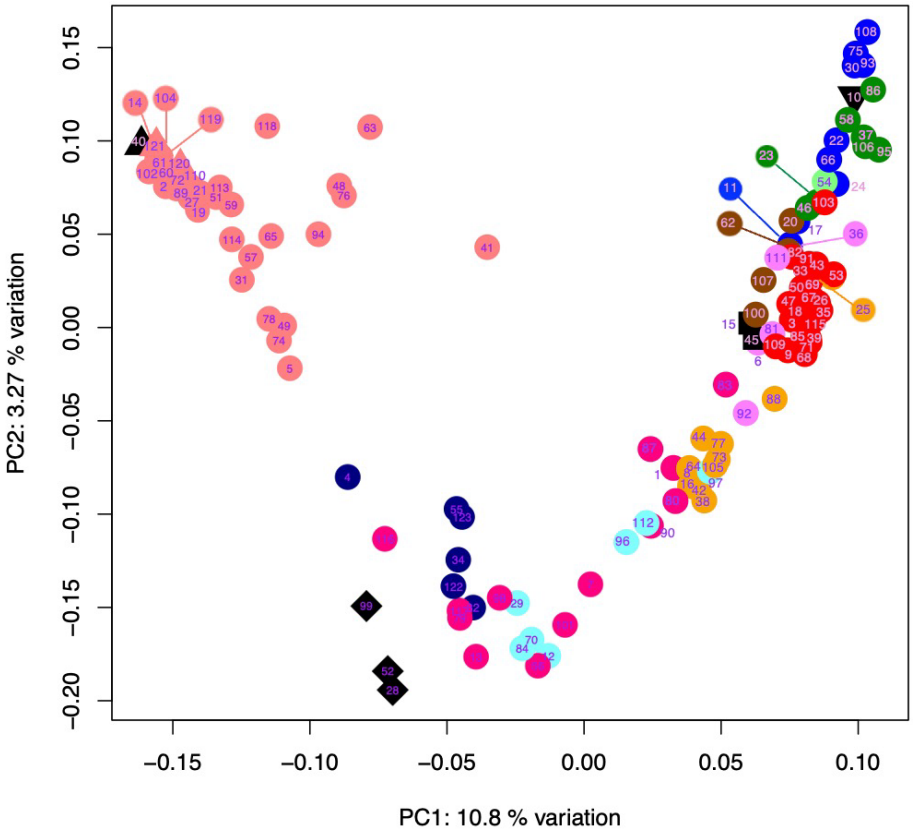

Figure 5.3. PCA of British hedgehogs coloured and labelled as for Figure 5.2; and Appendix II, Table 5.1.

A neighbour-joining (NJ) tree rooted with the Danish hedgehog identified two genetic clades (Figure 5.4), with the northern clade comprising individuals sampled only from Scotland, and the southern genetic clade including individuals mostly sampled from England and Wales, and Orkney (human-translocated). Overall, the patterns revealed by the NJ tree are consistent with the division between south and north as suggested by the PCA.

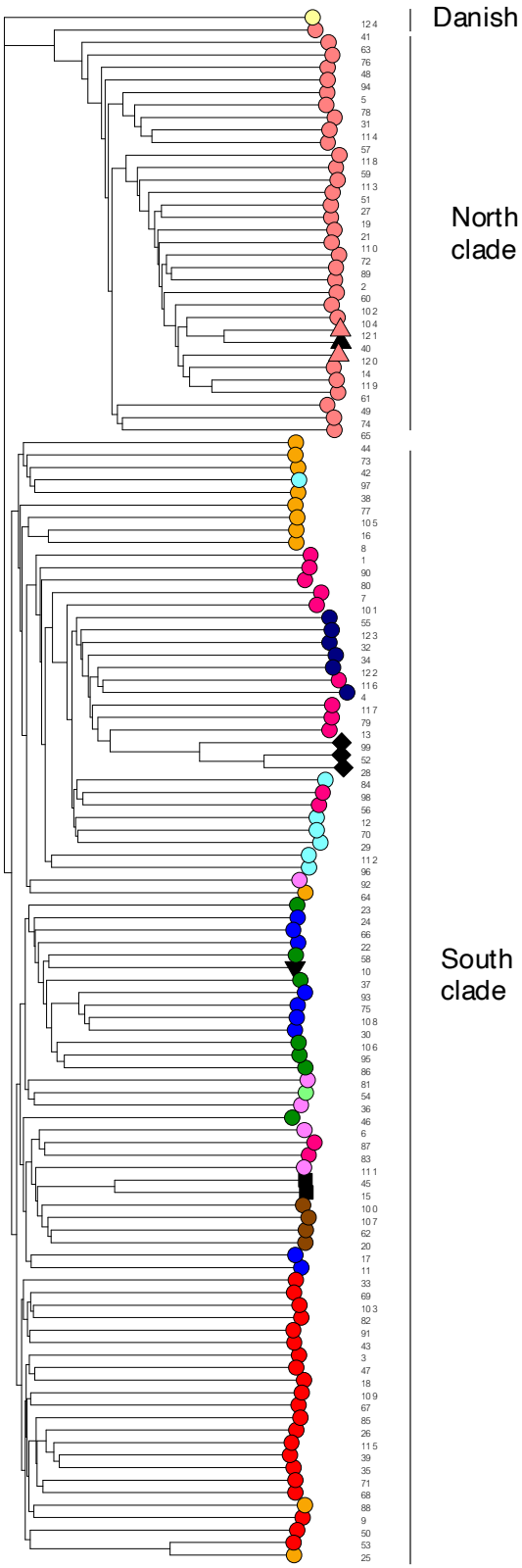

Figure 5.4. Neighbour-joining tree of British hedgehogs coloured and labelled as for Appendix II, Table 5.1.

We then estimated per-individual admixture proportions of British hedgehogs by using NGSadmix. This method is suggested to be particularly useful when a population is well represented by a small number of relatively distinct clusters, possibly with recent admixture. Admixture proportions were calculated for three *a priori* numbers of population clusters (K = 2, K = 3, and K = 4). The results are shown in Figure 5.5 c. At K = 2, clusters broadly north and south are observed. Although admixture seems to have occurred in both directions, it permeates southwards to a greater extent from a putative contact zone, likely at Hadrian's Wall that separated north and south Britain. At K = 3, a potential differentiation of a population in northern England is suggested, but this is not as distinct as the overall north/south divide, and not especially evident in the PCA (Figure 5.3) or NJ tree (Figure 5.4). At K = 4, the fourth cluster almost entirely comprises admixed individuals, indicating insufficiency in the data to accurately assign individuals to four populations.

Individual pairwise genetic distance was significantly positively related to geographic distance (Student's *t*-test, $p < 0.001$; Figure 5.5 a). The results suggest isolation by distance and support a single northward post-LGM colonisation and all present-day hedgehogs descend from a same pre-LGM ancestral population (Hewitt, 1996).

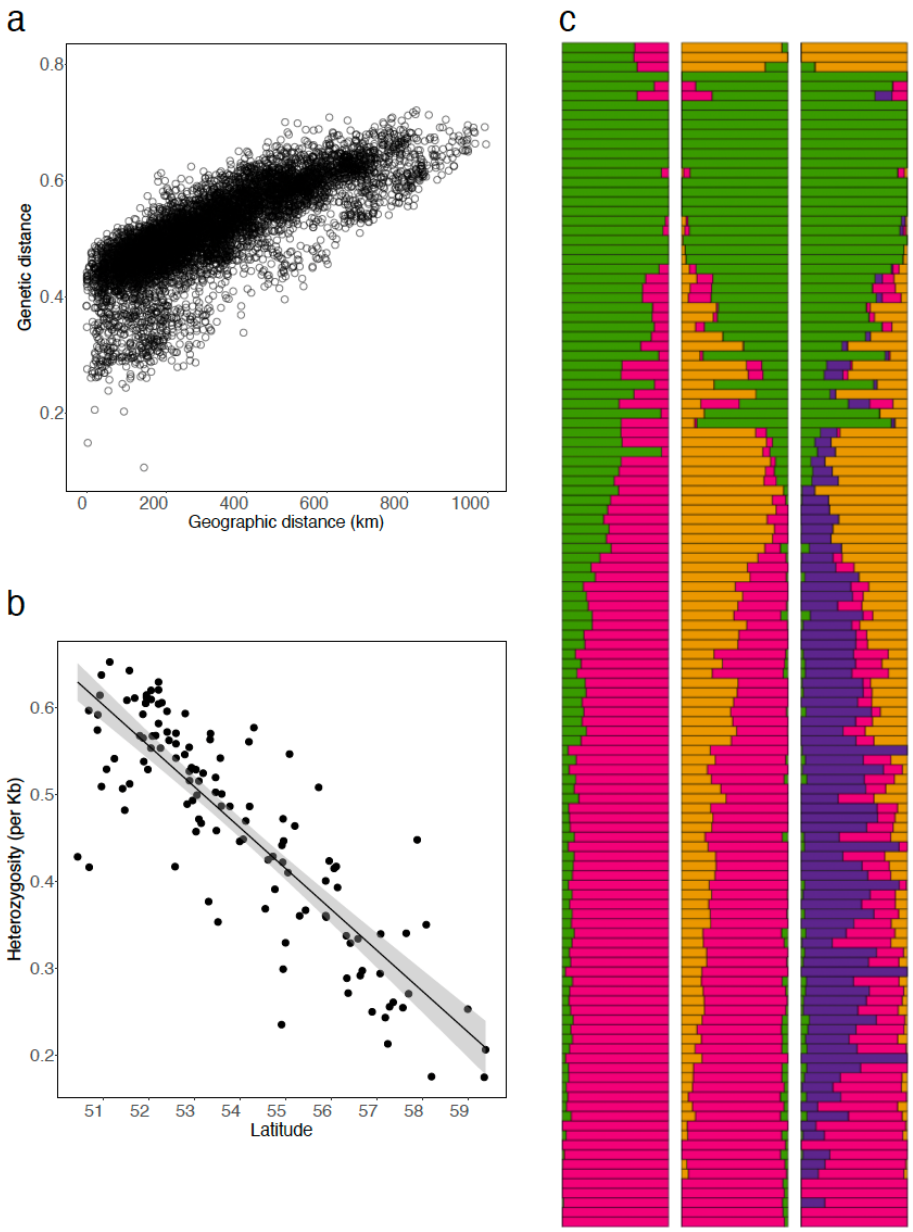

Figure 5.5. a, Isolation by distance plot for pairs of individuals. **b**, Linear regression of latitude and individual genome-wide autosomal heterozygosity (heterozygous sites per Kb). Shading indicates the 95% confidence intervals of the regression lines. Heterozygosity = 3.007 - 0.047*latitude (EPSG: 4326), R = 0.722, P < 0.001). **c**, Assignment of individual genomes (horizontal bars) to K = 2, 3 and 4 population clusters allowing admixture. Samples are arranged from top to bottom by descending latitude.

Effective migration patterns between local demes were investigated using Estimation Effective Migration Surfaces (EEMS). The results revealed complex patterns of effective migration over the country (Figure 5.6 a–c). The putative contact zone between northern and southern clusters is indicated as a region of reduced effective migration, consistent with the results of PCA and NJ tree. Marine areas separating islands and neighbouring mainland were also generally associated with low effective migration rates, as expected. A number of additional potential barriers are also observed, likely related to large urban centres and the motorways connecting them, including: Liverpool, Manchester, Leeds, and York, and M64, which are linked together separating north England and south England/Wales, likely reflecting the genetic substructure suggested by NGSadmix; Cardiff, Bristol, and London, and M4; and Glasgow, and Edinburgh, and M8. Some presumed natural barriers including mountains and rivers are not visible in our results, for example, the region in the Pennine Hills which was found to reduce human effective migration (Gilbert et al., 2017) was not suggested here, in line with a lack of long-term strong barriers for hedgehogs as suggested by the results of PCA and NJ tree.

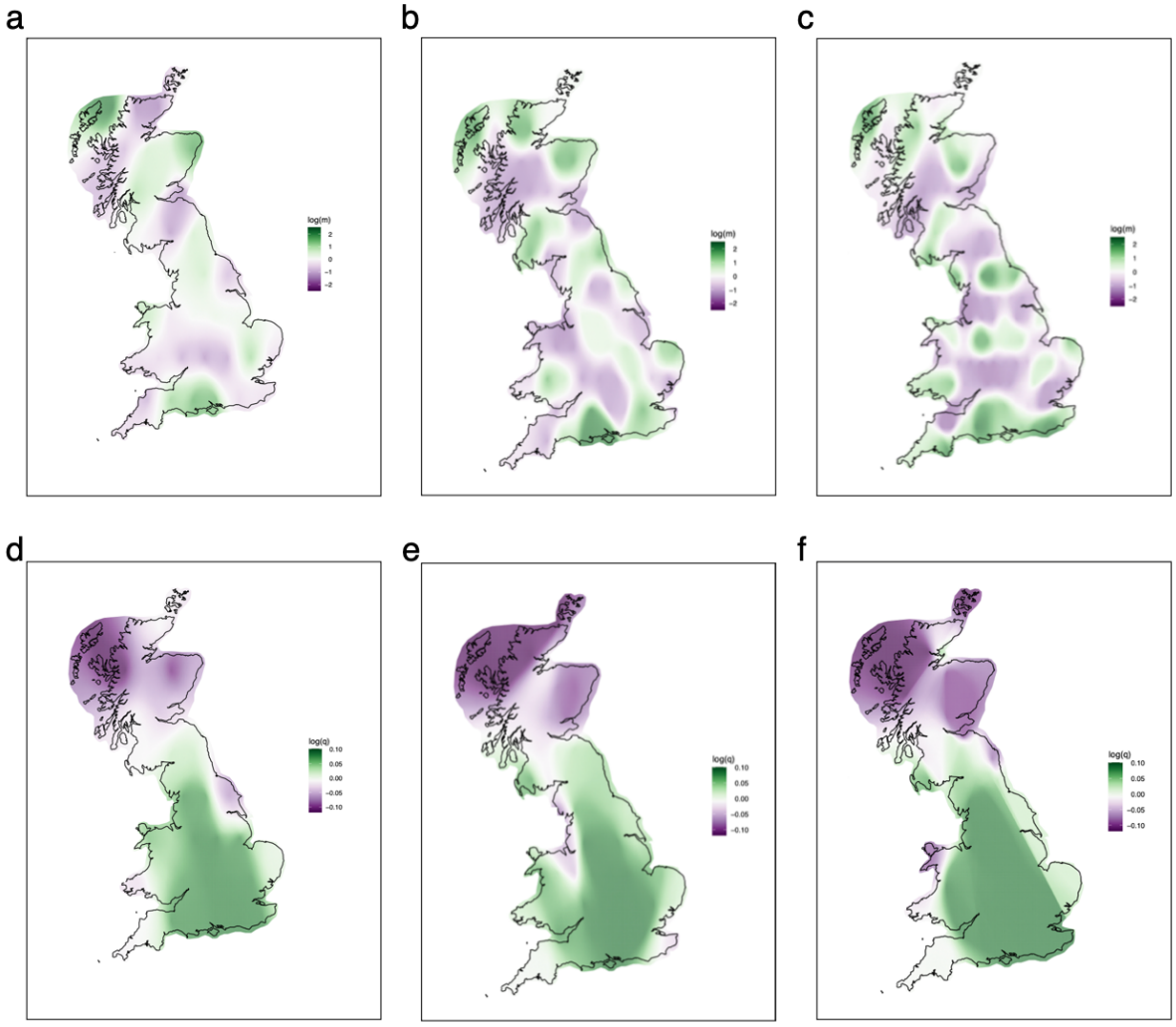

Figure 5.6. Estimated migration and diversity surface. Migration (**a**) and diversity (**d**) surfaces with 50 demes; migration (**b**) and diversity (**e**) surfaces with 100 demes; migration (**c**) and diversity (**f**) surfaces with 200 demes. Colours represent relative rates of migration (**a**–**c**), and genetic diversity (**d**–**f**), ranging from low (purple) to high (green).

5.3.3 Genetic diversity

Genetic diversity values within local demes, calculated in EEMS (Figure 5.6 d–f), were generally lower in north and higher in south. Individual heterozygosity values (corrected to 28x data; see methods; Figure 5.7) were averaged at 0.465 (heterozygous sites per Kb) across Great Britain, with the highest seen in Wiltshire, South West (averaged 0.648; n = 2). Mean individual heterozygosity was 0.319 (heterozygous sites per Kb) in Scotland (n = 36), and 0.526 in England and Wales combined (n = 87). Island hedgehogs had lower heterozygosity

compared to their mainland neighbours. Heterozygosity values in Orkney (mean 0.211; n = 3) and Outer Hebrides (0.175, n = 1) hedgehogs were extremely low. An overall south-to-north decrease in individual heterozygosity was found (Figure 5.5 b), with heterozygosity being significantly negatively related to latitude (Heterozygosity = 3.007 - 0.047*latitude (EPSG: 4326), R = 0.722, P < 0.001), suggesting serial founder effects following northward post-glacial expansion from a single ancestral population (Ramachandran et al., 2005).

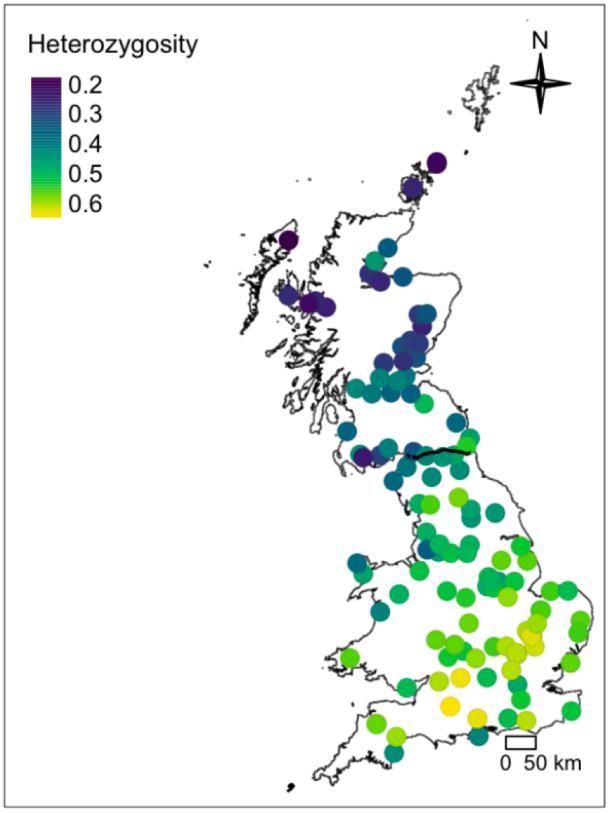

Figure 5.7. Individual heterozygosity map. Map of samples coloured by genome-wide autosomal heterozygosity (heterozygous positions per kb).

The inbreeding coefficients, measured as FROH (the fraction of genome in ROH, representing the overall inbreeding), were significantly positively correlated with latitude (Student's *t*-test, p < 0.001), being lower in England/Wales (average 21%) and higher in Scotland (average 36%), but with some disruptions around the border of Scotland and England (Figure 5.8). The longest ROH segment was found to be 4.35 Mb (sample order 64, from Nottinghamshire, East Midlands), probably indicating inbreeding from a common ancestor around 5–10 generations ago. This suggests that breeding between close relatives is not especially prevalent in hedgehogs which in turn indicates that most of our observed ROHs should reflect local population sizes. Short ROHs (100kb–1.5 Mb, corresponding to

around 660–40 years ago, see methods) in different length bins were more abundant in genomes of hedgehogs from north than south, reflecting the increased overall inbreeding (Figure 5.8; Figure 5.9). Long ROHs (> 1.5 Mb, generally corresponding to the last 40 years), however, did not follow the general south-to-north pattern, but varied substantially, with some locations showing enhanced recent inbreeding, likely due to reduced local population sizes.

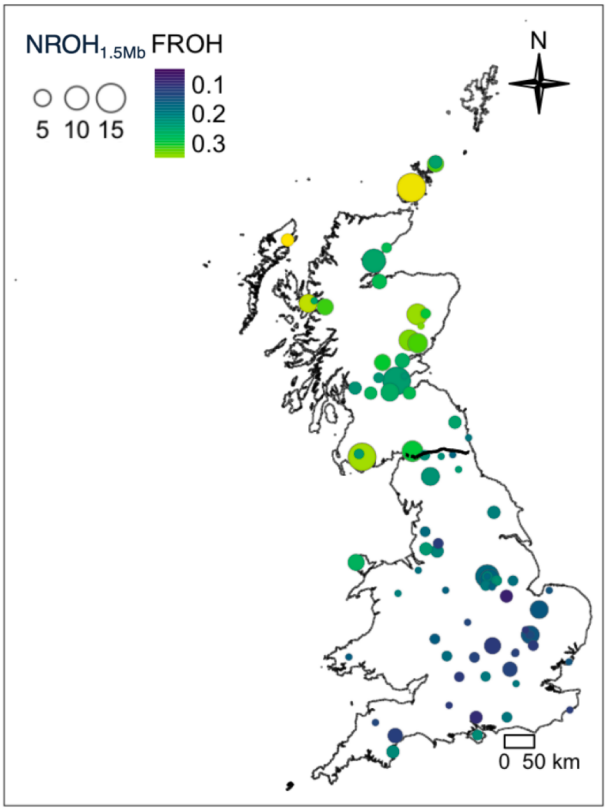

Figure 5.8. Sample map showing measure of inbreeding. Points are coloured by FROH, representing the overall inbreeding, and sized according to the number of ROHs longer than 1.5 MB, representing the more recent episodes of inbreeding.

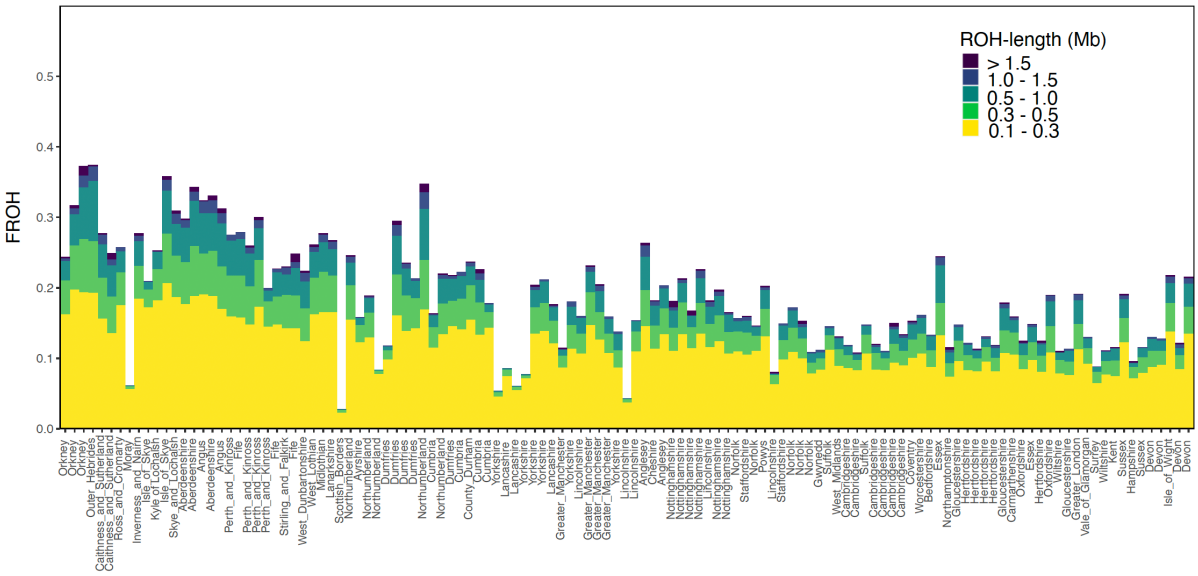

Figure 5.9. Length distribution of individual runs of homozygosity. Individuals are arranged from left to right by descending latitude, labelled by County name (Appendix II, Table 5.1).

5.3.4 Demographic inference

The population trend inferred from PSMC with the downsampled 8x data (Figure 5.10 b) from the New Zealand hedgehog remains the same as that with 28x data (Figure 5.10 a) from the same individual (although is flattened and left-shifted when with 8x data), enabling us to use our newly generated low coverage data for the comparison of population trajectories. Northern (Figure 5.10 f, h), and southern (Figure 5.10 e, g) British hedgehogs with similar coverages show very similar plots, which match the New Zealand plots (Figure 5.10 a, b), supporting they were all from the same historical population. In contrast, the Danish hedgehog (Figure 5.10 c) has markedly different demographic trajectories, supporting the deep differentiation between the Danish and British hedgehogs (separated before1.7–2.2 million years ago based on mitochondrial DNA; Seddon et al., 2001).

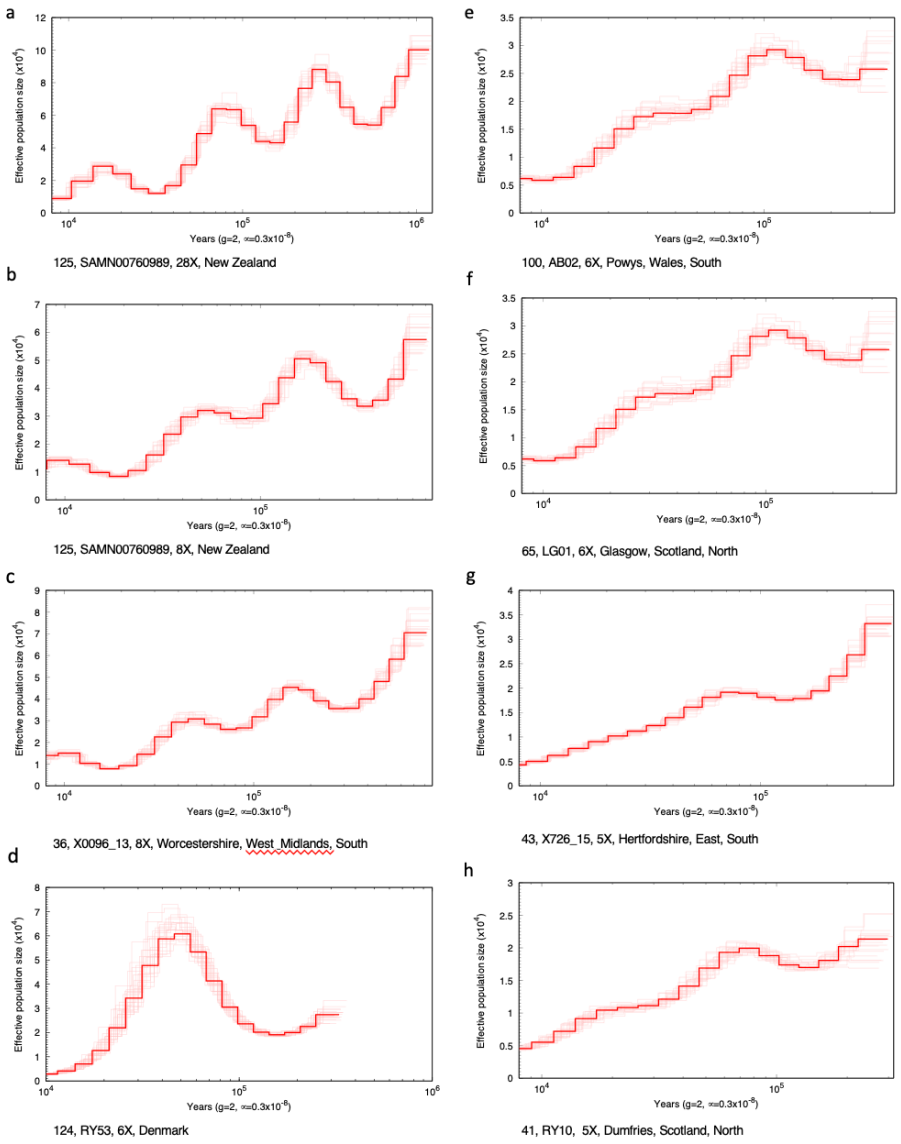

Figure 5.10. Demographic history (8,000 years ago) inferred from PSMC. a-c, Historical N_e of the British hedgehog, with 28x data from the New Zealand hedgehog (descended from the translocated hedgehogs from Great Britain around 150 years ago) to represent the final N_e of the historical British hedgehog (**a**), with 8x downsampled data from the New Zealand hedgehog (**b**), and 8X from a British hedgehog (**c**) to show the same trends between them, and to compare to the 28x (**a**) result to show the how N_e changed with the mapped read depth. **d-h**, Historical N_e with 5-6x data, to show that the British hedgehog from both south (**e, g**) and north (**f, h**) of the wall shared the same N_e trends when analysed with the same coverage data, which are different with the Danish hedgehog (**d**).

As suggested in the structure analysis the north/south differentiation, we hypothesised that Hadrian's Wall acted as a barrier and led to the differentiation. We inferred the demographic trajectories of the northern and southern hedgehogs, with respect to the wall, and expected

that if a single event structuring north/south, a common signal of N_e reduction in both north and south populations could be detected. We observed that for both south and north clusters, the N_e inferred from Stairway plots 2 (Figure 5.11 a) declined at around 2 kya, coinciding with the construction of Hadrian's Wall, in around 121–128 CE, by the Romans when they colonised Britain (Hingley, 2012). The N_e then bounced back hundreds of years later, with south being substantially higher than north (the confidence intervals were largely not overlapped). After being nearly stabilised for hundreds of years, the N_e slightly increased, which might indicate the admixture of two populations following the decay of Hadrian's Wall.

Recent new waves of effective population size decline started a few centuries ago and accelerated around 100 years ago (Figure 5.11 a). Specifically, the N_e in south was 245k (75% CI, 142–513k) 200 years ago, dropped by 40% during 200–100 years ago, and then further decreased by 55% in the last 100 years, to the near present 67k (75% CI, 33–159); the N_e in north was 153k (75% CI, 97–266k) 200 years ago, which was lost by 13% during 200–100 years ago, and then a further 31% in the recent 100 years, to the near present 91k (75% CI, 31–177).

Longer ROH segments were observed in the northern population (Figure 5.11 b), consistent with lower effective population sizes. It is notable that both the total autosomal content of runs of homozygosity (FROH) and the average length of ROH (Mean length of ROHs (Kb)) show considerable overlap between northern and southern populations, separated by Hadrian's Wall, suggesting a single ancestral population of both populations, with some southern individuals showing high levels of inbreeding, which may reflect more recent bottlenecks.

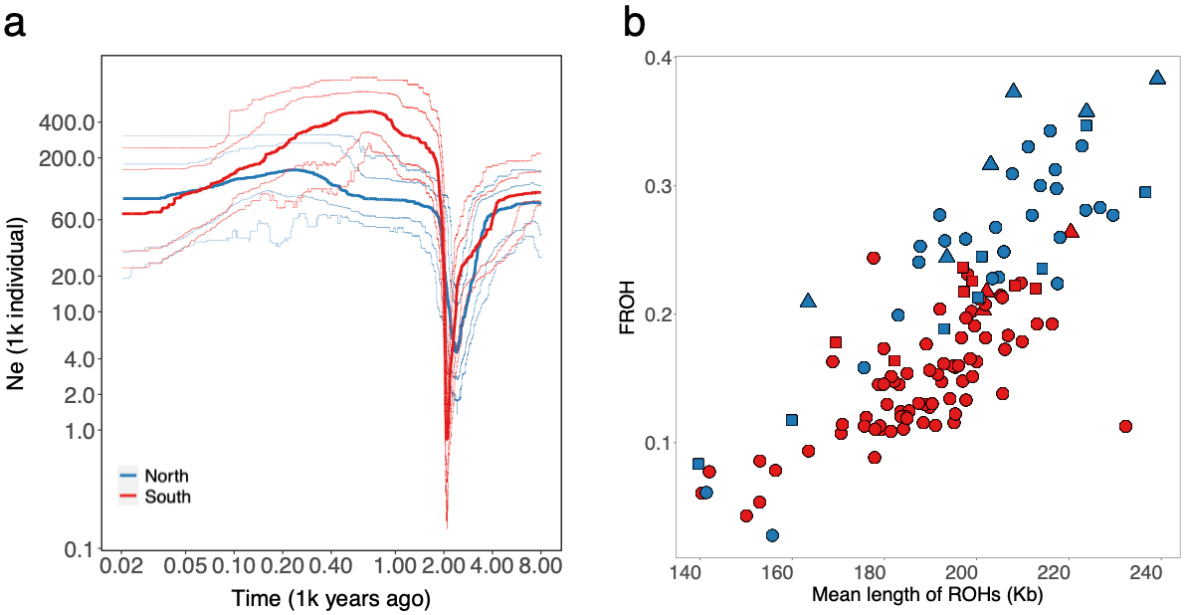

Figure 5.11. Demographic history. a, Demographic reconstructions for hedgehog populations north (blue) and south (red) of Hadrian's Wall generated by Stairway Plots 2, assuming a mutation rate μ as 3.03×10^{-9} and a generation time of 2 years. Time before present is on a log-scale x-axis, and effective population size (Ne) on the y-axis. The estimate is the median (thick line) of 200 bootstrap replicates with 2.5%, 12.5%, 87.5%, and 97.5% confidence intervals (four thin lines for each). **b**, Relationship between total autosomal content of runs of homozygosity (FROH) and the average length of ROH (Mean length of ROHs (Kb)) for hedgehogs sampled north (blue) and south (red) of Hadrian's Wall.

5.4 Discussion

The main goal of this study was to provide a comprehensive overview of the population history, population structure, and genetic diversity patterns of the British hedgehog, based on whole-genome sequencing of 123 hedgehogs evenly distributed across Britain. Our analysis suggests a natural post-LGM colonisation history of the hedgehog into Britain. A relatively continuous genetic variation distribution was observed, highlighting the gene flow on large geographic scales. Moderate genetic diversity was retained. Recent bottlenecks and recently disrupted inbreeding coefficients (measured as the FROH) were detected, yet the revealed genetic diversity and gene flow provide positive messages relating to the conservation status of the hedgehog.

5.4.1 Population history and genomic diversity

We found a generally continuous genetic pattern of the hedgehog across Britain, shown as the significant correlation between individual heterozygosity and latitude, and between pairwise individual genetic distance and geographic distance, supporting a single northward post-LGM colonisation and all present-day hedgehogs descend from a same pre-LGM ancestral population (Hewitt, 1996). This is concordant with our first hypothesis stating that hedgehogs colonised Britain naturally, rather than by human-induced introduction.

The averaged individual heterozygosity (heterozygous sites per Kb) was 0.526 in England/Wales, close to that in the Danish hedgehog sequenced in this study (0.583), and in European humans (0.595; Westbury, et al., 2018), potentially indicating a large number of founders of the hedgehog population in Britain. The averaged individual heterozygosity decreased to 0.319 in Scotland, likely primarily due to the serial founder effects along their northward expansion routes (like that in humans; Ramachandran et al., 2005). The individual inbreeding coefficient, measured as the FROH (the fraction of homozygous segments within the individual genome), shows a similar continuous pattern, being low in England/Wales (averaged 21%) and high in Scotland (averaged 36%), but overall (averaged 26%) is not high compared to some other mammals (e.g., ROH segments longer than 1.5 Mb comprised of 37.8% of the autosomes in the Scottish killer Whale, *Orcinus orca*; Foote et al., 2021). Individual heterozygosity and inbreeding coefficient were both significantly correlated with latitude, showing relatively continuous genetic patterns (although with some disruptions around the Scottish border), and supporting our hypothesis two, i.e., historically nearly ubiquitous distribution of the hedgehog in large parts of mainland Britain.

5.4.2 Population structure

As stated in hypothesis three, we expected to observe a complex genetic structure of the hedgehog, corresponding to rivers, mountains, agricultural lands, city centres, and roads. However, little differentiation was found to correspond to these presumed barriers. A significant positive correlation between genetic distance to geographic distance further suggests that these landscape features did not form strong barriers to the hedgehog gene flow. This is unexpected, given that hedgehogs' normal home ranges are small (Pettett et al., 2017),

and their distribution and movement are found to be limited by those landscape features in field studies (e.g., Huijser and Bergers, 2000; Rondinini and Doncaster, 2002; Hubert et al., 2011), but likely in accordance with its large population size, i.e., at least some individuals can surmount these barriers or have historically been able to live in these areas.

The low differentiation across large regions as shown in our results of PCA (Figure 5.3), NJ tree (Figure 5.4), and NGSadmix (Figure 5.5 c) analyses, contradicts the distinct differentiation found in some previous hedgehog genetics studies based on microsatellites, e.g., in Oxfordshire, UK in Becher and Griffiths (1998), and in Zurich, Switzerland in Braaker et al. (2017). This was unexpected as population genomics is likely to be more powerful in detecting even subtle structure (McMahon, Teeling and Höglund, 2014) than microsatellites. Thus, the genetic differentiation found between local populations in some studies might be due to ascertainment biases in sampling or analysis, e.g., due to uneven sampling, or involvement of related individuals (Meirmans, 2015), which requires further research. The results from the present study, therefore, provide positive messages for hedgehog conservation, that the genetic consequences of an inability to disperse across moderately fragmented habitats might have not significantly affected the population. However, some large urban centres and motorways potentially restrict recent hedgehog gene flow, as suggested in the EEMS analyses, which warrants further investigation. Further, a differentiation was observed around the Scotland/England border, structuring the northern and southern hedgehogs of Great Britain. We did not find any geographic barriers that would correspond to this, as rivers and mountains do not appear to form such strong barriers. The contact zone seems to match Hadrian's Wall, which was built ~1900 years ago by Romans after they colonised Britain and fell into disrepair after ~300 years following the Romans' retreat from Britain (Hingley, 2012), with only fragments remaining today. We analysed the demographic history separately for north and south groups in relation to Hadrian's Wall, and the results support that the two groups were likely formed ~2000 years ago, likely due to being separated by Hadian's Wall. This is interesting, showing the magnitude of the effects of ancient large-scale human-induced infrastructure in an otherwise continuous large population.

5.4.3 Recent demography

Recent population declines were shown (Figure 5.11), supporting our fourth hypothesis, and in line with the reported national hedgehog declines based on field surveys (Roos, Johnston, and Noble, 2012). The decline based on our genomic analyses was found started a few centuries ago and was exacerbated around the 1950s, likely coinciding with the agricultural expansion from the 17th century to the 1950s, when a large portion of natural grasslands was converted to arable lands and cultivated pastures (Pretty, 1991), followed by crop intensification post-1950s (Robinson and Sutherland, 2002; Amar et al., 2010). Recent studies have also shown that present-day hedgehogs have a patchy and discontinuous distribution (Williams et al., 2018), tend to avoid agricultural lands and select urban areas (Hubert et al., 2011; Parrott, Etherington and Dendy, 2014; Pettett et al., 2017; Schaus et al., 2020). It has been argued that hedgehogs might have always been associated with areas where humans have been since historical times. Nevertheless, if hedgehogs were likely ubiquitous in historical times (Morris, 2018; this study), then it is likely that our observed population decline in Ne is at least partially related to agricultural intensification (reviewed in Yarnell and Pettett, 2020).

To further investigate the potential human-induced effects on the hedgehog populations, temporal inbreeding coefficients based on individual ROH distribution were calculated. We found that short ROHs, corresponding to more historical times (Thompson, 2013), were more abundant in genomes of hedgehogs from the north than the south, reflecting increased background inbreeding following expansion routes. Nevertheless, long ROHs, corresponding to recent decades, did not follow the general south-to-north cline but varied substantially. Individuals with long ROHs were found in some arable-dominated areas, e.g., East and Middle East England (Robinson and Sutherland, 2002), but also in some other areas where arable density was low, suggesting complex drivers underly the recent population patterns. Future studies may increase the local sample density in different areas to better understand these patterns.

Although our inferred demographic patterns (with PSMC, Figure 5.10; stairway plot 2, Figure 5.11 a; ROH analyses, Figure 5.8, Figure 5.9, and Figure 5.11 b) generally agree with historical events, we caution that these estimates could be biased by the mutation rate and the generation time we used, e.g., a higher mutation rate or a shorter generation time would lower our time estimation (and on the opposite end, a lower mutation rate or a longer generation time would increase our time estimation; Liu and Fu, 2020). Further, the assumption to

estimate effective population sizes is based on drift-gene flow equilibrium, which is not likely possible in the current hedgehog populations due to the ongoing demographic changes. However, we are confident that we mitigated these effects as best as we could and we caution against over-interpretation of exact timing and effective population sizes, and rather encourage focusing on the broader trends presented by the data.

5.4.4 Island hedgehogs

As expected, island hedgehogs generally had lower heterozygosity and higher inbreeding compared to their mainland neighbours. Heterozygosity values in Orkney (0.211) and Outer Hebrides (0.175) were among the lowest reported in mammals (e.g., 0.176, Iberian Lynx, *Lynx pardinus*; Westbury et al., 2018). Hedgehogs in Orkney also have the highest recent inbreeding among all our samples. The Orkney hedgehogs are genetically closest to the hedgehogs in North West, England, where their ancestors might have been translocated from. All other island hedgehogs were genetically-closest to their mainland neighbours (i.e., the hedgehogs found on the Outer Hebrides were closely clustered to those individuals sampled on the west of mainland Scotland).

5.5 Conclusion

Our results provide new insights into the evolution history of the British hedgehog, supporting the natural colonisation of the hedgehog in Britain with a single northward expansion across Great Britain. A generally continuous genetic variation distribution was observed, highlighting gene flow on large geographic scales. Moderate genetic diversity was retained, likely because of its population-level expansion ability and large effective population sizes maintained during most of its evolutionary time. Our study supports the reported recent population decline, which was found to have started a few centuries ago, coinciding with the agricultural intensification, while bringing a greater resolution to our understanding of the magnitude and the potential causes of the decline and providing positive messages relating to the conservation status of the hedgehog.

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5.7 Appendix II: Extended results for Chapter 5

Table 5.1. Details of 125 hedgehog samples. * Mapping coverage in Great Britain (GB) (Mapped_Gb), read depth for individual hedgehog sequences mapped to the hedgehog reference genome post-filtering (calculated using both SAMTools v. 1.17 and ANGSD v. 0935). County and ITL (the International Territorial Levels) administrative area (ITL_area) of the hedgehogs from GB (order 1–123) is indicated. The sampling area of the hedgehog from Denmark (Order 124) and from New Zealand (Order 125) are listed under the column County. The latitude and longitude of all samples are listed (EPSG: 4326).

Order	Mapped_ Gb	Depth (SAM Tools)	Depth (AN GSD)	County	ITL_area	latitude	longitude
1	13.842	5.712	5	Greater_Manchester	North_West	53.487114	-2.378233
2	12.860	5.296	4	Angus	Scotland	56.899581	-2.813264
3	12.377	5.107	4	Cambridgeshire	East	52.219655	0.091909
4	12.320	5.087	4	Northumberland	North_East	55.440269	-1.900333
5	6.413	2.988	1	Scottish_Borders	Scotland	55.728102	-2.762718
6	11.661	4.809	4	Staffordshire	West_Midlands	52.894353	-2.150086
7	12.054	4.983	4	Lancashire	North_West	53.782282	-2.699587
8	15.417	6.327	5	Nottinghamshire	East_Midlands	53.103864	-1.029941
9	12.994	5.376	4	Cambridgeshire	East	52.293305	-0.007273
10	11.920	4.906	4	Isle_of_Wight	Isle_of_Wight	50.700303	-1.296631
11	13.295	5.475	5	Gloucestershire	South_West	51.986100	-1.710178
12	13.401	5.502	4	Yorkshire	Yorkshire_Humber	54.075476	-0.855256
13	11.007	4.644	4	Cumbria	North_West	54.558986	-3.578953
14	11.643	4.862	4	Ross_and_Cromarty	Scotland	57.698747	-4.228589
15	12.349	5.096	4	Anglesey	Anglesey	53.155179	-4.390662
16	12.177	5.02	4	Nottinghamshire	East_Midlands	53.103958	-1.033835
17	13.596	5.595	5	Gloucestershire	South_West	51.896489	-2.117667
18	13.568	5.567	4	Cambridgeshire	East	52.218869	0.135793
19	10.843	4.588	4	Perth_and_Kinross	Scotland	56.332609	-3.837226
20	10.306	4.364	3	Vale_of_Glamorgan	Wales	51.431603	-3.224104
21	12.270	5.035	4	Perth_and_Kinross	Scotland	56.589542	-3.353076
22	12.633	5.207	4	Wiltshire	South_West	51.150245	-2.058568
23	12.002	4.981	4	Oxfordshire	South_East	51.587229	-1.077628
24	13.269	5.448	4	Wiltshire	South_West	51.581663	-1.784916
25	10.155	4.249	3	Northamptonshire	East_Midlands	52.053185	-0.893058

26	13.000	5.362	4	Norfolk	East	52.892310	0.629633
27	12.748	5.262	4	Perth_and_Kinross	Scotland	56.345838	-3.843137
28	11.888	4.968	4	Orkney	Orkney	59.356429	-2.421111
29	13.269	5.468	5	Yorkshire	Yorkshire_Humber	53.998985	-1.498079
30	12.587	5.234	4	Devon	South_West	50.447310	-3.564728
31	10.778	4.484	3	Dumfries	Scotland	54.950053	-3.933166
32	16.486	6.74	6	County_Durham	North_East	54.724908	-1.803935
33	14.294	5.858	5	Hertfordshire	East	51.938214	-0.299457
34	11.705	4.878	4	Northumberland	North_East	54.948408	-1.960580
35	15.619	6.384	5	Suffolk	East	52.449236	1.427421
36	22.137	8.994	8	Worcestershire	West_Midlands	52.085339	-1.943038
37	13.186	5.429	5	Hampshire	South_East	50.968508	-1.332037
38	9.187	3.933	2	Lincolnshire	East_Midlands	53.358643	-0.690829
39	11.765	4.913	4	Norfolk	East	52.899177	1.091045
40	13.529	5.584	4	Outer_Hebrides	Outer_Hebrides	58.201055	-6.392909
41	13.224	5.453	5	Dumfries	Scotland	54.960240	-4.475195
42	15.268	6.274	5	Lincolnshire	East_Midlands	53.350097	0.000675
43	13.524	5.568	5	Hertfordshire	East	51.955764	-0.269687
44	13.434	5.521	5	Nottinghamshire	East_Midlands	53.040995	-0.778435
45	13.530	5.558	4	Anglesey	Anglesey	53.316561	-4.553472
46	11.641	4.843	4	Oxfordshire	South_East	51.876917	-1.376772
47	13.410	5.474	4	Cambridgeshire	East	52.219132	0.121165
48	13.038	5.356	4	West_Dunbartonshire	Scotland	55.958952	-4.580553
49	13.553	5.567	5	Midlothian	Scotland	55.882041	-3.120535
50	13.407	5.552	4	Essex	East	52.055813	0.200940
51	12.581	5.176	4	Fife	Scotland	56.422382	-2.974258
52	10.518	4.442	3	Orkney	Orkney	59.385205	-2.419726
53	12.648	5.194	4	Essex	East	51.809107	1.160951
54	11.467	4.777	4	Greater_London	London	51.479026	-0.258986
55	12.903	5.307	4	Northumberland	North_East	55.205989	-1.533550
56	9.887	4.261	3	Lancashire	North_West	54.214523	-2.893113
57	10.036	4.241	3	Ayrshire	Scotland	55.310155	-4.824416
58	14.132	5.764	5	Sussex	South_East	50.969857	-0.505976
59	12.842	5.409	4	Fife	Scotland	56.141303	-3.256815
60	15.186	6.208	5	Aberdeenshire	Scotland	57.075555	-2.910738
61	12.242	5.073	4	Skye_and_Lochalsh	Scotland	57.187552	-5.383146
62	12.389	5.108	4	Carmarthenshire	Wales	51.882443	-4.747462
63	13.198	5.399	4	Caithness_and_Sutherland	Scotland	57.887178	-4.077263

64	14.664	6.022	5	Nottinghamshire	East_Midlands	52.966048	-1.074049
65	16.206	6.625	6	Lanarkshire	Scotland	55.881265	-4.161888
66	11.305	4.665	4	Gloucestershire	South_West	51.527353	-2.361763
67	14.361	5.876	5	Cambridgeshire	East	52.219394	0.106537
68	12.939	5.315	4	Norfolk	East	52.601231	0.361096
69	13.040	5.36	4	Bedfordshire	East	52.058546	-0.557448
70	10.037	4.23	3	Yorkshire	Yorkshire_Humber	54.129941	-1.510393
71	14.265	5.874	5	Norfolk	East	52.601231	0.361096
72	13.981	5.738	5	Aberdeenshire	Scotland	56.683505	-3.124462
73	16.516	6.76	6	Lincolnshire	East_Midlands	53.039280	-0.343152
74	13.514	5.594	5	West_Lothian	Scotland	55.894072	-3.648600
75	11.556	4.807	4	Devon	South_West	50.690961	-3.502032
76	11.856	4.912	4	Fife	Scotland	56.067146	-3.463220
77	13.538	5.539	4	Nottinghamshire	East_Midlands	52.943297	-0.900242
78	11.872	4.969	3	Dumfries	Scotland	55.055661	-3.722505
79	10.859	4.525	4	Cumbria	North_West	54.769090	-3.226474
80	11.561	4.894	4	Greater_Manchester	North_West	53.521731	-2.680202
81	12.300	5.144	4	West_Midlands	West_Midlands	52.408188	-1.560420
82	13.038	5.422	4	Hertfordshire	East	51.937791	-0.270378
83	15.942	6.512	5	Cheshire	North_West	53.196791	-2.884610
84	9.647	4.11	2	Yorkshire	Yorkshire_Humber	54.309136	-1.838373
85	10.349	4.377	4	Norfolk	East	52.600936	0.375848
86	10.823	4.621	3	Kent	South_East	51.079963	1.182363
87	12.581	5.184	4	Greater_Manchester	North_West	53.469727	-2.046661
88	9.022	3.895	3	Lincolnshire	East_Midlands	52.804294	-0.518057
89	12.298	5.087	4	Angus	Scotland	56.638580	-2.898305
90	9.159	3.856	3	Greater_Manchester	North_West	53.604052	-2.349053
91	12.507	5.131	4	Hertfordshire	East	51.956188	-0.298777
92	11.375	4.711	4	Staffordshire	West_Midlands	52.795036	-1.645514
93	12.558	5.16	4	Devon	South_West	50.889735	-4.034502
94	13.804	5.702	5	Stirling_and_Falkirk	Scotland	56.114371	-3.947522
95	11.520	4.896	3	Surrey	South_East	51.252810	-0.167232
96	12.411	5.098	4	Yorkshire	Yorkshire_Humber	53.594664	-1.533099
97	11.968	4.943	4	Lincolnshire	Yorkshire_Humber	53.572655	-0.174167
98	13.087	5.356	4	Cumbria	North_West	54.618856	-2.559007
99	16.049	6.575	5	Orkney	Orkney	58.995282	-3.066384
100	15.985	6.563	6	Powys	Wales	52.847115	-3.428568

101	10.016	4.272	3	Lancashire	North_West	54.200056	-2.597760
102	8.946	3.901	2	Moray	Scotland	57.646532	-3.329790
103	12.302	5.043	4	Hertfordshire	East	51.697173	-0.424268
104	11.691	4.862	4	Inverness_and_Nairn	Scotland	57.573233	-3.927745
105	12.021	4.987	4	Nottinghamshire	East_Midlands	53.061739	-0.966796
106	14.315	5.904	5	Sussex	South_East	50.935703	-0.023167
107	12.845	5.301	4	Gwynedd	Wales	52.581981	-3.934859
108	12.129	4.987	4	Devon	South_West	50.880749	-4.034111
109	12.735	5.23	4	Cambridgeshire	East	52.405340	0.262405
110	10.545	4.4	3	Perth_and_Kinross	Scotland	56.374333	-3.313054
111	14.812	6.059	5	Coventry	West_Midlands	52.156456	-2.439931
112	11.287	4.69	3	Yorkshire	Yorkshire_Humber	53.469100	-1.609799
113	13.900	5.706	5	Aberdeenshire	Scotland	57.087157	-2.685959
114	11.642	4.81	4	Dumfries	Scotland	54.914768	-4.393836
115	14.054	5.75	5	Suffolk	East	52.262041	1.368980
116	12.824	5.031	4	Dumfries	North_West	55.002138	-3.040808
117	12.365	4.843	4	Cumbria	North_West	54.940753	-2.711744
118	11.586	4.575	4	Caithness_and_Sutherland	Scotland	58.085116	-3.735495
119	14.032	5.479	4	Kyle of Lochalsh	Scotland	57.281351	-5.676604
120	15.417	6.013	5	Isle_of_Skye	Isle_of_Skye	57.242347	-5.840906
121	11.886	4.688	4	Isle_of_Skye	Isle_of_Skye	57.361357	-6.399575
122	15.618	6.078	5	Northumberland	North_East	54.923295	-2.269627
123	12.874	5.035	4	Northumberland	North_East	55.089864	-1.619427
124	15.848	6.245	5	Roskilde, Denmark		55.643704	12.067644
125	81.510	31.765	28	Otago, New Zealand		-45.381676	170.430387

Table 5.2. Heterozygosity correction factors for mapped read depth based on downsampled data from the New Zealand hedgehog. * Fraction, the fraction of downsampling from the 28x dataset; Depth, mapped read depth; Het_low, and Het_28x indicate heterozygosity values calculated with the downsampled low coverage data, and the 28x data, respectively. The correction factor is calculated as Het_28x/Het_low.

Fraction	Depth	Het_low	Het_28x/Het_low	Het_28x
0.05	1	0.000257216	1.780099216	0.00045787
0.1	2	0.000284319	1.610409434	0.00045787
0.125	3	0.000309475	1.479505614	0.00045787
0.15	4	0.000325486	1.406727171	0.00045787
0.2	5	0.000347987	1.315767543	0.00045787
0.225	6	0.000367242	1.246780052	0.00045787
0.25	7	0.000367243	1.246776657	0.00045787
0.3	8	0.000381907	1.198904445	0.00045787
0.325	9	0.000388151	1.179618241	0.00045787
0.4	11	0.000403544	1.134622247	0.00045787
0.6	17	0.000429661	1.065654085	0.00045787
0.8	23	0.000446127	1.026322101	0.00045787
1	28	0.00045787	1	0.00045787

Chapter 6: General discussion and conclusions

6.1 Overview

The Western European hedgehog (*Erinaceus europaeus*; hereafter termed hedgehog) is declining in the UK with the main causes largely uncertain (Roos, Johnston and Noble, 2012; Wilson and Wembridge, 2018). To gain further insights into the reported population decline and to inform conservation, this thesis has focused on demography and connectivity of the hedgehogs from the local to the national scales. The Brackenhurst hedgehog monitoring based on long-term spatial capture-recapture (SCR) presents the first long-term SCR-based density and apparent survival estimates of the hedgehog, and provides an important example of a relatively stable population in the wider decline. The results show that juvenile and adult hedgehogs had similarly stable trends in densities and were subject to similar effects of habitats, i.e., being significantly positively related to soil permeability, edge density, proximity to the nearest building, and distance from the nearest badger sett, except that juveniles were more associated with buildings than adults. Modelled annual population density was 13.3 hedgehogs/km^2, with juvenile female: adult female: juvenile male: adult male ratios being 1.7: 1.6: 1.3: 1, and on amenity: pasture: arable approximating to 14: 4: 1. Modelled annual apparent survival rate of adult hedgehogs was stable, being around 0.5, which seems to be enough for the population to maintain population size. To investigate whether agricultural lands act as barriers to gene flow and further infer landscape-scale movement, a genetic study based on microsatellite genotyping was conducted, and contemporary gene flow across an agricultural matrix (next to the Brackenhurst site) was evidenced, suggesting local fragmentation was limited. The gene flow was found primarily driven by long-range movements of male hedgehogs (at least 3 km), adding support for potential male dispersal which was also suggested by the SCR modelling of Brackenhurst hedgehogs. To infer demography, and gene flow on large scales, whole-genome sequencing of hedgehogs evenly sampled across Great Britain was conducted. The analysis revises our understanding of the genetic structure and diversity patterns of the hedgehog and highlights its large-scale gene flow. Moderate genetic diversity was retained, likely because of its population-level expansion ability and large effective population sizes maintained during most of its evolutionary time. Our study provides empirical evidence of the reported recent

population decline, which was found started a few centuries ago, coinciding with agricultural intensification (Pretty, 1991; Robinson and Sutherland, 2002; Amar et al., 2010). Collectively, the results from this study support that the hedgehogs in Great Britain declined in recent times but may be stabilising in some local areas, the decline is at least partially related to habitat disruption driven by agricultural intensification, but genetic connectivity across agricultural lands is likely largely maintained. The results suggest that improving local habitat amount and quality and maintaining large-scale genetic connectivity would be beneficial for hedgehogs.

6.2 Population status of British hedgehogs

The recent demography inferred based on the genomic analyses in this study (Chapter 5) supports the reported recent hedgehog declines in previous national surveys (e.g., Roos, Johnston and Noble, 2012). The decline was likely to be at least partially driven by habitat loss due to agricultural intensification: the recent decline started a few centuries ago and became severer around 100 years ago (Chapter 5), coinciding with agricultural expansion and intensification in Great Britain (Pretty, 1991; Robinson and Sutherland, 2002; Amar et al., 2010); enhanced recent inbreeding was shown in some individuals surrounded by high-density arable land, although the patterns were complex (Chapter 5); higher relatedness and lower allelic richness were found from small suburban patches surrounded by agricultural lands (Chapter 4); hedgehog density was extremely low on arable lands (with land margins and set aside included) and some pastures, likely related to both present and near present cropping (Chapter 2; Chapter 3).

On a local scale, the density of hedgehogs on the arable lands at Brackenhurst was found extremely low, suggesting arable lands on site might not be suitable for hedgehogs (Yarnell and Pettett, 2020). This is in accordance with findings in previous studies, e.g., in northeastern France in Hubert et al. (2011), hedgehogs were only occasionally found on arable land, and mostly on the margins which were close to amenity land or pastures (but see Haigh, Butler and O'Riordan, 2012). Density was found to be significantly positively associated with soil permeability. Future studies on more sites may help to better understand whether this was due to a real correlation or a coincidence as soil permeability on site was largely confounded with land use types. Density was significantly positively related to edge

density, and negatively related to distance to the nearest building, in line with previous findings (e.g., Rodriguez Recio et al., 2013; Bearman-Brown et al., 2020). A new badger sett was identified halfway through the study, resulting in a shift in hedgehog population activity centre (~300 m, from where the new badger sett is located to areas with more buildings), a decline in overall density which was then stabilised, supporting the field-scale spatial segregation (Lee, in preparation), likely due to the landscape of fear response of hedgehogs to badgers, and supporting the potential landscape-scale coexistence of both species.

The modelled annual apparent survival rate of Brackenhurst adult hedgehogs, with emigration (annually around 10%) at least partially accounted for, was relatively stable, being around 0.5, which seems to be enough for the population to maintain population size. Survival during winter hibernation was supported to be high compared to that during active seasons, consistent with what was found based on radio-tracking in Bearman-Brown et al. (2020; England) but contradicting what was explained in Kristiansen (1990; Sweden), in which all disappeared hedgehogs were assumed to have died during winter which might not be true. Future studies based on GPS-tracking of large numbers of hedgehogs can help to further investigate this. A wide range of mortality causes were recorded on our study site, of which the majority (52%) were human-related. Only 14% of the marked hedgehogs were found dead. Of these, roadkill and badger predation accounted for the majority of detected mortality, but as this is likely the most obvious mortality to find, the main driver of hedgehog mortality may be unknown, illustrating the difficulty in inferring population dynamics through recording mortality.

Natal dispersal in males (within one year since born) was supported by our SCR modelling, which is in line with the findings in Reeve (1994; England) and Haigh (2011; Ireland) in which exploration movements of hedgehogs were documented, but inconsistent with Rasmussen et al. (2019; Denmark) in which young hedgehogs were shown only had small home ranges. This might suggest that natal dispersal is context-specific, e.g., driven by increased badgers on our study site. Nevertheless, results from our landscape-scale genetic study proved that long-distance movement (at least 3 km) might be more frequent in hedgehogs than previously thought (Morris, 2018). The relatively continuous genomic patterns in British hedgehogs found in our study further reveal the ability of the species to maintain large-scale gene flow. Therefore, future studies can aim to better understand

landscape permeability, alongside the dispersal capabilities of hedgehogs, including what features and habitats they can navigate through so that they can be protected and maintained.

The average individual heterozygosity (heterozygous sites per Kb) was 0.526 in England/Wales, close to that of the Danish hedgehog (0.583; this study) and in European humans (0.595; Westbury, et al., 2018), although in Scotland heterozygosity was lower (0.319). The individual inbreeding coefficient, measured as the FROH (the fraction of homozygous segments within the individual genome), was generally lower in England/Wales (averaged 21%), and higher in Scotland (averaged 36%), but the overall is not high compared with some other mammals (e.g., ROH segments > 1.5 Mb comprised of 37.8% of the autosomes in the Scottish killer Whale, *Orcinus orca*; Foote et al., 2021). Both heterozygosity and inbreeding coefficient were relatively continuously distributed, likely suggesting an almost ubiquitous distribution across large scales during historical times. However, contrasting to the relatively continuous background patterns, the recent inbreeding coefficient was found to vary substantially, likely due to local population declines in recent decades. High recent inbreeding coefficient was found in some individuals in East and Middle East England, possibly at least partially due to agricultural intensification in these areas (Robinson and Sutherland, 2002). Nevertheless, higher recent inbreeding coefficient was also found in some other areas where arable density was low, suggesting the effects of other factors. Future studies may increase the sample density in different areas to better understand the regional demographic patterns and to identify factors driving population changes.

Island hedgehogs had lower heterozygosity and higher inbreeding coefficient compared to their mainland neighbours. Heterozygosity values in Orkney (0.211) and Outer Hebrides (0.175) hedgehogs were among the lowest in the reported mammals (e.g., 0.176, Iberian Lynx, *Lynx pardinus*; Westbury et al., 2018), suggesting long-term isolation and/or historical small numbers of founders. To compare, the New Zealand hedgehog has moderate heterozygosity (0.458; this study), likely because of its relatively large number of founders being translocated (from Britain around 150 years ago; Bolfíková et al., 2013). Hedgehogs in Orkney also had the highest recent inbreeding. The Orkney hedgehogs were genetically closer to the hedgehogs in North West, England, where their ancestors might have been translocated from. All other island hedgehogs were genetically closer to their immediate mainland neighbours, suggesting they were sourced from the nearby mainland areas (i.e., the

hedgehogs found on the Outer Hebrides were closely clustered to those individuals sampled on the west of mainland Scotland).

6.3 Hedgehog conservation and future research recommendations

Chapter 6 has reviewed the findings of this study and the conservation implications for hedgehogs in Great Britain and potentially other countries. A summary of the recommendations and potential avenues for future research on hedgehog conservation are as follows:

- Robust habitat-specific density estimates on more sites are necessary for evaluating the effects of covariates on hedgehog densities and monitoring these populations effectively. Methods that can account for detection and habitat heterogeneity are suggested, e.g., spatial capture-recapture modelling.
- Hedgehog densities on arable lands and some pastures at Brackenhurst, Nottinghamshire, were extremely low, in accordance with findings in previous studies (e.g., Hubert et al., 2011; but see Haigh, Butler and O'Riordan, 2012). Future studies can aim to investigate the mechanisms (e.g., soil quality) underlying the observed low hedgehog densities on agricultural lands.
- Only 14% of the marked hedgehogs were found dead. Of these, roadkill and badger predation accounted for the majority of detected mortality, but as this is likely the most obvious mortality to find, the main driver of hedgehog mortality may be unknown. This illustrates the complexity of inferring mortality rates and causes of mortality to population dynamics and highlights the need for conservation management to consider not mortality, but rather identification of drivers of population changes is needed through long-term studies.
- The four local populations in Nottinghamshire, separated by about 2–6 km of agricultural lands, were found to be connected by current gene flow. As most villages in the UK are within this distance and hedgehogs are still widely distributed, genetic connectivity among local populations is likely largely maintained. Future studies can aim to better understand landscape permeability, alongside the dispersal capabilities of hedgehogs, including what features and habitats they can navigate through so that they can be protected and maintained.

- The recent large-scale decline in effective population size was suggested to have started a few centuries ago, coinciding with the agricultural intensification. Population genomics combined with intensive sampling of local areas may help to better understand this in the future.
- Some island hedgehogs have extremely low genetic diversity and high recent inbreeding that future studies can aim to further investigate.

6.4 Wider implications for common species conservation

- *Accounting for spatiotemporal habitat heterogeneity in SCR density modelling*

In Chapter 2, a new application was extended by integrating both spatially and temporally changed covariates into one SCR framework, by incorporating custom specifications into an existing SCR model (Sutherland, Royle, and Linden, 2019). The application enables density to be more accurately estimated and covariate effects on the density to be directly quantified and compared. The results in this study show that when covariates were not included in the density models, the overall hedgehog density was inflated 1.5 times. This highlights the importance of using SCR with covariate heterogeneity modelling to provide greater insights into how covariate relates to hedgehog density and meet challenges emphasized by Mathews et al., 2018 with regard to a lack of robust density estimates making an evaluation of total hedgehog population size challenging. With the ongoing habitat alteration, and with the development of landscape-scale sampling, the application of integrating variation in within-population density into overall density estimation can be widely used for many species.

- *Individual-based sampling in population genomics*

Understanding the distribution of genetic variation among habitats and geographic regions is important for safeguarding the genetic diversity of the species. Range-wide sampling is often needed. While population-based sampling tends to be used in literature, Chapter 5 has demonstrated the efficacy of individual-based geographic-evenly sampling in quantifying genetic diversity, while accounting for the effects of geographic distance on the genetic variation. In this study, the genetic distance was highly related to the geographic distance, indicating the importance of taking into account geographic distance in hedgehog population

genetic studies to avoid ascertainment bias in sampling (Lotterhos and Whitlock, 2015; Meirmans, 2015). This sampling scheme allows the effects of various potential barriers to be compared over large scales, and based on this, a barrier to hedgehog movement related to Hadrian's Wall was successfully identified which would be challenging if population-based sampling was used.

6.5 Conclusions

The results from this study have direct conservation implications for hedgehogs. The density and survival of hedgehogs at Brackenhurst are the first long-term SCR-based estimates for the species. Annual apparent survival rate of adult hedgehogs was around 0.5, which seems to be enough for the population to maintain population size. The detected mortality only accounted for 14% of the population, highlighting the need for conservation management to consider not only mortality, but rather identification of drivers of population changes through long-term studies. Hedgehog density was found to be significantly positively associated with soil permeability, and edge density, and negatively related to distance to the nearest building. A new badger (*Meles meles*) sett was identified halfway through the study period, resulting in a shift in the hedgehog density-weighted population centre, a decline in the overall density which was then stabilised, but not a decline in the apparent survival, suggesting spatial segregation on the field scale, due to the landscape of fear response of hedgehogs to badgers, and potential coexistence on the landscape scale of both species. Density was significantly lower on agricultural lands, with that on amenity: pasture: arable being 14: 4: 1, suggesting arable lands on site might not be suitable for hedgehogs. Despite this, arable lands might still facilitate current gene flow between local populations centered on urban areas, as supported by the little genetic differentiation found among local populations in rural Nottinghamshire, based on population genetics analyses. The genomic study provides new insights into the evolution history of the British hedgehog, supporting a natural colonisation of hedgehogs in Britain, with a single expansion from south to north. The genomic analyses revise our understanding of the genetic structure and diversity patterns of the hedgehog and highlight its large-scale gene slow and relatively high genetic diversity, likely related to its large effective population sizes maintained during most of its evolutionary time. The results provide empirical evidence of the reported recent national population decline, which was found to have started a few centuries ago, likely coinciding with agricultural intensification, while

bringing a greater resolution to our understanding of the magnitude and the potential causes of the decline and providing positive messages relating to the conservation status of the hedgehog. As a model species in agroecosystems for informing rural habitat quality and connectivity, the results of the hedgehog have wider conservation implications for other common mammals. The study highlights the importance of combining large fieldwork and molecular datasets and cutting-edge analytical approaches to provide empirical evidence for conservation.

6.6 References

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