BUFFERING CLIMATE DRIVEN EXTINCTIONS 
IN A MONTANE BUTTERFLY: ARE THERE 
GENETIC REFUGES?

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Abstract

Current genetic diversity in cold-adapted species has been shaped by historical processes over millions of years, including through Pleistocene climatic changes, and anthropogenic climatic change will continue to shape genetic diversity in the future. I used Species Distribution Models to predict past, present and future distributions of the montane butterfly *Erebia epiphron* and mtDNA sequencing to examine Europe-wide genetic diversity. These analyses revealed that *E. epiphron* survived in disjunct long-term refugia in continental Europe during previous glacial cycles, resulting in unique genetic diversity which is at risk of being lost in the future. Using mtDNA and ddRAD sequencing, I show that current populations in England and Scotland were colonised separately after the last glacial, experiencing population bottlenecks during colonisation of Britain (Chapter 2 & 3). I also find that the English populations are genetically distinct but have 17% less genetic diversity than Scotland, which is linked to more severe historical bottlenecks in England during post-glacial colonisation (Chapter 3). Morphological studies of body size show that English populations of *E. epiphron* are ~7-8% smaller than Scottish populations, and smaller individuals occur at warmer locations (Chapter 4). Finally, I used questionnaires to gather opinions on Gene Conservation Units (GCUs) and other genetic conservation measures, and found that UK land managers are supportive of integrating genetic considerations into conservation of wild populations (Chapter 5). Overall, my thesis shows that English populations represent at-risk populations (unique lineage, low genetic diversity, predicted to be lost). In contrast, Scottish populations may have the capacity to act as genetic refuges (higher genetic diversity, predicted to persist). Translocations of at-risk populations could be implemented to ensure species persistence and conserve unique genetic diversity, but further research is required in order to understand more about the types of genetic diversity (uniqueness, diversity) to conserve.
# List of contents

Abstract ................................................................................................................. 2  
List of contents ..................................................................................................... 3  
List of Tables ......................................................................................................... 6  
List of Figures ....................................................................................................... 7  
Acknowledgments ................................................................................................. 8  
Declaration ............................................................................................................ 10  

**Chapter 1 – General Introduction** ........................................................................ 12  
1.1 The importance of biodiversity ...................................................................... 12  
1.2 Climate induced range shifts ......................................................................... 13  
1.3 Genetic consequences of range shifts .............................................................. 16  
1.4 Cold-adapted Lepidoptera and *Erebia epiphron* as a study species ............... 17  
1.5 Rationale for thesis ......................................................................................... 19  

**Chapter 2 - Past, current and potential future distributions of unique genetic diversity in a cold-adapted mountain butterfly** ................................................................. 23  
2.1 Abstract ........................................................................................................ 23  
2.2 Introduction .................................................................................................... 24  
2.3 Methods ......................................................................................................... 25  
2.4 Results .......................................................................................................... 28  
2.5 Discussion ..................................................................................................... 34  
2.6 Conclusion .................................................................................................... 40  

**Chapter 3 - Genetic consequences of post-glacial colonisations in montane species in Britain** ................................................................................................................................. 41  
3.1 Abstract ........................................................................................................ 41  
3.2 Introduction .................................................................................................... 42  
3.3 Methods ......................................................................................................... 44  
3.4 Results .......................................................................................................... 47  
3.5 Discussion ..................................................................................................... 51  
3.6 Conclusions and conservation implications ..................................................... 54  

**Chapter 4 - Smaller montane butterflies at warm range boundaries may affect persistence under future climate change** ......................................................................................... 55
List of tables

Table 2.1: Current genetic diversity, and projected loss of climate suitability and haplotype loss in the future (2070)……………………………………………………………………………………………………34

Table 5.1: Case study species of UK conservation importance used to create selection criteria for GCU………………………………………………………………………………………………………71
List of Figures

Figure 1.1: A) Photograph of female *Erebia epiphron*, the mountain ringlet butterfly. B) Upland grassland typical of *E. epiphron* habitat.…………………………………………………………………………18

Figure 1.2: Geographic distribution of *Erebia epiphron* across Europe.………………………………………..19

Figure 2.1: Current distribution of genetic diversity of *E. epiphron* and historical divergence.…..30

Figure 2.2: Current and past projected distributions of *E. epiphron*.………………………………………..32

Figure 2.3: Projecting future climate suitability for *E. epiphron* in 2070 under two RCP climate change scenarios, and associated projected loss of genetic diversity.………………………………33

Figure 3.1: *Erebia epiphron* populations sampled (red crosses) and British current distribution (blue circles) in England and Scotland.…………………………………………………………………..44

Figure 3.2: The ddRAD data split British *E. epiphron* populations into three genetic structures.48

Figure 3.3: Genetic variation between the three genetic clusters, showing that English populations contain significantly less genetic variation than Scotland.…………………………………………………49

Figure 3.4: Pair-wise genetic distance (*Fst*) between populations within England and Scotland.50

Figure 3.5: *E. epiphron* population size (*N_e*) over the last 40,000 years plotted with information on climate fluctuations and tree cover.……………………………………………………………………………………51

Figure 4.1: *Erebia epiphron* populations sampled (red crosses) and British current distribution (blue circles) in England and Scotland.…………………………………………………………………..57

Figure 4.2: Forewing mean length variation shows a relationship with region and temperature in field and museum material.………………………………………………………………………………60

Figure 5.1: Current *in situ* genetic conservation implemented by conservationists and land managers.………………………………………………………………………………………………67

Figure 5.2: Questionnaire responses of 60 conservationists and land managers to test the feasibility, risks and benefits of extending the GCU concept to other species.………………………………68
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*For Colin Graham Minter*
Declaration

I declare that this thesis is a presentation of original work and I am the sole author. This work has not previously been presented for an award at this, or any other, University. All sources are acknowledged as References.

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Chapter 1: General Introduction

Biodiversity that exists today is the result of the continual creation and loss of genetic diversity over millions of years. Interspecific (between species) and intraspecific (within species) genetic diversity has been altered in response to biological (biotic) and physical (abiotic) changes in the environment over time. During the last two million years of the Pleistocene, the Earth has been dominated by climate fluctuations from cold glacial periods to warm interglacial periods shaping the intraspecific genetic diversity of current species. In the past 50 years, human activities has changed the climate at a rate faster than at any time in the last 11,000 years, resulting in climate-driven range shifts. Cold-adapted species are the most at risk under climate changes, as well as their genetic diversity, which needs to be safeguarded to ensure future persistence of these species.

1.1 The importance of biodiversity

Biodiversity has evolved over millions of years resulting in genetic and phenotypic variation between and within species. Within species, population variation in genetic and phenotypic traits results from physical and biological changes in the environment, and local adaptation to specific environmental conditions (Denoel & Winandy, 2015; Moritz, 2002). This resulting intra-specific genetic diversity determines the fitness of populations and the ability of species to adapt to novel environmental conditions (Hoffmann, Sgro, & Kristensen, 2017), potentially increasing their resilience to future environmental change. High genetic diversity has been associated with increased ability to colonise novel environments (Crawford & Whitney, 2010) and recovery following extreme climatic events (Reusch, Ehlers, Hammerli, & Worm, 2005). In contrast, low genetic diversity can lead to lower fitness (Reed & Frankham, 2003), detrimental changes to physiology (Roelke, Martenson, & Obrien, 1993), and higher loads of pathogens and infectious diseases (Cunningham et al., 2008). Therefore low genetic diversity can impact persistence and increase the risk of population decline. Genetic diversity can be lost in a single generation, but can take many generations to be replenished (Nei, Maruyama, & Chakraborty, 1975), and so conserving genetic diversity is likely to be important for conserving biodiversity under future climate change (Leigh, Hendry, Vázquez-Domínguez, & Friesen, 2019).

Anthropogenic activities which have caused climate change (McCarty, 2001; Walther et al., 2002) and land-use change (Stoate et al., 2001) threaten biodiversity. These activities have resulted in changes in biodiversity, with cold-adapted species likely to be vulnerable to declines. In order to halt such biodiversity declines, most countries have signed up to the United Nations Convention on Biological Diversity (CBD, https://www.cbd.int/), and pledged to take action towards conserving biodiversity, and set ‘Aichi’ targets for conserving species and ecosystems. These CBD targets included safeguarding genetic diversity (Aichi target 13: Genetic diversity maintained) (CBD, 2011), although this target specified “cultivated plants and farmed and domesticated animals and of wild
relatives, including other socio-economically as well as culturally valuable species” (CBD, 2011). Thus the focus was primarily agricultural species such as crops and livestock, and ex situ genetic conservation (e.g. gene/seed banks) (Hoban et al., 2021), rather than in wild populations. However, in situ methods exist to protect genetic diversity in wild populations, such as assigning and protecting Gene Conservation Units. This approach has focused on forest trees (Maxted, Hawkes, Ford-Lloyd, & Williams, 2000) but its wider relevance has not been examined beyond plant species. Genetic diversity, including genetic and trait differences within wild species, has rarely been a focus of in situ conservation policy and practice (Laikre, 2010) but proposed future targets post-2020 specify the protection of genetic diversity in wild populations (Hoban et al., 2020). Thus, quantifying current levels of genetic diversity across species ranges is needed to help to understand levels of inbreeding (Fredrickson, Siminski, Woolf, & Hedrick, 2007), population declines (Charman, Sears, Green, & Bourke, 2010) and connectivity (Jangjoo, Matter, Roland, & Keyghobadi, 2016). Genetic diversity would provide information about populations which may be isolated and suffering inbreeding (at risk populations) or populations with sufficient genetic diversity and connectivity with other populations (genetic refugia). Conserving genetic diversity of wild populations is vital for conserving biodiversity, and a better understanding of how historical processes have helped shaped current patterns of genetic variation in extant populations, will help to identify genetic refuges and at-risk populations for conservation management.

1.2 Climate induced range shifts

When species are faced with changes in climate during climate change, populations can respond in three ways (Davis, Shaw, & Etterson, 2005). Firstly, they can escape the deteriorating conditions by dispersing to more suitable habitats and shifting their range; secondly they can remain and adjust to the changing conditions through phenotypic plasticity; or thirdly, they can adapt to the conditions with genetic changes (Gienapp, Teplitsky, Alho, Mills, & Merila, 2008). If species cannot adapt, disperse or adjust through plasticity, then populations could decline, leading to extinctions at their range edge (Thomas, Franco, & Hill, 2006). Species ranges are underpinned by their fundamental and realised niche (Franklin, 2010), and their distributions can be represented by their “climate-envelope” which is the set of climatic conditions (measures of temperature and precipitation) where the species persists (Quintero & Wiens, 2013; Walther et al., 2002). As the climate envelope shifts with climatic changes, species track these movements with geographic range shifts through expansions or retractions. Current patterns of genetic diversity are a consequence of historical changes in species’ distributions during climatic changes in the Pleistocene. Continued range shifts under recent and future anthropogenic climate change could result in range retractions and subsequently genetic loss in cold-adapted species.
Pleistocene range shifts

The Pleistocene (2.58 mya – 11,700 ya) was an epoch dominated by repeated glacial and interglacial periods, including the relatively recent Last Glacial Maximum (LGM) about 21,000 years ago (Crowley & North, 1991). Over the last glacial cycle, ice sheets in the northern hemisphere were at their maximum extent during the LGM (Crowley & North, 1991; Ray & Adams, 2001). In Europe, the region considered in this thesis, had an extensive ice sheet which covered Ireland, northern Britain, Scandinavia and parts of Russia (Patton et al., 2017). During this time, many warm-adapted species retracted during glacial periods, and were confined to southerly refugia where climate conditions were sufficiently mild at the LGM (e.g. in Iberia, Italy and the Balkans in Europe) (Hewitt, 2000; Hewitt, 1999; Schmitt, 2007). These species subsequently expanded northwards as the climate warmed at the beginning of the Holocene epoch. In contrast, cold-adapted species were thought to have shown the opposite pattern, expanding in glacial periods and retracting into mountain refuges and towards the arctic during interglacial warm periods (Schmitt, 2007). However, recent evidence suggests that the distributions of some montane species remained disjunct in mountain refuges throughout glacial and interglacial periods (Haubrich & Schmitt, 2007; Huck, Budel, & Schmitt, 2012; Louy, Habel, Ulrich, & Schmitt, 2014; Schmitt, 2007, 2009; Schmitt, Hewitt, & Muller, 2006), suggesting their ranges shifted downhill and uphill within mountain refuges as temperatures changed. Whether cold-adapted species remained disjunct or expanded during glacial periods will impact the current genetic diversity of extant populations and should be examined in more montane species.

There is also evidence that some warm-adapted species survived in isolated northern cryptic refugia (e.g., warm microclimates) during these climate fluctuations, potentially in locations where they no longer survive (Provan & Bennett, 2008; Stewart & Lister, 2001). By contrast, the possible existence of cryptic refugia in cold-adapted species has not received similar attention. Populations of cold-adapted species which presently occur in northern latitudes in locations that were under ice sheets ~20,000 years ago must represent post-glacial colonisations (Hughes, Gyllencreutz, Lohne, Mangerud, & Svendsen, 2016). However, it is unknown whether these populations represent colonisations from areas of long-term survival in European mountains or from unknown cryptic refugia (i.e., areas of population that existed at the LGM but where populations are no longer present). There is no current evidence for the latter in the literature, but if undetected areas with historical populations existed for warm-adapted species, it is equally plausible that they did so for cold-adapted species. The consequences of past distribution changes and locations of refugia are reflected in current patterns of genetic diversity (Hewitt, 2000; Hewitt, 2004). Thus examining the distribution of genetic diversity will help determine whether currently-unknown populations of this kind existed, and provide insights into the extent of possible genetic loss under future climate change (McCallum, Guerin, Breed, & Lowe, 2014; Wroblewska & Mirski, 2018).
Anthropocene range shifts

In the Anthropocene, the current epoch, humans have become a global geophysical force (Steffen, Crutzen, & McNeill, 2007). Following the industrial revolution which began in the late 19th century, levels of CO₂ have increased in the atmosphere (Hook & Tang, 2013). Since these greenhouse gases started to increase, global average temperatures have also increased by 1.1°C since pre-industrial levels (IPCC, 2021). During this time, cold-adapted species have been experiencing shifts in their geographic range. Some montane species have been shown to be expanding their range upwards in elevation (Parmesan, 2006), including plants (Beckage et al., 2008; Jump, Matyas, & Penuelas, 2009; Kelly & Goulden, 2008; Lamprecht, Semenchuk, Steinbauer, Winkler, & Pauli, 2018; Penuelas & Boada, 2003; Rosbakh, Bernhardt-Romermann, & Poschlod, 2014), small mammals (Moritz et al., 2008) and butterflies (Rodder, Schmitt, Gros, Ulrich, & Habel, 2021; Wilson, Gutierrez, Gutierrez, & Monserrat, 2007). In addition to range expansions, range retractions have also been documented in cold-adapted species, experiencing local extinctions at their low-elevation or latitude range edge (Thomas et al., 2006). This includes climate-driven retractions in montane butterflies in Sierra de Guadarrama mountain range in central Spain (Wilson et al., 2005) where the low elevation boundary has moved on average 212 m uphill. Range retractions have also be documented in northern montane butterflies in Britain (Franco et al., 2006) where the range of the northerly distributed butterflies (Erebia aethiops, Coenonympha tullia) retracted 70-100 km north and the montane butterfly Erebia epiphron retracted 130-150 m uphill. The literature around range retractions is relatively sparse in comparison to range expansions, because of methodological difficulties, such as long-term monitoring in harsh/remote environments (Jump et al., 2009) and the difficulty in detecting declines (Thomas et al., 2006). Even if a decline and/or local extinction is detected, attributing the change to climate can be difficult, when an extinction could be attributed by a number of causes such as habitat loss or invasive species (Thomas et al., 2006). Therefore, it is likely that more cold-adapted species are experiencing range retractions than reported in the literature, and these extinctions could continue into the future.

Anthropogenic climate change has already created risks for biodiversity, and these risks will be amplified with levels of projected further climatic warming (IPCC, 2021). Under future climatic changes, species distributions are predicted to continue to shift. The mountain regions which are predicted to be most severely impacted by climate change are tropical mountains and mountains at high northern latitude (Still, Foster, & Schneider, 1999; Williams, Jackson, & Kutzbach, 2007). Cold-adapted species are more likely to suffer declines under continued warming and future predictions have been made in some species suggesting loss of geographic range (La Sorte & Jetz, 2010; Smith, Gregory, Anderson, & Thomas, 2013). Further predictions of cold-adapted species distributions must be examined in order to understand the extent of range retractions and extinction risk under future climate change scenarios.
1.3 Genetic consequences of range shifts

Responses to past climate changes and historical range shifts have resulted in the patterns of genetic diversity currently found in extant populations (Hewitt, 2004). In cold-adapted species, if montane regions provided refuges for their long-term survival (i.e. in the foothills during glacial periods, and retracting uphill during interglacial periods), and isolation on separate mountain ranges would result in genetic differentiation, with new alleles emerging over time resulting in unique lineages associated with each range (Haubrich & Schmitt, 2007; Huck et al., 2012; Louy, Habel, Ulrich, et al., 2014; Schmitt et al., 2006). In contrast, if cold-adapted species expanded through the lowlands during glacial periods, then there would be evidence of gene flow and shared lineages (Hewitt, 2004). During colonisations and founder events (small subset of large population colonise new area), populations can experience demographic bottlenecks, greatly reducing the population size leading to the loss of genetic diversity (Comps, Gomory, Letouzey, Thiebaut, & Petit, 2001; Hewitt, 2000; Holliday, Yuen, Ritland, & Aitken, 2010). Therefore, in many warm adapted species, a south-north trend of genetic loss can be shown, with populations in northern latitudes containing lower genetic diversity (Schmitt & Seitz, 2002). However, if colonised populations are from cryptic refugia, then these populations may contain unique genetic diversity no longer present elsewhere in extant populations (Bhagwat & Willis, 2008; Provan & Bennett, 2008), resulting in more complex latitudinal patterns of diversity. Compared with warm-adapted species, colonisation patterns of cold-adapted species are less well studied, and it is unclear how cold-adapted montane species currently inhabiting northern latitudes were colonised and where from, and the genetic consequences of colonisation.

After colonisation, further genetic and phenotypic trait changes may occur, resulting in differences between populations if gene flow is restricted. For example, genetic changes may occur if species are isolated on different mountains, resulting in genetic divergence between populations if valleys act as barriers to dispersal (Monsen & Blouin, 2004; Sexton, Hangartner, & Hoffmann, 2014; Velo-Anton, Parra, Parra-Olea, & Zamudio, 2013). This isolation can result in inbreeding if populations are small, which can reduce genetic diversity and the persistence of populations (Broquet et al., 2010; Saccheri et al., 1998). Therefore, potential genetic changes following colonisation may lead to negative genetic impacts, and influences the ability for populations to adapt and persist. Examining the genetic consequences of post-glacial colonisation and potential isolation has not been examined in cold-adapted species, but it is important to quantify to understand potential persistence. Populations may also experience different local environmental conditions and selective pressures, leading to variation in traits through local adaptation or phenotypic plasticity (Kawecki & Ebert, 2004; Valladares et al., 2014). This can lead to variation in traits such as body size (Bai, Dong, Guan, Xie, & Xu, 2016; Gunter et al., 2019), development time (Robinson & Partridge, 2001), and growth rates (Van Doorslaer & Stoks, 2005). Body size varies across latitude and altitude, and can be influenced by temperature (Atkinson, 1994). For example, some montane species located in warm lower altitude
areas have smaller body size (Brehm, Zeuss, & Colwell, 2019). Small size may reduce fecundity and dispersal (Gao et al., 2016; Lopez, McClanahan, Graham, & Hoddle, 2014) and the ability for individuals to colonise new environments (Hill, Thomas, & Blakeley, 1999). Therefore body size variation in montane species may have implications for their fitness and persistence in the future. Genetic diversity is important for the ability of species to adapt and persist, and phenotypic variation such as body size may have implications for fitness. Therefore quantifying genetic and phenotypic consequences of northern colonised populations will provide more understanding for implications for persistence and identifying genetic refugia.

Under future climate changes, predicted range retractions and extinctions may result in the loss of genetic diversity in cold-adapted species. The loss of genetic diversity has been predicted under future climate change scenarios in cold-adapted species (Yannic et al., 2014), and linking past, present and future distributions of genetic diversity has rarely been investigated (Alsos, Alm, Normand, & Brochmann, 2009). Therefore to project future genetic diversity in cold-adapted species, there is a need to understand how the past shaped the present genetic diversity (Wroblewska & Mirski, 2018) in order predict genetic loss of cold-adapted species in the future. This, coupled with understanding of the genetic and phenotypic consequences of northern latitude colonisations, will provide more understanding into the potential persistence of cold-adapted species, including identifying at-risk populations and areas of genetic refugia.

1.4 Cold-adapted Lepidoptera and Erebia epiphron as a study species

Lepidoptera are poikilothermic and so are sensitive to changes in climate, and cold-adapted Lepidoptera are especially vulnerable to changes in climate (Deutsch et al., 2008; Elsen & Tingley, 2015). This is because these montane cold-adapted butterflies already have limited suitable climate space which will eventually ‘run out’ under future climatic changes. The Erebia genus are the most diverse butterfly genus in the Palaearctic region, with high morphological variation within and between species (~ 1000 species described) (Albre, Gers, & Legal, 2008; Cupedo, 2010; Tennent, 2008). The species in this genus inhabit cold environments and have arctic-alpine distributions (Tennent, 2008) and occur in a number of different habitats including alpine grassland, mountain meadows, marshlands, shrubby tundra, screes and sparsely forested areas (Pena, Witthauer, Kleckova, Fric, & Wahlberg, 2015). The high diversity of Erebia in Europe is thought to be due to isolation and diversification of populations within the Alps, Carpathian and Iberian mountain systems, arising from Pleistocene range shifts and allopatric speciation (Pena et al., 2015). In some Erebia species, it has been found that there is genetic divergence between mountain systems resulting from long-term separation (Louy, Habel, Ulrich, et al., 2014; Schmitt, Habel, Rodder, & Louy, 2014; Schmitt et al., 2006). However, the genetic diversity of northern post-glacial populations of Erebia have not yet been studied. Recent climate-driven range retractions have been documented in Erebia epiphron and
Erebia aethiops in Britain (Franco et al., 2006) and in Erebia cassioides in the Apennines (Scalercio, Bonacci, Mazzei, Pizzolotto, & Brandmayr, 2014). Erebia species are predicted to lose substantial areas of their ranges as suitable climate deteriorates under future climate change (Settele et al., 2008), which could lead to genetic losses in these species. However it’s currently unclear how future climate change may impact the genetic diversity of this genus, and whether northern populations of Erebia have currently low genetic diversity following post-glacial colonisations. Therefore, the Erebia are a good model species to understand the genetic consequences of Pleistocene range shifts and whether genetic diversity is predicted to be lost under future climate change.

*Figure 1.1:* A) Photograph of female Erebia epiphron, the mountain ringlet butterfly. B) Upland grassland typical of E. epiphron habitat. Both photographs taken at Irton Fell, England. Photo credit: Melissa Minter.

Erebia epiphron (mountain ringlet butterfly), is a small brown butterfly with orange eye spots and black pupils on both forewings and hindwings (Figure 1.1A). E. epiphron is a montane specialist and occurs across Europe, including the Alps, Carpathians, Apennines, Balkans, Tatras, Pyrenees, Cantabrians, Massif Central and Vosges; and in Britain is found in the Lake District, England and the Scottish highlands (Figure 1.2). This species can be found in upland and montane grassland (Figure 1.1B) using Nardus stricta and Festuca ovina as the main larval food plants (Ewing, Menendez, Schofield, & Bradbury, 2020). In Europe, E. epiphron is in flight mainly between July and August (Konvicka et al., 2021), and in Britain between June and July. E. epiphron appears to be the only montane Erebia which colonised Britain after the last glacial, however there are six Erebia species which colonised Scandinavia (Settele et al., 2008). There is genetic differentiation among E. epiphron populations in Europe (Schmitt et al., 2006), however the areas which may act as long-term refugia have not been identified. E. epiphron which occur in Britain are post-glacial colonisations, but the origin of these populations is unknown. In Britain, there have been extinctions of E. epiphron at the warm low elevation range edge (Franco et al., 2006) and so quantifying genetic diversity of these at
risk populations is important for future conservation. Therefore *E. epiphron* represents a good model species to understand how Pleistocene climate change has shaped genetic diversity across Europe, and the genetic and phenotypic consequences of post-glacial colonisations of Britain, to identify at-risk populations and to explore genetic conservation management for this species.

![Geographic distribution of *Erebia epiphron* across Europe](image)

**Figure 1.2:** Geographic distribution of *Erebia epiphron* across Europe, black dots represent 50 x 50 km square grids where *E. epiphron* has been recorded as present. Distribution data from the Distribution Atlas of European butterflies (http://www.ufz.de/european-butterflies/index.php?en=42605).

### 1.5 Rationale for thesis

In this thesis, I examine the genetic diversity of *E. epiphron* across Europe, to understand consequences of climate-driven post-glacial range shifts. To do this, I investigate past, present and future demographic changes in *E. epiphron* and genetic consequences across Europe, along with the resulting genetic and phenotypic diversity in Britain following post-glacial colonisation; and genetic conservation techniques to safeguard genetic diversity. My three objectives for this thesis include:

1) to examine how past and future climate-induced range shifts influence the patterns of genetic diversity in a cold-adapted species in Europe;
2) to examine the genetic and morphological consequences of post-glacial colonisation of Britain by a cold-adapted species;

3) to explore whether Gene Conservation Units (GCUs) could be implemented more widely to safeguard genetic diversity in wild populations.

In Chapter 2, I examine climate-driven range shifts by predicting the past, current and future distributions of *E. epiphron*, identifying glacial refugia, patterns of post-glacial colonisations and future loss of genetic diversity. From this analysis, I conclude that current populations in England and Scotland are genetically dissimilar, and therefore *E. epiphron* colonised Britain in two separate colonisation events. Therefore, in Chapter 3 I examine the genetic consequences of post-glacial colonisations of Britain by *E. epiphron*, to quantify genetic differences in England and Scotland populations in further detail, and identify at-risk populations. The two populations in Britain may also contain morphological variation, therefore, in Chapter 4, I examine body size variation in *E. epiphron* in Britain in relation to region and temperature variation. I find that there are predicted losses in the future in *E. epiphron* and in other cold-adapted species, therefore, in Chapter 5, I investigate the current implementation of Gene Conservation Units (GCUs) in Britain, and whether GCUs could be extended to conserve genetic diversity of a wide range of wild species. The key aims of each of these chapters are outlined below:

**Chapter 2: Past, current and potential future distributions of unique genetic diversity in a cold-adapted mountain butterfly** (published in Ecology and Evolution, Minter et al., 2020)

In this chapter, I aim to understand how past climate range shifts have shaped current genetic diversity and whether this is at risk of being lost under future climate change. To do this, I use mtDNA sequencing to map the current distribution of genetic diversity of *E. epiphron*, and I also use species distribution modelling to project past, current and future distributions of the species.

**Key objectives:**

1) Identify areas of glacial refugia for *E. epiphron* in Europe and source populations of post-glacial colonisations;

2) Identify genetic haplotype diversity across populations in mountain regions in continental Europe and Britain;

3) Examine potential loss of genetic diversity under future climate change scenarios.

**Chapter 3: Genetic consequences of post-glacial colonisations in montane species in Britain**

In this chapter, I aim to examine the genetic differences of English and Scottish populations following post-glacial colonisation by *E. epiphron* of Britain, and subsequent isolation into mountain populations. I use ddRAD data to examine the differences in genetic diversity and isolation in
mountain regions in England and Scotland, along with predicting population sizes during post-glacial colonisation.

Key objectives:

1) Examine genetic structure and diversity in England and Scotland;
2) Quantify genetic connectivity among populations and regions, and the extent to which populations are genetically isolated with respect to geographic and environmental distance;
3) Infer variation in population size over the past 40,000 years, to explore evidence for population bottlenecks following colonisation of Britain.

Chapter 4: Smaller montane butterflies at warm range boundaries may affect persistence under future climate change

In this chapter, I aim to examine body size of *E. epiphron* in relation to temperature, testing whether individuals from warmer areas and those emerging in warmer years are smaller. To do this I examine variation in body size (between and within populations) between England and Scotland, and use museum material, to test whether spatial and temporal variation in body size is related to temperature.

Key objectives:

1) Examine body size variation in populations of *E. epiphron* in England and Scotland in relation to temperature;
2) Examine body size variation in museum specimens over ~100 years in relation to temperature;
3) Examine differences in within-population body size variation among regions, to identify differences in plasticity.

Chapter 5: Exploring the potential for ‘Gene Conservation Units’ to conserve genetic diversity in wild populations (CASE placement with NatureScot, and published in Ecological Solutions and Evidence, Minter et al., 2021)

In this chapter, I aim to understand the current extent GCUs and other genetic conservation techniques in practise, and examine whether GCUs could be implemented more widely. I discuss current global application of in situ genetic conservation management techniques using a systematic literature review. I then explore whether the GCU approach could be effective for conserving evolutionary potential in a wide range of plant and animal taxa, using a questionnaire and four case study species.

Key objectives:

1) Review the current implementation of GCUs and other genetic conservation techniques using a systematic literature review;
2) Use a structured questionnaire to canvass conservationists’ and land managers’ opinions for adopting a system of GCUs in Britain;

3) Test whether existing methods for voluntary accreditation of GCUs for trees are appropriate for application to other taxa, using case study species.
Chapter 2: Past, current and potential future distributions of unique genetic diversity in a cold-adapted mountain butterfly

2.1 Abstract

Aim Climatic changes throughout the Pleistocene have strongly modified species distributions. We examine how these range shifts have affected the genetic diversity of a montane butterfly species, and whether the genetic diversity in the extant populations is threatened by future climate change.

Methods We analysed mtDNA to map current genetic diversity and differentiation of *E. epiphron* across Europe to identify population refugia and post-glacial range shifts. We used species distribution modelling (SDMs) to hindcast distributions over the last 21,000 years to identify source locations of extant populations, and to project distributions into the future (2070) to predict potential losses in genetic diversity.

Results We found substantial genetic diversity unique to specific regions within Europe (total number of haplotypes = 31, number of unique haplotypes = 27, $H_d = 0.9$). Genetic data and SDM hindcasting suggest long-term separation and survival of discrete populations. Particularly high rates of unique diversity in post-glacially colonised sites in England ($H_d = 0.64$), suggests this population was colonised from a now extinct cryptic refugium. Under future climate change, SDMs predict loss of climate suitability for *E. epiphron*, particularly at lower elevations (< 1000 metres above sea level) equating to 1 to 12 unique haplotypes being at risk under climate scenarios projecting 1 °C and 2-3 °C increases respectfully in global temperature by 2070.

Main conclusions Our results suggest that historical range expansion and retraction processes by a cold-adapted mountain species caused diversification between populations, resulting in unique genetic diversity which may be at risk if distributions of cold-adapted species shrink in future. Assisted colonisations of individuals from at-risk populations into climatically-suitable unoccupied habitat might help conserve unique genetic diversity, and translocations into remaining populations might increase their genetic diversity and hence their ability to adapt to future climate change.
2.2 Introduction

Projecting the future geographic distribution of genetic variation within species’ ranges, and the potential loss of genetic variation from anthropogenic climate change, requires understanding of the past, present and future distributions of species (Wroblewska & Mirski, 2018). Geographic variation in the distribution of genetic variation across a species’ range results from a combination of historical and current conditions, which influence patterns of genetic differentiation among populations that are, or have been, geographically isolated, and from colonisation bottlenecks during range shifts (Hewitt, 2004). These range shifts and their genetic consequences have primarily been driven by the fundamental niche of a species, or their ‘climate-envelope’, and species’ ranges shift to track environmental changes, altering the location of populations and their genetic structure (Thomas, 2010) (Hewitt, 2004; McCallum, Guerin, Breed, & Lowe, 2014; Thomas, 2010). The Earth has gone through many climate fluctuations, including glaciations in the Pleistocene and human-induced climate change in the current Anthropocene (Hewitt, 2004; Santer et al., 2019). Future anthropogenic climate warming may further impact species through distribution changes, genetic erosion and extinctions (Botkin et al., 2007). Cold-adapted/mountain species may be especially vulnerable to future climate changes as they are already restricted to mountain ecosystems where suitable climate space is limited, and loss of genetic diversity within these range-restricted cold-adapted species may reduce their ability to adapt to future changes (Elsen & Tingley, 2015). Understanding how past climatic changes have impacted current genetic structure may allow us to make predictions for the likely extent of genetic loss under future climate change, and thereby prioritise at-risk populations for conservation management (McCallum et al., 2014; Wroblewska & Mirski, 2018).

During the last ice age, ice sheets were at their greatest extension 20,000-21,000 years ago, during the last glacial maximum (LGM) (Crowley & North, 1991; Ray & Adams, 2001). During the LGM, species were thought to persist where climatic conditions were buffered, at lower elevations or in more southerly regions (Dapporto et al., 2019; Morelli et al., 2016), however some studies have shown evidence of species surviving in northern isolated refugia (Provan & Bennett, 2008; Schmitt & Varga, 2012; Stewart & Lister, 2001). Cool-adapted species which currently occur in mountain ecosystems were probably more widespread during the LGM and only became isolated in their current interglacial populations after climate-induced range retraction, although some cold-adapted species were already restricted to isolated glacial refugia during the LGM (Schmitt, 2009; Schmitt, Hewitt, & Muller, 2006). The consequences of past distribution changes will be reflected in current genetic diversity, because contractions and expansions from long-term refugia leave a genetic signature of high diversity in refugia compared to lower diversity in recently-colonised populations (Hewitt, 2000; Keppel et al., 2012; Morelli et al., 2016). Thus understanding historical interactions of cold-adapted species with climate can help us understand current genetic structure and diversity of populations.
Lepidoptera are poikilothermic and therefore sensitive to changes in climate, and those species which are cold-adapted are particularly vulnerable to warmer conditions (Deutsch et al., 2008; Elsen & Tingley, 2015). Some cold-adapted Lepidoptera are experiencing extinctions at their low latitude/elevation margins as the climate deteriorates for these species (Franco et al., 2006; Wilson, Gutierrez, Gutierrez, & Monserrat, 2007). The Mountain Ringlet *Erebia epiphron* is a butterfly found in the mountains of continental Europe and Britain, and its distribution has retracted 130-150 m uphill in Britain over the past five decades due to climate warming (Franco et al., 2006). Therefore *E. epiphron* represents a good model organism to understand how past climate-induced changes have impacted current genetic structures of populations, and whether genetic diversity may be lost with further climate-induced local extinctions.

Species distribution models (SDMs) are commonly used to project future distributions of species under climate change scenarios (Guo et al., 2017; Urban, 2015), and to develop climate adaptation conservation strategies. These modelling approaches have also been used with palaeoclimate data to hindcast past distributions and to understand how they shape current population structures (Smith et al., 2013). Phylogeography with genetic techniques can be used to identify divergence between populations and to infer historical distribution patterns and colonization routes (Luquet et al., 2019). Previous studies have shown how a combination of species distribution modelling and phylogeography can provide better understanding of past, present and future distributions of species, and predict the potential loss of genetic diversity resulting from climatic warming (Schmitt, Habel, Rodder, & Louy, 2014; Wroblewska & Mirski, 2018; Yannic et al., 2014).

In this study, we use mtDNA sequencing to map the current distribution of genetic diversity of the cold-adapted butterfly, *E. epiphron*, and also use species distribution modelling to project current, past, and future distributions of the species. We use this genetic and modelling information to determine the distribution of *E. epiphron* in continental Europe during the last glacial maximum, the locations of glacial refugia, and patterns of subsequent postglacial expansion into northerly latitudes in Britain. We identify populations with unique genetic diversity and examine potential loss of genetic diversity under future climate change scenarios in order to prioritise populations for protection.

### 2.3 Methods

*Genetic analyses to map current haplotype diversity*

We sampled 146 adults of *E. epiphron* from 13 mountain regions across continental Europe and Britain. European populations (76 adults) were sampled between July - August 2002-2014, populations in England and Scotland (74 adults) were sampled in June-July 2016-2019, and adults preserved in 100% ethanol at -20°C. All relevant fieldwork permissions were obtained. DNA was extracted from 111 individuals with Omega bio-tek E.Z.N.A.® DNA Isolation Kit following the
manufacturer’s protocol. For each individual, the head and antennae were removed and placed in 1.5 ml tubes with CLT buffer and Proteinase K and homogenised with pellet pestles. A 658-bp fragment of the mitochondrial cytochrome oxidase-I (COI) gene was amplified using the primers LepF (5’-ATTCAACCAATCATAAGATATTGG-3’) and LepR (5’- TAAACTTCTGGATGTCCAAAAATCA-3’) (Hajibabaei, Janzen, Burns, Hallwachs, & Hebert, 2006). PCR amplification of individual DNA samples was carried out in 20 µl reactions which included 1.8 µl of template DNA, 1x PCR reaction buffer (Promega), 1.5 mM MgCl₂, 0.2 mM of dNTPs and 1U of Taq DNA polymerase (Promega GoTaq®). PCR conditions used the following profile: 94°C for 2 minutes (one cycle), 2 minute at 94°C, 58°C for 45s and 72°C for 1 minute (35 cycles), followed by a final extension step of 75°C for 5 minutes. PCR products were purified and Sanger sequenced with forward and reverse primers using © Eurofins Scientific PlateSeq service and LightRun Tube service. Chromatograms were checked visually using SeqTrace (Stucky, 2012). Additional COI sequences were obtained from a panel of 39 samples collected in England in June 2016 as a part of a whole genome resequencing project (NERC Highlight project NE/N015797/1). Briefly, the complete mitochondrial genome was assembled for each individual sample using the MitoZ toolkit (Meng et al. 2019) and annotated using the mitos2 webserver (Bernt et al. 2013). Low coverage regions (<10) were masked to avoid introducing low quality SNPs and the COI region was extracted for further analyses. These 150 sequences along with 65 existing COI sequences from Genbank were combined to create a data set of 215 COI sequences from 13 mountain regions across the species’ European range (for sample information see Appendix S1.1 and map of mountain regions see Appendix S1.2). These sequences were aligned with ClustalX implemented in MEGA-X (Kumar, Stecher, Li, Knyaz, & Tamura, 2018) and the alignment checked by eye and cropped to the same length (649 bp). Haplotypes were identified and genetic diversity measures were determined using DnaSP6 (Rozas et al., 2017). Genetic diversity measures included number of haplotypes (H_s), number of unique haplotypes (H_u), haplotype diversity (H_d, the probability that two randomly sampled alleles are different) and nucleotide diversity (π, the average number of nucleotide differences per site between sequences (Nei, 1987). A TCS network (Templeton, Crandall, & Sing, 1992) of all haplotypes was constructed using PopArt (Leigh & Bryant, 2015). A COI phylogenetic tree was constructed in BEAST (Suchard et al., 2018) of the Erebia genus, outgroups and the E. epiphron populations. The same methods and COI sequences were used from (Pena et al., 2015) using a log-normal relaxed molecular clock, with a birth-death incomplete speciation model for the randomly generated tree prior, and then an uncorrelated log-normal relaxed molecular clock and all the programs other default settings to model the rate of evolution. The age between Erebia and its sister taxa was set at 37.4± 2 Myr, (Pena et al., 2015) to estimate age in divergence between E. epiphron subpopulations.
Using species distribution modelling (SDMs) to map current distribution of *E. epiphron*

Current distribution data for *E. epiphron* (50 x 50 km grid resolution) were obtained from the Distribution Atlas of European butterflies (http://www.ufz.de/european-butterflies/index.php?en=42605). Current (1970-2000) climate data were downloaded from WorldClim (http://www.worldclim.org/) at a resolution of 2.5 arc minutes (~4.5 km grid cell resolution). Climate variables for inclusion in SDMs were selected to reflect climate limitations and extremes of cool-adapted species, which are likely to be most limited by climatic conditions during the coldest and hottest times of the year. We therefore included climate data on annual mean temperature and mean precipitation of the coldest quarter (December to February) and warmest quarter (June to August) of the year (Smith et al., 2013). Spatial autocorrelation was tested using Moran’s I in R. The butterfly distribution data were at 50 km grid resolution, but the species is likely to be restricted by local climate conditions in each grid square (Smith et al., 2013). Thus, we included in models only the coldest/warmest and wettest/driest cells (4.5 km resolution) within each 50 km grid, resulting in a total of eight climatic variables being incorporated into our SDMs (see Appendix S1.3). 50 x 50 km grid cell resolution data are appropriate for our model building to address biogeographic questions at regional scales, because we are interested in changes in the distribution of the study species over long periods of time (i.e. millennia), rather than shorter-term changes at individual sites. This 50km spatial resolution also ensures that the pseudo-absences (i.e. locations where *E. epiphron* is assumed to be absent) are more accurate representations of true absences, because these grids have been visited by butterfly recorders but *E. epiphron* was not recorded as present. In addition, 50km data for presences cover the entire global distribution of *E. epiphron* at this spatial resolution. Butterfly distributions were modelled using an ensemble approach (R package BIOMOD2; (Thuiller, Lafourcade, Engler, & Araujo, 2009), combining outputs from the models; Generalised Linear Models (GLM), Multiple Adaptive Regression Splines (MARS), Maximum Entropy (MAXENT,Phillips), Generalized Additive Model (GAM), Boosted Regression Trees (GBM), Classification Tree Analysis (CTA), Artificial Neural Network (ANN), Surface Range Envelope (SRE), Flexible Discriminant Analysis (FDA) and Random Forest (RF). We used the mean Receiver Operating Characteristic (ROC) value to evaluate each model, with a threshold of ROC > 0.85 for inclusion of models within the ensemble model. We restricted pseudo-absences to locations within a buffer of 250 km around presence data points to avoid placing absences in mountain systems with potentially suitable climate space that are not currently occupied by the species (e.g. Scandinavia) (Akcakaya & Atwood, 1997; Hirzel, Helfer, & Metral, 2001). Models were generated using 70% training data and 30% testing data (Franklin, 2010; Huberty, 1994).
Hindcasting past distributions and identifying glacial refugia

We incorporated paleoclimate data into our ensemble SDM for the eight climate variables representing the coldest/hottest and driest/wettest locations within each 50 km grid square. Data for climate projections over the last 21,000 years were downloaded from Paleoview (2.5 × 2.5° (latitude/longitude) grid) (Fordham et al., 2017), and downscaled to match the resolution of the current climate data (2.5 arc minutes), using established methods (Mitasa & Mitas, 1993; Platts, Omeny, & Marchant, 2015; Ramirez-Villegas & Jarvis, 2010). We projected climate suitability for *E. epiphron* every 1,000 years from the LGM to 1,000 years before present, generating 21 outputs, which were each clipped using Eurasian ice sheet data (Hughes et al., 2016). Long-term climate suitability of 50 km grid squares was calculated by overlaying the 22 output maps and summing the climate-suitability probability values of each grid, and then designating the top 30% of grids with highest probability values as areas of highest long-term climate stability for the study species (Chan, Brown, & Yoder, 2011).

Projecting future distributions and loss of genetic diversity

Future climate projections for 2070 were obtained from IPCC 5th Assessment Report (Complete Coupled System Model, CCSM4 global climate models) from WorldClim (http://www.worldclim.org/; 2.5 arc minutes resolution) for high (RCP 8.5, ~2-3°C warming) and low (RCP 2.6, ~1°C warming) future climate scenarios. Unique haplotypes were assumed to be at risk if all 50 km grid squares in one of the 13 mountain regions were predicted to become climatically unsuitable in the future (based on binary presence or absence threshold probability values from the ensemble SDM output). We set the threshold value as the probability value associated with the low elevation climatic range edge *E. epiphron* in its current range (low elevation range boundary in England; threshold probability = 0.49). Using this threshold, model probabilities were converted into presence/absence to show grid squares with no change over time (i.e., population persistence), grids predicted to become climatically unsuitable (i.e., extinction), and grids predicted to become climatically suitable (i.e., colonisation). Haplotype risk (*Hr*) was calculated as the number of unique haplotypes at risk in each of the 13 mountain regions (Figure 1A) due to projected loss of all climatically suitable areas within a region in the future.

2.4 Results

Current haplotype diversity across 13 mountain regions in Europe

From our 215 mtDNA samples, we identified 31 mtDNA haplotypes across Europe, including 27 haplotypes unique to a specific mountain region (Figure 2.1A, Table 2.1). The high frequency of unique haplotypes across Europe suggests low levels of allele-sharing. There was also high genetic
differentiation between populations (AMOVA, \( \phi = 0.76, p < 0.001 \)) and the divergence between some of these populations is dated before the last glacial maximum (phylogenetic tree: see Appendix S1.5). The mountain regions containing the highest haplotype diversity include the Pyrenees \((H_d = 0.63)\) the western Alps \((H_d = 0.91)\) and England \((H_d = 0.64)\) (Table 2.1). The mountain regions containing only unique haplotypes include the Carpathians \((H_u = 2)\) and the Tatras \((H_u = 2)\). Populations in England \((H_u = 6)\) and the western Alps \((H_u = 6)\) not only had the highest number of unique haplotypes but also contained some shared haplotypes with other regions (Figure 2.1A). There are six unique haplotypes in England which diverged from haplotype 8 (Figure 2.1B), which is present in England, Scotland, Vosges and the western Alps. Scotland, in addition to the shared haplotype 8, contains one unique haplotype \((\text{haplotype } 30)\), which has diverged from haplotype 8 by 1 substitution and shares haplotype 10 with the Apennines (Figure 2.1). Despite evidence that regions are differentiated, shared haplotypes also provide evidence of historical gene flow across Pyrenees and Cantabrians, and between the Alps and Balkans (Figure 2.1). The Massif Central population shares one haplotype \((\text{haplotype } 16)\) with the Pyrenees and Cantabrian Mountains, and has one unique haplotype \((\text{haplotype } 29)\) which diverged from haplotype 16 by one substitution (Figure 2.1B).
Figure 2.1: Current distribution of genetic diversity of *E. epiphron* and historical divergence. A) Frequency pie charts of haplotypes across the species’ European range, including the current observed distribution of *E. epiphron* (white circles; 50 km resolution) in 13 mountain regions, with number of samples (individuals) in brackets. B) TCS network of all 31 identified haplotypes. Size of circle
represents number of individuals containing that haplotype and tick marks represent a nucleotide substitution.

**Modelling the current distribution of *E. epiphron***

Our ensemble SDM was a good fit to the current distribution of *E. epiphron* (95.4% of presences predicted correctly, 76.3% of pseudo-absences predicted correctly (based on the total presence data), ROC = 0.9) (Figure 2.2A). Areas predicted to be climatically suitable but currently uninhabited by *E. epiphron* include Wales, Scandinavian mountains and eastern Balkans, the latter of which is currently occupied by *Erebia orientalis*. The model rated the minimum temperature of the warmest quarter of the year (June – August) as the most important variable for predicting climate suitability for the species (average importance of this variable across models = 0.73; importance rated from 0-1), probably because this is an important variable in identifying high elevation areas within a 50 km grid square.
Figure 2.2: Current and past projected distributions of *E. epiphron*, A) current probability of climate suitability and current distribution records (white circles). Past climate suitability B) 6,000 years ago, C) 11,000 years ago, D) 16,000 years ago E) 21,000 years ago (i.e. LGM; blue shading shows the extent of the ice sheet (from Hughes et al., 2016)). Probability values of occurrence for b-e scaled from 0 (unsuitable, white) to 1 (suitable, black). Panel f shows climate stability over time since the LGM produced by summing 22 outputs from SDMs for the last 21,000 years, plus the output for the present (summed probability values scaled from 0.73 (white) to 20 (black), with the top 30% of grids shown as white circles. See Appendix S1.4 for all output maps.

Hindcasting past distributions of *E. epiphron* and identifying areas of long-term survival

Climate suitability in the LGM (21,000 years before present) showed overlap of climatically suitable areas (at 50 km grid resolution) with many locations currently occupied by *E. epiphron*, as well as some southerly locations (Figure 2.2E). This overlap was confirmed when all 21 SDM outputs for each 1000-year time period up to the present day were combined to show long-term climatic stability since the LGM (Figure 2.2F). These climate stability maps provided evidence that the locations of
glacial refugia were in areas of high topographic variation within the species’ current distribution in continental Europe.

Figure 2.3: Projecting future climate suitability for *E. epiphron* in 2070 under two RCP climate change scenarios, and associated projected loss of genetic diversity. A) low RCP 2.6 climate scenario (~1°C increase by 2070), and C) high RCP 8.5 scenario (~2-3°C increase by 2070) showing grids projected to remain climatically suitable (black), become unsuitable (orange), and become suitable (blue). B) low RCP 2.6 scenario haplotype map with predicted lost haplotypes coloured in white (2 regions lost, 1 unique haplotype lost), and D) high RCP 8.5 haplotype map with predicted lost haplotypes coloured in white (5 regions lost, 12 unique haplotypes lost).

**Projecting future distributions and loss of genetic diversity**

As expected for a cold-adapted species, SDM outputs from both high and low future climate change scenarios project that many extant *E. epiphron* areas will have reduced climate suitability in the future (38-64% loss of 7,000 km² occupied grids across Europe) (Figure 2.3, Table 2.1). The loss of climate suitability is most severe in lower elevation sites, as shown by significant linear regressions between change in probability over time and average elevation of the 50 km grid square (low scenario: \( p < 0.001, R^2: 0.27, F_{150} = 56.51 \), high scenario: \( p < 0.001, R^2: 0.13, F_{150} = 22.86 \)). The mountain regions predicted to experience the greatest reduction in range size are the Vosges (100% loss of grid squares under both scenarios) and Apennines (100% loss of grid squares under both scenarios), followed by
the Balkans (75-100% loss), Carpathians (70-100% loss), England (50-100% lost) and Cantabrians (63-81% loss) (Figure 2.3, Table 2.1). These range retractions result in the potential loss of 1 haplotype under the low climate change scenario (RCP 2.6); and the total loss of 12 unique haplotypes under the high climate change scenario (Figure 2.3, Table 2.1). Many of the haplotypes predicted to be lost are a single substitution from their nearest haplotype, however the haplotypes in the Carpathians are more genetically distinct (Figure 2.1B). By contrast, range sizes in the Alps and Scotland are projected to remain relatively stable, assuming the species colonises sites at higher elevations that are predicted to become climatically suitable in these regions. Under both scenarios, areas north of Scotland and England become suitable in the future. Although *E. epiphron* does not currently occur in Scandinavia, our models predict that this area will remain stable in climate suitability in the future.

**Table 2.1**: Current genetic diversity, and projected loss of climate suitability and haplotype loss in the future (2070). $H_n$ = number of haplotypes; $H_u$ = number of unique haplotypes; $\pi$ = Nei nucleotide diversity ($\pi_i$); % range change = % change in range size (number of occupied 50km grid squares) in the future compared with current distribution, and $H_r$ = number of unique haplotypes at risk in the future, under RCP 2.6 (low) and 8.5 (high) climate scenarios.

<table>
<thead>
<tr>
<th>Region</th>
<th>$H_n$</th>
<th>$H_u$</th>
<th>$H_d$</th>
<th>$\pi$</th>
<th>% Range change (low)</th>
<th>% Range change (high)</th>
<th>$H_r$ (low)</th>
<th>$H_r$ (high)</th>
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<tbody>
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<td>No change</td>
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<td></td>
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<td>0.0015</td>
<td>-50%</td>
<td>-100%</td>
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<td>-100%</td>
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<td>-75%</td>
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<td>0.0059</td>
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<td>-81.8%</td>
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<td>0.0024</td>
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<td>-100%</td>
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<td>0.303</td>
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<td>-100%</td>
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<td>1</td>
</tr>
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<td>0.0043</td>
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<td>-41.3% (all Alps)</td>
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<td>Alps East</td>
<td>2</td>
<td>1</td>
<td>0.303</td>
<td>0.0005</td>
<td>-14.3% (all Alps)</td>
<td>-41.3% (all Alps)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alps Central</td>
<td>2</td>
<td>1</td>
<td>0.182</td>
<td>0.0006</td>
<td>-14.3% (all Alps)</td>
<td>-41.3% (all Alps)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### 2.5 Discussion

By using species distribution modelling and mtDNA analyses, we explore the past, present and potential future distributions of genetic diversity in the cold-adapted species *E. epiphron*. We identify high levels of genetic differentiation across Europe, and found evidence of long-term climate suitability in many of these regions since the LGM, which suggests these climatically stable regions...
were refugial areas of long-term survival by our study species over the last 21,000 years, and potentially longer-term areas of persistence over previous glacial-interglacial cycles. Our study focuses on a single mountain species but our findings are likely to be widely applicable to other mountain species where populations contain unique genetic diversity as a consequence of past climate fluctuations, and which may be at risk under future climate warming. These areas of long-term survival are within topographically heterogeneous landscapes, allowing populations to shift to the foothills during glacial periods. Our analyses also revealed that populations in the Massif Central, Vosges and Britain are presumed postglacial colonisations (Figure 2.1, Figure 2.2F) due to low climate suitability over time, shared haplotypes and the fact that Britain was under an ice sheet during the LGM. Britain was apparently colonised via two different routes, with the Scottish populations likely originating from populations in Vosges/Alps mountain regions due to the high prevalence of shared haplotype 8. By contrast, the English population has high levels of unique genetic diversity, and no evidence that any of the six unique haplotypes are shared with other extant populations (although there is one shared haplotype present), suggesting the English population has separated from the western Alps before the last glacial maximum (given the large number of nucleotide substitutions; Figure 2.1B), and colonised Britain via a different route, from a cryptic refugium in an area where the study species survived during the glacial period but where it no longer exists. Under future climate change scenarios, we predict 38-64% loss of range size, which equate to 1 unique haplotype to 12 unique haplotypes being at risk of loss under climate scenarios projecting 1 °C and 2-3 °C increases respectfully.

Limitations

This study has potential limitations, which are inherent in species distribution modelling, especially when projecting into different climates (Buisson, Thuiller, Casajus, Lek, & Grenouillet, 2010). We did not have suitable data to include sampling effort formally into our models and so the areas outside of the current *E. epiphron* distribution are considered ‘pseudo-absences’ rather than ‘true’ absences. However, other butterfly species have been recorded in these squares (Lepidopterists have visited these squares) without recording *E. epiphron* as present, and hence the proportion of false absences in the data is likely to be very low at the spatial (50 km across the whole of Europe) and temporal (accumulation of Lepidoptera records over 3 decades) scales considered here. We consider that our modelling approach robustly describes the bioclimatic conditions occupied by *E. epiphron* at a continental scale (the species’ global distribution). Future work could use sampling effort to account for imperfect species detection, with standardised sampling and occupancy modelling providing additional insight into (especially) within-region distributions and dynamics.

For future projections, the loss of populations and consequently genetic diversity was based on a probability threshold to define butterfly presence or absence. This threshold was based on the
probability value for English populations, given that this region represented the lowest elevational range edge for the study species. However, currently realised and fundamental niche characteristics may differ among regions (i.e. thresholds may differ), and hence caution should be taken with our predictions. The difference between using two different thresholds (either the lowest elevation versus a threshold calculated by the Biomod2 program), affects whether or not the entire English and Apennines regions are lost, and hence there is some uncertainty about the level of genetic diversity at risk. Nonetheless, the relatively low probability of future persistence in both of these regions suggests that these populations are at the climatic range limit for the species and therefore at risk. While regional adaptations may differ, we have no evidence that haplotypes are individually adaptive to climate variables and hence we use them as markers of colonisation rather than as adaptive traits. For the same reason, we did not model the specific niches of individual haplotypes when considering the potential future loss of genetic variation (Breiner, Nobis, Bergamini & Guisan et al., 2018). Future work could use next generation sequencing to further test our hypotheses; and to model specific genetic-climatic relationships in the future (see Bay et al., 2018).

Our analyses suggest that entire mountain regions of the butterfly’s distribution could be lost under future climatic change, but it is possible that isolated populations could survive in particular microhabitats, at least temporarily. However, these localised populations may not contain all of the genetic variation currently present in the wider region, and overtime these refugial populations may gradually lose genetic variation and viability (e.g., through inbreeding), and so they may not persist in the longer term due to their isolation (metapopulation failure). A variety of processes may lead to the loss of genetic diversity following isolation, and there can sometimes be a delay in genetic loss following population decline (Kadlec, Vrba, Kepka, Schmitt, & Konvicka, 2010). For example, the sister species of *E. epiphron*, *Erebia orientalis*, is very localised and currently occurs only in the Eastern Balkans and is genetically homogeneous, potentially putting it at risk of inbreeding depression (Hinojosa, Monasterio, Escobes, Dinca, & Vila, 2019). Therefore, our model projections should be seen as representing much longer-term regional-scale expectations, rather than short-term predictions at the local population or microhabitat scale. We believe that our conclusions about the long-term (LGM to present) continental-scale dynamics of *E. epiphron* are robust, and that this knowledge of the past helps frame future risks and provides information for conservation management.

*Long-term survival resulting in unique genetic diversity in cool-adapted species*

SDM outputs provide evidence that our exemplar cold-adapted study species occurred in disjunct regions throughout the period from the LGM to the present day, based on the distribution of suitable climate; the genetic data confirm likely separation not only since the LGM, but most probably over much longer periods and successive glacial-interglacial cycles. For mountain species, limited gene
flow between the disjunctive parts of their range during glacial and interglacial periods results in divergence and unique haplotypes, unlike lowland European species which colonised northwards from their glacial refugia, and where large parts of the current geographic ranges often share haplotypes (Hewitt, 2004). Only limited areas of postglacial expansions and retractions are evident in *E. epiphron*, and the British populations would be susceptible to extinction if the climate was to return to LGM conditions at some time in the future. Similarly, our SDM outputs suggest that additional populations of *E. epiphron* could have existed further south in southern Europe at the LGM (Figure 2.2E) but as they no longer exist a northwards translocation of the range might have taken place under interglacial conditions. If cold-adapted species such as *E. epiphron* were more widespread during glacial periods, then the current divergence could be associated with subsequent losses of genetic diversity (e.g., due to selection, or random drift during population bottlenecks), or a failure of our analyses to detect localised or rare haplotype variation. However, this alternative hypothesis seems unlikely because our estimates of times of genetic divergence (phylogenetic tree: see Appendix S1.5) imply that most splits occurred before the LGM. However, other divergence dates between *E. epiphron* and *E. orientalis* have been reported (e.g. 1.53 (+0.65) Mya (Hinojosa et al., 2019)). However they still reported strong mtDNA divergence and long term separation (Hinojosa et al., 2019) and therefore different assumptions of divergence dates do not impact the interpretation of our results. Hence we conclude that populations of *E. epiphron* survived as allopatric populations in mainland Europe during the LGM, with postglacial colonisations from these regions into the Massif Central, Vosges, Scotland and England.

High genetic differentiation is observed among populations of other mountain *Erebia* species, supporting the hypothesis that they also survived as allopatric populations during the LGM (Haubrich & Schmitt, 2007; Louy, Habel, Abadjiev, et al., 2014; Louy, Habel, Ulrich, & Schmitt, 2014; Martin, Gilles, Lortscher, & Descimon, 2002; Schmitt et al., 2014; Schmitt, Louy, Zimmermann, & Habel, 2016; Schmitt & Seitz, 2001). LGM separation of populations has also been identified in mountain plants and other invertebrates (Bettin, Cornejo, Edwards, & Holderegger, 2007; Huck, Budel, & Schmitt, 2012; Margraf, Verdon, Rahier, & Naisbit, 2007; Pauls, Lumbsch, & Haase, 2006). The numbers of glacial-interglacial cycles over which populations have remained disjunct remains unclear, but some studies have indicated divergence dates covering several glacial-interglacial cycles or even predating the Pleistocene (Hewitt, 2000). The reality is likely to be more complex with areas of persistent separation, but with occasional links between them (i.e. rare gene flow or brief periods of connection), as indicated by the distributions and relatedness of haplotypes in Figure 2.1.

*Unique haplotypes in populations derived from northern cryptic refugia*

Following the LGM, the ice retreated in northern Europe and many species colonised northwards, for example via the land bridge between continental Europe and Britain, which was present until sea level
rise ~7,000 years before present (Sturt, Garrow, & Bradley, 2013). The locations of southerly glacial refugia, which are thought to be the main sources of colonisations, have been debated extensively, with proposed glacial refugia in the Iberian Peninsula, Italy and the Balkans (Hewitt, 2000) and this has recently been reinforced in European butterflies (Dapporto et al., 2019). However, there is also evidence for more northern cryptic refugia based on fossil, pollen and genetic evidence (Birks & Willis, 2008; Provan and Bennett, 2008; Stewart and Lister, 2001), where species apparently persisted at higher latitudes in sheltered locations with suitable microclimates (Stewart, Lister, Barnes, & Dalen, 2010). However, most cryptic refugia described to date have been for relatively warm-adapted species. Here, we present evidence for the existence of northern cryptic population(s) for cold-adapted species during the LGM, based on high unique genetic diversity of the present-day *E. epiphron* populations in England, an area that was beneath an ice sheet at the LGM (Hughes et al., 2016). The high genetic uniqueness of populations in England, together with a single shared haplotype with Scotland/Vosges/Alps (haplotype 8; Figure 2.1b), is consistent with northern colonisations from the Alps, but distinct separate colonisation of Britain via two routes, although there are alternative explanations. For example, the 6 unique haplotypes in populations in England might occur elsewhere but were not detected in this study. Alternatively, the six unique haplotypes identified in England could have diverged from the shared haplotype in Scotland, Vosges and Alps populations (haplotype 8; Figure 2.1b) since the LGM, although this seems highly unlikely given the short time period for one to three mutations to occur (Figure 2.1b). It is possible that these LGM populations were situated on land that is currently below sea level, at an edge of the glacier, or in sheltered low elevation microclimates on land. Multiple colonisation events have also been shown in other taxa in the UK (Piertney et al., 2005), and the locations of cryptic refugia during the LGM are assumed to be ice free areas in southern England (Bocherens, Fogel, Tuross, & Zeder, 1995; Lister, 1984), northern Scotland (Bennett, 1984) and southern Ireland (Montgomery et al., 2014). Evidence for cryptic refugia for insects in Britain also comes from cold-adapted beetles (see Appendix S1.6 (Buckland & Buckland, 2006)), which currently have mountain or northern distributions in the UK, but were found as sub-fossil remains in southern England 18,000-26,000 years BP, providing evidence of cold-adapted insects surviving in ice-free locations in Britain in the LGM. It is, therefore, possible that the current population of *E. epiphron* in England survived elsewhere in Britain during the LGM as populations which no longer exists.

**Future loss of unique genetic diversity in cold-adapted species**

High levels of genetic diversity are important in relation to the capacity for populations and species to adapt to changing environmental conditions, including climate change (Balint et al., 2011; Hoffmann & Sgro, 2011). Cold-adapted species that have been shaped by diversification across mountain systems during the Pleistocene contain high levels of genetic diversity and unique populations, and are under threat from climate warming. Populations with unique genetic diversity may have evolved
independently to be adapted to their local environment (Weeks, Stoklosa, & Hoffmann, 2016) and thus may be particularly vulnerable to future climatic changes. Our SDMs project loss of suitable climate for *E. epiphron* in many locations in Europe, especially in regions with predominantly low elevation populations and few opportunities to shift uphill to high elevation, which could result in loss of genetic diversity. However, our projections of range retraction do not take into account any potential of populations to adapt to warmer temperatures *in situ* (Franks & Hoffmann, 2012). Future loss of genetic diversity has also been predicted in other species (Alsos et al., 2012; Beatty & Provan, 2011; Yannic et al., 2014), and rates of loss of genetic diversity in wild populations since the industrial revolution (Leigh, Hendry, Vázquez-Domínguez, & Friesen, 2019) are consistent with our projections.

**Conservation interventions to mitigate climate-driven genetic erosion**

Conservation management and adaptation could protect cold-adapted populations and safeguard unique genetic diversity from climate change (Mawdsley, O'Malley, & Ojima, 2009). Options include translocation or assisted colonisation to areas that have, or are predicted to have, suitable climate and habitat in the future (Hoegh-Guldberg et al., 2008). Translocations are a controversial topic due to the fear that translocated species may become ‘invasive’ in their new ranges, posing threats to ecosystems including disturbance, disrupting ecological interactions, disease spread, competition and extinctions (Ricciardi & Simberloff, 2009). However, others argue that the arrival of new species is typical of ecosystem changes in the Anthropocene, and that translocations mirror colonisations occurring as a consequence of current environmental change (Thomas, 2011). Translocations of *E. epiphron* and other butterflies into unoccupied but climatically-suitable areas have been successful (Cizek, Bakesova, Kuras, Benes, & Konvicka, 2003; Willis et al., 2009), and cold-adapted insects may represent good targets for translocations given that the climate is rapidly deteriorating for them in many parts of their range, and they may find it difficult to colonise new areas across inhospitable landscapes (Thomas, 2011). For *E. epiphron*, our SDMs reveal areas in Scandinavia to be climatically suitable, although the species does not occur there, and climate is predicted to increase in suitability in future in Scandinavia for *E. epiphron* (Figure 2.3) and for other *Erebia* species (Settele et al., 2008). However, although Scandinavia may have suitable climate, it may not have the required habitat for *E. epiphron*. Local translocations within mountain systems that are currently occupied by *E. epiphron* could also be implemented, for example moving individuals to areas of colder climate at higher elevation, or neighbouring mountains which are too isolated for the species to colonise naturally. However there may be very few areas of unoccupied but climatically-suitable habitats within some mountain systems occupied by *E. epiphron*, particularly if the species already occurs at high elevations in these regions. Future work could include finer scale country specific SDMs with additional land use and genetic data on habitat availability could be used to locate areas for potential translocations.
As well as translocating individuals to new sites, it might be possible to consider translocating genes or ‘genetic rescue’ by moving individuals among existing populations. Not only might this conserve unique genetic diversity at risk from local extinction of populations, but might increase the adaptive capacity of populations by increasing their genetic diversity (Aitken & Whitlock, 2013). This could involve moving warm-adapted individuals into cooler populations to increase their adaptive capacity as the climate warms (Weeks et al., 2011). However, moving locally-adapted populations may result in outbreeding depression and maladaptation, negatively impacting populations (Weeks et al., 2011), although some genetic rescue interventions have resulted in increases in populations, and alleles associated with local adaptation were not lost following gene flow (Fitzpatrick et al., 2020). Genetic conservation interventions for insects, and specifically butterflies, has been rarely implemented, although increasing habitat connectivity has led to genetic rescue of populations (Jangjoo et al., 2016) and genetic data have been used to inform on reintroductions (Dinca et al., 2018). There is no evidence of attempted genetic rescue via translocations of butterflies, although translocating individuals is a genetic conservation strategy which may be important in ensuring future survival and adaptability of populations under climate change. As with translocations, these conservation options may also be controversial, but could remove the need for on-going intervention and management at sites with declining populations (Weeks et al., 2011). We recommend that before the implementation of any climate adaptation strategy, populations are closely monitored to determine if populations are retracting and likely to become extinct in areas that are becoming too warm for the species. In addition, individual species’ assessments are required to assess the genetic diversity of populations and any local adaptation, which would determine the most appropriate conservation strategy.

2.6 Conclusions

The genetic diversification of cool-adapted mountain species, as demonstrated in our study species *E. epiphron*, has been shaped by Pleistocene glaciations, the locations of long-term survival of populations, and colonisation patterns after the LGM, resulting in unique genetic diversity in isolated populations. Mountain and cold-adapted species are vulnerable to future climate warming, and we predict *E. epiphron* will lose 38-64% of its range in the future, especially at low elevations. The uniqueness of genetic diversity contained in these populations could be at risk depending on the severity of future climate change. Conservation strategies such as translocation could ensure the survival of these cold-adapted species, but more research is needed on the likely effectiveness of such approaches.
Chapter 3: Genetic consequences of post-glacial colonisations in montane species in Britain

3.1 Abstract

1. The consequences of Pleistocene climatic changes and post-glacial range expansions and retreats are reflected in present-day genetic diversity, which will have consequences for species persistence under future climate change. Cold-adapted montane species that are now restricted to isolated interglacial refugia are vulnerable to extinction from climate change, and so it is important to examine demographic history and resulting genetic diversity.

2. Using genome-wide SNPs (ddRAD sequencing) I examine the genetic consequences of post-glacial expansion in a cold-adapted montane grassland butterfly, Erebia epiphron, which colonised Britain after the Eurasian ice sheet retracted ~10kya and now occupies montane grassland sites in England’s Lake District and Scotland’s Grampian Mountains. I compared genetic diversity, genetic structure, and connectivity in populations in England and Scotland, and modelled changes in population sizes over time to understand how post-glacial population changes have influenced current genetic diversity.

3. My ddRAD data reveal distinct English and Scottish populations, confirming different post-glacial colonisation routes originating from large (i.e. high $N_e$) glacial-maximum populations in Europe. Genetic structure and distance measures show further genetic separation of England’s Lake District into east and west populations. English populations harbour 17% less genetic diversity than the larger Scottish populations, which is linked to more severe bottlenecks in England following post-glacial colonisation, as shown by stairway plot analyses. After post-colonisation bottlenecks, both populations experienced population growth in line with Anthropogenic tree clearances, potentially increasing suitable grassland habitat.

4. My discovery of three genetic populations in Britain reveals the consequences of variation in prevailing climatic conditions over the last 40ky for current patterns of genetic diversity, and highlights the importance of conserving genetically distinct populations. My results may be typical of other cold-adapted species that have undergone population bottlenecks following post-glacial colonisations, and reduced genetic diversity may contribute to local extinction of isolated montane populations under future climate change.
3.2 Introduction

Climatic changes over the past 2 million years of the Pleistocene have caused species to shift their ranges, causing range retractions, expansions and colonisations over time, and resulting in a variety of characteristic biogeographic patterns of genetic diversity seen in present-day ranges (Hewitt, 2000; Hewitt, 2004; McCallum et al., 2014). Warm-adapted species typically retracted during glacial periods and then expanded from low latitude genetically diverse refugia (e.g. Iberia, Apennines, Balkans in Europe) during warm interglacial periods (Hewitt, 2000). By contrast, many cold-adapted species that currently occur in montane regions (Elsen & Tingley, 2015) showed opposite patterns, expanding to the foothills during cooler periods and retreating uphill into interglacial refugia as the climate warmed, resulting in genetic structuring among populations in disjunct montane regions (Haubrich & Schmitt, 2007; Huck et al., 2012; Louy, Habel, Abadjiev, et al., 2014; Minter et al., 2020; Schmitt et al., 2006). Thus montane species which presently occur in northern latitudes in Europe represent Holocene post-glacial colonisations (Crowley & North, 1991; Hughes et al., 2016) and the genetic consequences of these Holocene colonisations of montane species is important to quantify to understand historical demographic changes.

In Europe, northern latitudes were covered by ice sheets during the last glacial period ~40-10 thousand years ago (Hughes et al., 2016) and therefore cold-adapted species which currently occur in these northern areas represent post-glacial colonisations from areas of long-term survival. However, colonisations may arise from Pleistocene populations that no longer survive (‘cryptic refugia’; Stewart & Lister, 2001), and so populations of cold-adapted species at northern latitudes may contain unique genetic diversity no longer present elsewhere in extant populations (Bhagwat & Willis, 2008; Minter et al., 2020; Provan & Bennett, 2008). Genetic and fossil evidence for cryptic refugia at northern latitudes have been found in a number of mammals (Montgomery, Provan, McCabe, & Yalden, 2014; Stewart & Lister, 2001), amphibians (Teacher, Garner, & Nichols, 2009), plants (Leipold, Tausch, Poschlod, & Reisch, 2017; Stewart & Lister, 2001) and recent evidence in a cold-adapted species (Minter et al., 2020). Post-glacial colonisations of cold-adapted species to northern latitudes may arise from long-term or cryptic refugia, and may have caused demographic changes resulting in altered genetic diversity of extant populations.

Following colonisations of northern latitudes, populations can undergo demographic changes, the consequences of which can be found in current genetic diversity. Populations may experience demographic bottlenecks, from an event which drastically reduces the population leading to loss of genetic diversity (Broquet et al., 2010; Landergott, Holderegger, Kozlowski, & Schneller, 2001; Liu, Zhang, Wang, & Ma, 2020) such as long-distance colonisation events (Comps et al., 2001; Holliday et al., 2010). After colonisation, other genetic changes may occur in populations due to genetic drift or local adaptation if populations become isolated or small (Poirier, Colman, Pelletier, Jorgenson, &
Festa-Bianchet, 2019). Once populations become isolated, a reduction in gene flow can lead to genetic isolation, which can be due to the geographic distance between populations or the environment, for example unsuitable habitat/climate between the populations (Sexton et al., 2014). Without gene flow, small populations could be impacted by inbreeding depression, reducing genetic diversity and the persistence probability (Broquet et al., 2010; Saccheri et al., 1998). It is common in montane species to show genetic isolation between mountains (Monsen & Blouin, 2004; Velo-Anton et al., 2013) and thus more at risk of reduced genetic diversity. Genetic diversity is important in wild populations in determining fitness and ability to adapt (Hoffmann et al., 2017) and so it is important to quantify the genetic consequences of colonisations to inform conservation management in cold-adapted species.

Current anthropogenic climate change has resulted in range retractions of cold-adapted species in northern montane regions. Cold-adapted species such as the mountain ringlet butterfly Erebia epiphron have experienced local extinctions at warm low altitude/latitude range margins in Britain (Franco et al., 2006; Thomas et al., 2006), and unique genetic diversity could be lost under projected range retractions under future climate change (Minter et al., 2020). Erebia epiphron persisted as disjunct populations in several mountain regions in central Europe during the last glacial, and colonised Britain via two separate colonisation routes (Minter et al., 2020; Schmitt et al., 2006). These separate colonisations have resulted in two extant populations in Britain; in the Lake District in England (from a cryptic refugium, location unknown), and in the Grampian mountains in Scotland (from a long-term refugium in Western Alps) (Minter et al., 2020). English and Scottish populations are genetically dissimilar implying they may have not interacted for thousands of years (Minter et al., 2020). Previous studies have shown genetic divergence between English and Scottish populations due to different source refugia, but it is not clear if differences in genetic diversity also arise from historical population changes and differentiation within regions following colonisations and subsequent isolation.

This chapter examines the genetic consequences of post-glacial colonisation of Britain by Erebia epiphron and its subsequent isolation in two interglacial refugia in mountain regions in England and Scotland. I collect Erebia epiphron from populations in England and Scotland, and use double-digest restriction site associated DNA (ddRAD) sequencing: 1) to examine genetic structure and diversity in England and Scotland; 2) to quantify genetic connectivity among populations and whether populations are genetically isolated with respect to geographic distance and spatial distribution of suitable climate; and 3) to infer variation in population size over the past 40,000 years, to explore evidence for population bottlenecks following colonisation, and associations between population size, climate and grassland habitat availability. Taken together, this information will help to understand the genetic consequences of past climate variation and post-glacial colonisation of Erebia epiphron in Britain, which will help to inform the conservation of this species.
3.3 Methods

Field sampling

I sampled male *E. epiphron* from 19 populations in England and Scotland during summer (June-July) 2018 and 2019 (6-15 individuals per population; total = 192 individuals). I selected populations from a wide range of geographic locations, to sample genetic diversity from across the England and Scotland regions (Figure 3.1). Butterflies were euthanized in a -20°C freezer, and their bodies stored in ethanol at -20°C until DNA extraction.

![Map of England and Scotland with sampled populations and current distribution of *E. epiphron*](image)

**Figure 3.1:** *Erebia epiphron* populations sampled (red crosses) and British current distribution (blue circles) in England and Scotland. Elevation (meters above sea level) is shown at 90m x 90m grid resolution. Populations were sampled (England n = 9, Scotland n = 10, 6-15 males per population) at sites with elevations between 380 m and 780 m above sea level and annual mean temperatures between 5°C and 7.6°C. Butterfly distribution data were acquired from the Butterflies for the New Millennium recording scheme courtesy of Butterfly Conservation (Fox et al., 2015). Temperature data were from [http://www.worldclim.org](http://www.worldclim.org) and elevation data were from: [https://data.globalchange.gov/dataset/nasa-srtm-90m-digital-elevation-data](https://data.globalchange.gov/dataset/nasa-srtm-90m-digital-elevation-data)

DNA extraction and ddRAD library preparation

I extracted DNA from the thorax of 192 *E. epiphron* from the 19 populations. The DNA was extracted from the thorax using the Qiagen Blood and Tissue kit following the manufacturer’s standard protocol. I measured DNA quantity with a Qubit fluorometer and we checked DNA integrity using agarose gel electrophoresis. I then created double digest RAD libraries following the protocol of Da
Costa & Sorenson, (2014). I double digested 500ng DNA per sample using PstI and EcoRI enzymes (New England Biolabs) at 37°C for 18 hours followed by a 20 minute enzyme deactivation at 65°C. I barcoded each individual using unique combinations of P2 and P1 adapters (Appendix S2.2) with NEBuffer, rATP and T4 ligase (New England Biolabs), and ligated at 24°C for 30 minutes followed by enzyme deactivation for 20 minutes at 65°C. I size selected samples with agarose gel electrophoresis to 300-450bp, and DNA extracted with a QIAGEN MinElute Gel Extraction Kit. I then amplified DNA using PCR which was conducted with 10ul of gel extracted DNA, forward and reverse RAD primers (Appendix S2.3), Phusion high-fidelity PCR master mix (Thermo Scientific™) using these cycling conditions; 30 seconds at 98°C, 24 cycles of 10 seconds at 98°C, 30 seconds of 60°C and 40 seconds at 72°C, followed by 5 minutes at 72°C. I purified PCR products using AMPure beads and quantified using qPCR (KAPA Biosystems) and then pooled to a final concentration of 5nM. I sent ddRAD libraries to Liverpool Centre for Genomic Research (UK) and sequenced on one lane on the Illumina NovaSeq SP using paired-end 2x150bp sequencing. I then cleaned the raw sequencing data and removed Illumina adapters using Trimmomatic (Bolger, Lohse, & Usadel, 2014), removed PCR duplicates using Stacks:Clone filter (Catchen, Hohenlohe, Bassham, Amores, & Cresko, 2013), removed the DBR region using CutAdapt (https://doi.org/10.14806/ej.17.1.200), de-multiplexed using Stacks:process radtags (Catchen et al., 2013) and aligned to a E. epiphron reference genome using gsnap (Wu & Nacu, 2010). The reference genome was assembled from ~80x coverage 250bp paired-end Illumina sequence using MaSuRCa. This highly-fragmented assembly was improved using RagTag and the high-quality chromosomal Erebia lygia genome assembly as a guide. The final reference guided E. erebia genome assembly was 421Mb long, and had the following Lepidopteran BUSCO score (C:94.7%[S:94.2%,D:0.5%],F:1.6%,M:3.7%,n:5286). On average, 98% of ddRAD reads mapped to this assembly, and ~90% of ddRAD reads were properly paired.

Genetic structure and diversity between regions

In order to understand the differences in genetic diversity between the English and Scottish regions, and population structure within regions, I extracted single nucleotide polymorphisms (SNPs), which were identified and individuals were genotyped using Stacks:gstacks software (Catchen et al., 2013) which built 44, 544 SNPs. SNPs were then filtered using bcftools (https://github.com/samtools/bcftools), selecting SNPs with: two alleles, a minimum depth of coverage of 5 (number of reads which align), a minimum genotype quality of 30, and at least two copies of the minor allele (i.e. excluding singleton SNPs which have a higher likelihood of being genotyping errors) across samples. Low coverage individuals were also removed. After filtering, this provided a final set of 17, 488 SNPs from 185 individuals which were used for further genetic analyses. (See Appendix S2.1 for population level information on samples). In order to quantify genetic diversity in England and Scotland, I used Stacks: populations software (Catchen et al., 2013) to create summary statistics of genetic diversity (observed heterozygosity, nucleotide diversity (π)) for
each of the 19 populations (Appendix S2.1). In order to quantify the genetic structure of populations in Britain, I created a Principal Components Analysis (PCA) of genetic structure using plink (Chang et al., 2015) and a structure plot of ancestry proportions using ‘Snfm’ function in LEA R package (Frichot & Francois, 2015).

**Genetic connectivity with distance and climate availability**

To quantify genetic connectivity among populations within the England and Scotland regions, I calculated pair-wise genetic differentiation (F_{ST}) between populations using Stacks:Populations software (Catchen et al., 2013). I calculated mean F_{ST} for each population using the mean of all pair-wise F_{ST} values which included the focal population. I tested whether pair-wise genetic connectivity among populations in England and Scotland was best predicted by geographic or environmental (climate) distance between populations. I calculated geographic distance between populations using the proximity tool in ArcGIS Pro. I calculated environmental distance as the least-cost distance using a gridded map of climate suitability created using a species distribution model implemented with land cover. To do this, I downloaded current (1970–2000) climate data from WorldClim (http://www.worldclim.org/) at a resolution of 30 arc seconds (~1km grid cell resolution) and extracted climate data (mean temperature and precipitation of summer and winter) for each of the 19 populations. I created a species distribution model for *E. epiphron* using methods described in Chapter 2 (Minter et al., 2020), and applied to Britain using 1km species distribution data (Butterflies for the New Millennium recording scheme courtesy of Butterfly Conservation (Fox et al., 2015)) and 1km climate data. The model was used to project current climate suitability (probability score 0.01 – 0.96) and combined with land use (*E. epiphron* habitat is acid grassland, score = 1, any other land -use score = 0) and inverted into a friction layer using ArcGIS Pro. Pair-wise least cost distances were calculated between populations using SDMtoolbox (Brown, Bennett, & French, 2017). These pair-wise least cost distances represent the distances between populations according to suitable climate and availability of acid grassland habitat, creating a measure of environmental distance between populations. I used Mantel tests to examine associations between genetic distance, geographic distance, and environmental distance.

**Inferring historical population sizes**

To estimate population size (N_e) over the past 40,000 years, i.e. a period spanning the last glacial maximum and post-glacial colonisations of England and Scotland, I created SNP frequency spectra (SFS) using vcf2sfs software to infer historical population changes (Liu, Ferchaud, Gronkjaer, Nygaard, & Hansen, 2018). I created stairway plots, which infer demographic histories using SNP frequency, using Stairway Plot v2.1 (Liu & Fu, 2020) with SFS, and using the mutation rate of *Heliconius melpomene* of 2.9 × 10^{-9} (Keightley et al., 2015) and a length of sequencing data of 8,196,096 base pairs. This length was calculated using number of loci (44,544) in the filtered
sequencing data multiplied by the mean length (bp) of site per loci (184 bp). Separate stairway plots were created for western Lake District, eastern Lake District and Scotland because of population structure. I downloaded high resolution climate record for the northern hemisphere, the ratio of stable isotopes oxygen-18 and oxygen-16 (δ18O) over the period ~40kya until present from (Andersen et al., 2004) and compared variation in climate with changes in E. epiphron population sizes from stairway plots, to explore associations between climate fluctuations and effective breeding population (N_e) size over time. E. epiphron is dependent on open grassland habitat, and I also examined associations between population sizes of E. epiphron and historic tree clearing in Britain since the LGM using tree cover information (% of grid square with tree cover) from 12 kya to present (https://doi.org/10.1594/PANGAEA.886656 (Zanon et al., 2018b)) at 5 arc minutes resolution (~ 8 km x 8 km). I used these data to calculate average annual tree cover (%) for England and Scotland and plotted this information with population size (N_e) and climate (δ18O), to infer factors affecting E. epiphron population size during post-glacial colonisation of Britain.

3.4 Results

Genetic structure and diversity of populations

The output of the PCA revealed that the 19 populations I sampled are split into three genetic groups in Britain: east and west Lake District in England, and Scotland (Figure 3.2). PC1 explained 18.3% of the variation in the data and separated Scotland and England populations, while and PC2 explained 8.96 % of variation and separated the eastern and western Lake District populations; Figure 3.2A). This genetic separation into three groups is also shown in the ancestry proportions, which splits the genetic data into three groups based on similar genetic variants and therefore ancestry (Figure 3.2B).
Figure 3.2: My ddRAD data split British *E. epiphron* populations into three genetic structures. A) PCA of SNP data explains 27.26% of the genetic variation. Data points are plotted by region (east Lake District = circles, west Lake District = squares, Scotland = triangles) and the 19 populations we sampled are represented by different colours (see Figure 1 for map of locations). B) Structure plot showing proportions of inferred ancestry, grouped by similar genetic variants into three genetic clusters (genetic clusters denoted by white, grey and black) for each of the 192 individuals I analysed, grouped by region.

English populations harbour ~17% less genetic variation (Observed heterozygosity) than in Scotland (ANOVA of mean population genetic diversity by region (England versus Scotland), $F = 132.7, p < 0.001$) (Figure 3.3). I found similar results for lower nucleotide diversity (Pi) in England ($F = 151.3, p < 0.001$).
Figure 3.3: Genetic variation between the three genetic clusters, showing that English populations contain significantly less genetic variation than Scotland. A) Observed Heterozygosity and B) Nucleotide diversity (Pi).

**Genetic isolation is influenced by distance and environment**

I found that pair-wise population genetic distance ($F_{ST}$) was positively associated with geographic distance, with populations further apart geographically more genetically dissimilar, a pattern found in both England and Scotland (Mantel test: England: $R = 0.76$, $p = 0.003$, Scotland: $R = 0.85$, $p = 0.001$, Figure 3.4A). Genetic distance was also positively associated with environmental distance (Mantel test: England: $R = 0.78$, $p = 0.001$, Scotland: $R = 0.83$, $p = 0.001$, Figure 3.4B). However both geographic and environmental distance were positively correlated (Mantel test: England: $R = 0.95$, $p = 0.001$, Scotland: $R = 0.93$, $p = 0.001$). Overall, the slope of genetic distance with geographic distance is steeper in England than in Scotland (Figure 3.4A-B), indicating more reduced gene flow among populations within England than Scotland.
Figure 3.4: Pair-wise genetic distance (FST) between populations within England and Scotland. A) Genetic distance is positively associated with A) geographic distance (km) (England: $R = 0.76$, $p = 0.003$, Scotland: $R = 0.85$, $p = 0.001$), and B) environmental distance using climate suitability and land use corridors in England (grey circles) and Scotland (black triangles) (England: $R = 0.78$, $p = 0.001$, Scotland: $R = 0.83$, $p = 0.001$).

Population declines following post-glacial colonisation

During the last glacial maximum (~21kya), both English populations and Scottish populations of the cold-adapted butterfly had their largest effective population sizes, $N_e \approx 100,000$ (Figure 3.5B), which then declined in size from about ~11kya onwards, when the global climate began to warm (Figure 3.5A). Tree cover also increased in Britain (Figure 5C) during this period after the ice sheet retracted, reducing suitable breeding habitat for the butterfly. These decreases in breeding population size were apparently more severe in both English populations, which experienced longer and more severe bottlenecks, reducing $N_e$ to ~5,000 (Figure 3.5B), with the eastern population declines occurred over a much longer period (~11 to 2 kya). Whereas, in Scotland a single bottleneck reduced $N_e$ to ~20,000 $N_e$. The low $N_e$ in all regions in the early-mid Holocene ~5-6 kya was then followed by increases in population size between 4 – 5 kya (for Scotland and England west) and 2-3 kya for England east, associated with loss of tree cover at this time (Figure 3.5B-C) and thus availability of more open habitats suitable for *E. epiphron* breeding. However, these population increases did not result in English populations returning to their pre-LGM sizes, whereas Scottish populations do appear to recover, and population size of Scottish populations ($N_e = 80,000$) has been about twice that of England ($N_e = 40,000$) for the last ~6 kya (Figure 3.5B). Hence both regions suffered population bottlenecks following post-glacial colonisation, with more severe population size declines in England which is linked to the lower genetic diversity currently present in this region (Figure 3.3).
Figure 3.5: *E. epiphron* population size ($N_e$) over the last 40,000 years plotted with information on climate fluctuations and tree cover. A) Climate fluctuations of the oxygen isotopic composition of the ice ($\delta^{18}O$) from the NGRIP ice core from ~45kya to 50 years before present (Andersen et al., 2004). B) Stairway plot showing median effective population size ($N_e$) of west England (light blue line), east England (dark blue line) and Scotland (red line) predicted from ~40kya to 50 years before present. Stairway plots for west and east sub-regions of England show the same patterns, and are in Appendix S2.4. C) Mean tree cover (%) (Zanon et al., 2018b) for England (blue line) and Scotland (red line) from 12kya until 50 years before present.

3.5 Discussion

My findings confirm that present-day populations of the cold-adapted butterfly *E. epiphron* apparently colonised Britain via at least two different routes from LGM large ($N_e$) source populations in Europe. Following colonisation of Britain, my stairway plots show that population bottlenecks occurred in both populations during the Holocene, but that the bottleneck was much more severe in England. This severe bottleneck in England is linked to the lower genetic diversity that is currently present in England. After these bottlenecks, populations in both regions increased associated with tree-clearing during the early-mid Holocene, but the population size has generally been lower in England than Scotland for the last 6,000 years until very recently. I also find that genetic distance between present
populations is associated with geographic distance, with isolation more severe in England separating east and western Lake District populations.

*Genetic differences in British E. epiphron*

Both mitochondrial DNA haplotypes and my ddRAD data show genetic separation of English and Scottish regions (Minter et al., 2020) however my data further reveals genetic separation of English populations into west and east populations. My results based on ddRAD data also show higher genetic diversity in Scottish populations than in English populations, although previous studies reveal more unique mitochondrial DNA haplotypes in English populations (Minter et al., 2020). The presence of unique haplotypes in England suggest colonisation from an extinct source refugium (cryptic refugium), whereas Scottish populations have fewer haplotypes, which are shared with populations in locations that were LGM refuges in the Vosges mountains (France) and Western Alps (Minter et al., 2020). Therefore, haplotype diversity reveals information about longer term divergence between England and Scotland and unique linages, and ddRAD data in this study reveal lower diversity in England. These differences could arise if English populations originated from cryptic refugia with lower diversity, however our stair plot analyses suggest that the English populations may have lost more variation since colonisation during the Holocene compared with Scotland.

*Genetic isolation is associated with geographic and environmental distance*

My finding of two genetic populations in England may be a result of reduced gene flow and isolation following colonisation of the Lake District (Phylogenetic tree; Appendix S2.5), as found in other isolated montane populations of cold-adapted species with restricted gene flow (Savage, Fremier, & Shaffer, 2010; Valbuena-Urena et al., 2018). The east and west populations in the Lake District are separated by a low elevation valley (Figure 3.1), which may be a barrier to dispersal. Previous studies have shown that areas of unsuitable climate can result in genetic isolation of populations (Jiang et al., 2019; Sexton et al., 2014), which could be likely in *E. epiphron* if does not disperse across low elevation warm areas. Habitats of European *E. epiphron* are uniform and isolated, which benefits within habitat movement but means that dispersal between populations is highly unlikely (Kuras, Benes, Fric, & Konvicka, 2003). This lack of dispersal between populations is probable in the English populations resulting in genetic isolation which could have negative impacts on these populations. Genetic isolation can result in increased risk of inbreeding as shown in *Erebia orientalis* (Hinojosa, Monasterio, Escobes, Dinca, & Vila, 2019) which may impact the persistence of these species, especially with additional risks such as climate change. It has been shown in *E. epiphron* and other British northern butterflies, that recent climate change has caused populations declines, retracting their range uphill or north (Franco et al., 2006). Under future projected climate change, *E. epiphron* is predicted to experience declines across its range along with other montane species (La Sorte & Jetz, 2010; Minter et al., 2020). Anthropogenic climate change induced isolation in *E. epiphron* and other
montane species could increase in the future with negative genetic consequences such as inbreeding impacting the persistence of these species.

**Population declines of cold-adapted species following post-glacial colonisation**

My demographic analyses show that during the LGM, population sizes of *E. epiphron* were at their highest levels ($N_e \approx 100,000$). This high $N_e$ during the last glacial suggests that populations of this cold-adapted species flourished at that time, and that more areas had suitable climate resulting in larger population sizes during the LGM than currently. Many cold-adapted species survived the LGM at the foothills of their current ranges in Europe (Bettin, Cornejo, Edwards, & Holderegger, 2007; Huck et al., 2012; Louy, Habel, Abadjiev, et al., 2014; Minter et al., 2020; Schmitt et al., 2014; Schmitt et al., 2006) where population sizes may have been much larger than currently, but subsequently retracting uphill climate warmed. Our study provides some of the first demographic information about a cold-adapted species during the LGM and post-glacial colonisation of northern latitudes, providing evidence of large population sizes during the LGM. This is in contrast to warm-adapted species which may have undergone population declines during the LGM, followed by population increases during post-glacial range expansions (Imai et al., 2021; Liu et al., 2020; Ma et al., 2021). Following the LGM, *E. epiphron* colonised Britain along at least two separate routes (Minter et al., 2020) and during this time, all *E. epiphron* populations experienced population declines from ~10 kya which coincides with the retraction of the Eurasian ice sheet from Britain ~11 kya (Hughes et al., 2016). Post-glacial population declines and reduction in genetic diversity has also been found following a founder event in the cold-adapted Muskox on its way to Greenland (Hansen et al., 2018), however our study is the first evidence of population declines in a montane species during post-glacial colonisation.

**Population growth with Holocene tree clearances**

I show that from ~5kya onwards, *E. epiphron* populations increased in size coinciding with the reduction in tree cover that occurred during the Neolithic (Zanon, Davis, Marquer, Brewer, & Kaplan, 2018a). *E. epiphron* is a species of *Nardus* dominated grassland (Ewing et al., 2020) and during the time of population declines, Britain was mainly tree-covered (Zanon et al., 2018a) with only the highest elevation areas above the tree line appropriate habitat for *E. epiphron*. The Mesolithic-Neolithic agricultural transition around 6 kya involved land clearance, burning and deforestation for grazing (Woodbridge et al., 2014), which may have provided suitable breeding habitat for *E. epiphron*, together with the development of blanket bogs throughout the Holocene, which may also have contributed to increased landscape openness (Fyfe et al., 2013; Tipping, 2008). Hence, tree clearing and sheep grazing may have increased the availability of suitable habitat for *E. epiphron*, which is supported by evidence that *E. epiphron* is associated with sheep grazing on upland sites in the present-day. Moreover, sheep avoid *Nardus stricta*, the main larval host plant of *E. epiphron*, but
graze other grass species, thereby increasing the abundance of *E. epiphron*’s food plant (Grant, Torvell, Sim, Small, & Armstrong, 1996). In addition, sheep grazing maintains short, sparse grass swards which is preferred by egg-laying females (Ewing et al., 2020). Thus this suggests that Anthropogenic tree clearances may have created more favourable habitat, allowing for *E. epiphron* population increases during the Holocene.

### 3.6 Conclusions and conservation implications

I have shown that *E. epiphron* had larger source population sizes during the LGM, but underwent population bottlenecks during post-glacial colonisation of Britain as the climate warmed. Cold-adapted species such as *E. epiphron* persisted during previous warm inter-glacial periods, but are now experiencing Anthropogenic warming that threatens their persistence. Although English populations of *E. epiphron* populations harbour less genetic diversity than Scotland, they support unique haplotypes that are at risk from future warming (Minter et al., 2020), and so these populations need to be prioritised for conservation management. Our results show distinct populations in England, and both east and west populations need protecting to capture both genetic linages. In order to conserve these species, conservation management options could include translocations of individuals to more suitable climate (Thomas, 2011), or moving genes to increase genetic diversity via genetic rescue (Weeks et al., 2011). Populations could also be assigned as Gene Conservation Units (GCUs), to protect and potentially monitor genetic diversity in assigned areas (Minter et al., 2021). Habitat management in sites could also be implemented to increase variation in microclimates (Ewing et al., 2020), allowing for areas of cool microclimates. Conservation management will be appropriate to ensure the persistence of *E. epiphron* and other cold-adapted species at risk under future climate change.
Chapter 4: Smaller montane butterflies at warm range boundaries may affect persistence under future climate change

4.1 Abstract

1. Intra-specific variation in insect size is related to temperature during development, and may affect the persistence of populations under future climate change if small individuals have reduced fitness. Montane species are particularly vulnerable to climate-driven local extinctions at their warm range margins, and so I examined spatial and temporal variation in body size in the butterfly *Erebia epiphron* in the UK, where it is restricted to two montane regions in England and Scotland.

2. I sampled 19 populations (6-15 individuals per population) spanning elevations from 380-720 m, and also examined museum specimens collected between 1890 and 1980. I examined variation in body size (between and within populations) between England and Scotland, and tested whether body size is related to local temperature of sites, and temporal variation in temperature during larval development over the last century.

3. Adult body size (forewing length) of individuals in England were on average 7-8% smaller than in Scotland (e.g. field material, England, mean = 14.9 mm, Scotland, mean = 15.9 mm), and warmer sites also had smaller individuals (wing size decreasing by 0.2mm per 1°C increase in local site mean temperature). However, I found no differences in body size variation (coefficient of variation) among populations, and no effect of temperature during larval development over time.

4. I found that *E. epiphron* were smaller in England, and at warm range edge populations. The implications of size variation are unclear, but an ability for species to ‘shrink’ under warmer temperatures may support shifts from 2-year to annual life cycles. However, if smaller individuals have lower fitness, then climate change impacts on body size may contribute to local extinctions at warm sites in future.
4.2 Introduction

Cold-adapted montane insect species are predicted to be at risk from future climate change (Minter et al., 2020), and local extinctions have already been documented at warm trailing-edge range margins due to recent climate change (Franco et al., 2006). These cold-adapted insects are restricted to mountain ecosystems with limited opportunities to shift their ranges into new climatically-suitable areas, and so deteriorating climate conditions within their current range makes them extremely vulnerable to local extinctions (Elsen & Tingley, 2015). Montane species experience heterogeneity in local climate conditions within their ranges due to variation in altitude and latitude, which is likely to result in variation in traits, such as body size, that are influenced by temperature (Gunter et al., 2019). Variation in these traits may have implications for the persistence of local populations under future warming, and so are important to study.

Body size in ectothermic species shows variation along gradients in latitude and altitude, and can be influenced by temperature. Warmer temperatures can result in smaller body size, known as the ‘temperature-size rule’ (Atkinson, 1994), which can reduce fecundity and dispersal, if smaller individuals have reduced flight ability (Gao et al., 2016; Lopez et al., 2014), and smaller individuals may be less able to colonise new environments (Hill et al., 1999). Therefore, it is important to quantify body size variation in montane species, and to determine if individuals in warm range-edge populations are smaller, which could have implications on their fitness and persistence under future climate change.

In this study, I examine the Mountain Ringlet butterfly *Erebia epiphron* (Knoch, 1783) which in Britain occurs in two discrete mountain regions: the Lake District in England and the Scottish highlands (Figure 4.1). Populations in the two regions are genetically dissimilar, due to different post-glacial colonisation routes of Britain (Minter et al., 2020). Current populations also differ in local temperature conditions due to variation in latitude and altitude of populations in different regions, which will have impacts on their future persistence (Minter et al., 2020). In this study, I compare body size (measured as forewing length) in England and Scotland, and examine variation in body size within and among populations in relation to local temperature of sites. I also use museum material to examine variation in body size over ~100 years of temporal variation in temperature during butterfly development. Using field-caught and museum material, I test: (1) whether warmer regions (i.e. England) and populations in warm low-elevation range boundaries have smaller individuals; (2) whether over the past century, smaller individuals emerge in years with warmer temperatures, and (3) whether within-population variation in size differs among regions, indicating potential differences in plasticity.
4.3 Methods

Study area and sampling of field material

I collected male *E. epiphron* individuals from 19 populations in England and Scotland (6-15 males per population) during summer 2018 and 2019. Populations were selected to represent a wide range of local elevation and temperature gradients (Figure 4.1). *E. epiphron* individuals were frozen, and then the wings removed and electronically scanned with a scale bar.

**Figure 4.1:** *E. epiphron* sample populations 100m x 100m grid square (red crosses) for field-caught material, and current distribution at 1km x 1km grid square resolution (blue circles) in England and Scotland. Elevation data at 90m x 90m grid (meters above sea level) are also plotted. Populations were sampled (England n = 9 populations, Scotland n = 10, 6-15 males per population) at elevations between 380 m and 780 m above sea level, corresponding to average mean annual temperature between 5°C and 7.6°C. Butterfly distribution data were acquired by the Butterflies for the New Millennium recording scheme courtesy of Butterfly Conservation (Fox et al., 2015). Elevation data were acquired from: [https://data.globalchange.gov/dataset/nasa-srtm-90m-digital-elevation-data](https://data.globalchange.gov/dataset/nasa-srtm-90m-digital-elevation-data)

Museum material

I acquired photographs of museum specimens from the online data portal ([https://data.nhm.ac.uk/](https://data.nhm.ac.uk/)) from the Natural History Museum, London (2014) and downloaded for measurement. I selected specimens based the year of collection, and up to 5 male specimens were measured every year with specimens collected between 1890 to 1987 (England n = 127 individuals, Scotland n = 100).
**Measuring size**

All wing scans/photos (field and museum material) included a scale bar. I measured forewing length, which is a widely accepted proxy for body size (Brehm et al., 2019; Graca et al., 2016). I opened the images on Image J software (https://imagej.nih.gov/ij/index.html) and we used the ‘draw line’ tool to draw a line between the cell base and v10 wing margin veins (Appendix S3.1). The length of the line was our measure of forewing length, and was measured in pixels and then converted into millimetres using the scale bar. Only relatively undamaged wings where all veins and cell bases were visible were used for measurements. We measured both left and right forewings, and calculated the average length of the two forewings for each individual. To account for measurement error, 10 individuals were selected at random and forewing length was measured 3 times. I calculated the differences between repeated measurements, and found the rate of error was 0.3mm, and so our methods were robust for detecting differences among populations that we report.

**Climate data**

I analysed spatial variation in temperature at field location as mean annual temperature, and temporal variation in temperature for museum material as temperature during larval development. In order to quantify temperature at *E. epiphron* field locations, I downloaded mean annual temperature data (1970-2000) (°C) from http://worldclim.org at a resolution of 30 seconds (~1 km grid). For analyses of museum specimens, we downloaded historical climatic data from the MetOffice UK climate series (https://www.metoffice.gov.uk/research/climate/maps-and-data/about/archives) as UK average monthly temperatures at regional resolution (region data: North England and Scotland) from 1880 to 1987. I computed mean temperature (°C) during larval development (i.e. average monthly mean temperature for five months of larval development; August – September in the previous year and March – May in the year of collection). I used regional resolution temperature data for analyses of museum material due to lack of more fine-scale specific locality information on specimen labels.

**Statistical analysis**

In order to test for regional differences in forewing length in the field and museum data, I used two-sample t-tests, comparing data from England versus Scotland. I examined effects of annual temperature on forewing length in the field data using a GLM, with forewing length as the response variable, with region (England or Scotland) and annual mean temperature of sample sites (N = 19 sites) as predictors. I created a GLM for the museum data, with forewing length as the response variable, and larval development temperature of England and Scotland as the predictor variable. I calculated the coefficient of variation for forewing length in field data as a measure of within population wing size variation, and I tested for differences in wing size variation between England and Scotland populations by analysing the coefficient of variation in wing size using a two sample t-test.
4.4 Results

I found that the forewing lengths of field-collected individuals from English populations (mean = 14.9± 0.05 mm) were ~7% smaller than individuals from Scotland (mean = 15.9± 0.05 mm; Figure 4.2A) (T-test: $T_{(247.7)} = -15, P < 0.001^{***}$). This finding was supported by museum material, which also showed that specimens from English populations (mean = 14.8± 0.08 mm) were ~8% smaller than individuals from Scottish populations (mean = 16.2± 0.09 mm) (T-test: $T_{(212.4)} = -11.4, P < 0.001$) (Figure 4.2B). From field data, I also found that smaller individuals occur at warm low elevation range edge populations ($F = 117.5, R^2 = 0.48, P < 0.001$), with wing size decreasing by 0.2 mm per 1°C increase in annual mean temperature (Appendix S3.2, Figure 4.2C). However, I found no change in body size over the past century in museum material; there was no effect of annual temperature during larval development on body size, and region (i.e. England or Scotland) was the only the significant predictor of body size ($F = 66.6, R^2 = 0.36, P < 0.001$, Figure 4.2D), (Appendix S3.2). I found there were also no differences in the coefficient of variation in forewing length between England and Scotland (T-test: $T_{(12.6)} = 0.2, P = 0.84$), suggesting similar levels of within-population size variation.
Figure 4.2: Forewing mean length variation shows a relationship with region and temperature in field and museum material. A) Boxplot showing English populations have smaller forewing size than Scottish populations in modern specimens ($P < 0.001^{***}$), B) and museum specimens ($P < 0.001^{***}$). C) Forewing mean length shows a negative relationship with annual mean temperature (${^o}C$) in modern specimens ($P = 0.01^*$). D) Forewing mean length shows a non-significant relationship with mean temperature during active larval period (${^o}C$) in museum specimens ($P = 0.11$).

4.5 Discussion

I find that English *E. epiphron* are smaller than Scottish individuals, and that the smallest individuals are found at the lowest elevation. Hence, I conclude that some variation in body size that we observe is associated with temperature. My findings are consistent with other Lepidoptera which are smaller at lower altitudes/latitudes (Brehm et al., 2019; Taylor-Cox et al., 2020) although this is not always the case (Gunter et al., 2019; Shrestha et al., 2020). There was no relationship between development temperature and size, whereas some insect species have smaller wings under warmer seasonal temperatures (Wonglersak, Fenberg, Langdon, Brooks, & Price, 2020). This could be due to the coarse resolution of yearly climate and location information on museum specimens, and individuals may be more susceptible to finer scale changes in development temperature. I also do not find any differences in within-population variation, suggesting similar levels of plasticity across Britain, and could imply that the warm range edge does not have limited plasticity.

The mechanism of this size difference is unclear but could be caused by a combination of environmental and genetic factors. The English and Scottish populations are genetically distinct and have not interacted for thousands of years since before the post-glacial colonisation of Britain (Minter et al., 2020), and these genetic differences and separate colonisation history of Britain could be associated with the morphological differences in body size we currently observe between the two regions. There may also be reduced predation at higher elevations/latitudes, allowing for larger body size to evolve in these areas (Brehm et al., 2019). These regional differences may also reflect environmental variation in local resource availability (Pineda-Munoz, Evans, & Alroy, 2016) (e.g. smaller individuals from areas which contained low-quality food plants (Dempster, King, & Lakhani, 1976)), as well as regional variation in direct and indirect impacts of temperature variation.

The smaller body sizes I observed in populations at warm range margins may be caused by the direct impact of warmer ambient temperature, resulting in faster insect development (Atkinson, 1994). Small body size may also be a result of temperature impacts on voltinism. Some insect species have variable life-cycles, which depend on temperature (Macgregor et al., 2019). For example, some species in Britain have annual life cycles in the south, but 2-year life cycles in cooler northern areas (Kempe, Wrightham, Trust, & Heritage, 2006). These 2-year life cycles may allow longer feeding and development times during cooler conditions, which result in larger body size compared with annual
life cycles (Everall, Johnson, Wilby, & Bennett, 2015). It has been suggested that *E. epiphron* may have a biennial cycle in Scotland, based on breeding information in captivity (Wheeler, 1982), which is observed in other *Erebia* butterflies in Europe (Kleckova, Vrba, & Konvicka, 2015). Confirming whether Scottish *E. epiphron* are larger as a result of 2-year life cycles would require further study and rearing in captivity.

The implications of this size variation is complex and it is unclear how this ‘shrinkage’ might impact the ability of populations to persist under environmental and climate change. The body sizes of other species have ‘shrunk’ in response to recent climate change (Gardner, Peters, Kearney, Joseph, & Heinsohn, 2011; Wu et al., 2019), which could be due to shifting to annual life cycles in response to warmer temperatures, resulting in smaller size (Everall et al., 2015). Hence some species with flexible voltinism may benefit from warmer temperatures by developing through more generations each year and achieving higher local abundances (Macgregor et al., 2019), even if this results in smaller size. In the absence of any change in voltinism, smaller individuals may be due to direct effects of temperature on development rates, and/or poorer quality larval host plants in warmer conditions, leading to smaller individuals (Atkinson, 1994; Beerli, Bartschi, Ballesteros-Mejia, Kitching, & Beck, 2019; Pineda-Munoz et al., 2016). In this case, smaller body sizes could be detrimental for populations if they reduce fecundity, as smaller female insects have been shown to lay fewer eggs (Gao et al., 2016), and are associated with decreased flight ability (Lopez et al., 2014), resulting in reduced ability to colonise sites through meta-population dynamic processes (Hill et al., 1999; Taylor-Cox et al., 2020). Larger sized individuals, in contrast, may be more able to buffer against harsher environments and may be more resilient to starvation and desiccation (Ashton, 2002; Cushman, Lawton, & Manly, 1993). While there are some potential implications of body size variation, not all species will respond the same to temperature changes, with the relative influences of local environmental and genetic effects impacting how different species respond, and the consequences.

### 4.6 Conclusions

To conclude, I find that English *E. epiphron* are smaller than Scottish individuals, which is associated with temperature differences between regions, and different post-glacial histories of colonisation. I also find smaller individuals at warmer sites. The mechanism for this is unclear, but could be due to differences in life-cycle length in response to local climate conditions, and/or other direct environmental impacts on development rates resulting in smaller individuals in these populations. How this ‘shrinkage’ may impact the ability for species to adapt to future climate change is unclear. I found no change in within-population variation, implying plasticity was not reduced at warmer sites, and so populations may have the capacity to ‘shrink’ further under future climate change. However, it is unclear whether this shrinkage is beneficial (e.g. responding to warmer temperatures by developing
through more generations per year) or detrimental, if it leads to reduced fecundity, dispersal and temperature buffering ability.
Chapter 5: Exploring the potential for ‘Gene Conservation Units’ to conserve genetic diversity in wild populations

5.1 Abstract

1. Genetic diversity is important for species persistence and Gene Conservation Units (GCUs) have been implemented for forest trees to protect genetic diversity and evolutionary processes in situ. The Convention on Biological Diversity stipulates the protection of genetic diversity as an Aichi target, and so we explore the potential for GCUs to be implemented more widely.

2. Our global systematic review showed that GCUs are currently implemented primarily for plant species of economic importance (109/158 species studied), but a questionnaire sent to land managers and conservationists (60 UK participants) revealed strong support for fully integrating genetic information into conservation management (90% agree), and for creating GCUs for other plant and animal taxa.

3. Using four case studies of UK species of conservation importance which vary in genetic threat and population dynamics (two insect species, a fungus and a plant), we highlight that GCU implementation criteria need to be flexible to account for variation in effective breeding population size and geographic extent of target species. The wider uptake of GCUs would ensure that threatened genetic diversity is protected and support evolutionary processes that aid adaptation to changing environments.
5.2 Introduction

Intra-specific genetic diversity is key in providing populations with the capacity to adapt to changing environmental conditions and to challenges from novel pests and diseases (Barrett & Schluter, 2008; Hoffmann & Sgro, 2011). Genetic diversity may be neutral (no effect on fitness) or adaptive (Holderegger, Kamm, & Gugerli, 2006), and loss of genetic diversity can lead to lower fitness (Reed & Frankham, 2003), changes to physiology (Roelke, Martenson, & O’Brien, 1993), and higher loads of pathogens and infectious diseases (Cunningham et al., 2008). Therefore, conserving genetic diversity is important for mitigating biodiversity loss (Reed & Frankham, 2003) and enabling species to respond to changing environments (Wernberg et al., 2018). Despite its importance, conservation of genetic diversity, and hence local adaptation, is rarely included in policy and conservation management (Laikre, 2010). However, under the Convention of Biological Diversity (CBD), maintenance of genetic variation is an Aichi target (target 13) (CBD, 2011). A recent analysis showed that although many CBD country reports mentioned maintaining genetic variation, this mainly focused on agricultural or forestry species, and used primarily ex situ approaches to genetic conservation (Hoban et al., 2021), such as captive breeding and seed banks. Ex situ approaches are usually implemented as a last resort, and only contain a ‘snapshot’ of a species’ genetic diversity (Koskela et al., 2013). Thus, more attention to genetic conservation in wild species is needed, especially given proposed targets for CBD’s post-2020 biodiversity framework to maintain genetic diversity within wild species (Hoban et al., 2020).

To meet these CBD targets, in situ conservation approaches must be designed to maintain genetic variation. For example, conserving populations deemed to be Evolutionary Significant Units (ESUs) (de Guia & Saitoh, 2007), e.g. Coho salmon Oncorhynchus kisutch (National Marines Fisheries Service, 2012), implementing genetic rescue and translocations to increase genetic diversity in populations (Fredrickson et al., 2007; Johnson et al., 2010; Whiteley, Fitzpatrick, Funk, & Tallmon, 2015) or improving connectivity (i.e. dispersal and gene flow) between populations (Jangjoo, Matter, Roland, & Keyghobadi, 2016). These methods aim to conserve distinct populations in situ (ESU) or to increase genetic diversity in small wild populations. There are also methods that specifically use genetic data to prioritise objectives for conservation management such as to prioritise connectivity or evolutionary potential (Nielsen et al., 2020). In situ conservation through Gene Conservation Units (GCUs) focuses on managing for genetic diversity in wild populations within defined areas (Maxted, Hawkes, Ford-Lloyd, & Williams, 2000). ‘Dynamic gene conservation’ is promoted in these areas by maintaining and managing populations in their natural habitats to allow adaptation to environmental changes through natural selection. By designating GCUs across the ecological range of a species, and managing these sites to allow reproduction and dynamic evolution, the GCUs conserve the adaptive genetic variation within species, and allow ongoing evolution and change. GCUs are novel in their emphasis on encouraging natural genetic adaptation, allowing populations in the wild to persist and
adapt to future change, this dynamic process is particularly important in environments that are undergoing change. For current GCUs for trees, specific criteria are given including the population size and geographic size, to allow for dynamic gene conservation through natural regeneration (Koskela et al., 2013). However, this operationalization may not be applicable to other taxa and in different habitats.

In this policy perspective paper, we discuss current global application of in situ genetic conservation management techniques, considering whether the GCU approach could be effective for conserving evolutionary potential in a wide range of other taxa. We review current implementation of GCUs and use a structured questionnaire to canvass conservationists’ and land managers’ opinions concerning adopting a system of GCUs to protect biodiversity. We then test whether existing methods for voluntary accreditation of GCUs for trees (Koskela et al., 2013) are appropriate for application to other taxa, and recommend alterations to these methods, illustrating these recommendations for four case study species (Erebia epiphron (butterfly), Bombus distinguendus (bee), Campanula rotundifolia (plant) and Hypocreopsis rhododendri (fungus)). Our paper focuses on the UK, but the policy recommendations we develop are relevant for creating GCU networks across Europe and beyond.

5.3 Current implementation of GCUs and other in situ genetic conservation techniques

Firstly, we aimed to gain a better understanding of the taxa that are currently the focus of GCUs globally (we refer to any areas managed for genetic conservation as GCUs) and other in situ conservation programmes including types of species and their socio-economic importance. Our literature review included published papers and ‘grey literature’ such as government/NGO reports. We extracted information on the focal species, the in situ genetic conservation method applied, and the reason for conservation action (economic or conservation importance) (see more information in Appendix S4.1). We found genetic conservation implemented in 158 species, mostly trees and other plants (Appendix S4.2). The most common programme was establishment of a GCU (72.8%), followed by assigning an ESU (without official ratification; 15.8%), and genetic rescue by translocation (8.9%), captive breeding (1.9%) or habitat connectivity (0.6%) (Appendix S4.2). GCUs were selected to protect genetic resources of economically important plant species including about 100 tree species, and 10 species of crop wild relatives (Appendix S4.2), such as citrus, wheat, maize and chilli. The European Forest Genetic Resources Programme (EUFORGEN) (www.euforgen.org) promotes conservation of genetic resources through a pan-European strategy for the establishment of GCUs (Koskela et al., 2013), resulting in over 3,200 GCUs harbouring more than 4,000 populations of about 100 tree species. A subsample of these form a core network which aims to capture current genetic diversity across Europe for a number of forest tree species by representing populations from different local climate and environmental conditions. Therefore, GCUs have been successfully used to protect genetic diversity in mainly economically important plant species in the wild. The proposed
future CBD targets focus on protecting genetic diversity within all wild species (Hoban et al., 2020), making it vital to explore the potential to extend the GCU approach to other plant and animal taxa.

5.4 Exploring the scope for implementing GCUs more widely as a technique to conserve genetic diversity

We used a structured questionnaire to canvass conservationists’ and land managers’ opinions concerning adopting a system of GCUs to protect biodiversity. We want this GCU method to be something that is co-developed with stakeholders so that it is something that practitioners and land managers are willing to sign up for, and therefore any concerns and benefits were important for us to understand. Our experience suggests that a co-development approach is likely to appeal to land managers as it gives them greater ownership of the process (O’Brien et al. 2021). We received responses from 60 UK participants including researchers (26%), non-governmental organisations (33%), private land managers (7%), government/non-departmental public bodies (24%) and others (4%) (Appendix S4.3). Responses provided information on current genetic practises and support for developing GCUs for species conservation, including opinions on perceived risks, benefits and feasibility of GCUs (see methods in Appendix S4.1). This information provided insight into the scope for GCU implementation, and whether existing methods could be applied to other species. Genetic conservation is valued in the UK (Appendix S4.4, S4.5, S4.6) and in situ genetic conservation management has focused on plant species (Figure 5.1B, 5.1C), confirming the findings from our literature review. Most organisations surveyed do not have a genetic conservation policy (Appendix S4.5) although many participants considered that genetic information should be more integrated into conservation in the future (Figure 5.1A). The main perceived barriers to implementing genetic conservation management are lack of specific knowledge and financial constraints (Figure 5.1D). These hamper progress, despite support for integrating genetic information into conservation management in the UK. Therefore, there is merit in exploring the feasibility of extending GCU policy to include all species so that, when accompanied by simple guidelines, GCUs may serve as a genetic conservation technique which could be implemented by land managers.
Figure 5.1: Current in situ genetic conservation implemented by conservationists and land managers
A) Opinions of current and future implementation of genetic conservation, responses to statements were collected in a Likert scale, B) Types and frequency of genetic conservation management currently implemented, C) Type and frequency of taxa included in genetic conservation management and D) Perceived barriers to implementing genetic conservation management.

Conservationists and landowners listed several perceived benefits of GCUs (Figure 5.2A, 5.1B). The most frequently mentioned was maintaining genetic diversity and adaptability of populations, allowing them to persist and continue to adapt in response to environmental changes and other challenges. The most frequently cited benefits for landowners related to financial gains (e.g. benefits to economically exploited species, attracting public funding), prestige and pride that land managers experienced when conserving their land for species resilience, and wider conservation benefits (e.g. increasing connectivity, GCUs acting as gene banks). The role of GCUs in raising awareness of the importance of species conservation was often mentioned as a general benefit or a benefit to landowners, with a recognition that more awareness and engagement on the importance of genetic diversity and adaptability could promote genetic conservation activities in the future. Respondents also suggested several potential risks of designating populations as GCUs (Figure 5.2C), including neglecting non-target species, overlooking populations outside of the GCU and negative genetic consequences, including inbreeding. There were mainly positive responses regarding the potential to recognise GCUs for more mobile target species such as large mammals, insects and birds (Figure 5.2D). Respondents considered that to make them applicable to more mobile species, GCU boundaries should be flexible, accounting for dispersal distances, with adaptable criteria to suit
species’ characteristics such as population size and geographical scale. Another concern was that future climate change may displace populations uphill or to more northern latitudes (i.e. poleward), and that GCUs may need to move with them.

**Figure 5.2:** Questionnaire responses of 60 conservationists and land managers to test the feasibility, risks and benefits of extending the GCU concept to other species, (open ended answers grouped into broad categories) A) Perceived benefits of GCUs, B) Perceived benefits of GCUs specifically to land managers, C) Perceived risks of GCUs, D) Perceived feasibility of extending GCUs to include more mobile species E) Perceived conflicts of GCU management plans with existing management plans

There were mixed responses regarding the potential for GCU management to conflict with current management actions (Figure 5.2E). While some stated that the GCU would enhance the existing management plans, others stated that there could be conflicts if the area was not already managed for the conservation of the focal species. Other conflicts raised included concerns that current management plans might fail to recognise genetic diversity and evolutionary processes, for example if ‘pure bred’ conservation measures are in force e.g. deliberately removing hybrids. Similarly, some responses expressed concern for ‘keeping things apart’ rather than allowing mixing and gene flow in the area. However, although one objective of GCUs for trees is to protect adaptive traits, other objectives are to encourage dynamic gene conservation, through natural processes which may involve
mixing and connecting-up habitats. Similarly, a new objective for GCUs for other taxa may be to increase genetic diversity, thereby introducing new genes through captive breeding or translocations from elsewhere. Most respondents whose answers were grouped into ‘yes’ or ‘possibly’ gave some advice to reduce these potential conflicts, including having flexible criteria, and working alongside land managers to fully integrate the GCU management plan into existing plans. Some respondents also expressed concern for yet another system of registering sites of high conservation interest, and suggested that instead of a standalone scheme, GCUs could be integrated with current practises.

Therefore, responses indicate general support from conservationists and land managers for the GCU approach for other taxa, as well as raising some concerns. To address these concerns, we propose a flexible approach, including voluntary certification (not statutory designation) with simple standardised selection criteria that can be adapted for each target species or group of target species. This would allow GCU boundaries to move, for example if populations are displaced uphill or northwards under future climate change. To explore how GCU criteria may need to be tailored to suit particular species, we consider four exemplar case study species.

5.5 Developing GCU guidance to protect a wide range of species: four case study species

EUFORGEN has developed minimum criteria for registering populations as GCUs on the publicly available EUFGIS database (Koskela et al. 2013). GCUs for forest tree species must have a management plan, at least one target species, with a breeding population of at least 50 (marginal or scattered tree populations) or 500 (stand-forming conifer or broadleaf species) individuals. To explore the feasibility of developing GCUs for species other than forest trees, we selected four species to act as test cases and developed criteria specific to each. These case study species differ in their level of genetic risk and population dynamics, but are all of conservation importance in the UK (Table 5.1). These differences between species highlighted the need to retain certain criteria and to revise or introduce others.

Deciding on the effective population size for GCU

The minimum size of a genetically viable population (or breeding population) is defined as $N_e = 500$ where the goal is to maintain long-term evolutionary potential in a population (Franklin, 1980), and this is incorporated into the GCU forest guidelines to protect genetic diversity and ensure continued evolutionary processes (Koskela et al. 2013). An $N_e$ of 500 is also suggested for any initiative for the conservation of genetic diversity in wild populations (Hoban et al., 2020). $N_e$ can be inferred from $N_c$ which represents a population census, and a $N_e$ of 500 roughly equates to an $N_c$ of 5,000, however there is variation in this ratio among taxa (Hoban et al., 2020). A universal ‘rule of thumb’ $N_e$ or $N_c$ for inclusion in a GCU would be difficult to put into practice as these numbers will vary considerably among taxa. For example, breeding populations may represent individuals, however, in eusocial species such as bumblebees, each nest represents one breeding unit. In practice identifying 5,000
individuals in an area would be unrealistic for many species. Thus, rather than providing a set $Ne$ or $Nc$ value, we suggest that the population size threshold for inclusion in a GCU needs to be taxon specific and calculated using information on the species biology.

**Recommended GCU criteria appropriate for each case study species**

**Bombus distinguendus**

The number of great yellow bumblebee *Bombus distinguendus* breeding colonies among different sites across its distribution range from 12 – 63, with a mean of 25 (Charman, Sears, Green, & Bourke, 2010). The population density of the great yellow bumblebee is 19.3 nests per km$^2$ of suitable habitat (Charman et al., 2010). Gene flow occurs within Scottish island groups (Appendix S4.7A), but little occurs between them (Charman et al., 2010), therefore it would be appropriate to designate a GCU for each island group (Orkney, Outer Hebrides, Inner Hebrides) and the mainland population. Therefore, GCUs could be designated to incorporate the total area of occupied suitable habitat (> 2km$^2$) in the islands and mainland group, with conservation management to increase gene flow within each group.

**Erebia epiphron**

The mountain ringlet butterfly, *Erebia epiphron*, (UK distribution: Appendix S4.7B) occurs in discrete colonies where they are locally abundant, but with little dispersal between populations (Czech populations; Kuras, Benes, Fric, & Konvicka, 2003). Designated GCUs should include the entire metapopulation (e.g. Eastern Lake District, England or Ben Lawers, Scotland) and should contain suitable upland habitat, with appropriate grazing regimes (Ewing et al., 2020).

**Hypocreopsis rhododendri**

Hazelgloves, *Hypocreopsis rhododendri* (UK distribution: Appendix S4.7C) is a parasitic ascomycete fungus which requires abundant host populations, the wood decaying ‘glue fungus’ *Pseudochaete corrugata* (Grundy, Woodward, Genney, & Taylor, 2012). The number of breeding individuals is unknown but the presence of the host fungus may be used as an effective proxy to indicate the population number for the parasite. Further understanding of this species’ biology, along with demographic and genetic data for the host fungus, are required before GCU design can be considered. This case study species highlights the importance of information on species’ biology to design GCUs.

**Campanula rotundifolia**

Harebells *Campanula rotundifolia* are widespread but declining (UK distribution: Appendix S4.7D) and form four cytotypes (differences in the number of sets of chromosomes), three of which occur in the UK: tetraploid, pentaploid and hexaploid (Wilson et al., 2020). GCUs could be created in different areas of the range to incorporate different cytotypes. *C. rotundifolia* is locally common in tall-herb
grassland habitats (Stevens, Wilson, & McAllister, 2012), so we suggest a GCU area which incorporates the entire occupied grassland in a particular site.

**Table 5.1:** Case study species of UK conservation importance used to create selection criteria for GCU. The four case study species vary in genetic risk, population dynamics and taxa to understand whether criteria can be designed for different species of varying genetic importance. GCU criteria is suggested for all species, with Hazelgloves requiring more demographic data to determine GCU criteria. References: Mountain ringlet: Franco et al., (2006), Minter et al., (2020) Hazelgloves: Grundy et al., (2012); Great yellow bumblebee: Charman et al., (2010), Harebell: Stevens, Wilson, & McAllister (2012), Wilson et al., (2020). Genetic risk derived from Hollingsworth et al., (2020).

<table>
<thead>
<tr>
<th>Species &amp; genetic risk</th>
<th>UK population threats</th>
<th>Contribution of UK population to species diversity</th>
<th>Genetic risks</th>
<th>GCU selection criteria</th>
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<tbody>
<tr>
<td>Mountain ringlet <em>Erebia apphiron</em>; Moderate</td>
<td>Climate change</td>
<td>High unique genetic diversity in England</td>
<td>Risk of loss of English genetic diversity</td>
<td>Area to include metapopulation</td>
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<td>Hazel gloves <em>Rhododendron</em>; Negligible</td>
<td>Heavy grazing, Habitat change, Invasive spp</td>
<td>Bottleneck in Scottish populations, Source populations in N America higher genetic diversity</td>
<td>No evidence of adaptive variation, Little risk as Scottish genetic diversity is a subset found elsewhere</td>
<td>Area = could not be determined, More data required</td>
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<tr>
<td>Great yellow bumblebee <em>Bombyx</em>; Serious</td>
<td>Habitat loss, Climate change</td>
<td>Scotland holds last remaining genetic diversity from UK</td>
<td>Genetic diversity already lost due to declines, Low genetic diversity</td>
<td>Area to include total area of suitable habitat, GCU's in within each island group and mainland</td>
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<tr>
<td>Harebell or Scottish bluebell <em>Campanula rotundifolia</em>; Moderate</td>
<td>Habitat loss (through agricultural intensification &amp; woodland regeneration)</td>
<td>UK contains three cytotypes: tetraploid, pentaploid and hexaploid</td>
<td>Potential hybridisation with non-native genotypes, Declines in Scotland would impact hexaploid cytotype</td>
<td>Area to include the entire grassland habitat, GCU's across the different cytotypes</td>
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5.6 Management recommendations

Considerable time and thought have been invested in developing the concept of GCUs for *in situ* conservation of forest tree species and here we explore the support for, and the feasibility of, using this approach across a wider range of species as a means of achieving the CBD Aichi target of maintaining genetic variation. Our study suggests that GCUs could conserve genetic diversity in a wide range of target species and we present guidelines for the minimum qualification criteria that must be met for GCU certification (Box 5.1). As such GCUs could be classed as ‘other effective area-based conservation measures’ (OECMs): areas that are achieving effective *in situ* conservation of biodiversity outside of protected areas (CBD, 2018).

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<thead>
<tr>
<th>Box 5.1: Gene Conservation Unit criteria for terrestrial species</th>
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<tr>
<td><strong>Criterion A: At least 1 target species must be included in the GCU</strong></td>
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<td><em>Multiple target species can be included if they meet species criteria</em></td>
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<td><strong>Criterion B: Conservation objective</strong></td>
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<tr>
<td>Bi) To maintain genetic diversity</td>
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<td>Bii) To conserve adaptive or other traits in distinct population</td>
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<td>Biii) To increase genetic diversity (with additional methods e.g. captive breeding or translocation)</td>
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<td><strong>Criterion C: Population size</strong></td>
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<td>Breeding population should be tailored to species specific requirements and depending on distribution (Criterion D) and biological characteristics</td>
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<td><strong>Criterion D: Distribution</strong></td>
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<td>Di) Distinct or local</td>
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<td>Dii) Metapopulation</td>
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<td>Diii) Continuous distribution</td>
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<td><strong>Criterion E: Land area</strong></td>
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<td>Land area must contain the appropriate breeding populations of target species and appropriate habitat cover</td>
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<td><strong>Criterion F: Management objectives</strong></td>
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<td>Fij) Maintaining genetic diversity must be key management goal</td>
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<td>Fii) Ensure continued existence of target species</td>
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<td>Fiii) Create favourable conditions and actions to mitigate genetic threats for target species through habitat management</td>
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<td><strong>Criterion G: Monitoring</strong></td>
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<td>Gij) Field surveys are undertaken to monitor population size of target species</td>
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<tr>
<td>Gii) Field visits to ensure favourable conditions for target species are maintained</td>
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<td><strong>Criterion H: Database</strong></td>
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<td>GCU must be listed on a publicly accessible database which has clear definitions of the data to ensure consistency.</td>
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Some GCU criteria used for forest trees remain appropriate for GCUs for other taxa (Box 5.1, Criterion A, B, F & G) (Koskela et al. 2013). However, other criteria must be tailored to particular species (Box 5.1, Criterion C, D & E). Firstly, the breeding population size (Ne) of the target species must be calculated species-specifically, and it is not appropriate to apply a single ‘rule of thumb’ Ne for multiple taxa (Box 5.1, Criterion C). Secondly, the land area of a GCU should be inferred by the space required to support a minimum breeding population, and will differ depending on the target species’ mobility and dispersal characteristics (Box 5.1, Criterion E). The distribution of the breeding population for inclusion in the GCUs will depend on the species distribution type (distinct or local, metapopulation or continuously distributed) (Box 5.1, Criterion D), which can be identified on the basis of genetic, demographic or ecoregion data. GCUs for species with continuous populations can be identified using ecoregions (different climatic zones). Genetic data could be used to identify genetic diversity ‘hotspots’, or to select populations based on the objective to prioritise connectivity or evolutionary potential (see Nielsen et al., 2020). As with GCUs for forest trees, those for other taxa will not be statutory designations and therefore there will be flexibility as long as the minimum viable population is maintained.

The operationalization of a GCU for trees is to encourage dynamic gene conservation by recognising appropriate breeding populations in a geographic area to manage these populations to promote regular cycles of natural regeneration to occur. For other taxa, the operationalization of GCUs must similarly promote the occurrence of natural regeneration or reproduction. This will be achieved through conservation management actions listed in the management plan that promote persistence of the focal species, and mitigate genetic threats. Depending on the conservation objective of the GCU (Box 5.1, Criterion B), this may for example involve connecting up habitats to increase gene flow, or translocating individuals (genetic rescue) into the GCU to increase genetic diversity. Genetic and population monitoring of focal populations would also be appropriate to ensure sufficient population sizes for reproduction and healthy genetic diversity.

Although we have described some enthusiasm for the efficacy and feasibility of the GCU system for multiple taxa, alternatives to this method were suggested by some respondents to our questionnaire. Some individuals stated that rather than a stand-alone scheme, the GCU objectives could instead be integrated into existing land protection methods. However, a caveat to this suggestion is that GCUs would be a voluntary certification, allowing more land owners and conservation bodies to register their land if it meets the GCU general criteria.

We have highlighted how existing methods for GCU designation could be altered for other taxa, however deciding which taxa should be the focus of a GCU is something which needs to be further explored, and is beyond the scope of this paper. Whether GCUs could be used for multiple taxa or may be more species-specific, along with the types of species to include, are all issues which need to
be further discussed with stakeholders. Species prioritisation tools could be used, such as selecting species based on their socio-economic and/or cultural value (Hollingsworth et al., 2020) or combining criteria based on species value, management costs, and threat status (Joseph et al., 2009).

5.7 Conclusions and next steps

There is a need to develop a system for in situ genetic conservation. By building on the GCU approach successfully applied to trees in Europe, it will be possible to develop a system that is low cost to participants and that can coexist with current management practices, and one that aligns with proposed expansion of ‘other effective area-based conservation measures’ (CBD, 2018). For land managers to register sites as GCUs, funds are required to establish and maintain an international database, such as EUFGIS for tree species, where common criteria are applied for the listing of GCUs of a given species and the same descriptors are used to characterise the selected populations. These data could then be used to select populations to establish a core network of GCUs for each species that would capture the diversity across its distribution range. Additionally, further investigation into the application of GCUs for other taxa requires additional discussion about how to prioritise species for GCUs, for which we have set up a Gene Conservation Unit working group, to facilitate discussion and make key decisions on taking this approach forward to implement the first non-tree GCU.
Chapter 6: General Discussion

6.1 Abstract

The results presented in this thesis have increased our understanding of how climate driven range shifts influence the genetic diversity of cold-adapted species. My key findings include, that: 1) unique genetic diversity, shaped by Pleistocene range shifts, is at risk of being lost under future climate change; 2) in Britain, English populations contain smaller individuals, lower genetic diversity and many populations are predicted to be lost under future climate change (at-risk populations), whilst Scottish populations contain larger individuals, higher genetic diversity and are predicted to persist (genetic refugia); and 3) Gene Conservation Units (GCUs) can be a used to conserve genetic diversity of threatened species in the wild. Cold-adapted species are at risk from future climate change, with at-risk populations and genes isolated to the mountain tops. In this Chapter, I discuss conservation management practices for montane species, such as translocating unique populations or specific genes, and explore knowledge gaps for implementing these actions. There are likely to be changes in upland communities as a consequence of population declines and extinctions in many cold-adapted species, but uplands will also be refuges for species to expand into from the lowlands. My thesis explored how changes from the past can influence the present, and museum collections provide a valuable resource of knowledge of the past, leading to better understanding of morphology, plasticity and genetic changes over time. Overall, my findings reveal how knowledge of past distributions of species, and their responses to climate change is key to projecting how biodiversity, including genetic diversity, may change in future.
6.2 Summary of thesis findings

My thesis examined genetic diversity of *E. epiphron* across Europe, in order to understand the consequences of climate-driven post-glacial range shifts. I had three objectives to my project: 1) to examine how past and future climate-induced range shifts influence the patterns of genetic diversity in a cold-adapted species in Europe; 2) to examine the genetic and morphological consequences of post-glacial colonisation of Britain by a cold-adapted species; and 3) to explore whether Gene Conservation Units (GCUs) could be implemented more widely to safeguard genetic diversity in wild populations. Here, I review the key findings from each chapter in more detail, and then go on to discuss some wider issues emerging from my findings, including conservation management options, the future of upland biodiversity, and museum collections to expand this research.

Chapter 2: Past, current and potential future distributions of unique genetic diversity in a cold-adapted mountain butterfly.

In this chapter, I examined how past climate-driven range shifts have shaped current patterns of genetic diversity of *E. epiphron* in Britain, and whether this genetic diversity is at risk of being lost under future climate change. I used mtDNA sequencing to map the current distribution of genetic diversity of *E. epiphron* across mountain regions in Europe, and used species distribution modelling to project current, past, and future distributions of the species. My specific objectives were to:

1) Identify glacial refugia for *E. epiphron* in Europe and source populations of post-glacial colonisations of Britain;
2) Identify genetic haplotype diversity across populations in mountain regions in continental Europe and Britain;
3) Examine potential loss of genetic diversity under future climate change scenarios.

I found unique genetic diversity and regions of long-term climatic suitability in southern Europe, suggesting that *E. epiphron* persisted in disjunct populations during the last glacial cycle (or possibly longer) in mountain regions, which provided long-term refugia. These disjunct populations may have expanded downhill to the foothills of their mountain systems during glacial periods, retracting uphill as the climate warmed. This lack of gene flow between mountain systems meant that these populations diverged, resulting in haplotypes unique to specific regions. This haplotype uniqueness of populations arising from long-term separation is evident in several *Erebia* species of butterflies in Europe, and likely to occur in other cold-adapted species. I find that Scottish populations have shared haplotypes with the Vosges and western Alps mountains, suggesting a likely post-glacial colonisation route from these sources. English populations in contrast, contain a large number of unique haplotypes not found elsewhere in Europe, suggesting separate colonisation of Britain via another route, from a source population that is no longer extant (i.e. originating from a cryptic refugium). My SDM models do not identify areas in northern Europe or Britain which could have acted as areas of
genetic refugia, but other studies have identified cryptic refugia in northern Europe and Britain. For example, sub-fossil remains of beetles which currently have montane/northern distributions in Britain were found south of the ice sheet during the last glacial, and *E. epiphron* could also have occurred in the past in northern cryptic refugia. To understand more about the consequences of separate colonisations of Britain, in Chapter 3, I examine genetic differences between English and Scottish populations in more detail with ddRAD data, and in Chapter 4, I examine morphological differences between English and Scottish populations in relation to local temperature. Results from Chapter 2 reveal that under best- and worst-case future climate change scenarios, I predict 38-64% loss of *E. epiphron*’s European range size, which equates to 1-12 unique haplotypes being at risk of loss under +1 °C (best-case) and +2-3 °C (worst-case) increases in temperature. Many other cold-adapted montane species are at risk from climate change, and there may also be a high risk of loss of genetic diversity in these species too. Based on these findings, in this Chapter, I discuss conservation options for cold-adapted montane species, including translocations of populations and genes.

Chapter 3: Genetic consequences of post-glacial colonisations in montane species in Britain

Findings from the previous Chapter 2 revealed genetic differentiation between English and Scottish populations and that *E. epiphron* colonised Britain via two separate routes after the last glacial maximum. In Chapter 3, I examine the genetic consequences of these separate post-glacial colonisations of Britain by *E. epiphron*, and its subsequent isolation in two interglacial refugia in mountain regions in England and Scotland. I used ddRAD sequencing to examine genetic differences in diversity and isolation between the two regions, and modelled changes in *E. epiphron* population sizes during post-glacial colonisations, and evidence of population bottlenecks. My objectives were to:

1) Examine genetic structure and diversity in England and Scotland;
2) Quantify genetic connectivity among populations and regions, and the extent to which populations are genetically isolated with respect to geographic and environmental distance;
3) Infer variation in population size over the past 40,000 years, to explore evidence for population bottlenecks following post-glacial colonisation of Britain.

The ddRAD sequencing supports the findings from Chapter 2, confirming that English and Scottish populations colonised Britain from different source refugia, which supported very large populations ($N_e$) during the LGM. My study provides some of the first evidence of large population sizes of a cold-adapted species during the last glacial, with my stairway analyses suggesting populations experienced bottlenecks during post-glacial colonisation of Britain, with more severe and prolonged bottlenecks in England. Subsequent population increases may be associated with Neolithic tree-clearing during the Holocene, which would have increased openness and provided suitable habitat for
E. epiphron. Current populations contain 17% less genetic diversity in England than Scotland, with English populations suffering a more severe historical population bottlenecks, as revealed from the stairway plot analyses, and it can take many generations to recover from severe genetic bottleneck. In addition to genetic separation between English and Scottish regions, the ddRAD analyses revealed further genetic separation of populations England into east and west Lake District populations. These results imply further isolation between these two areas of the Lake District, and lack of gene flow across valleys. I find that genetic distance between populations is related to the distance between populations in terms of geographic distance and spatial distribution of climatically suitable areas. This isolation between mountains in the same region may be typical of other cold-adapted species with limited dispersal and barriers to gene flow from inhospitable warm valleys. I conclude that conservation efforts should be focused on at-risk English populations, to conserve genetic diversity and promote gene flow where appropriate.

Chapter 4: Smaller montane butterflies at warm range boundaries may affect persistence under future climate change

The previous Chapters highlight separate post-glacial colonisation of England and Scotland and so I examined differences in morphology between these two regions, related to differences in temperature. Using field-collected material and museum specimens, I measured variation in body size of E. epiphron in relation to temperature, testing whether individuals from warmer sites and regions, and those emerging in warmer years are smaller. My specific objectives were to:

1) Examine body size variation in populations of E. epiphron in England and Scotland in relation to temperature;
2) Examine body size variation in museum specimens over ~100 years in relation to temperature;
3) Examine differences in within-population body size variation among regions, to identify differences in plasticity.

I found that English E. epiphron are 7-8% smaller than Scottish individuals. also found smaller individuals at warm low elevation range edge populations, with the smallest individuals found at the lowest elevation site in England, but no change in body size of museum material over time. I also found similar levels of within-population variation in size among sites, suggesting similar levels of plasticity across Britain. Variation in size could be due to genetic differences and separate colonisation history (Chapter 2 and 3) or the effects of local temperature on development, with warmer temperatures resulting in faster development and smaller adult body size. The size differences could also reflect differences in voltinism between England and Scotland. It has been suggested that E. epiphron in Scotland may have a two-year life cycle, similar to other Erebia butterflies in Europe, which would result in longer periods of larval feeding, and therefore larger adults. It is unclear what
the consequences of size difference could mean in the context of persistence of populations under climate change. For example, shrinkage could be beneficial if by responding to warmer temperatures *E. epiphron* can develop through more generations per year and increase abundance. Alternatively, shrinkage could be detrimental, if it leads to reduced fecundity, dispersal and temperature buffering ability.

Chapter 5: Exploring the potential for ‘Gene Conservation Units’ to conserve genetic diversity in wild populations (CASE placement with NatureScot)

My findings from Chapter 2 indicate that there is a substantial risk of loss of unique haplotypes under climate change. In order to safeguard genetic diversity in wild populations, *in situ* methods of genetic conservation are needed and so I examined the current extent of Gene Conservation Units (GCUs) and other genetic conservation techniques, and whether GCUs could be implemented more widely to protect genetic diversity *in situ* in a wider range of animal and plant species. My specific objectives were to:

1) Review the current implementation of GCUs and other genetic conservation techniques using a systematic literature review;
2) Use a structured questionnaire to canvass conservationists’ and land managers’ opinions for adopting a system of GCUs in Britain;
3) Test whether existing methods for voluntary accreditation of GCUs for trees are appropriate for application to other taxa, using case study species.

My literature review and questionnaire revealed that the most common form of genetic conservation management was GCUs, but GCUs were focused on conserving plant genetic diversity, including forestry trees and wild relatives of crop species. Thus, genetic conservation has been more focused on species of economic importance and less on species for conservation value. My questionnaire revealed that land managers and conservationists considered that genetic information should be better integrated into conservation in the future. There were mainly positive responses regarding the potential to recognise GCUs for more mobile species, but that GCU criteria would need to be co-designed with respect to the focal species being protected. Using four case study species (great yellow bumblebee *Bombus distinguendus*, mountain ringlet *E. epiphron*, hazelgloves fungus *Hypocreopsis rhododendri* and harebells *Campanula rotundifolia*), I explored the feasibility of developing GCUs for these species and developed criteria for each. I found that some GCU criteria need revising to be applicable to non-plant species, and I created management recommendations to reflect this. These revisions included consideration of the breeding population size (*Ne*) and the land area required to support a breeding population, and key decisions about species prioritisation and application must be developed with key stakeholders.
Overall, my thesis reveals that range-shifts by species in the Pleistocene shaped current patterns of genetic diversity, resulting in unique genetic diversity in isolated populations which may be at risk in the future. I find that in Britain, English populations are predicted to be more at-risk (Chapter 2), have lower genetic diversity (Chapter 3), and contain smaller individuals (Chapter 4). In contrast, Scottish populations are predicted to be likely to persist in future (Chapter 2), have higher genetic diversity (Chapter 3), and contain larger individuals (Chapter 4), and so these represent potential genetic refugia. My thesis highlights the future range shifts of cold-adapted species and the need to conserve biodiversity in the uplands. In this Chapter, I discuss the conservation options that would be appropriate for cold-adapted species, including conservation management of current populations, as well as translocations of populations and genes, and knowledge gaps for their implementation. I discuss the future of the uplands, and changes in upland biodiversity through range shifts, both colonisations and extinctions, and changing land management practices. My thesis shows how the past is important for predicting the future, and so I discuss how museum collections could expand our knowledge of biodiversity changes over time.

6.3 Conservation of *E. epiphron* and other cold-adapted species

I find that *E. epiphron* populations were larger during the last glacial, suggesting that colder climates at the last glacial maximum were more suitable for the species, and other cold-adapted species may have thrived at this time. Currently, *E. epiphron* and other cold-adapted species are restricted to interglacial cold refugia on mountain tops, and dispersal prevented by warm valleys form a barrier to movement among mountain regions. Conservation therefore needs to focus on preventing local extinctions as well as translocating populations to new cooler locations that they cannot naturally disperse to. Here, I discuss conservation management options that could be appropriate for cold-adapted species restricted to mountain tops.

*Situ habitat management

My projections of range retractions and genetic losses under projected future climate change are based on a best-case and worse-case climate scenarios, representing +1 °C and +2-3 °C increase in average temperatures. If temperature increases are kept to a minimum, then *in situ* habitat management could be implemented in order to attempt to slow down population declines of cold-adapted species. In upland areas, habitat management could increase microclimate variability (Greenwood, Mossman, Suggitt, Curtis, & Maclean, 2016), providing cooler microhabitats (Suggitt et al., 2011) through manipulation of the vegetation structure and sward height. This could be achieved through afforestation and reducing grazing of livestock, and ensuring protection of north-facing areas (Greenwood et al., 2016). Trees can create cooler areas, and reduction in grazing would allow for variation in sward heights, and presence of scrubs would create shaded areas (Natural England and RSPB, 2019). These *in situ* methods may slow down population declines in cold-adapted species and
are also relatively low-risk strategies to implement as they do not involve moving populations, but may not be a successful long-term solution. Given worse-case climate scenarios for warming, more drastic methods may need to be implemented to enable the persistence of cold-adapted species.

Translocations and assisted colonisations

Lack of gene flow between mountain regions results in unique haplotypes and linages, which are at-risk from future climate change. This loss could be through extinctions of unique populations on isolated mountain tops e.g. Erebia pandrose sevoensis in the Apennines (Sistri et al., 2021), or through inbreeding in very small populations, e.g. Erebia orientalis (Hinojosa et al., 2019). Therefore, to conserve specific population linages and uniqueness, translocations could be an effective conservation strategy. Translocations involve moving individuals to areas which are currently unoccupied, but have suitable climate and habitat but are beyond the reach of dispersing individuals (Hoegh-Guldberg et al., 2008). There are only a few instances of success translocations in the wild (Cizek et al., 2003; Willis et al., 2009), but may be the best option for ensuring the persistence of cold-adapted species.

Translocations of E. epiphron could involve moving individuals from at-risk English populations into new cooler areas in Britain which are climatically suitable. Translocations of this kind are associated with a number of risks, such as inter-specific competition with ‘native’ species in the new area, introducing diseases, and harming source populations by reducing their size (IUCN/SSC, 2013). At-risk populations may be uniquely adapted to their local ‘home’ environment (Crandall, Bininda-Emonds, Mace, & Wayne, 2000) and so could have reduced fitness in new sites. However, if unique genetic diversity is non-adaptive, resulting from random genetic drift due to isolation (Weeks et al., 2016), then translocations could have negative genetic effects if it increases inbreeding in small isolated populations. To reduce this risk, translocating individuals from multiple source populations would be beneficial (IUCN/SSC, 2013). Ensuring that translocations are into locations with suitable climate and habitat is vital, and large founder populations to ensure genetic diversity and future persistence.

Translocations for reinforcement

In contrast to translocating populations to new un-occupied areas, individuals and their genes can be translocated to reinforce current populations. The aim of this may be to increase genetic diversity, known as genetic rescue (Whiteley et al., 2015), or to increase adaptability to climate change (Weeks et al., 2011). Genetic rescue involves translocating individuals into a population with low genetic diversity in order to increase genetic diversity. For example, E. epiphron individuals from Scottish populations could be translocated into English populations to increase genetic diversity. In addition to translocations to reinforce genetic diversity of populations, individuals and their genes can be translocated to increase adaptability of populations (Weeks et al., 2011). In E. epiphron, for example,
this could involve translocation of warm-adapted English populations into Scotland, potentially increasing the adaptability of cooler areas to future warming. Increasing the prevalence of warm-adapted genes could increase the adaptive capacity of populations in colder areas to persist under warmer temperatures (Weeks et al., 2011).

However, there is little practical guidance for implementing genetic rescue in the wild e.g. on the minimum number of breeding individuals required for a genetically viable population. Genetic rescue has been implemented a number of times in the wild, mainly for large vertebrates, but only a single instance in insects (Jangjoo et al., 2016), although this was by increasing connectivity through habitat management rather than physically moving individuals. Therefore, it is unclear whether translocations and genetic rescue would be successful in insects which have relatively large population sizes. There is also no evidence of translocation to increase adaptability in populations being implemented in practise and whether warm-adapted genes would be maintained in the new population. For example, translocating a small number of English _E. epiphron_ into a large Scottish population may have minimal impact on adaptability of the recipient populations unless strong selection is acting on beneficial alleles from source population.

There are risks associated with mixing individuals from genetically diverged populations, resulting in outbreeding depression (Frankham et al., 2011; Weeks et al., 2011). Current guidance on mixing populations for translocations suggests populations should not be mixed if they have been separated for more than 500 years (Frankham et al., 2011) or occupied ecologically divergent habitats for more than 20 generations (DEFRA, 2021). But hybridisation of some closely-related species can result in beneficial genes being transferred through adaptive introgression, for example introgression of genes associated with pesticide resistance in _Helicoverpa_ moth pests (Valencia-Montoya et al., 2020). Hybridisation in the wild has occurred between species as a result of changes in range under climate change (Larson, Tinghitella & Taylor, 2019; Mallet, Wynne & Thomas, 2011) and some argue that adaptive introgression between closely-related species may increase their ability to respond to climate change (Hamilton & Miller, 2016). Therefore, mixing of individuals from diverged populations could result in the transfer of genes beneficial for climate change adaptation, and represents an interesting research area to provide valuable information about implementing gene translocations for climate change adaptation.

**Knowledge gaps**

Cold-adapted species and their genetic diversity are restricted to isolated mountains, and further information is required before implementation of translocations. Firstly, to identify climatically suitable new areas for translocations, detailed knowledge of the habitat and climatic requirements of species is required. For example, winter snow cover is important for winter survival of _E. epiphron_ (Konvicka et al., 2021) with vegetation structure and microclimates suitable for oviposition (Ewing et
If translocating to reinforce genetic diversity (genetic rescue) then the outcomes of mixing divergent populations needs to be investigated, which would include controlled mating *ex situ*, testing for reproductive success and potential outbreeding in offspring. Similarly, if translocating genes is the conservation objective, then offspring need to be tested for the presence of genes of interest after mixing of populations. In all these conservation actions, information about local adaptation could be investigated through environmental association analysis (Rellstab, Gugerli, Eckert, Hancock, & Holderegger, 2015), genome wide association analysis (Korte & Farlow, 2013) or through common garden experiments (Savolainen, Lascoux, & Merila, 2013). More research is required if translocating populations and/or genes for climate change adaptation is to be a successful and viable management strategy.

### 6.4 The future of upland biodiversity

Many cold-adapted species, such as *E. epiphron*, will undergo population declines and local extinctions under future climate change. Here, I discuss how these losses, along with potential gains from colonising species from lowland areas, will change the communities of upland areas. In Britain, the very high montane specialists species are likely to be the first species lost under future warming. For example, the cold-adapted beetle *Nebria nivalis*, is restricted to mountain tops, and in England and Wales it only occurs on Scafell Pike and Snowden (Telfer, 2016). This species probably had a much larger historical range in Britain, but retracted to the mountain tops during the Holocene, and represent species that are likely be lost in future from upland communities. Current levels of biodiversity could be maintained, or enhanced, if warm-adapted species undergo range expansions to colonise the high mountain tops, replacing species that go extinct. Some species which currently occur in the uplands were once widespread but have retracted to the uplands due to habitat loss or human disturbances. For example, the black grouse *Tetrao tetrix*, was once widespread in England, but after significant population declines this species is now restricted to upland areas (Baines & Hudson, 1995). Therefore, the uplands may also represent refuges for species which are not necessarily restricted by climate, but have suffered declines in the lowlands. Uplands may receive species whose ranges are expanding uphill (Chen et al., 2011; Wilson et al., 2007), providing climate change refugia (Ashcroft, 2010), assuming sufficient habitat connectivity for species to colonise new areas at higher elevation. There may also be negative impacts if the colonists compete with existing occupants or bring new diseases (IUCN/SSC, 2013). Turnover of species and community composition is expected under future climate change in upland areas, losing high montane specialists, which may be replaced by colonists from lower elevations areas.

The future of the uplands and the biodiversity they support will also be determined by the consequences of changing land management, such as increased rewilding and other Nature-based Solutions to enhance carbon and biodiversity. The British uplands have been heavily modified by
people, managed for food production (sheep, cattle), forestry, and management for economically important activities (e.g. grouse moors) (Sandom et al., 2019; Thompson, Macdonald, Marsden, & Galbraith, 1995). However, concerns about climate and biodiversity has led to calls to change land management practises. Rewilding can include leaving land to nature, restoring degraded ecosystems, and the movement of species to restore functional communities (Sandom et al., 2019). Some of these approaches overlap with the aims of Nature-based Solutions, for enhancing nature to address societal challenges (Seddon, Turner, Berry, Chausson, & Girardin, 2019), supporting climate change adaptation through flood protection, water quality regulation, and removing carbon (e.g. tree planting) (Griscom et al., 2017; Seddon et al., 2019). Approaches that are being implemented in the uplands include peatland restoration and protection, and afforestation. Tree-planting across the uplands would contribute to creating cooler microclimates and enhancing variation in microclimates, helping to support some cold-adapted species (Natural England and RSPB, 2019). However, such practises to increase tree cover are unlikely to benefit open habitat and grassland species, such as E. epiphron, where recovery from bottlenecks and population increases were associated with Holocene tree clearances and increased ‘openness’. In contrast, increasing areas of forest and peatland would provide the opportunities for other species dependent on these habitats to expand and thrive in upland regions. These potential changes in land management to mitigate climate change impact could alter the composition of upland communities, arising from species losses and gains.

6.5 Understanding change over time from museum specimens

Understanding the past is important for understanding current distributions of species and their genetic diversity, and for predicting future changes in species. In this thesis, I projected distributions of E. epiphron over the last 21,000 years in order to understand more about the current distribution of genetic diversity and implications of future climate change. I identified areas of long-term refugia which were sources of present day populations and their unique genetic diversity. Using Natural History Museum specimens allowed me to examine evidence for changes on body size of E. epiphron over ~100 years. Hence, studying past processes is important for understanding the current distribution of diversity, and how it may change in the future.

Museum collections, such as the Natural History Museum (NHM) London, provide opportunities to investigate ecological questions from the past, and helping to understand how species may respond in future. These museums house millions of specimens collected over decades to centuries. For example, the NHM collection of Lepidoptera contains ~13.5 million specimens dating from the 18th century (Natural History Museum, 2014). These collections provide a valuable resource to measure changes in morphology, genetic diversity and plasticity over time. Museum collections have been used to assess changes in wing melanism (MacLean, Nielsen, Kingsolver, & Buckley, 2019), shrinkage and changes in wingspan in birds (Weeks et al., 2020), shrinkage of tropical moths (Wu et al., 2019) and moths in
high arctic regions (Bowden et al., 2015), allowing shrinkage to be associated with climate warming over the last century (Sheridan & Bickford, 2011). Museum specimens have also been used to examine size plasticity of dragonflies and damselflies to temperature changes during development (Wonglersak et al., 2020). Testing whether species show phenotypically plastic responses in warm years could provide information on limits to plasticity and if species will be able to future warmer temperatures and extreme events, such as heatwaves. Museum specimens could also be used to quantify changes in plasticity over time, and whether variation in morphological traits changes over time. Hence museum specimens provide a huge data resource to exploit to understand changes in morphology and plasticity over time.

With costs of DNA sequencing reduced and the technology improving over the last decade, it is possible to use museum material to examine genetic changes over time (Nakahama, 2021). For example, genetic diversity of extinct populations and species could be examined (Nakahama, 2021), to explore genetic factors associated with extinction, such as in-breading and low genetic diversity, (Bijlsma, Bundgaard, & Boerema, 2000; Saccheri et al., 1998) and quantify loss of genetic diversity and unique haplotypes. If museum material is used in combination with wild-caught material, then changes in genetic diversity over time in extant populations can be examined (Nakahama, 2021), to quantify any loss of genetic diversity over time due to climate change and/or habitat fragmentation. Using museum material to explore how historical processes have influenced genetic diversity of current populations would provide better understanding of the genetic consequences of future climate change.

6.6 Conclusion

In this thesis, I find that isolated populations of *E. epiphron* are at risk from future climate change, and identify genetic refugia in Britain. Climate-driven range shifts of cold-adapted species, such as *E. epiphron*, have resulted in the post-glacial colonisation of Britain, where they are currently restricted to montane regions, and are vulnerable to future climate warming. Changes in community composition are likely in upland areas. Climate change and land management changes could result in population extinctions, but the uplands may also act as a refuge for species colonising from lowland areas. Translocations could safeguard the unique diversity of at-risk populations and to increase adaptability of populations, but there are knowledge gaps which need to be addressed to ensure translocations were to be successful. Museum collections provide information about changes over the last century, and are a valuable data resource for exploring changes in morphology, genetic diversity and phenotypic plasticity over time. Using these historical resources to further understand past changes in cold-adapted species would provide more information on whether they may persist in future.
Appendix 1: Past, current and potential future distributions of unique genetic diversity in a cold-adapted mountain butterfly: Supporting Information
**Appendix S1.1:** mtDNA haplotype sample information and Genbank/BOLD accession codes

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Appendix S1.2: Population locations for all individuals used in mtDNA analysis from 13 mountain regions.

Appendix S1.3: Bioclimatic variables used in SDMs to predict climate suitability for *E. epiphron*, extracted from mean temperature and precipitation data between 1970 and 2000 (http://www.worldclim.org/). 'Cells' are 2.5 arc minute (~4.5 km) resolution data extracted from within a 50km grid at the same spatial extent as distribution data. ‘Season’ refers to mean data from summer (June, July, August) and winter (December, January, February).

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**Appendix S1.4:** All SDM outputs showing probability of climate suitability from present-day to 21,000 years ago (22 outputs in total). Probability values of occurrence for all panels are scaled from 0 (unsuitable, white) to 1 (suitable, black). Ice sheets (from Hughes et al., 2016), blue shading) are present from 21,000 years BP to 10,000 years BP.

Present

1,000 years BP

2,000 years BP
6,000 years BP

7,000 years BP

8,000 years BP
12,000 years BP

13,000 years BP

14,000 years BP
15,000 years BP

16,000 years BP

17,000 years BP
18,000 years BP

19,000 years BP

20,000 years BP
21,000 years BP
Appendix S1.5: CO1 phylogenetic tree of the Erebia genus, outgroups and E. epiphron populations. Phylogenetic tree analyses were performed in Beast using methods described by Pena, Withthauer, Kleckova, Fric, & Wahlberg, (2015). Outgroup and Erebia genus data were accessed from Genbank using accession numbers in Pena et al., (2015). Age of split between Erebia and sister taxa of 37.41 Myr (Pena et al., 2015) was used to calibrate the age split between Erebia epiphron and E orientalis. Scale bar represents age of tree in million years before present. Node number represent estimated age of node with blue error bars.
Appendix S1.6: Locations and ages of remains in the UK of the cool-adapted beetle species *Partobus septentrionis, Amara alpina, Amara quenseli* and *Notaris aethiops*, with corresponding most credible ice sheet extent. Beetle fossil data acquired from BugsCEP (Buckland & Buckland, 2006), ice sheet data from (Hughes, Gyllencreutz, Lohne, Mangerud, & Svendsen, 2016).
Appendix 2: Genetic consequences of post-glacial colonisations in montane species in Britain: Supporting Information

Appendix S2.1: Population level genetic variation measures and number of samples included in final SNP file

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Appendix S2.2: P1 and P2 adapters for ddRAD ligation

P1 top
5'- AATGATACGGCGACCAGACGGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCT

P1 bottom
3'- TTACTATGCGCTCGTGGCTCTCTGATGTGAGAAAGGGATGTGCTGCGAGAAGGCTAGA

P2 top
5'- Phos-AATTCCTIINNNNAGATCGGAGAAGAAGACACGTCTGAATCTCCAGTCACCGAGAAGCTAGA

P2 bottom
3'- GGMMHNNNNCTAGCCTTCTCGTCGAGACGTTCAGTCGAAGGCTTAGACGATAACGCGAGAAGGAAGCTAGA

Appendix S2.3: RAD forward and reverse primers for PCR amplification

RAD1.F*: 5'-AATGATACGGCGACCACCCGAG-3'

RAD2.R*: 5'-CAAGCAGAAGACGCCGATACCCGAG-3'
Appendix S2.4: Stairway plots of effective population size (Ne) since 60kya. Red lines show the medians of the inferred populations, dark grey lines show 12.5% and 87.5% percentiles of inferred populations and light grey lines show the 2.5 and 97.5 percentiles of the inferred populations. A) Scottish populations, B) English populations, C) East Lake District populations and D) West Lake District populations.
**Appendix S2.5**: Phylogenetic tree of all ddRAD samples aligned to *Erebia ligea* genome, coloured labels indicate the genetic population from Structure analysis (England West = Red, England East = Green, Vosges mountains, France = Blue, Scotland = purple and *E. ligea* outgroup = grey). Tree was made using RAxML-NG (Stamatakis, 2014) and plotted using the ggtree package in R (Yu et al., 2017).
Appendix 3: Smaller montane butterflies at warm range boundaries may affect persistence under future climate change: Supporting Information

Appendix S3.1: Morphological measurements taken using Image J. A) Forewing length was measured in field scanned images as the distance between the cell base and vein 10 B) Forewing length was measured in Natural History Museum photographs as the distance between the cell base (near to thorax) and vein 10.
**Appendix S3.2**: Model outputs of GLMs to explain forewing mean length with regional and temperature affects in modern and museum material. Tables present the estimate (slope), the standard error, t value and P value of the explanatory variables. Adjusted R-squared and F-statistic of overall model presented below A) Model output of modern population forewing mean length with region and annual mean temperature (1km) (°C) and B) Model output of museum material forewing mean length with region and mean temperature during larval development (1km) (°C). Models for other wing measurements can be found in Supplementary Materials SM4. Asterisk denotes whether the test was significant, * P ≤ 0.05, ** P ≤ 0.01, *** P ≤ 0.001.

### A) Modern

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Appendix 4: Exploring the potential for ‘Gene Conservation Units’ to conserve genetic diversity in wild populations: Supporting Information

Appendix S4.1 Methods for systematic literature review and questionnaire

*Literature review*

We conducted a systematic literature review (published papers and ‘grey’ literature) to search for evidence of genetic conservation in the literature. Only studies where the main or one of the main purposes of the conservation was to protect or increase genetic diversity were included. Searches in the literature include ‘genetic rescue’, ‘gene reserves’, ‘genetic conservation unit’, ‘evolutionary significant unit’. Further literature was obtained through references within this literature. For each study, the name of the species along with the type of genetic conservation management was extracted. The species were then categorised into species group (trees, mammals, plants (not trees), birds, reptiles, amphibians and fish) and socio-economic value (conservation, timber, craft, medicinal, game, fisheries, agriculture and ornamental). Data from the literature review is available from the Dyrad Digital Repository: [https://doi.org/10.5061/dryad.3j9kd51hm](https://doi.org/10.5061/dryad.3j9kd51hm) (Minter et al., 2021).

*Questionnaire*

To understand how different stakeholders including conservationists and other land managers perceive the importance of genetic diversity, and to gather information about current approaches to genetic conservation in the UK a questionnaire was designed. The questionnaire was made up of four sections: 1) information about the participant (organisation sector), 2) their perceptions of genetic conservation (including perceived importance, perceived impact of genetics on UK conservation), 3) their understanding of current genetic conservation in the UK (if genetic data were used to inform conservation management, what genetic conservation has been implemented, which species this had been focussed on) and 4) their understanding of the concept of Gene Conservation Areas (including risks and benefits) (see Questionnaire Appendix S4.8). Sections 1-3 are mostly made up of questions with standardized answers, and section 4 contains questions with open-ended questions to get detailed input from participants. A variety of stakeholders was targeted for this study, including NGOs, land managers (i.e. including farming and estate management), government/non-departmental public bodies and research institutes/universities.
Appendix S4.2: A) Number of species which *in situ* genetic conservation management strategies have been implemented on, grouped by taxon and management type. B) And split by management type and reason for conservation action, either species of conservation value or economic value, such as agriculture, forestry, game etc.
**Appendix S4.3:** Questionnaire respondents and their employer $n=60$

**Appendix S4.4:** Word cloud of the open ended answers to Question 6 of the Questionnaire: “What do you think are the benefits of conserving genetic diversity?” Size of word represents frequency of word mention. Word cloud created at https://wordart.com/
Appendix S4.5: Questionnaire response to questions about the use of genetic information and research to guide management practices in the UK

Appendix S4.6: The reasons for which genetic information has been incorporated into species conservation in the UK
Appendix S4.8: Questionnaire which was sent to conservationists and land managers in the UK including information for participants, consent form and the Questionnaire

Genetic conservation in the UK: Gene Conservation Areas (GCA), broadening the concept beyond trees

1. Information for participants

We would like to invite you to take part in our research study. Before you decide we would like you to understand why the research is being done and what it would involve for you. We understand that this is a difficult time under the current COVID-19 pandemic, and so we understand if this is not an appropriate time to be involved in this research study which is part of a PhD project at the University of York and Scottish Natural Heritage.

In our study we aim to understand perceptions of genetic conservation in the UK, the role of genetics in conservation management, and to explore whether, and how, Gene Conservation Areas (GCA) could be used as a genetic conservation management tool for other species beyond trees.

Genetic information from DNA sequencing of wild populations has increased over the last 30 years, along with understanding the role of genetics in supporting the resilience of species and habitats. We want to understand how people perceive the role of genetics in conservation management. We also want to understand whether you are using genetic data in conservation management, or implementing specific management to protect genetic diversity or to increase genetic health.

The first Gene Conservation Area (also known as Gene Conservation Unit for trees) in the UK was designated at Beinn Eighe in Scotland in 2019, to protect the genetic diversity of population of Scots pine tree. For information on this genetic reserve please see the following BBC article:

[Genetic reserve in Wester Ross to protect Scotland's national tree](https://www.bbc.co.uk/news/uk-scotland-47633399)
The concept of Gene Conservation Units (GCU) for trees was established over 20 years ago, and these reserves can be found all over Europe. GCUs were established to allow dynamic gene conservation to take place, which means the protection of natural processes in the area, and allowing genetic changes to develop naturally in response to changes in the natural environment e.g. climate change. Forest Research has put together guidelines for establishing and managing Gene Conservation Units for trees which can be found here: https://www.forestresearch.gov.uk/research/establishing-and-managing-gene-conservation-units/.

In this survey, we want to gather information on Gene Conservation Areas (GCA), which would extend the GCU approach to other species. We want to explore whether this same approach could be used on other species beyond trees, and therefore we want to gather information on the perceived risks and opportunities of this. If this could be applied to other species, then a registration scheme would be produced and used to inform future management guidelines. We will use your responses in this survey to develop these ideas.

2. What information is being collected?
We are gathering information from different stakeholders including conservationists and other land managers. The questionnaire is divided into 4 main sections, containing 22 questions and should take about 20 minutes to complete. There are no wrong or right answers, we are primarily interested in your personal opinions (except section 3 on current genetic conservation):

Part 1: Information about you
Part 2: Your perceptions of genetic conservation
Part 3: Your understanding of current genetic conservation in the UK
Part 4: Your understanding of the concept of Gene Conservation Areas

3. Why do we need your personal information, and how will it be used?
Neither you nor your organisation will be identified in any output (e.g. report) arising from the research. Your name, contact email and company name will be kept separate from the rest of the data, and would only be used 1) (to identify your response) in the event that you wished to withdraw your response after submission and 2) if there is any follow-up study where we may invite you to participate. We request information on your employer type (e.g. NGO, research institution, land agency, estate company etc) so that we can compare responses between sectors. Both the pseudonymised research data, and your personal information, will be stored securely to ensure confidentiality. All personal data will be destroyed upon completion of the PhD project, in 2022. Participants can withdraw from the survey at any time and request their data to be withdrawn. We request written informed consent (electronic form) at the start of the questionnaire where you will also find our privacy statement. The survey conforms to all ethical approvals required by University of York Department of Biology Ethics Committee.

If you have any questions about the project or data collection, you can email Melissa Minter mm1874@york.ac.uk.

Consent form

Please also see the privacy notice statement

I confirm that I have read and understand the information sheet explaining the research project and I have had the opportunity to ask questions (by email if required) about the project.
I give permission for the PhD student and the PhD student’s supervisor to have access to my pseudonymised responses and personal data kept separately. I understand that my name will not be linked with the research materials, and I will not be identified or identifiable in the report or reports that result from the research. I understand that my responses will be kept strictly confidential.

I understand that my participation is voluntary and that I am free to withdraw at any time (until the completion of the project) without giving any reason and without there being any negative consequences. In addition, should I not wish to answer any particular question or questions, I am free to decline. I can indicate a wish to withdraw by informing Melissa Minter (mm1874@york.ac.uk). At the start of 2022 all personal data will be destroyed, and the data will become fully anonymised. After this point it will no longer be possible to withdraw your response.

I agree for my personal information to be stored securely, separate from the pseudonymised data. I am happy to be contacted by the email I provided if any follow-up study was conducted before all personal data is destroyed in 2022.

I agree for the data collected from me to be stored and used in relevant future research in an pseudonymised form

I understand that relevant sections of the data collected during the study may be looked at in pseudonymised aggregated format by individuals from Scottish Natural Heritage or University of York. The data will be aggregated to ensure individuals or organisations could not be identified from questionnaire responses. I give permission for these individuals to have access to my pseudonymised aggregated data.
Please enter your initials and date to complete consent

**Questionnaire**

**Part 1: Information about you**

1. *What is your name?*

2. *What is your contact email?*

3. *What is the name of your organisation/employer?*

4. *Please select the most relevant to your organisation/employer*
   
   NGO (Conservation)
   NGO (Other)
   Land management (i.e. including farming and estate management)
   Government/Non-departmental public body
   Research institute/University
   Self-employed
   Other

   *If ‘Other’ please specify*

**Part 2: Your perception of genetic conservation**

5. *Do you think genetic diversity is important to species survival?*
   
   0 Don’t know
   1 Very important
   2 Important
   3 Neutral
   4 Less important
   5 Not important

6. *What do you think are the benefits of conserving genetic diversity?*

7. *Please state how much you agree or disagree with the following statement: Genetic information has had a strong impact on conservation in the UK.*
   
   0 Don’t know
   1 Strongly agree
   2 Agree
   3 Neutral
   4 Disagree
   5 Strongly disagree
8. Please state how much you agree or disagree with the following statement: Genetic information should be more integrated into biodiversity conservation in the future.

0 Don’t know
1 Strongly agree
2 Agree
3 Neutral
4 Disagree
5 Strongly disagree

Part 3: Implementation of genetic conservation

This section is to understand how genetics is being used currently in the UK within conservation. This section may be more applicable to those who work in land and conservation management. If this section is not applicable to you, please skip to part 4.

9. Have you or your organisation incorporated genetic information or techniques into species conservation?

Yes
No
Don’t know

10. If the answer to question 9 was ‘Yes,’ what did this conservation action seek to address? Please tick all that apply

- Re-introductions of species where they had previously gone extinct
- Translocations of species beyond their current range
- Improving population health
- Improving habitat connectivity between populations
- Addressing inbreeding
- Taxonomic identification
- Other

If ‘Other’ please specify

Any additional comments:

11. Have you or your organisation used genetic information to guide your management recommendations?

Yes
No
Don’t know

Any additional comments:
12. Have you or your organisation genetic scientific research to guide your management recommendations?

Yes
No
Don’t know

Any additional comments:

13. Have you or your organisation implemented any conservation management to specifically conserve genetic diversity?

Yes
No
Don’t know

14. If the answer to question 13 was ‘Yes’ please specify what kind of conservation management this was:

Genetic rescue (via Introductions of captive populations into the wild to increase genetic diversity)
Genetic rescue (via translocating populations to increase genetic diversity)
Captive breeding
Establishing a Gene Conservation Unit
Protection of locally adapted population
Designating an Evolutionary Significant Unit (ESU) (population of organisms that is considered distinct to the rest of the species)
Other

If ‘Other’ please specify

Any additional comments:

15. If the answer to questions 13 was ‘Yes’ what species group was this conservation action focused on? Please tick all that apply

Plants (not trees)
Trees
Mammals
Invertebrates
Birds
Reptiles
Amphibians
Fish
Other
16. Does your organisation have a genetic policy in respect to conservation?

Yes
No
Don’t know

If ‘Yes’ can you please specify

17. What are the barriers to implementing genetic conservation management for you or your organisation?

Financial reasons
Not appropriate
Other priorities
Lack of specific knowledge
Lack of communication with specialists in this field
Other

If ‘Other’ please specify

Any additional comments:

Part 4: Gene Conservation Areas: a concept beyond trees?

In this section, the questions are open-ended, as we wish to gather your opinions on the concept of GCAs for genetic conservation of species. If GCA certification is implemented for species other than trees, we want to ensure this would benefit land owners and would not conflict with existing conservation management plans.

18. Please can you describe the potential benefits of GCAs for gene conservation

19. Please can you describe the potential risks of GCAs for gene conservation

20. Please can you describe the benefits of GCA certification for landowners

21. GCAs have been established to protect trees and ‘crop wild relatives’ (a wild plant closely related to crop species). Do you think this GCA concept could work in more mobile species such as mammals, insects, birds etc?

22. Do you think implementing a GCA management plan could conflict with existing conservation management plans?
23. Please add any further comments.
References


Blackburn, K B (1952), The dating of a deposit containing an elk skeleton found at Neasham near Darlington, County Durham. *New Phyt. 51,* 364.


128


134


