Impacts of climate driven range changes on the genetics and morphology of butterflies

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Abstract

This thesis studied the genetic responses of butterflies to climate induced distribution shifts in terms of patterns of genetic diversity at expanding and contracting range margins, the relative importance of genes versus environment on adaptations to dispersal and local adaptation to temperature during range expansion.

Loss of genetic diversity during range expansion in *Pararge aegeria* was confirmed using neutral genetic markers (AFLPs). High reductions of genetic diversity were discovered at the range margin relative to the distribution core. Range margin populations exhibit a nearly 50% reduction in neutral genetic diversity, and lower genetic divergence between sites.

The contracting southern range margin of the butterfly *Erebia aethiops* has not suffered a reduction in genetic diversity relative to the distribution core. As genetic diversity remains relatively high population extinction is unlikely to be exacerbated by inbreeding or reduced fitness from low genetic diversity during range contraction.

Contrary to results from laboratory reared butterflies, wild male *P. aegeria* do not have significant differences in flight morphology between core and margin sites. This suggests developmental influences suppress the expression of genetic adaptations to dispersal. Wild butterflies also represent a smaller range of phenotypes possibly indicating balancing selection on morphological traits.

Little to no evidence for local adaptation to temperature is apparent at the expanding range margin of *P. aegeria*. Neither was there evidence for reduced fitness due to lower genetic diversity, as F_2 butterflies from core sites had poorer survival rates than the less genetically diverse margin sites.

This study found that neutral genetic diversity is unlikely to affect species during distribution shifts as even high losses during distribution expansion do not appear to affect survival rates. Also adaptation to dispersal and temperature may be limited during range expansion both by environmental constraints and limited selection pressure respectively.

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On a personal note I would like to thank my family for all the emotional support that was needed during this experience, so a massive thanks to Dorothy Harper, Raymond Harper and Alan Harper.

Declaration

I declare that the work presented in this thesis represents my own work, and is written by me, except where outlined below:

Chapter 3 was a collaborative effort; wild material was collected and weighed/measured by Luke Smallman while the data on laboratory reared *P. aegeria* came from the work of Claire Hughes (previously published in (Hughes 2004)) and was used with permission. All data analysis presented was conducted by me and the chapter was written by me.

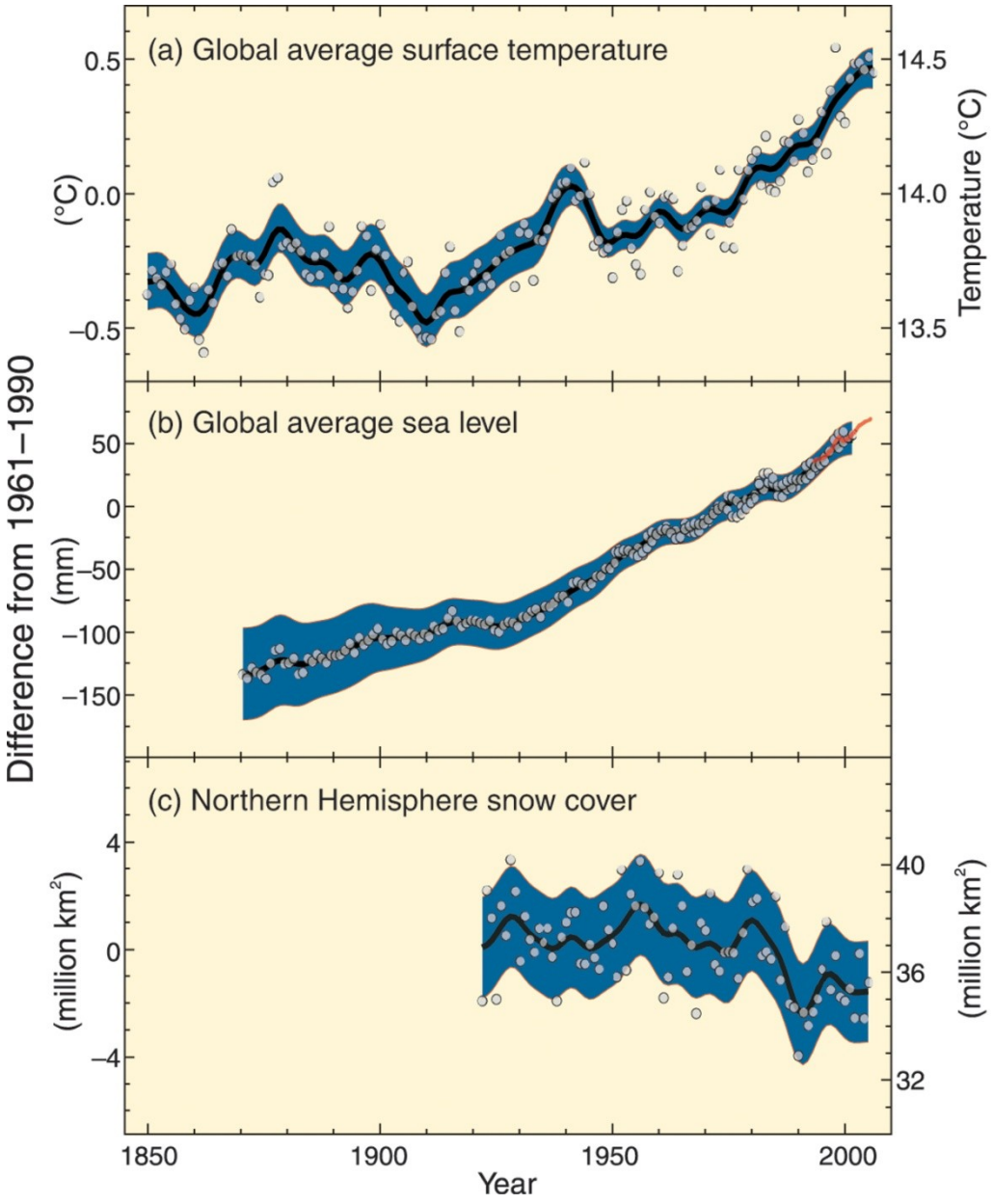
Chapter 1 – General Introduction

1.1 Thesis introduction

There has been a marked change in the climate of the earth during the last century. This climatic change is often characterised by the increase of the global mean temperature, which has risen by 0.74^{o}C in the hundred years between 1906 and 2005 (IPCC 2007a). Also eleven of the twelve years between 1995 and 2006 were among the twelve hottest years on record as of 2007 (IPCC 2007a). The increasing temperature is occurring in parallel with rising global sea levels and reduced snow cover in the northern hemisphere (Figure 1.1). The climatic changes are believed to be associated with wide ranging changes in physical and biological processes. Predictions of the future climate suggest further warming of 1.1^{o}C to 6.4^{o}C by 2099, relative to the 1980-1999 average (IPCC 2007a). This would result in more than a doubling of the increase in temperature already experienced under most scenarios, and is likely to exacerbate any impacts already occurring. Global temperature changes of 2^{o}C or more are projected to have significant ecological impacts (Root & Schneider 2002).

The Intergovernmental Panel on Climate Change (IPCC 2007a) concludes there is 'very high confidence' for wide ranging impacts on terrestrial biological systems and 'high confidence' of impacts on species in marine/freshwater environments as a result of climate change and associated physical processes. The effect of climate change was also highlighted in the Millennium Ecosystem Assessment (MEA 2005). The assessment suggests that although some species and environments will benefit from the changing climate many will suffer, particularly those at risk from other drivers of biodiversity loss. It is worth remembering that the majority of multicellular species on earth are insects or other invertebrates which are commonly poikilothermic or ectothermic and unable to regulate their own body temperature (Gullan & Cranston 2001). Therefore many species biology is heavily influenced by climatic variation. Extinction is the most dramatic outcome for a species due to the changing climate, but even where this extreme does not occur many species will have to respond in some form. Either species have to change their distribution to track their climatic niche or adapt to the new environment.

Figure 1.1 – Global trend in temperature, sea level and snow cover. Plots show the divergence from the average of 1961 to 1990 on the left hand axis (units shown). Circles show yearly values, the black line represents the decadal average value and the blue area represents the uncertainty of the decadal estimate. Reproduced from IPCC (2007a), pp31, without modification.



Much work is needed to understand the full impacts that climate change may have on species ecology. This thesis aims to expand our knowledge in this regard by investigating distribution changes in response to climate change in butterflies, more specifically the impact of distribution change on genetic diversity and selection for adaptation to dispersal and temperature.

1.2 Climate change

The earth's climate is not static and has oscillated between periods of warmth and periods of cold over the course of the earth's history. We are currently in an interglacial period within an ice age; the current interglacial period, known as the Holocene, has lasted approximately 11,600yrs (IPCC 2007b). Glacial-interglacial cycles, within the current ice age, are indicated by ice cores dating back over 740,000yrs (IPCC 2007b). With glacial periods characterised by increased continental ice cover and colder global temperatures. These fluctuations mean the average global temperature has been both hotter and colder than present. For example during the period known as the Holocene optimum, 5,000yrs to 9,000yrs before the present, summer temperatures would have been 2^{o}C warmer than present in Europe (Huntley & Prentice 1988).

The current period of warming is of interest due to the relative warmth and speed of warming compared to the last few thousand years. Northern hemisphere temperatures in the last half of the 20th century are likely to be the warmest in the last 1300yrs (IPCC 2007a). Modelling of current rates of warming suggest that increases in temperature during the latter part of the 20th century are faster than those experienced during the last interglacial and faster than any point during the last 1000 years (Crowley 2000). More important though is the mechanism believed to be inducing the warming of the climate. It is widely accepted that human activity is now altering the climate through the production of greenhouse gases (though it is still a contentious issue in public and political debates). Greenhouse gases (GHGs) such as carbon dioxide (CO_2), methane (CH_4), nitrous oxide (N_2O) and halocarbons are produced by human activities including combustion of fossil fuels and agriculture. The atmospheric concentrations of these gases are at the highest levels in the last 650,000yrs (IPCC 2007a). Increased concentrations of these gases are believed to increase global temperatures by absorbing additional solar radiation. Light form the sun normally heats the earth and is then re-emitted as infra-red radiation that would radiate back into space but higher concentrations of GHGs absorb more of the infra-red radiation trapping it as heat in the atmosphere. The complete picture is much more complex as there are various other factors regulating the climate for example heat absorption by the oceans, patterns of cloud cover, sunspot activity and the various unknowns of the glacial cycles.

The evidence that current warming is due to anthropogenic factors is believed to be strong (Crowley 2000; IPCC 2007a). Climate modelling suggests the current changes are

strongly influenced by GHGs and that this impact exceeds natural variability in the climate cycles (Crowley 2000). As concentrations of GHGs are still increasing further increases in temperature and associated changes in climate are expected. Estimates of future average temperatures indicate an increase of 1.1^{o}C to 6.4^{o}C depending on the rate of GHG emissions (IPCC 2007a). Beyond the change in temperature global patterns of other features of the climate are also expected to change, for example in the UK the intensity of winter rainfall is believed to have increased (Maraun *et al.* 2008). Increases in precipitation are expected across the northern hemisphere and reductions at lower latitudes (IPCC 2007a), the frequency and intensity of tropical storm systems is also expected to increase. The impacts of the changing climate vary between different regions and a detailed account can be found in IPCC (2007c).

1.3 Impacts of climate change

As already discussed large widespread climate changes are a normal feature of the environment on the earth. Therefore species will have had to undergo some adaptation to changing climates in the past. Over geological time scales fluctuations in the climate have had dramatic impacts on the flora and fauna of the planet, for example the 'runaway greenhouse' effect (Benton & Twitchett 2003) at the end of the Permian is linked to a mass extinction event. Examination of the fossil record over the last 520Myrs has linked reductions in global biodiversity, in terms of numbers of families and genera, to temperature maxima (Mayhew *et al.* 2008). The warm periods examined in this work also correlate to both increased extinction and origination rates of lineages. These studies highlight the potentially significant impacts of climatic oscillations on global biodiversity and that further warming may lead to increased extinction rates. It should be remembered though that extinction rates are not purely a function of the climate. Habitat loss and invasive species have been widely recognised as major drivers in current losses of biodiversity (MEA 2005). Therefore the impact of current climate change on biodiversity will involve the interaction between changing climate patterns, habitat availability and changing community interactions.

Data from beetles and pollen have indicated large distribution changes as a response to periods of glaciation (Coope 1977; Atkinson *et al.* 1987; Debeaulieu & Reille 1992). Vegetation patterns correlate with climate fluctuations indicating they are the

primary driver of vegetation patterns over millennial timescales (Guiot 1997; Whitlock & Bartlein 1997; Ferris *et al.* 1999). Also the main response of woody trees to changes in climate over the Quarternary is believed to have been shifts in distribution (Davis & Shaw 2001). This demonstrates it is not uncommon for distribution shifts to occur, during a species history, to reflect the global climate. The coleopteran communities observed in the UK in different interglacial periods are consistent with limited extinction (Coope & Wilkins 1994), suggesting communities adapted to warm or cold environments have typically tracked the appropriate climate over these time scales. During the last glacial maxima many species now common in central and northern Europe/North America would have been restricted to refuga south of their current distributions (Hewitt 2000; Hewitt 2004). Once the current interglacial began they would have expanded from these locations to establish the communities we observe today. The current Lepidopteran community of the UK are believed to have expanded from southern refuges following the last glacial period (Dennis 1992).

This information on historic responses to climate change is useful in extrapolating the possible long term consequences of current climatic changes. Inferences from historic climate change must be made with some caution though. This is due to both the apparent speed of the current increase in temperature and the anthropogenic pressures currently affecting species (MEA 2005); which would not have been present during previous phases of climatic instability. Also historic distribution patterns and extinction rates do not illustrate the short term adaptive responses that species may employ. Much work has gone into investigating the ecological consequences of current climate change, such that there are now several reviews of the subject (Bale *et al.* 2002; Walther *et al.* 2002; Walther 2004; Parmesan 2006; Hill *et al.* 2011). Some species are likely to benefit but there will also be others which will suffer and it remains a continuing effort to establish what the full effects will be. Predictions of extinction rates due to current climate change suggest that 18-35% of species, across a range of taxa, will become 'committed to extinction' depending on the future rate of warming (Thomas *et al.* 2004). The evidence for species declines, which may lead to extinctions, is growing but is probably underestimated due to the problems of attributing a cause to species declines and limited data on species distributions and abundance (Thomas *et al.* 2006). Species affected by the changing environment are obliged to adapt or disperse and the sections below illustrate some of the common ecological responses attributed to current climate change.

1.3.1 Phenology

Phenology refers to the timing of life history events and is commonly regulated by environmental signals including temperature. Phenological changes are one of the most widely reported impacts of the warming climate (Walther *et al.* 2002; Root *et al.* 2003; Walther 2004; Parmesan 2007). Changes are apparent in a wide range of taxanoimc groups including; earlier breeding or first song in birds (Cotton 2003), advancement of shooting and flowering in plants (Walther *et al.* 2002; Walther 2004), earlier emergence in insects (Roy & Sparks 2000; Bradshaw & Holzapfel 2001; Stefanescu *et al.* 2003; Hassall *et al.* 2007) and earlier spawning in amphibians (Beebee 1995; Parmesan 2007). The study by Hassall *et al.* (2007), which investigated emergence in odonata, shows that changes in phenology are apparent even in aquatic environments, which experience dampened temperature changes. These changes in phenology can be a result of plastic responses to the altered environment or be genetically controlled, as is the case of the pitcher-plant mosquito (*Wyeomyia smithii*) (Bradshaw & Holzapfel 2001). The most important knock on effects of changes in phenology will occur in species that have some form of interaction. Where one species alters the timing of its life history and another does not adapt it could result in reduced fitness due to loss of a food source or increased competition. Experimental manipulations of three Mongolian grasshopper species demonstrated that warming will result in advancement of egg hatching and eclosion in all three species (Guo *et al.* 2009). This manipulation also suggested that there will be convergence of the emergence times of the studied species toward the middle of the growing season, which could lead to increased resource competition (Guo *et al.* 2009). Altered species dynamics due to changes in phenology have also been highlighted in interactions between plants and their insect herbivores (van Asch *et al.* 2007; van Asch & Visser 2007). Large differences are apparent in the magnitude of the advancement of traits between taxanomic groups (Parmesan 2007), which suggests that altered species interactions may become more common.

Earlier emergence and first sightings appear to be common responses to the increasing temperature among butterflies (Roy & Sparks 2000; Stefanescu *et al.* 2003). It has been estimated that an increase of $1^{o}C$ would lead to the advancement of emergence in British butterflies by 2-10 days (Roy & Sparks 2000). This response in butterflies is likely to be due to higher temperatures leading to faster development rates, and could increase the probability of multiple broods in multivoltine species.

1.3.2 Distribution

If a species cannot either adapt their climatic niche through phenotypic plasticity or evolutionary change they need to track changes in the environment by shifting their distribution. These climate induced distribution changes have been observed across a range of taxa including amphibians, birds, butterflies, fish, and plants (Hill *et al.* 1999b; Parmesan *et al.* 1999; Warren *et al.* 2001; Parmesan & Yohe 2003; Karban & Strauss 2004; Hickling *et al.* 2005; Perry *et al.* 2005; Franco *et al.* 2006; Hickling *et al.* 2006; Thomas *et al.* 2006). There is evidence of both expansions at leading-edge high-latitude/high-elevation range margins and contractions at trailing-edge low-elevation/low-latitude range margins. The majority of the recorded changes in distribution are range expansions towards the poles at an average rate of 6.1km per decade (Parmesan & Yohe 2003). Many species distributions are limited by physiological constraints on their ability to adapt to variations in temperature or precipitation (Walther *et al.* 2002). Therefore the apparently widespread changes in species distribution are to be expected, as species respond to changes in the climate by moving into areas that become climatically suitable for them. Changing the average global temperature by 3^{o}C is estimated to result in a shift of 300-400km in latitude among isotherms (Hughes 2000). As the projected future warming is from 1.1^{o}C to 6.4^{o}C this suggests there could be large changes in the distributions of many species. Work on butterflies in the UK suggests that there are discrepancies between the responses of generalist and specialist species such that specialist species are failing to shift their distributions in response to the climate (Warren *et al.* 2001). This is probably due to the lack of available habitat and continued climate change may lead to selection for a fauna of mobile generalist species, as specialists fail to track the climate due to limited available habitat.

The majority of observed distribution changes are at species expanding cool edge margins but there is also evidence of range contractions occurring at warm edge range margins (Parmesan *et al.* 1999; Wilson *et al.* 2005; Franco *et al.* 2006). In contrast species warm edge range boundaries may be more limited by biotic factors than climate (Thomas *et al.* 2006), and may also be more stable than cool edge margins (Parmesan *et al.* 1999; Hampe & Petit 2005). Time lags in climate-induced extinction of local populations, and failure to monitor species at sufficient temporal and spatial resolution to detect local extinctions may also account for the limited observations of distribution declines (Thomas *et al.* 2006).

1.3.3 Other impacts and adaptations

In addition to changes in phenology and distribution there are other ecological impacts on species due to the effect of changes in temperature, precipitation or CO_2 concentrations. Some are direct impacts on species normal biological functions such as physiology, metabolism or development. For example development rate in butterflies is linked to temperature, commonly faster development at higher temperatures but linked to greater mortality (Braby & Lyonns 2003; Gibbs *et al.* 2010). Therefore increased global temperatures could affect development rates when dispersal does not occur. High temperatures are also expected to result in decreased survival and fecundity in northern and montane butterfly species in the UK (Dennis & Shreeve 1991). Though some species may benefit as butterfly abundance has previously been shown to have a positive correlation with warm summers and increased winter precipitation (Pollard 1988). In the case of the woody species *Fagus sylvatica* growth rate declines at the southern range margin have occurred as a result of warmer temperatures and increased drought (Jump *et al.* 2006b). This has resulted in selection for adaptive genetic variation related to temperature performance (Jump *et al.* 2006a), though the species is still declining. Seedling establishment in artificial drought treatments by the shrub *Fumana thymifolia* leads to non-random genetic differentiation, indicating selection for drought resistance (Jump *et al.* 2008). These results indicate there is scope for some species to undergo genetic adaptation to environmental changes. Though this is more likely in cases where species mobility is limited otherwise selection will be weakened by the ability to move away from unfavourable environments. Evolutionary changes may also occur as a consequence of climate induced distribution shifts, when selection favours improvements in dispersal ability. An example of this phenomenon is found in crickets (both *Conocephalus discolour* and *Metrioptera roeselii*) where greater frequencies of long winged, more dispersive, individuals have been observed at newly founded sites following range expansion (Simmons & Thomas 2004). Also butterflies from the expanding range margin of the butterfly *Pararge aegeria* allocate more mass in their thoraxes which is believed to be associated with improved dispersal ability (Hughes *et al.* 2003). Evidence has also been found for changes in habitat use, the butterfly *Polygonia c-album* has begun to make use of a greater range of larval host plants following its climate induced distribution shift (Braschler & Hill 2007). Other butterflies have also changed their habitat use; *Aricia agestis* uses additional larval food plants at its expanding distribution margin and *Hesperia comma* is no longer restricted to south/south-west facing hillsides in southern England (Thomas *et al.* 2001).

1.4 Genetic Impacts of distribution change

The previous sections have described some of the adaptive responses that have occurred in response to the changing climate. This section focuses on the impacts of historic and current climate change on neutral genetic diversity.

1.4.1 Postglacial genetic changes

As already discussed fluctuations between glacial and interglacial periods has caused repeated expansions and contractions of species distributions. These distribution changes have had distinct impacts on both patterns of genetic diversity and divergence (Comes & Kadereit 1998; Hewitt 2000; Schmitt & Hewitt 2004). Glacial refuges become isolated over extensive periods of time allowing genetic drift, mutation and local patterns of selection to promote genetic differentiation. Subsequent expansion from these refuges then determines continental patterns of genetic differentiation, as populations retain markers common to the refuge from which they originated. For example the distribution of genetic divergence in the pearly heath butterfly (*Coenonympha arcania*) is largely explained by divergence between two glacial refuges, and the pattern of expansion from them during the current interglacial (Besold *et al.* 2008). Similarly genetic differentiation of the meadow brown butterfly (*Maniola jurtina*) in Europe is largely due to the pattern of colonisation from two glacial refuges (Schmitt *et al.* 2005).

Colonisation history not only affects patterns of population differentiation but many species show predictable declines in genetic diversity with increasing distance from their glacial refuge (Cwynar & Macdonald 1987; Suyama *et al.* 1997; Hewitt 1999; Schmitt & Seitz 2002). For example *Polyommatus coridon* exhibits a decline in genetic diversity from south to north along its post glacial colonisation route (Schmitt & Seitz 2002). This is due to the impact of founder events during the distribution expansion. Dispersal into newly available habitat is generally conducted by a small number of individuals, representing a fraction of the original gene pool. These populations are more likely to then establish additional new populations further compounding the loss of genetic diversity. This series of repeated founder events leads to a loss of genetic diversity, the magnitude of which is related to the distance from the refuge population and the mitigating effect of gene flow from the refuge.

1.4.2 Current genetic changes

The same factors that lead to reductions in genetic diversity following post glacial distribution shifts affect current shifts in distribution. Therefore reductions in neutral genetic diversity are likely to result from current climate induced distribution shifts. Some evidence is available from allozymes that this has happened during the distribution shift of *P. aegeria* (Hill *et al.* 2006). Though in the same study no loss of genetic diversity was observed in a less habitat specific species (*Pyronia tithonus*), the implication being that habitat availability affects loss of genetic diversity due to distribution change. As habitat loss is a major driver of current biodiversity loss (MEA 2005) it is probable that more species will lose genetic diversity during distribution shifts, than under historic conditions. Reduced genetic diversity and high levels of inbreeding have detrimental impacts on population fitness, egg hatching success, longevity, and population extinction risk (Saccheri *et al.* 1996; Saccheri *et al.* 1998; Nieminen *et al.* 2001; Reed & Frankham 2003). Therefore species which lose genetic diversity during current distribution changes are at greater risk from future environmental changes. It has also been argued that they will be less able to adapt to anthropogenic climate change (Jump *et al.* 2009).

1.5 Thesis rational & outline

The focus of this thesis is the impact of climate induced distribution changes on butterflies in the UK, particularly the impacts on genetic diversity and evolutionary change. This thesis principally aims to investigate changes in the genetic diversity of satyrine butterflies, at both expanding (*Pararge aegeria*; Figure 1.2) and contracting range margins (*Erebia aethiops*; Figure 1.3). It also aims to investigate what impacts the selective forces of dispersal and temperature are having on populations of *P. aegeria* during its distribution expansion.

Chapter 2 investigates patterns of genetic diversity and divergence between the expanding range margin and distribution core of the butterfly *P. aegeria*.

Chapter 3 compares investment in flight related morphology between core and margin populations of *P. aegeria*, and contrasts observations of laboratory reared and wild caught

butterflies to investigate the importance of environmental versus genetic factors during development.

Chapter 4 investigates local adaptation to temperature in *P. aegeria* during range expansion at core and margin sites.

Chapter 5 examines whether genetic diversity is being lost at the contracting distribution margin of the butterfly *E. aethiops*.

Chapter 6 discusses the significance of the previous Chapters findings for the understanding of the impacts of climate change on genetic diversity and morphological evolution.

Figure 1.2 – Male *Pararge aegeria* (Photograph taken at Bishops Wood near Selby, Yorkshire (Ordnance survey ref SE53) by Neil Harper).

Figure 1.3 – Female *Erebia aethiops* (Photograph taken at Glen Affric near Inverness, Inverness-shire (Ordnance survey ref NH12) by Neil Harper).

Chapter 2 – Impacts of climate driven range expansion on genetic diversity

2.1 Abstract

Distribution shifts in response to recent climatic warming are evident in a wide range of taxonomic groups, including many insects. We investigated changes in genetic diversity associated with recent range expansion in the satyrine butterfly *Pararge aegeria*, and compared genetic changes with a non-expanding, 'control' satyrine butterfly *Maniola jurtina*. Reduced genetic diversity can affect fitness, evolutionary potential, and population extinction rates. We aimed to confirm the findings of a previous study and tested the hypothesis that genetic diversity was lower at the expanding range margin of *P. aegeria* due to repeated founder effects during colonization, but that such effects were not evident in *M. jurtina*. We sampled *P. aegeria* (n = 137 individuals) and *Maniola jurtina* (n = 120 individuals) from six sites in the UK; three 'core' sites in southern England within the main UK distributions of both species, and three sites at the range margin of *P. aegeria* in northern England where *M. jurtina* also occurs (~ 200 km from core sites). Analyses using AFLPs indicated approximately twice the proportion of polymorphic loci in core (56.8%) compared with margin populations (28.1%) of *P. aegeria*. Heterozygosity was also higher in core (0.203) than margin (0.125) populations of *P. aegeria*. No differences were observed between populations of the non-expanding species *M. jurtina*. Recent range expansions reduce genetic diversity at range margins, with ~ 50% loss of genetic diversity in *P. aegeria* populations established within the past 15 years, a significantly greater reduction than previously reported from allozyme analyses. Human-induced habitat loss may lead to more pronounced founder effects than during historical range expansions, and reduced genetic diversity may affect species ability to persist in newly-colonised sites.

2.2 Introduction

The recent warming of the earth's climate has had many ecological impacts including changes in species' phenology, habitat use, behaviour and distribution (Parmesan 2006). Distribution changes have been linked to climate across a range of taxa (Hill *et al.* 1999b; Parmesan *et al.* 1999; Warren *et al.* 2001; Parmesan & Yohe 2003; Karban & Strauss 2004; Hickling *et al.* 2005; Hickling *et al.* 2006; Poyry *et al.* 2009). Many studies report range expansions at species' cool-high-latitude/high-elevation range margins (Hickling *et al.* 2006), and there is also evidence of climate driven range contractions at species' warm-trailing-edge margins (Wilson *et al.* 2005; Franco *et al.* 2006; Thomas *et al.* 2006). Without sufficient habitat to allow distributions to track the climate, species long term persistence could be affected as they become restricted to smaller areas of suitable habitat. Distribution shifts can affect neutral genetic diversity and act as selective forces leading to evolutionary changes. For example, increased dispersal ability has been observed at expanding range margins of butterflies in terms of changes in morphology and metabolism (Hill *et al.* 1999a; Thomas *et al.* 2001; Simmons & Thomas 2004; Mitikka & Hanski 2010). This study focuses on the impact of range expansion on neutral genetic diversity. As reduced genetic diversity can have detrimental effects on population survival (Saccheri *et al.* 1998) and the potential of species to adapt to environmental changes, making it important to study in the context of climate change. Even species able to track changes in climate could be affected leaving them vulnerable to future environmental challenges.

Distribution shifts are not a novel response of species to climatic changes, and post-glacial expansions from low latitude refuges have left a genetic legacy in many species (Hewitt 1996; Comes & Kadereit 1998; Petit *et al.* 2004; Besold *et al.* 2008). Latitudinal clines in genetic diversity are evident in some species (Cwynar & Macdonald 1987; Suyama *et al.* 1997), although this is not a universal consequence of post-glacial range expansion (Petit *et al.* 2004). Reductions in genetic diversity during range expansion arise from repeated founder events, population bottlenecks and genetic drift (Frankham *et al.* 2002), and observed losses of genetic diversity are supported by theoretical work on the spread of lineages and neutral mutations during the migration of populations (Ibrahim *et al.* 1996; Bialozyt *et al.* 2006; Klopfstein *et al.* 2006; McInerny *et al.* 2009). Loss of genetic diversity can be mitigated by mutation, gene flow, and the migration of individuals among populations, particularly long distance dispersal events (Bialozyt *et al.* 2006). Current distribution shifts differ from post glacial changes due to reduced habitat availability in the landscapes that species are colonizing, due to human-induced habitat destruction. This is

likely to exacerbate contemporary losses of genetic diversity compared with post-glacial expansions; as habitat loss has reduced genetic diversity in many species (Berwaerts *et al.* 1998; Butcher *et al.* 2009; Collier *et al.* 2010), and is likely to reduce gene flow among populations during range expansion (Hill *et al.* 2006). Reduced genetic diversity has been linked to lower fitness, increased expression of deleterious mutations, and lower evolutionary potential in species (Saccheri *et al.* 1998; Frankham *et al.* 2002; Beebee & Rowe 2004; Markert *et al.* 2010). Thus recent climate-driven range changes may have impacts on genetic diversity and species long-term persistence, but data are lacking.

Previous studies have shown reduced genetic diversity in margin populations of expanding butterfly species (Hill *et al.* 2006). This work indicated that loss of genetic diversity was associated with habitat specificity; as only *Pararge aegeria*, the species with the most restricted habitat requirements, exhibited a loss of genetic diversity at its range margin. However, this previous study examined allozymes which have limited power to resolve genetic differences (Beebee & Rowe 2004), and may not be neutral markers (Goulson 1993; Eanes 1999; Dahlhoff & Rank 2000; Haag *et al.* 2005; Karl *et al.* 2010). In this study we aimed to confirm the findings of Hill *et al.* (2006) using Amplified Fragment Length Polymorphisms (AFLPs), which can produce large numbers of markers without *a priori* sequence information (Mueller & Wolfenbarger 1999; Meudt & Clarke 2007). They also have similar power to resolve recent genetic divergence and genetic diversity to codominant techniques such as microsatellites (Meudt & Clarke 2007). We examine changes in genetic diversity between core and margin populations of a range expanding butterfly *Pararge aegeria*, in the UK. To control for potential confounding latitudinal effects on genetic diversity, we also sampled a non-expanding 'control' species at the same study sites. The control species was the closely-related satyrine *Maniola jurtina*, which has a stable distribution and is more or less ubiquitous throughout the UK. We tested the hypotheses that genetic diversity is lower in margin versus core populations of *P. aegeria*, but that this difference is not evident in *M. jurtina*. We also examine population structure by comparing pairwise F_{ST} values and a Bayesian clustering method (Pritchard *et al.* 2000) to determine if margin populations shared a common ancestry.

2.3 Methods

2.3.1 Sample Collection

Individuals of *P. aegeria* and *M. jurtina* were collected from six sites; three sites within the core and three sites at the margin of the current distribution of *P. aegeria* in England. Both *P. aegeria* and *M. jurtina* colonized Britain after the last Ice Age from refuges further south in Europe. *Pararge aegeria* underwent a major range retraction during the 19th century and became confined to south west England and Wales, but with a relic population in western Scotland (Asher *et al.* 2001). Since the mid-20th century, *Pararge aegeria* has re-expanded its distribution in Britain as a consequence of recent climate warming (Hill *et al.* 1999b), although it has not yet recolonised all historically-occupied sites. By contrast, the distribution of *M. jurtina* has apparently been stable during the past 200+ years, and this species occurs throughout most of Britain, with the exception of high elevation sites (Asher *et al.* 2001).

Each site represents a single 10km^2 ordnance survey grid cell and was at least > 30 km from any other site (Figure 2.1). This is greater than the normal dispersal range of *P. aegeria*; based on mark recapture data, mean movement within habitat fragments is ~ 90 m and up to 450m between fragments (Van Dyck *et al.* 1997). Core and margin sites were > 210 km apart. Core sites were occupied by both study species in 1970-82 (Asher *et al.* 2001). Margin sites were occupied by *M. jurtina* in 1970-82, and estimated to have been colonized by *P. aegeria* no earlier than 1992 (i.e. ~ 15 years prior to the study, based on national survey data supplied by Butterfly Conservation). Twenty adult male *P. aegeria* and *M. jurtina* were collected from each site during the adult flight period (July and August) in 2007 - 2008. Some unexpected results were obtained from one of our study sites (C3) for *P. aegeria* during this first period of sampling, and so an additional sample of 20 male *P. aegeria* was collected from this site in 2009. All samples were stored at -80^{o}C prior to dissection and extraction of DNA.

2.3.2 AFLP protocol and analysis

DNA was extracted from approximately one third of the thorax of each individual using an ammonium acetate based ethanol precipitation method (see Appendix 1). The concentration of the extracted DNA was determined using a Nanodrop Spectrophotometer

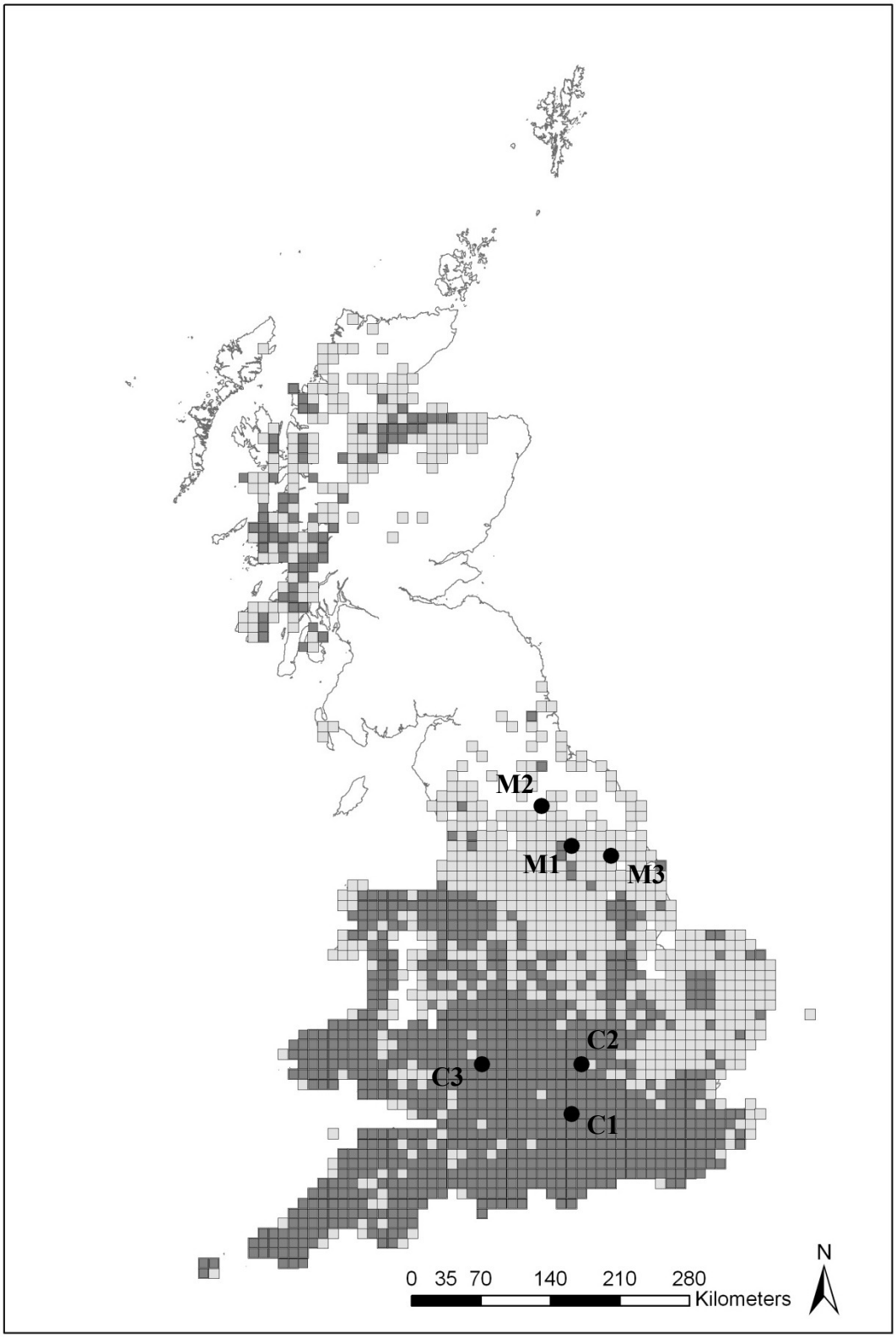


Figure 2.1 – Distribution of *P. aegeria* in the UK at a 10km grid cell resolution. Mid grey squares show sites occupied prior to 1982; light grey squares show sites colonised since 1983. Black circles represent sampling locations, site names are indicated. Ordnance survey grid locations; C1 = SU56; C2 = SP61; C3 = SO61; M1 = SE53; M2 = SE27; M3 = SE92 (sites represent a single $10km^2$ ordnance survey grid cell).

and then diluted to 10ng/µl prior to AFLP fingerprinting. The AFLP protocol was modified from Vos *et al.* (1995) as described in Whitlock *et al.* (2008a). In addition, the ligation of the adaptor sequences was conducted at 8^{o}C overnight, and a total reaction volume of 10µl was used during the pre-selective PCR, which did not include any formamide. Two separate pairs of pre-selective PCR primers were used per sample (*Eco*RI primer (A) 5'-GACTGCGTACCAATTCT-3' & *Mse*I primer 5'-GATGAGTCCTGAGTAAC-3'; *Eco*RI primer (B) 5'-GACTGCGTACCAATTCA-3' & *Mse*I primer 5'-GATGAGTCCTGAGTAAC-3'). The template DNA was then diluted 1 part in 50 before the selective PCR. The selective PCR was also conducted in a total volume of 10µl without formamide. Three primer pairs were used to generate AFLP markers for *P. aegeria* (*Eco*RI-TCT and *Mse*I-CC, *Eco*RI-TGA and *Mse*I-CT, *Eco*RI-ATC and *Mse*I-TC). Two primer pairs were used for *M. jurtina* (*Eco*RI-TCT and *Mse*I-CGA, *Eco*RI-TGA and *Mse*I-CCC). Positive and negative controls were included with all batches of samples to ensure accuracy.

The selective *Eco*RI primers were labelled with 5' fluorescent dyes (Applied BioSystems – 6FAM, LIZ & PET) to allow AFLP fingerprints to be produced by capillary electrophoresis using an ABI 3130 Genetic Analyser and the LIZ600 size standard (Applied BioSystems). Profiles were then visualized using GeneMapper v4.0, and peak height tables were generated. The R script AFLPscore v1.3 (Whitlock *et al.* 2008b) was used to convert the peak height profiles into binary presence/absence genotypes. The script allows the minimization of error rates and removes the subjectivity of manually editing AFLP loci (Whitlock *et al.* 2008b) by selecting thresholds to retain loci and score alleles. Twenty replicated samples were used to estimate the mismatch error rates, which were 1.91% (*P. aegeria*) and 2.23% (*M. jurtina*).

2.3.3 Data analysis

In order to examine genetic differences among sites, the proportion of polymorphic loci at the 95% level and the expected heterozygosity at sites (He) were determined using AFLP-SURV v1.0 (Vekemans 2002). A re-sampling technique was used to test for significance among sites where individuals were randomly assigned to populations (i.e. sites) and the difference in the proportion of polymorphic loci between the core and margin populations was then determined. This provides an estimate of the absolute probability of the regions having different genetic diversity. Sites were assumed to be significantly different if ≤ 5% of the 10,000 replicates were greater or equal to the observed difference between core and

margin populations. The randomization procedure was conducted in MATLAB v7.8 and the script is available from the authors (NEH). Standard ANOVAs were also conducted to test the difference between the regions in terms of the proportion of polymorphic loci and expected heterozygosity at sites within the regions. The proportions of polymorphic loci were arcsin square root transformed prior to ANOVA analysis. Genetic divergence among sites was investigated using nested Analysis of Molecular Variance (AMOVA) as implemented in Arlequin v3.1 (Excoffier *et al.* 2005), with individuals nested within sites, and sites nested within regions (core/margin). Pairwise estimates of F_{ST} and significance tests for population differentiation were also conducted in Arlequin. Isolation by distance was investigated with a Mantel test in Arlequin which compared pairwise F_{ST} estimates between sites against geographic distance (km) calculated from the Ordnance Survey grid reference of each site. Samples of *P. aegeria* from site C3 collected in 2008 and 2009 were grouped together for the Mantel Test to counter the confounding effect of zero distances. Population structure was further investigated with the Bayesian clustering method used in STRUCTURE v2.3 (Pritchard *et al.* 2000), to assign individuals probabilistically to populations of origin, and determine the origin of the marginal populations. This version of the program specifically treats dominant genetic data such as obtained by AFLP (Falush *et al.* 2007). The admixture based ancestry model was used with a burn in length of 20000 and 10000 simulations, to allow convergence of α. The true value of k, number of populations sampled, was estimated using the method of Evanno *et al.* (2005), based on the second order rate of change (Δk). Twenty runs for each k value, from 1 to 9, were used to determine the modal Δk.

2.4 Results

2.4.1 Core versus margin sites

We obtained AFLP genotypes from a total 137 individuals of *P. aegeria* from six study sites (Table 2.1), resulting in 173 AFLP loci (97.7% segregating fragments) ranging in length from 60 to 378bp. We also obtained data from 120 individuals of *M. jurtina* which produced 311 AFLP loci (99.2% segregating fragments), ranging in length from 60 to 500bp (Table 2.1). In *P. aegeria*, which is currently expanding its range, there was a significantly greater proportion of polymorphic loci among the core populations (mean =

Table 2.1 – Genetic diversity in populations of *P. aegeria* and *M. jurtina* based on AFLP genotypes. 'Core' sites lie within the main distribution of *P. aegeria* in southern England. 'Margin' sites are locations that have been colonized by *P. aegeria* in the past 15 years.

Species	Region	Site code	N	Proportion of polymorphic loci	Expected heterozygosity (He)	SE (He)
Pararge aegeria	Core	C1	20	65.3	0.201	±0.0141
		C2	20	67.1	0.220	±0.0141
		C3 (2008)	20	30.6	0.139	±0.0115
		C3 (2009)	17	64.2	0.250	±0.0144
		Mean		56.8	0.203	±0.0135
	Margin	M1	20	28.3	0.122	±0.0118
		M2	20	28.9	0.129	±0.0120
		M3	20	27.2	0.123	±0.0113
		Mean		28.1	0.125	±0.0117
Maniola jurtina	Core	C1	20	39.3	0.110	±0.0070
		C2	20	39.5	0.114	±0.0071
		C3	20	35.5	0.108	±0.0073
		Mean		38.1	0.111	±0.0071
	Margin	M1	20	41.9	0.120	±0.0074
		M2	20	36.9	0.110	±0.0072
		M3	20	40.6	0.117	±0.0071
		Mean		39.8	0.116	±0.0072

56.8%) compared with margin populations (mean = 28.1%; re-sampling statistic, p = 0.018; ANOVA $F_{1,6} = 7.514$, p = 0.04). Estimates of expected heterozygosity were also much greater among core populations (He = 0.203) compared with margin populations (He = 0.125) in *P. aegeria* (ANOVA $F_{1,6} = 7.837$, p = 0.038). The samples collected from core site C3 for *P. aegeria* during the first sampling period exhibited much lower values of proportion of polymorphic loci and expected heterozygosity compared with other core sites, and so an additional sample of *P. aegeria* was collected from site C3 in 2009. This second sample showed levels of genetic diversity comparable to the other core populations (Table 2.1) supporting the notion of reduced genetic diversity in margin sites in range-

expanding species. There was little difference in genetic diversity between the sites for the non-expanding control species *M. jurtina*, collected from the core and margin of *P. aegeria's* distribution (Table 2.1; re-sampling statistic, p = 0.46; ANOVA $F_{1,5} = 0.740$, p = 0.44).

2.4.2 Genetic differentiation among sites

Analysis using AMOVA revealed significant differentiation within and between sites but

Table 2.2 – AMOVA results for *P. aegeria* and *M. jurtina*. Sources of genetic variation are nested; within sites, between sites in regions and between regions. Analysis of *P. aegeria* data include both samples from site C3 and so d.f.s are greater for the among site analysis than for *M. jurtina*.

Species	Source of Variation	d.f.	Sum of squares	Variance components	Proportion of variation	P value
P. aegeria	Among regions	1	128.32	0.77	5.29	0.111
	Among sites within regions	5	378.50	3.34	22.93	< 0.001
	Within sites	130	1359.59	10.46	71.78	< 0.001
M. jurtina	Among regions	1	33.24	-0.01	-0.04	0.399
	Among sites within regions	4	135.70	0.34	1.23	< 0.001
	Within sites	114	3096.55	27.96	98.81	< 0.001

Table 2.3 – Population pairwise F_{ST} estimates for *P. aegeria*. Site names are given in the top row and first column. Values below the diagonal are F_{ST} estimates; stars above the diagonal indicate pairs of sites significantly more differentiated than by chance (at the 5% level) and X indicates non-significantly differentiated pairs of sites.

	C1	C2	C3 (2008)	C3 (2009)	M1	M2	M3
C1	-	X	*	*	*	*	*
C2	-0.002	-	*	*	*	*	*
C3 (2008)	0.267	0.254	-	*	*	*	*
C3 (2009)	0.397	0.390	0.358	-	*	*	*
M1	0.269	0.264	0.124	0.437	-	*	*
M2	0.242	0.228	0.124	0.427	0.071	-	*
M3	0.242	0.239	0.113	0.431	0.053	0.054	-

not between core and margin regions in both study species (Table 2.2). For *P. aegeria*, the F_{ST} value for the total sample was 0.282, and pairwise estimates ranged from -0.002 to 0.437. With the exception of core sites C1 and C2 (F_{ST} = -0.002, p = 0.474), all the pair-wise estimates of F_{ST} were significantly more differentiated than a random assemblage of individuals (Table 2.3). Site C3(2008) was most similar to the margin sites, and as differentiated from the other core sites as they were from the margin. The second sample at site C3 in 2009 was the most differentiated from all other sites (including the sample from C3 in 2008; Table 2.3) Margin sites had low pairwise F_{ST} values (ranging from 0.053 to 0.071) showing that margin sites were more similar to each other than were core sites. In *M. jurtina* all estimates of pairwise F_{ST} were low (range -0.001 to 0.031), and were much lower than in *P. aegeria*.

There was a significant, positive, isolation by distance effect in *P. aegeria* (Mantel

Figure 2.2 – Effect of geographic distance on genetic differentiation between pairs of populations. Distance is the straight line distance (km) between sites, and genetic differentiation is represented by pairwise F_{ST} estimates.

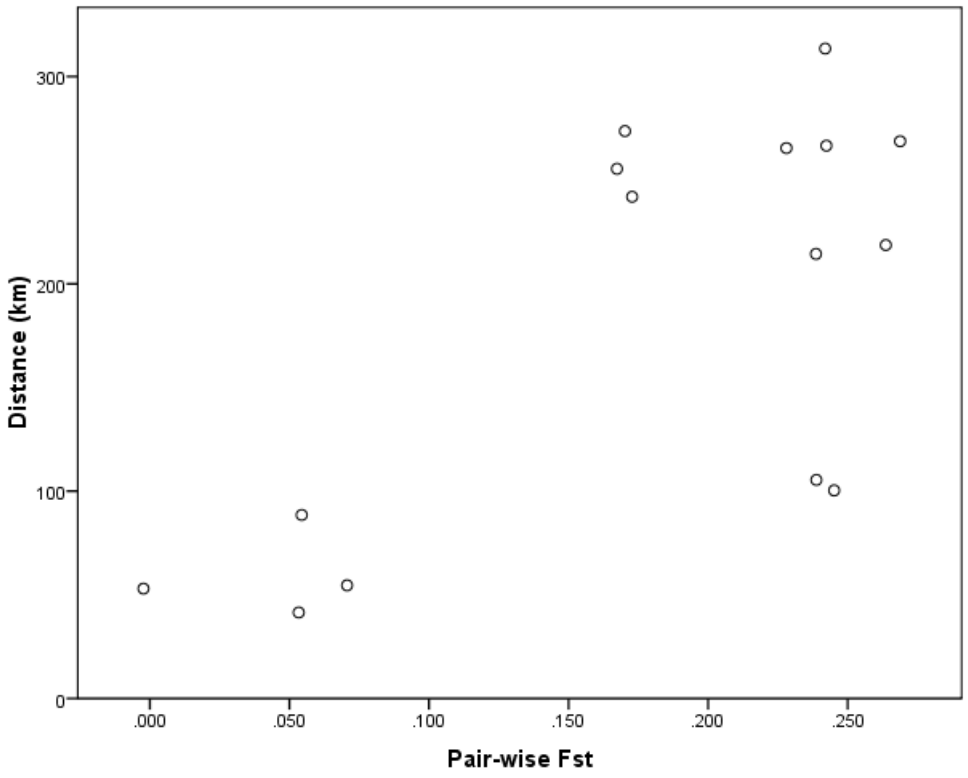
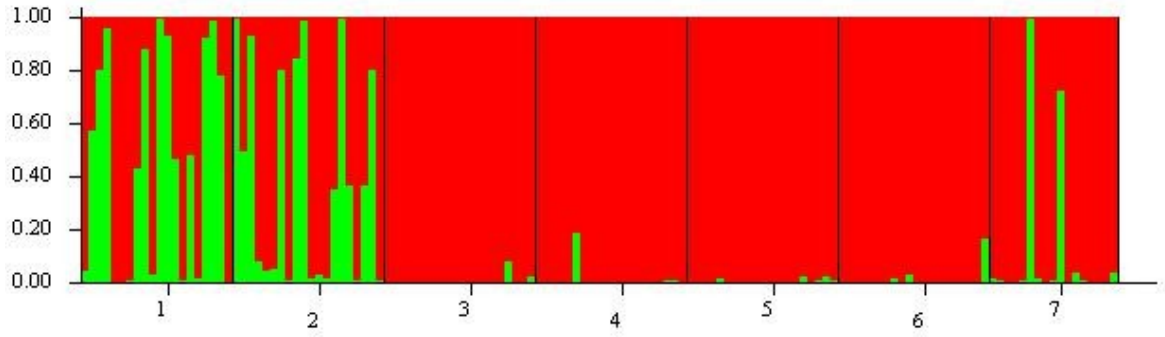
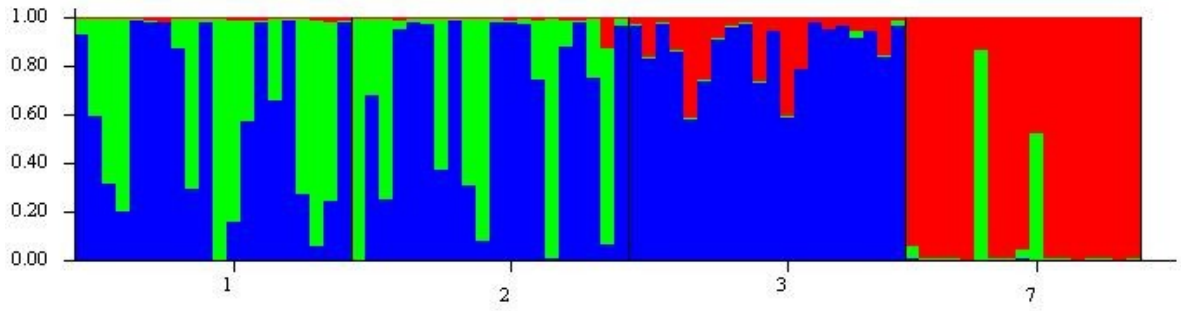


Figure 2.3 – Probabilities of assignment of *Pararge aegeria* individuals to population clusters; a) all individuals analysed together (assuming k = 2 clusters), b) individuals from core sites only (k = 3 clusters). Sampling locations are represented as follows; 1 = C1, 2 = C2, 3 = C3 sampled in 2008, 7 = C3 sampled in 2009; 4 = M1, 5 = M2, 6 = M3. Colours indicate different clusters and the proportion of each colour in a column indicates the probability of assignment of an individual to that population. Colours are not consistent between images and so do not infer relationships between the populations in the two images. Black lines separate each of the clusters.

a)



b)



test; r = 0.695, p = 0.012), showing that genetic similarity decreased with increased geographic distance between sites (Figure 2.2). The two points with low distance but high F_{ST} relate to the comparisons between the core site C3 and the other two core sites (C1 & C2) (Figure 2.2). Output from the Bayesian clustering, suggested that two clusters were present when all sites were analysed together, Figure 2.3a illustrates each individual's probability of assignment to these two populations. The individuals from the range margin sites were all strongly clustered into the same population. Individuals from core sites C1 and C2 were assigned to each of the two clusters in roughly equal proportions, whilst C3(2008) were grouped with the same cluster as the margin sites. The pairwise estimates of F_{ST} (Table 2.3) had indicated more population structure than this result implied, and so core and margin populations were analysed separately to see if any additional population

structure was detected. This analysis confirmed that almost all margin individuals were assigned to a single cluster regardless of the maximum value of k tested, but core sites did exhibit additional population structure (Figure 2.3b). When considering only core sites, individuals from sites C1 and C2 were again assigned to two clusters, and individuals from site C3 were also assigned to different clusters according to the year of sample. Samples collected in the first time period (2008) were strongly grouped with a cluster found in all the core sites, whereas samples from the second time period (2009) formed a separate fourth cluster.

2.5 Discussion

The pattern of genetic diversity we found in this study was consistent with the prediction that recent range expansion in *P. aegeria* during a period of climate warming has led to greatly reduced genetic diversity at the range margin. The proportion of polymorphic loci was 50% lower at range margin sites compared with core sites, and the expected heterozygosity was reduced by a similarly large amount (39%). Minimum distances between core and margin sites were ~ 210 km, and so these genetic effects were evident over a relatively short distance. Our results for AFLPs suggest losses in genetic diversity almost twice those revealed by allozyme analysis (Hill *et al.* 2006), in which the average number of allozyme alleles per locus was reduced by about 25% in *P. aegeria* over a similar distance. By contrast no such loss of diversity was found among populations of the non-expanding control species, *M. jurtina* in this study or other studies (Hill *et al.* 2006). Our results confirm our original hypotheses for a reduction in genetic diversity during range expansion, with markers that more convincingly capture neutral genetic diversity than previous studies. They also indicate that these losses may be far greater than previously described.

Not only was there a loss of genetic diversity in sites at the range margin in *P. aegeria*, but populations of *P. aegeria* were strongly differentiated (F_{ST} = 0.282). There was clear divergence between sites, and over 20% of the variation among samples was attributed to differences between sites. By contrast, there was no significant differentiation between core and margin regions, which is not surprising given the relatively recent establishment of the margin sites. Northern populations of *P. aegeria* develop through 1-2 generations per year, and so margin sites that were colonized ~ 15 years ago represent

probably no more than 30 generations of *P. aegeria* development. These margin populations were much less differentiated, based on pairwise F_{ST} values, compared with most other core sites. The clustering program Structure identified three genetic clusters in individuals from core sites (Figure 2.3b) of which only one cluster appears to have been involved in *P. aegeria's* range expansion. This pattern of low genetic diversity and closely related margin populations is indicative of limited long distance dispersal (Bialozyt *et al.* 2006), typical of most butterflies particularly in fragmented landscapes, and reflect a shared ancestry from a single genetic cluster. Two of the core sites, C1 and C2, represent a freely interbreeding population, with no genetic differentiation, based on pairwise F_{ST} estimates. These sites are 52 km apart and *P. aegeria* often occurs in a wider range of habitats towards the core of its range (Oliver *et al.* 2009), contributing to high gene flow among core sites.

The first samples of *P. aegeria* collected from core site C3 in 2008 produced unexpectedly low estimates of genetic diversity (Table 2.1). Therefore, we resampled this site the following year (2009) and found that estimates of genetic diversity were higher and consistent with values expected from a core site for *P. aegeria*. Unexpectedly, the samples collected from this core site in 2008 were least differentiated from the margin sites (Table 2.3), with which they were more strongly clustered than with other core sites (Figure 2.3a). Samples from site C3 collected in 2009 showed the greatest differentiation from all sites, based on pairwise F_{ST} values (Table 2.3). We suggest that the observed strong genetic differentiation between samples from site C3 in 2008 and 2009 indicates two highly segregated clusters of individuals at this site. Site C3 is located in the Forest of Dean near Gloucester an area that may have been affected by very heavy rainfall during the summers of 2007 and 2008 resulting in widespread local flooding. The rainfall and flooding may have caused heavy mortality and a population crash resulting in the low genetic diversity in 2008. The results from 2009 may reflect recovery from this event, with immigration from elsewhere resulting in the high genetic divergence and genetic diversity. More fine scale spatial and temporal sampling might help understand the results from this site.

By contrast with the expanding species, there was very little genetic differentiation among populations of *M. jurtina*. Our findings of little differentiation between the populations of *M. jurtina* are in agreement with previous studies (Goulson 1993; Schmitt *et al.* 2005; Hill *et al.* 2006). However, caution should be taken when interpreting the data for *M. jurtina* because the large number of fragments generated per primer combination means that size fragment homoplasy may affect results (Caballero *et al.* 2008); reducing

estimates of genetic diversity and population divergence. Size fragment homoplasy occurs when multiple fragments of the same length from different loci are generated, the probability of which increases with the number of fragments generated per primer combination.

Our findings showing low genetic diversity and relatively low divergence among margin populations of *P. aegeria* supports the hypothesis that climate-driven range expansion is affecting genetic diversity. This appears to be due to selective expansion of a relatively small number of individuals from the range core, from which all the margin populations appear to originate (based on the low within region F_{ST} estimates amongst margin sites). This pattern mirrors that expected due to expansion resulting from a few long distance dispersal events (Bialozyt *et al.* 2006). *Pararge aegeria* is considered a generalist butterfly in the UK (Warren *et al.* 2001), but nonetheless its range expansion is restricted in heavily fragmented landscapes (Hill *et al.* 2001). Though the species is found in gardens and parks, which would support expanding populations, such sites do not support large populations. The butterfly is associated primarily with woodland habitats (Hill *et al.* 1999b) and this habitat often comprises <3% of the landscape in areas of northern England where it currently reaches its range margin. Therefore, the loss of genetic diversity will be compounded by colonisation of this heavily fragmented landscape. Other studies have shown that loss of genetic diversity is associated with increased extinction of local populations (Saccheri *et al.* 1998), and so loss of genetic diversity observed in *P. aegeria* may affect persistence of margin populations, which may also be affected by relatively cool years when distributions temporarily contract. Other studies have shown increased dispersal evolves at expanding range margins (Hughes *et al.* 2003; Hughes *et al.* 2007; Hill *et al.* 2011) and so reduced diversity recorded in this study may reflect an increased proportion of dispersive individuals in margin sites. Increased dispersal in margin sites is likely to help species shift their ranges, although the associated trade-off between flight and fecundity (Hughes *et al.* 2003) may reduce population growth rates in newly-colonized sites. Further work is required to determine what impacts reduced diversity might have on species growth and survival.

Chapter 3 – Comparing flight morphology between core and margin populations in a range expanding butterfly: A comparison between wild-caught and lab-reared individuals.

3.1 Abstract

Many species are shifting their ranges as a consequence of climate warming, and evolutionary increases in dispersal ability have been observed in populations at expanding range boundaries. These increases in dispersal ability may help species track climate, but it is unclear whether environmental factors also affect dispersal ability. We studied the speckled wood butterfly *Pararge aegeria* that is expanding its range in the UK, and compared the relative importance of genetic and environmental factors on adult flight morphology. Insect material was collected in 2003 (lab-reared material) and 2007 (wild-caught material) from locations in the core (n = 2 sites in both years, 30-22 individuals) and range margin (n = 1-2 sites; 45-29 individuals). We compared the flight morphology of wild-caught adults, and adult F1 butterflies reared under common lab conditions (photoperiod 16L:8D; 21^{o}C). Lab reared butterflies had greater total dry mass (mean = 15.09 mg) than wild-caught (mean = 13.53 mg). This was principally due to heavier abdomens amongst the lab-reared butterflies. In both cases, butterflies from the margin had ~ 10-11% heavier thoraxes than their core counterparts. Relative thorax mass was significantly greater at the range margin but only in lab-reared material ($p = 0.024$) and not in wild-caught individuals ($p = 0.105$). There was more variation in the relationship between total mass and thorax mass amongst wild-caught than lab-reared butterflies (wild-caught $r^2 = 0.544$; lab-reared $r^2 = 0.782$). The failure of this study to detect a significant difference in relative thorax mass between core and margin populations in the wild-caught material is likely due to impacts of environmental factors during insect development. These results imply that sub-optimal environmental conditions during larval development may outweigh evolutionary increases in dispersal ability among expanding populations at the range margin. Thus, previous conclusions that increased dispersal ability may help species track climate change may be less important than suggested from lab-based studies.

3.2 Introduction

Organisms can respond to climatic changes by either morphological/physiological adaptation *in situ* or by shifting their distributions (Hill *et al.* 2011). Clear evidence is available demonstrating shifts in the distributions of many species due to recent climatic changes (Parmesan *et al.* 1999; Warren *et al.* 2001; Hickling *et al.* 2005; Hickling *et al.* 2006; Parmesan 2006). Warming of the climate and the associated distribution changes of species have lead to a range of ecological and evolutionary changes; including phenology changes (Roy & Sparks 2000; Bradshaw & Holzapfel 2001; Hassall *et al.* 2007), changes in habitat or resource use (Thomas *et al.* 2001; Braschler & Hill 2007), and changes in dispersal ability (Hill *et al.* 2011). Theoretical studies on species responses to distribution changes indicate selection for, and evolution of, greater dispersal ability at species expanding margins (Travis & Dytham 2002; Hughes *et al.* 2007; Mustin *et al.* 2009; Travis *et al.* 2009). This is because founder events will favour individuals with greater dispersal ability during range expansion; therefore newly-founded populations will have greater proportions of dispersive individuals. This prediction has been supported by empirical studies demonstrating increased dispersal at the expanding margin of the invasive cane toad (*Bufo marinus*) in Australia (Phillips *et al.* 2010). Dispersal evolution is also evident in insects including; higher frequencies of dispersive female ants in recently colonised areas (*Petalomyrmex phylax* and *Cataulacus mckeyi*) (Leotard *et al.* 2009), and increased proportions of dispersive long-winged individuals in newly-founded populations of crickets (*Conocephalus discolour* and *Metrioptera roeselii*) (Thomas *et al.* 2001; Simmons & Thomas 2004). Hassall *et al.* (2009) demonstrated morphological changes, associated with increased dispersal ability, at the range margin of a rapidly expanding damselfly (*Calopteryx splendens*). Thus, evolutionary increases in dispersal are evident in a wide range of animal and plant taxa.

Butterflies are commonly used as model research organisms, and several examples exist of dispersal adaptations at expanding margins, and in newly-colonised populations. For example, Glanville fritillary butterflies (*Melitaea cinxia*), that exist as a metapopulation in the Åland Islands (Southern Finland), are most dispersive if they originate from newly colonised populations (Hanski *et al.* 2002). However, there is no significant morphological variation between these populations and differences in dispersal are due to physiological differences in flight metabolism. Non-morphological variation in flight ability is also apparent in the Map butterfly (*Araschnia levana*) where superior flight metabolic rates occur in individuals from populations at the species expanding range

margin (Mitikka & Hanski 2010). Some other butterfly species show morphological changes at expanding margins. The speckled wood (*Pararge aegeria*) is expanding its distribution in the UK (Hill *et al.* 1999b; Asher *et al.* 2001) and changes in flight morphology associated with improved flight performance have been observed in recently colonised populations (Hill *et al.* 1999a; Hughes *et al.* 2003). The most commonly reported change in flight morphology is greater relative thorax mass in individuals at the range margin, although changes in wing shape have also been reported (Hill *et al.* 2011). The thorax contains primarily flight muscles, and increased thorax mass in *P. aegeria* has been related to improved acceleration ability during flight (Berwaerts *et al.* 2002; Berwaerts *et al.* 2008); supporting the notion that changes in flight morphology in this species are associated with flight ability. Increased dispersal ability at the margin of this species is expected to increase the rate of range expansion (Hughes *et al.* 2003), thus helping species track climate and respond to climate changes.

However, studies of morphological variation have generally been studied in individuals reared under controlled conditions in order to examine genetic factors. What is not clear is the degree to which these morphological differences are evident in the field. There is some evidence that lab-reared individuals may show different patterns of morphological variation compared with wild-caught individuals (Braschler & Hill 2007), but data are lacking on the relative importance of genetic versus environmental factors on dispersal ability. Environmental factors during insect development may affect morphology (Braschler & Hill 2007), and changes in habitat, larval host plant quality and microclimate at margin sites may outweigh any genetic differences that evolve in recently-colonised sites.

This study compared variation in flight morphology between core and expanding range margin populations of *Pararge aegeria*. We examined the relative importance of genetic and environmental factors by testing whether morphological differences between core and margin populations were consistent in lab-reared and wild-caught populations. To do this we compared wild-caught material with an existing data set of lab-reared *P. aegeria*, described in Hughes (2004). The data from Hughes (2004) were reanalysed for this study. We tested the hypothesis that range margin populations contained individuals with flight morphology associated with increased flight ability (i.e. increased thorax mass, decreased abdomen mass and lower wing loading), and that these differences were evident in both lab-reared and wild-caught individuals.

3.3 Methods

3.3.1 Study species

Pararge aegeria was once distributed through much of the UK (Asher *et al.* 2001). During the 19th and early 20th century it suffered a major range retraction (Burrows 1916; Gibbs 1916; Asher *et al.* 2001), becoming restricted to a small population in Scotland and confined to southern Wales and south west England. The latter 20th century saw a marked change in the species' fortunes, during which time it re-colonised much of its former range in the UK (Fox *et al.* 2006). This re-expansion is a consequence of recent climatic warming (Hill *et al.* 1999b), although the species distribution lags behind the projected area of climatically -suitable habitat available for colonisation due to habitat fragmentation in northern England, making it difficult for colonising individuals to reach new habitats. Habitat fragmentation is thought to have contributed to the increased evolution of dispersal-related traits at the expanding margin of the species UK distribution because of increased founder effects (Hughes *et al.* 2003).

3.3.2 Sampling wild-caught material

In 2007, male *P. aegeria* were collected from two regions in the UK, two sites in the core of the species UK range (n = 29 individuals, Ordnance Survey references; SU56, SP61) and from two sites at the range margin (n = 22 individuals; OS references SE27, SE53) (Table 3.1; Figure 3.1). Sites within a region (core/margin) were > 50 km apart to ensure individuals came from separate populations, and margin populations were ~210 km north of core sites. Core sites have apparently been continuously occupied for at least 35 yrs, and probably longer (Asher *et al.* 2001), and margin sites had been established no more than 15 years prior to the collection date (based on survey records received from Butterfly Conservation UK). Adult butterflies were collected using a hand net and killed by freezing.

3.3.3 Lab-rearing study protocol

Data from a prior laboratory study (Hughes 2004) were reanalysed to provide a comparison for the wild-caught material. The collection and rearing protocol used to gather these data are detailed below.

Figure 3.1 – Distribution of *P. aegeria* in the UK at a 10km grid cell resolution. Mid grey squares show sites occupied prior to 1982; light grey squares show sites colonised since 1983. Black circles represent sampling locations for wild-caught material, triangles represent sampling locations for lab-reared material. Ordnance survey grid locations; C1 = SP02; C2 = SO61; C3 = SU56; C4 = SP61; M1 = SE53; M2 = SE27.

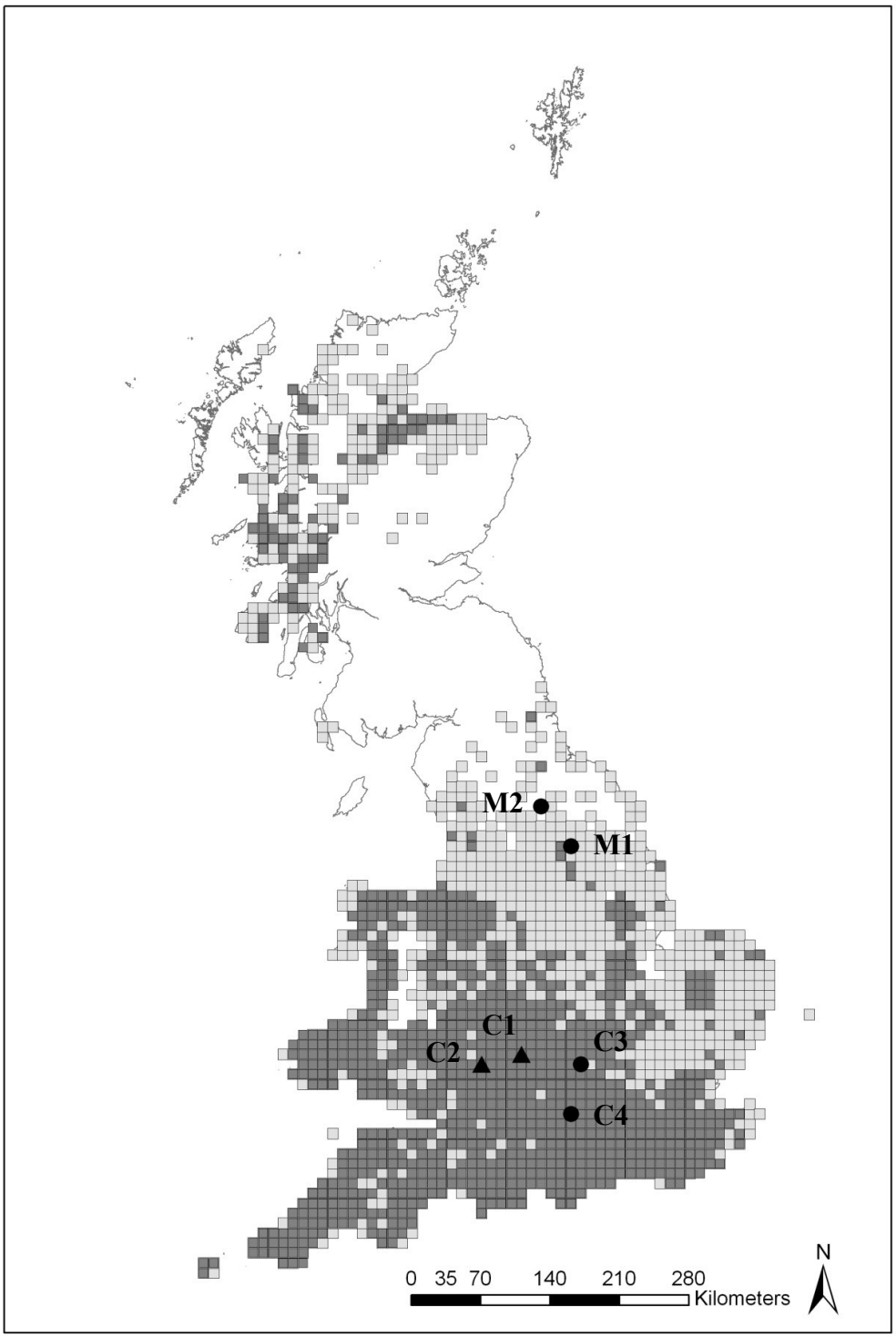


Table 3.1 – Location of study sites. 'Age since establishment' is estimated from the date of the first *P. aegeria* observation record for each 10 km grid square (records from Butterfly Conservation).

Study	Site Code	Region	Year sampled	OS Grid reference	Age since establishment (Years)
Lab reared	C1 (Cheltenham)	Core	2003	SP02	>30
	C2 (Ross-on-Wye)	Core	2003	SO61	>30
	M1 (Bishop Wood)	Margin	2003	SE53	14
Wild Caught	C3 (Rushbeds Wood)	Core	2007	SP61	>30
	C4 (Bowdown Woods)	Core	2007	SU56	>30
	M1 (Bishop Wood)	Margin	2007	SE53	14
	M2 (Nutwith Common)	Margin	2007	SE27	5

Insect material was collected in 2003 from two sites in the core region (Ordnance Survey grid reference SP02 and SO61) and from one margin site (OS grid reference SE53) (Table 3.1; Figure 3.1) from which wild-caught material had also been sampled (Table 3.1), The margin site had been established for ~10 years at the time of sampling (Hughes 2004). A total of 13 females were collected from each region (core and margin sites) and allowed to lay on potted *Poa pratensis* grass plants placed in breeding cages. Adults were kept alive by providing pads of cotton wool soaked in a honey/water solution. Data from the offspring of individuals from the two core sites were combined for subsequent analysis. A total of 10 F1 offspring per female were transferred to a fresh potted *Poa pratensis* plant (Hughes 2004) and then allowed to develop under constant environmental conditions (photoperiod 16L:8D, temperature $21^{o}C \pm 2^{o}C$). Fresh grass was provided as necessary to ensure no food shortage occurred. The F1 offspring of each female were reared together on the same plant under the same conditions and are considered a family in the analysis. Pupae were maintained under the same conditions until adult emergence when butterflies were killed by freezing, after expulsion of the meconium. Only adult males were used in the following analysis to allow comparison with the wild-caught specimens.

3.3.4 Dissection and Data Collection

Adults were thawed and dissected to remove wings, thorax, abdomen, head and legs. Following dissection, the samples were dried at $60^{o}C$ for 24 hours, and body parts were weighed to the nearest 0.01mg on a Sartorious electro balance (accurate to 1μg). Images of

the right forewing were taken, using a Canon EOS D30 digital camera with a 105mm macro lens, to determine wing area and forewing length, and images were analysed in SigmaScan Pro v5. Images were taken with the camera at a set stage height, and consistent focus, shutter speed, exposure levels, and flash.

3.3.5 Data analysis

Repeated measures were taken for each morphology variable to assess the reliability of measurement accuracy; all measures had less than 10% error which is consistent with error rates in Hughes (2004). Some wild-caught adults had damaged wings and were not included in analyses of wing size, and so sample sizes are not the same for all the variables within the wild-caught study. Aspect ratio (4 x forewing length^2/wing area) and wing loading (total dry mass/wing area) were computed, and all variables were $\log_{10}$ transformed prior to analysis. The $\log_{10}$ transformation was performed to improve the approximation of normality for the distributions of the variables and limit violations of this assumption of the ANOVA analyses. Variation in total dry mass, wing loading and aspect ratio of wild-caught individuals between the regions was examined using a nested ANOVA, with region (core/margin) as a fixed factor, and site nested within region. Lab-reared material was also analysed by ANOVA, with region as a fixed factor and family nested within region. Thorax and abdomen mass were analysed using nested ANCOVA, to account for allometry, with $\log_{10}$ total dry mass as a covariate and region as a fixed factor (with site nested with region for wild-caught material and family nested within region for lab-reared material). All analyses were performed using the statistical package PASW v18.

3.4 Results

A total of 51 wild-caught and 75 lab-reared individuals were measured. Overall, wild-caught individuals (mean = 13.53 mg, SD = ±1.96, N = 51) were smaller than lab-reared individuals (mean = 15.09 mg, SD = ±3.87, N = 75; ANOVA, wild-caught/lab-reared factor, $F_{1,123} = 5.877$, $p = 0.017$; Table 3.2). This difference was primarily because wild-caught individuals had small abdomens (wild-caught, mean abdomen mass = 2.87 mg; lab-reared, mean = 4.31 mg). The age of wild-caught individuals was not known, but older individuals may have already used much of their larval resources which are stored in the abdomen (Boggs 1997) (e.g. for flight, and mating) prior to being caught, and these

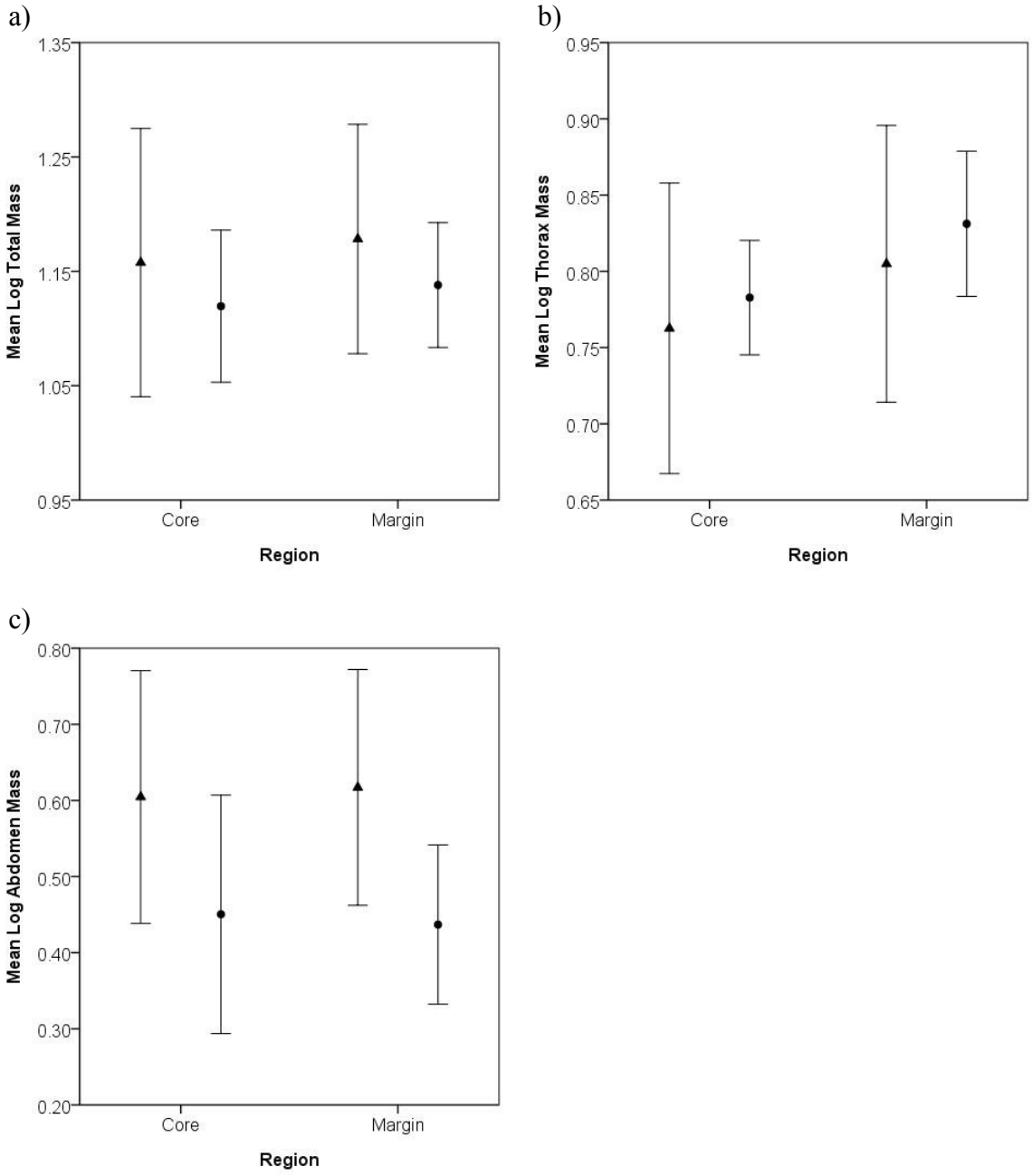
Table 3.2 – Morphological variation in lab-reared and wild-caught *P. aegeria* from core and margin sites. Values in brackets are Standard Errors (SE). '% change' is the % change in margin sites compared with core (positive values indicate larger values in margin sites). N = sample size. 'Residual thorax mass' refers to the residual value from the regression of thorax mass against total dry mass.

		Total dry Mass (mg)	**Thorax Mass (mg)**	**Abdomen Mass (mg)**	**Aspect Ratio**	**Wing Loading (mg/cm^2)**	**Residual Thorax Mass**
Lab reared, Means	Core	14.61 (±0.82) n=30	5.80 (±0.25) n=30	4.26 (±0.39) n=30	9.83 (±0.08) n=30	8.57 (±0.32) n=30	-0.017 (±0.009)
	Margin	15.46 (±0.51) n=45	6.52 (±0.20) n=45	4.39 (±0.22) n=45	9.52 (±0.09) n=45	8.39 (±0.22) n=45	0.012 (±0.006)
	% mean change	5.50	11.04	2.96	-3.26	-2.15	Na
Wild caught, Means	Core	13.57 (±0.39) n=22	6.09 (±0.11) n=21	3.01 (±0.27) n=21	9.87 (±0.11) n=21	8.34 (±0.24) n=21	-0.024 (±0.006)
	Margin	13.80 (±0.35) n=29	6.81 (±0.14) n=29	2.76 (±0.11) n=29	9.54 (±0.12) n=24	8.00 (±0.13) n=23	0.017 (±0.004)
	% mean change	1.67	10.57	-9.06	-3.78	-4.25	Na

resources are not replaced by adult feeding. In both lab-reared and wild-caught individuals, range margin individuals were larger and had larger thoraxes. Margin butterflies had smaller aspect ratios (~ 3 - 4% smaller) and wing loadings (~ 2 – 4% lighter) for both lab-reared and wild-caught samples. Margin individuals that had been lab-reared were ~ 5% larger in terms of total mass and thorax mass was ~ 11% larger compared to distribution core individuals (Table 3.2). Similarly, wild individuals from the margin were larger by ~ 1% in terms of total mass and ~ 10% heavier for thorax mass (Table 3.2). Figures 3.2 a & b show similar increases in total mass and thorax mass from core to margin in both lab-reared and wild-caught butterflies. Abdomen mass was larger amongst core individuals in the lab-reared material and smaller overall in wild-caught individuals (Figure 3.2c).

Total mass, abdomen mass, aspect ratio and wing loading showed no significant differences between the regions for either the lab-reared or wild-caught butterflies (based on $\log_{10}$ transformed data) ($p > 0.294$ for all analyses). However, lab-reared butterflies did exhibit significantly greater relative thorax mass at the range margin (ANCOVA; $F_{1,23.25}$ =

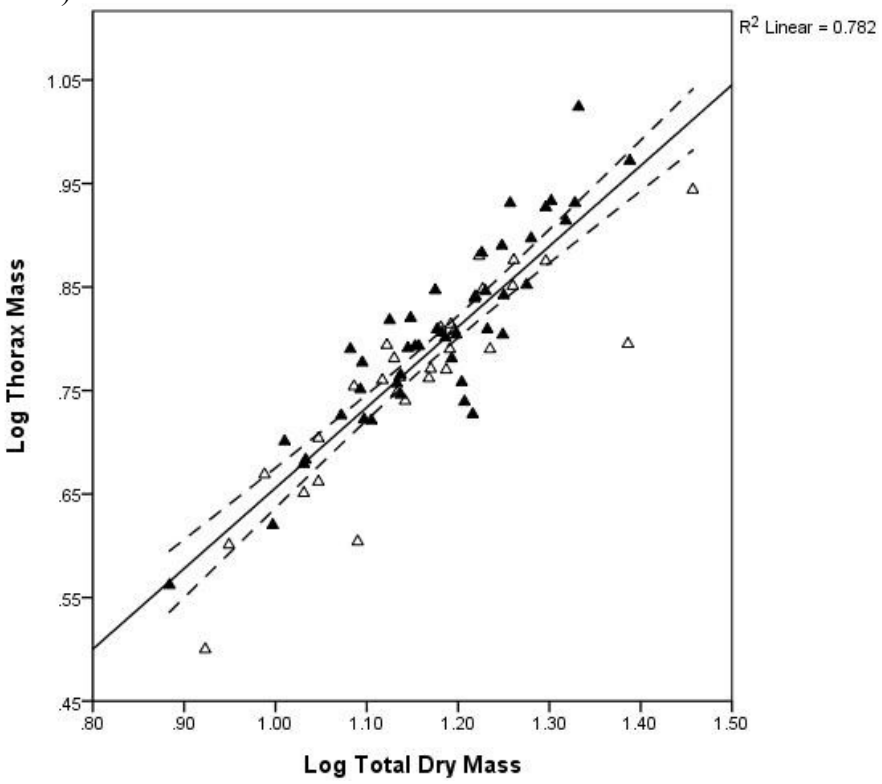
Figure 3.2 – Plot of a) mean total mass; b) mean thorax mass; c) mean abdomen mass; by region. Error bars represent standard deviations. Triangles represent lab-reared, and circles represent wild-caught insects.



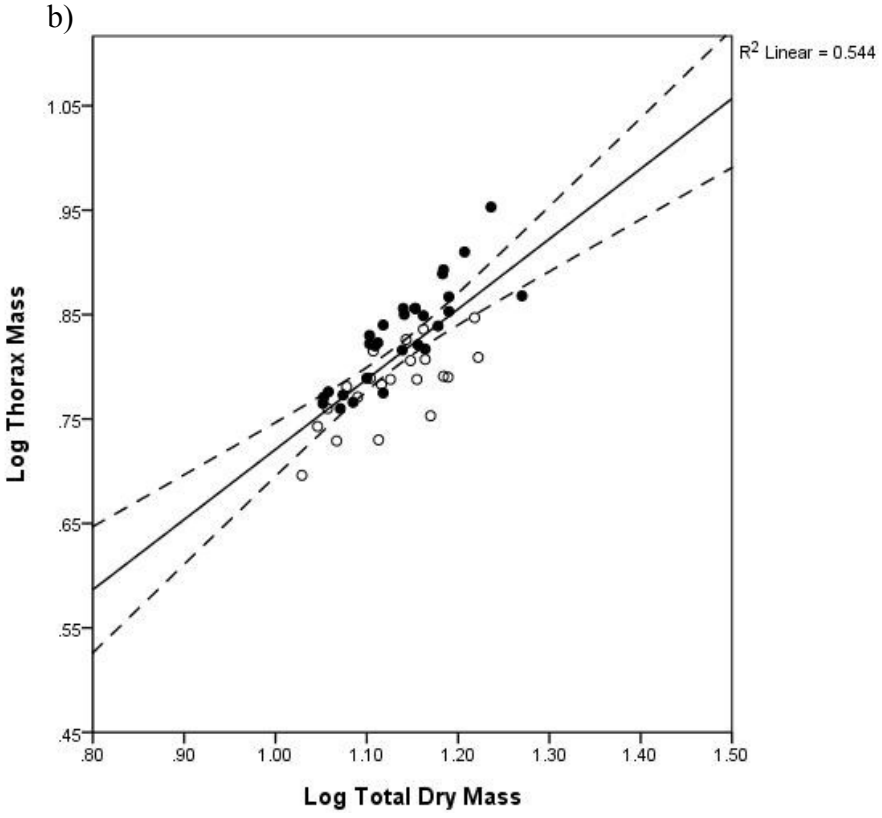
5.873, p = 0.024), consistent with increased investment in dispersal ability at the range margin. In contrast, wild-caught butterflies did not exhibit significantly greater relative thorax mass at the range margin (ANCOVA; $F_{1,1.97}$ = 8.212, p = 0.105). In lab-reared material, plotting thorax mass against total dry mass (using Log_{10} values) revealed a significant positive linear relationship (Figure 3.3a) which explained 78% of the variation in thorax mass. A similar regression of thorax mass against total mass for wild-caught butterflies also indicated a significant positive linear relationship (Figure 3.3b), but in this

Figure 3.3 – Plot of $\log_{10}$ thorax mass against $\log_{10}$ total mass for a) lab-reared butterflies; b) wild-caught butterflies. Triangles represent lab-reared butterflies, and circles represent wild-caught butterflies. Open symbols refer to butterflies from the core; filled symbols are margin butterflies. Regression lines are plotted in both cases, and the dashed lines indicate the 95% confidence intervals.

a)



b)



analysis the regression accounted for a lower percentage of the total variation in thorax mass (54%).

3.5 Discussion

Wild-caught butterflies were significantly lighter, in terms of total mass, than were lab-reared butterflies (1.56 mg difference between the mean values). The main reason for this difference appears to be the reduction in abdominal mass in wild-caught butterflies (1.44 mg reduction in mean abdominal mass of wild-caught butterflies compared with lab-reared butterflies). Abdominal mass is believed to decline with age in butterflies (Boggs 1997), as it is converted to energy for metabolic processes and not replaced by adult feeding. As stated in the Results section above, the age of the wild caught butterflies was unknown, although they are almost certain to be older than the lab-reared butterflies which were killed within ~24 hrs of emergence. Therefore the reduction in total mass in wild-caught butterflies is probably due to the relatively greater age of these individuals. In both the lab-reared and wild-caught butterflies the margin butterflies were larger in terms of total mass (Table 3.2), although no significant differences were detected between the regions (core and margin) for either total mass or abdominal mass.

Neither of the measures of wing morphology (aspect ratio and wing loading) varied much in terms of the absolute values; either between studies or between core/margin regions. Both variables have previously been related to butterfly flight ability (Berwaerts *et al.* 2002), in terms of acceleration capacity. Therefore the lack of differences in these measures suggests that they are not sensitive indicators of dispersal ability and not under selection during range expansion.

In contrast to the other variables examined, thorax mass was heavier in margin sites in both lab-reared and wild-caught butterflies (Table 3.2). ANCOVA analysis indicated that margin population had relatively larger thoraxes in the case of lab-reared insects ($p = 0.024$), but not in wild-caught material ($p = 0.105$). When thorax mass was regressed against total mass there was a significant positive relationship in both studies (larger individuals have larger thoraxes). The amount of variation explained in each analysis differed; 78% of the total variation was explained in the lab-reared material but only 54% in the wild-caught material. This suggests that environmental variation is masking the

genetic differences between the regions due to the lower fraction of the variation in thorax size that the regression explains. The mean residual variation at core and margin shows a larger gap between the mean values for the wild caught butterflies than the lab reared (Table 3.2). This data indicates that the increased environmental variation in the wild has decoupled the relationship governing the allocation of mass to thoracic tissue. It is also interesting to note that the Figure 3.3b shows a much smaller range of values than Figure 3.3a, which suggests among the wild material there has been selection against extreme phenotypes. Neither particularly large or small butterflies are apparent in the wild caught sample; possibly the result of some balancing selection where small butterflies suffer high mortality and large butterflies don't develop due to resource limitation.

One of the sites sampled at the margin was ~15yrs old, which is almost 23 generations at the range margin (assuming 1.5 generations per year) this could be enough time for adaptations to dispersal to be lost. Bush crickets that show higher proportions macroptery in recently-colonised sites, have then been observed to lose these adaptations within 5 to 10 years (Simmons & Thomas 2004). Once a site has been established selection will begin to favour traits related to reproduction. Therefore as a site ages the proportion of individuals with dispersive genotypes would be expected to decrease. Investment in thorax tissue is believed to involve a trade off with reproduction in female *P. aegeria* (Hughes *et al.* 2003), suggesting there would be selection pressure toward less dispersive genotypes after establishment. Therefore a control for the effect of time since establishment is necessary to establish whether the age of the sites surveyed will confound the results.

It has been suggested that morphological traits may be poor correlates to dispersal ability. Whilst greater thorax mass has been linked to flight performance, such as acceleration ability (Berwaerts *et al.* 2002; Berwaerts *et al.* 2006; Berwaerts *et al.* 2008), it has not been directly linked to dispersal ability and an increased colonisation rate. Long distance dispersal between habitat patches, as would be experienced during distribution shifts, may rely more on the energetic performance of an individual. Butterflies with the lowest energetic requirements during flight or those able to maintain flight the longest could have a better chance of arriving in new suitable habitat. Not all butterfly species exhibit morphological variation related to dispersal even in populations with higher dispersal rates, as is the case of the Glanville fritillary butterfly (*Melitaea cinxia*) (Hanski *et al.* 2002). In which case variation in metabolic performance is believed to be responsible for dispersal ability (Hanski *et al.* 2004), though no published references explore this in *P.*

aegeria. Further work to investigate the metabolic control of flight in *P. aegeria* would be highly valuable.

Of all the variables examined only thorax mass exhibited a significant difference between the regions and this was only the case for the lab-reared study. This appears to be due to a combination of the smaller sample size of the wild-caught study and the weaker relationship between total size and thorax mass. This suggests that environmental influences in wild populations inhibit the expression of genetic differences detected in the lab-reared study.

Chapter 4 – Evidence for local adaptation during climate-driven range expansion: role of temperature on development, survival and flight morphology of *Pararge aegeria*.

4.1 Abstract

Species are responding to climate change in many ways, including changes in phenology, morphology and distribution shifts. In ectotherms, populations at range margins are likely to be at the (cold) thermal limits of species distributions, and populations may exhibit localised adaptations to temperature. Such adaptations might affect species' responses to future changes in climate, and we examined whether there was evidence for adaptation to colder optimum temperatures at the expanding margin of the butterfly *Pararge aegeria*. We also investigated evidence for a link between variation in morphological variables and genetic diversity. Wild female butterflies were collected in 2009 from two locations at the core and margin of the species distribution in the UK. Margin sites had been colonised up to 16 years (~ 24 generations) previously, and were ~ 210km distant from continuously-occupied core sites. F2 offspring were reared at four temperatures (17^{o}C, 20^{o}C, 23^{o}C and 26^{o}C; photoperiod 16L:8D). There were few significant differences between the regions in adult flight morphology under different temperature treatments. Female wing aspect ratio was greater in margin sites ($p = 0.024$) indicating that wings were longer relative to their area in margin sites. In addition, there was an interaction between temperature and region in total mass of males ($p = 0.039$), such that core butterflies were lightest at 20^{o}C and 23^{o}C while margin butterflies had similar mass at all temperatures. There was significantly reduced survival among individuals from core sites at all temperatures ($p < 0.001$), and individuals from core populations also developed more slowly than their margin counterparts (male, $p = 0.024$; female, $p = 0.002$). Patterns of variation in the morphological traits in relation to temperature were consistent in both regions and gave little indication of being related to genetic diversity. These results suggest little or no morphological adaptation to temperature in populations of *P. aegeria* at their expanding range margin.

4.2 Introduction

The Earth's climate has warmed by 0.74^{o}C between 1906 and 2005 and based on various emissions scenarios is predicted to warm 1.1^{o}C to 6.4^{o}C by 2099, relative to the 1980 - 1990 average global temperature (IPCC 2007a). The warming of the climate is known to have clear and wide ranging impacts on species ecology (Walther *et al.* 2002; Parmesan & Yohe 2003). The vast majority of extant species are insects (Gullan & Cranston 2001), or other invertebrates, and are therefore poikilothermic (unable to regulate a constant temperature independent of the local environment). Thus changes to global temperatures are highly likely to influence the ecology of the majority of species in some form. There are two principal strategies for dealing with the changing climate, either dispersal or adaptation *in-situ*. The most widely reported response is shifts in species distribution, which have shown a consistent trend observed in many taxa both at latitudinal (Parmesan *et al.* 1999; Warren *et al.* 2001; Hickling *et al.* 2005; Franco *et al.* 2006; Hickling *et al.* 2006) and altitudinal distribution margins (Wilson *et al.* 2005). Though not all species are able to shift their distributions in response to climate changes, such as more specialised butterflies (Warren *et al.* 2001), and in these cases adaptation is the only alternative. Species are known to adapt to the changing climate though changes in phenology such as earlier bud burst and emergence (Root *et al.* 2003; Stefanescu *et al.* 2003; Edwards & Richardson 2004; Hassall *et al.* 2007; Parmesan 2007). Alternatively species could respond by adapting to temperature more directly; altering their thermal reaction norms to maximise fitness under warmer conditions. This type of direct adaptation to temperature has not been widely reported in the literature as a consequence of climate change (Hill *et al.* 2011).

Temperature does play an important role for the ecology of many species as work on the spittle bug, *Philaenus spumarius*, demonstrates (Karban & Strauss 2004). In this case laboratory studies demonstrated that high mortality occurs outside a limited range of optimum temperatures, and that natural population abundance is strongly correlated with local temperature; therefore physiological tolerance to climate is driving the reported distribution change in this species (Karban & Strauss 2004). Evidence is also available for local adaptation to temperatures in species such as the beetle *Chrysomela aeneicollis* (Dahlhoff & Rank 2000). Greater proportions of allozyme alleles associated with improved survival following cold shock have been reported following a period of cold, wet weather between 1988 and 1996, in *Chrysomela aeneicollis* (Rank & Dahlhoff 2002). Experimentally simulated climate change results in genetic change in the shrub *Fumana*

thymifolia (Jump *et al.* 2008). The loci represented in the stressed treatments violate assumptions of neutral loci indicating selection for drought resistance (Jump *et al.* 2008). Distribution expansion of introduced species into novel climatic environments can also result in adaptation to temperature as has been observed in the hemlock woody adelgid (*Adelges tsugae*) in eastern North America (Butin *et al.* 2005). Populations in newly colonised areas at the north of its expansion are more tolerant of cold shock than southern populations, from where the introduction originated. Adaptation to temperature is not a universal occurrence even when selection pressure may be perceived to be strong and there is apparent potential for adaptive change. Growth rates at the southern range margin of the shrub *Fagus sylvatica* are 49% lower than elsewhere in its distribution (Jump *et al.* 2006b), due to warming temperatures and associated dryer weather. Population declines are occurring at this species southern margin despite apparent genetic potential for adaptation to the new climatic environment (Jump *et al.* 2006a). Though the above examples are largely based on evolutionary responses to temperature it must be remembered that other forms of adaptation can occur including altered habitat use (Thomas *et al.* 2001; Davies *et al.* 2006; Braschler & Hill 2007) or expression of phenotypic plasticity, see Miner *et al.* (2005) for a review of the ecological impacts. Where the potential for similar responses to changing climate exist evolutionary changes could be restricted or absent.

This study aims to investigate the evidence for local evolutionary adaptation to temperature during climate driven distribution shifts in butterflies. Newly founded populations are likely to occur in less climatically suitable areas than experienced by the original populations, this would favour genotypes adapted to colder environments. If new populations are founded preferentially by individuals from the margin fixation for adaptation to cooler temperatures may be enhanced. As butterflies are ectothermic they will have to adapt to changes in climate in some form (distribution changes, altered habitat use or evolutionary change). Responses to temperature are commonly studied in butterflies and include; responses to temperature related to life-history stage (Bauerfeind *et al.* 2009); inter population variation in developmental response to temperature (Ayres & Scriber 1994); impact of temperature on egg size (Fischer *et al.* 2004; Fischer *et al.* 2006); flight ability related to body temperature (Berwaerts & Van Dyck 2004). This is a far from exhaustive list but illustrates the wide ranging impact temperature can have on butterflies and therefore the potential importance of changes in climate. In this study we use *Pararge aegeria* to test for localised adaptation to temperature. It has been demonstrated that *P. aegeria* is changing its distribution in response to climate (Hill *et al.* 1999b), allowing a comparison between the distribution core and range margin to be made. Importantly there

are known differences between populations of *P. aegeria* (Karlsson & Van Dyck 2005) in terms of reaction norms for fecundity and life time egg laying across a range of temperatures. This demonstrates that the species has the potential to adapt to different thermal environments. The distribution expansion in *P. aegeria* has two additional impacts that are relevant to this study. Firstly there is evidence of greater investment in traits linked with improved flight ability (Hughes *et al.* 2003) among margin populations and similar tradeoffs would be expected in this study (see Chapter 3). Secondly the range expansion has resulted in reduced genetic diversity at the range margin (Hill *et al.* 2006) (see Chapter 2). Low genetic diversity is linked with reduced population fitness (Reed & Frankham 2003; Reed *et al.* 2003) and therefore margin populations may be negatively impacted by the observed reductions in genetic diversity. The reduced genetic diversity could also lead to less phenotypic variation in margin populations.

We investigated differences between core and margin sites of *P. aegeria* in the UK under a range of temperatures to see if there was a difference in their morphology or reaction norms for survival and development speed. The hypotheses to be tested were as follows:

1) Margin populations will have longer development times as previously observed in Hughes (2004); but improved performance at cold temperatures.
2) Survival will be poorer at the range margin, due to reduced genetic diversity, and the optimum survival would be at a lower temperature due to adaptation to relatively cool conditions.
3) Greater investment in thorax mass and lower investment in abdominal mass was expected at the range margin, as observed in previous studies on margin populations of *P. aegeria* (Hughes *et al.* 2003).

A range of morphological variables were also examined to see if there were consistent patterns at the different temperatures indicating an adaptive morphological response between the regions.

4.3 Methods

4.3.1 Study species

Pararge aegeria's global distribution extends throughout Europe to the Urals, and from north Africa to southern Scandinavia (Asher *et al.* 2001). The species was historically distributed throughout much of the UK (Asher *et al.* 2001). However, during the 19th and early 20th century it suffered a major range retraction (Burrows 1916; Gibbs 1916; Asher *et al.* 2001), becoming essentially confined to Wales and south west England, but with small isolated populations in Scotland and northern England. During the 20th century, the species expanded its distribution and recolonised much of its former range in the UK (Fox *et al.* 2006). This re-expansion has been a consequence of recent climatic warming (Hill *et al.* 1999b), although the species' distribution currently lags behind the changing climate because human-caused habitat loss and fragmentation has made it difficult for colonising individuals to reach newly-available, climatically-suitable habitats beyond the range margin. The range edge moved northwards by ~ 107km between the 1940's and 1990's (Hill *et al.* 2001). This has involved repeated founder events due to expansion through fragmented habitat (Hill *et al.* 2001). These founder events have lead to a reduction in genetic diversity, of up to 50%, among margin sites relative to core (see Chapter 2; Table 4.1).

Table 4.1 – Study site information. 'Age since establishment' is estimated from the date of the first *P. aegeria* observation record for each 10 km grid square (records from Butterfly Conservation). The estimates of genetic diversity (Proportion of polymorphic loci and Heterozygosity) were obtained from butterflies collected in the same 10 km grid square and are detailed in chapter 2.

Site Code	Region	Year sampled	OS Grid reference	Age since establishment (Years)	Proportion of Polymorphic Loci	Heterozygosity (He)
C1	Core	2009	SU56	>30	65.3	0.201
C2	Core	2009	SP61	>30	67.1	0.220
M1	Margin	2009	SE53	16	28.3	0.122
M2	Margin	2009	SE92	9	27.2	0.123

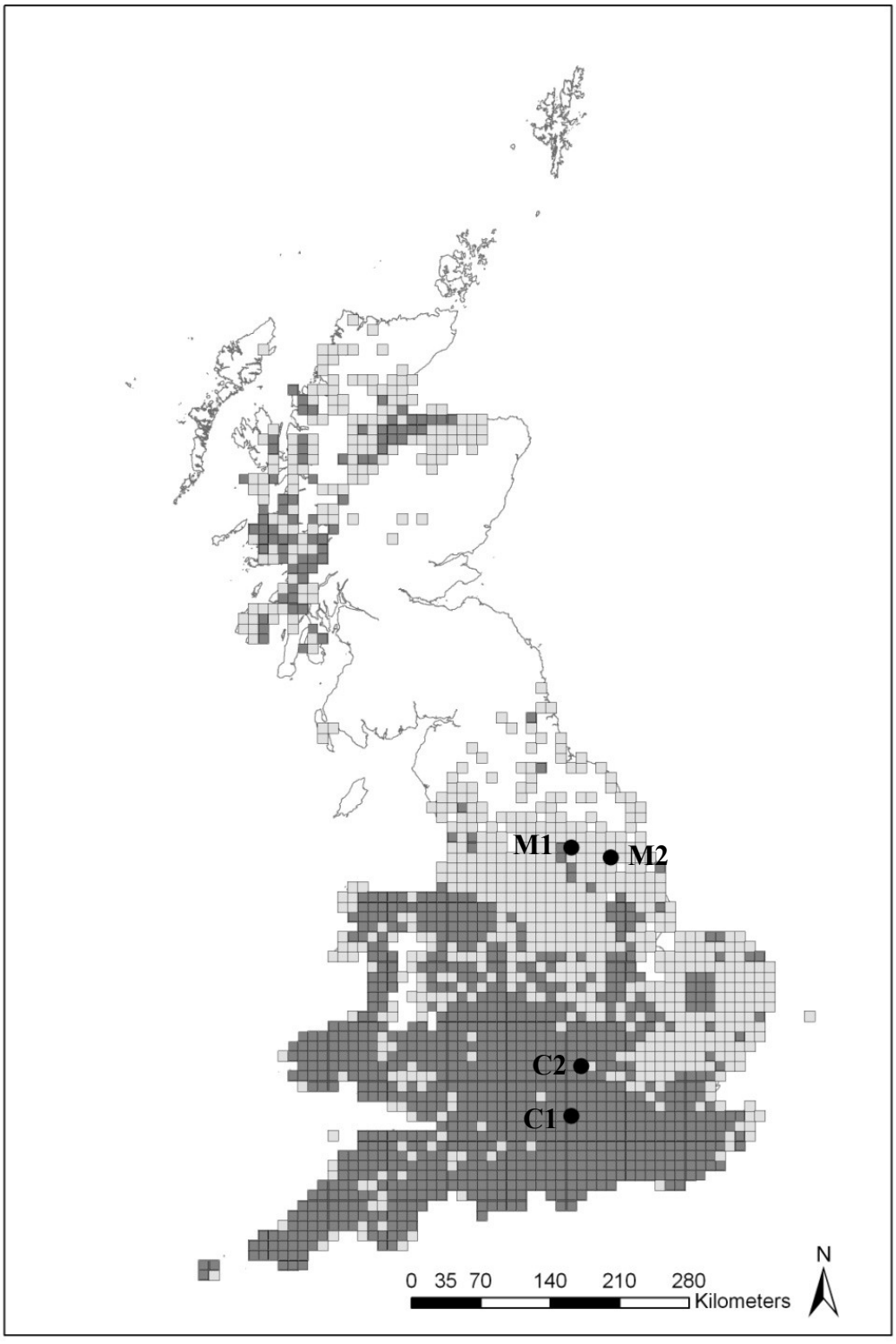
4.3.2 Sampling wild-caught material

In 2009, female *P. aegeria* were collected from four sites in the UK, two in the core of the species range (n = 20 individuals, Ordnance Survey references; SU56, SP61) and from two sites at the range margin (n = 22 individuals; OS references SE27, SE92) (Table 4.1; Figure 4.1). Sites within a region (core/margin) were > 40 km apart to ensure individuals came from separate populations; this was to provide replication within the region and limit the impact of site specific effects. Margin populations were ~210 km north of core sites. Core sites have been continuously occupied for at least 35 yrs, and probably longer (Asher *et al.* 2001), and margin sites had been established no more than 16 years (~ 24 generations; assuming 1.5 generations per year (Pollard *et al.* 1996)) prior to the collection date (based on survey records received from Butterfly Conservation UK). Adult female butterflies were collected using a hand net and transported back to the lab in keep nets.

4.3.3 Rearing protocol

Female butterflies were placed separately in cylinder breeding cages and allowed to lay on potted *Poa pratensis* grass plants. Adults were kept alive by providing pads of cotton wool soaked in a honey and water solution (~50:50). Ten days after collection, ten F1 offspring from each female were transferred to a fresh potted *Poa pratensis* plant and then allowed to develop under constant environmental conditions in a growth room (photoperiod 16L:8D, temperature 20^{o}C ± 2^{o}C). Larvae were transferred to fresh potted grass plants when necessary to ensure no food shortages occurred for larvae. The F1 offspring of each female were reared together on the same plant(s) under the same conditions, and pupae were transferred individually to separate clear plastic containers and maintained under the same rearing conditions until adult emergence. The F1 adults were paired for mating with another individual from the same site but from a different mother. Where possible, these matings took place ~24hrs after adult emergence. After mating, F1 females were allowed to lay eggs for 10 days on a fresh potted *Poa pratensis* plant. Rearing of F2 offspring was similar to F1 protocols; F2 larvae from each F1 family (female) were split between temperature treatments in a split-brood design (10 larvae per family per treatment; four temperature treatments, 17^{o}C, 20^{o}C, 23^{o}C and 26^{o}C; ± 2^{o}C), and reared on separate potted *Poa pratensis* plants. Fresh grass plants were provided when necessary so there were no food shortages. Plants provided were principally *Poa pratensis* but to account for a shortfall at the end of the experiment some wild growing *Dactylis glomerata* were also used (a known larval food plant (Shreeve 1986)). F2 offspring were used for the analysis to

Figure 4.1 – Distribution of *P. aegeria* in the UK at a 10km grid cell resolution. Mid grey squares show sites occupied prior to 1982; light grey squares show sites colonised since 1983. Black circles represent sampling locations, site names are indicated. Ordnance survey grid locations; C1 = SU56; C2 = SP61; M1 = SE53; M2 = SE92.



account for the potential impact of maternal effects on development, morphology and population dynamics (Benton *et al.* 2005). Treatments were checked daily for pupae, and pupae were placed individually into separate plastic pots and maintained under the same conditions until emergence of adult butterflies. Adults were killed by freezing, after expulsion of the meconium (~24hrs after emergence).

4.3.4 Dissection and Data Collection

Adults were thawed and dissected to remove wings, thorax, abdomen, head and legs. Following dissection, the samples were dried at 60^{o}C for 24 hours, and body parts were weighed to the nearest 0.01mg on a Sartorious electro balance (accurate to 1μg). Images of the forewings were taken, using a Nikon D5000 digital camera with an 18-55mm lens, to determine wing area and forewing length; images were analysed using ImageJ v1.43. Images were taken with the camera at a set stage height, and with consistent focus, shutter speed, exposure levels, and flash setting to ensure comparability among different wing images.

4.3.5 Data analysis

Repeated measures were taken for all adult morphology variables (wing area, wing length, total dry mass, thorax mass and abdomen mass) to assess the reliability of measurement accuracy; and all measures had less than 10% error. Only adults with two undamaged wings were included in analyses of wing size, and so sample sizes were not the same for all analyses. The mean of the right and left forewing length and forewing area were used. Aspect ratio (4 x (mean forewing length^2/mean wing area)) and wing loading (total dry mass/mean wing area) were calculated from these wing image data. Development time was calculated as the number of days between transfer of 1st instar larvae to a temperature treatment and adult emergence. Most eggs are laid within the first few days of a female's adult life (Hughes 2004) and so the transfer date provides a reasonable approximate start date for larval development. Survival was the percentage of a family (i.e. all larvae on a single plant) that survived to adulthood.

All variables (survival, development time, total dry mass, abdomen mass, thorax mass, aspect ratio and wing loading) were tested to determine if they were normally distributed prior to analysis (Kolmogorov–Smirnov tests). Total dry mass, thorax mass, abdomen mass, aspect ratio and wing loading were $\log_{10}$ transformed prior to analysis to

ensure normality. Survival was expressed as a proportion, and was arcsine transformed to improve normality (Kolmogorov–Smirnov test). Development time could not be normalised by transforming the data, and so confidence in any parametric statistical result must be reduced accordingly. As development time could not be normalised by transforming the data it was also analysed using a Cox regression, which is not constrained by assumptions of normality and can accommodate censored or missing data. Development time was the input variable, and region and temperature treatment were the covariates used as model parameters. In this case males and females were analysed together as the gender of censored cases is not known. For the other analyses low survival rates among the core families (mean of only 2 individuals per sex, per treatment) meant it would be inappropriate to include family as a factor in the analyses (due to the number of families represented by singletons). Therefore family mean values (or individual values where N = 1) were used in analyses, thus accounting for the non-independence of siblings. Male and female butterflies were analysed separately as their morphology is known to differ (Hughes 2004). Survival and development time were analysed using 2-Way-ANOVA (with region and temperature treatment as fixed factors). T-tests of the between-site family means revealed a significant difference between the margin sites in total mass (male, $p = 0.023$; female, $p = 0.024$), indicating significant within-region effects. To account for this within-region variation, a nested 2-way-ANOVA was used to analyse total mass, wing loading and aspect ratio; with region (core/margin) and temperature ($17^{o}C$, $20^{o}C$, $23^{o}C$ and $26^{o}C$) as fixed factors, and site nested within region. To account for allometry, thorax and abdomen mass were analysed using nested 2-way ANCOVA with region and temperature as fixed factors, site nested within region, and $\log_{10}$ total dry mass as a covariate. All analyses were performed using the statistical package PASW v18.

4.4 Results

A total of 423 F2 butterflies (231 males, 192 females) were analysed from the two regions (core, n = 155 individuals; margin, n = 268 individuals). These butterflies were offspring from 12 families from the margin sites (mean = 6 individuals per family per temperature treatment) and 11 families from the core sites (mean = 4 individuals per family per treatment). The core sites produced fewer individuals for analysis than the margin sites (Table 4.2), but for both analyses of male and female material, all combinations of temperature and region were represented by ≥5 families.

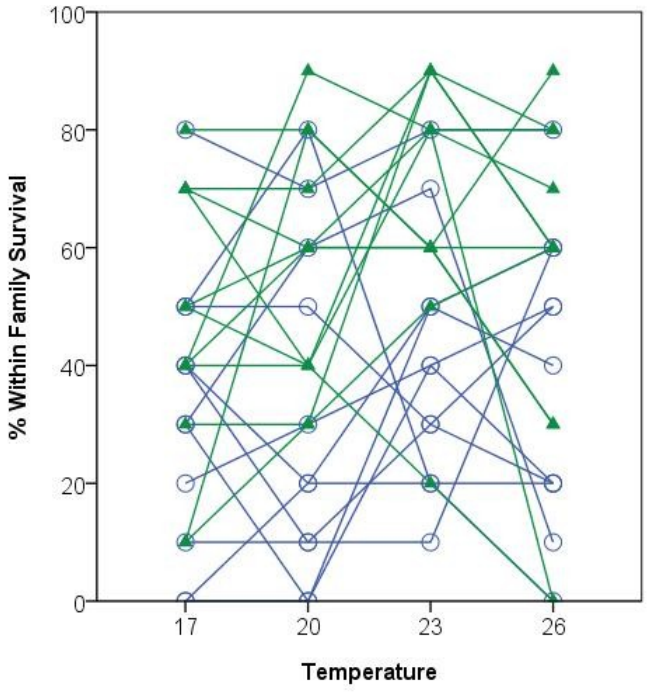
Table 4.2 – Sample size per treatment. Values in brackets indicate number of families.

Region	Sex	17°C	20°C	23°C	26°C	Total
Core	Female	20 (9)	12 (5)	20 (10)	15 (9)	67 (11)
	Male	15 (6)	23 (8)	24 (11)	26 (9)	88 (11)
	Total	35	35	44	41	155
Margin	Female	24 (10)	30 (12)	45 (10)	26 (10)	125 (12)
	Male	32 (11)	38 (12)	37 (12)	36 (9)	143 (12)
	Total	56	68	82	62	268

4.4.1 Survival and development

The analyses of morphology data exclude 45 individuals that were damaged during processing, although these individuals were included in the estimates of survival. Of the F2 core families, 176 of 440 larvae survived to adulthood across all treatments, while 292 of 480 individuals survived from the margin families. This was significantly lower survival in the core families (2-way ANOVA; $F_{1,84} = 16{,}682$, $p < 0.001$), but no significant effect of temperature on survival, or interaction between region and temperature was detected. The reaction norms plotted in Figure 4.2 show no consistent patterns for either region, and so there does not appear to be a strong genetic determinant of survival rates with temperature.

Figure 4.2 – Family mean survival reaction norms. Triangles represent margin, and circles core insects.



The longest development periods were observed at 17°C in both females (core; mean development = 81 days (SD ±20); margin; mean = 68 days (SD ±34); Figure 4.3a) and males (core; mean = 74 days (SD ±31); margin mean = 66 days (SD ±28); Figure 4.3b). There were significantly longer development times among the core families in females (2-way ANOVA, region effect, $F_{1,64} = 9.920$, $p = 0.002$) and males (2-way ANOVA, region effect, $F_{1,69} = 5.351$, $p = 0.024$). In both cases, there was also a significant temperature effect (females, $F_{3,64} = 78.334$, $p < 0.001$; males, $F_{3,64} = 68.286$, $p < 0.001$); visual assessment of the data (Figure 4.3a and 4.3b) shows this significant effect was primarily due to longer development times at 17°C. There appears to be a relatively strong genetic control of development time between 20°C and 26°C for both male and female butterflies (Figure 4.3c & d), and that the core butterflies have consistently longer development times. At 17°C the environmental impact on development time appears to be more pronounced.

The Cox regression survival analysis conducted on the development data used both region (core/margin) and temperature (17°C, 20°C, 23°C, 26°C) as covariates in the model building phase of the analysis. The first stage of the analysis is to build a model which is performed by adding covariates and determining if they significantly improve the model performance. Both of the variables used in this analysis were retained and used in the final model. The results indicated that there were significant differences between the regions and between the temperature treatments (Table 4.3) in terms of the cumulative survival probabilities (Figure 4.4). The cumulative survival plot for the regions (Figure 4.4a) indicates that the probability of a core butterfly surviving a given number of days before developing into an adult is almost always greater than a margin butterfly; therefore core butterflies developed more slowly than margin butterflies. The survival plot for temperature (Figure 4.4b) demonstrates there are similarly clear differences between each of the temperatures. Butterflies at 26°C developed fastest, then 23°C, then 20°C and those reared at 17°C developed most slowly as they have the greatest probability of surviving over any given time period before developing into an adult butterfly. These results support the conclusions of the ANOVA analysis of development time.

Figure 4.3 – Plot of mean development time against rearing temperature; a) female, b) male. Family mean development time reaction norms (only families with at least one individual in each temperature category are plotted); c) female, d) male. Error bars represent 95% confidence intervals. Triangles represent margin, and circles core insects.

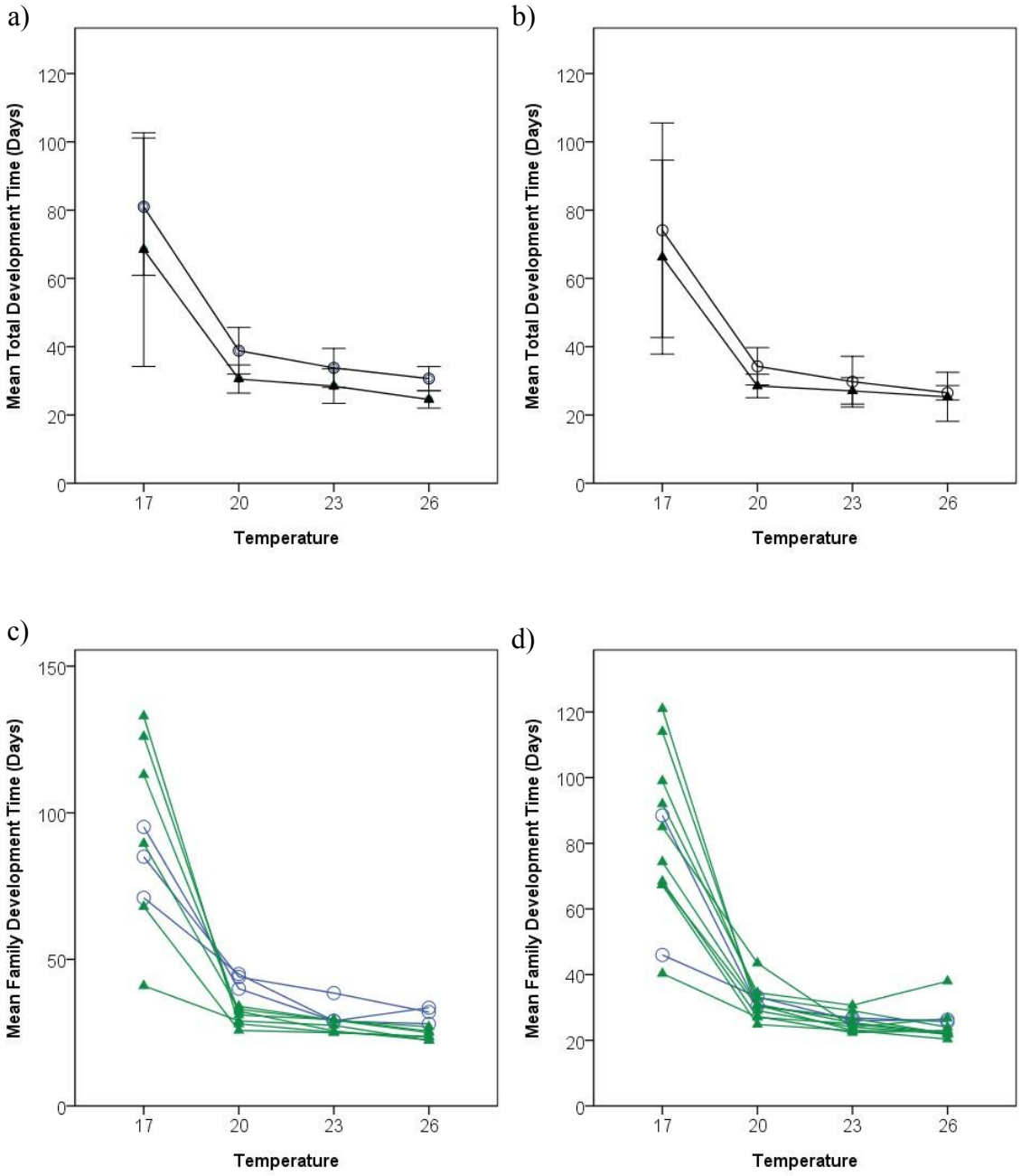


Table 4.3 – Cox Regression model output; variables in the final equation. The P values indicate if there is a significant difference between the categories of the variable. Exp (B) indicates the factor by which the average cumulative probability of a butterfly developing into an adult at a given time interval differs between the groups of the variable. For example Exp (B) for region is 0.572 which means that the probability of a margin butterfly developing into an adult at a given time period is on average 0.572 times the probability of a core butterfly.

Variable	**SE**	**Wald**	**d.f.**	**P value**	**Exp (B)**
Region	0.105	28.098	1	< 0.001	0.572
Temperature		198.771	3	< 0.001	
17°C compared to 26°C	0.218	192.983	1	< 0.001	0.049
20°C compared to 26°C	0.141	36.121	1	< 0.001	0.429
23°C compared to 26°C	0.134	15.268	1	< 0.001	0.593

Figure 4.4 – Cumulative Survival plots. Cumulative survival describes the probability of an average individual from the group surviving the given number of days before developing into an adult butterfly, grouped by; a) Region (Blue line = Core; Green line = Margin); b) Temperature (Blue line = 17°C; Green line = 20°C; Yellow line = 23°C; Red line = 26°C).

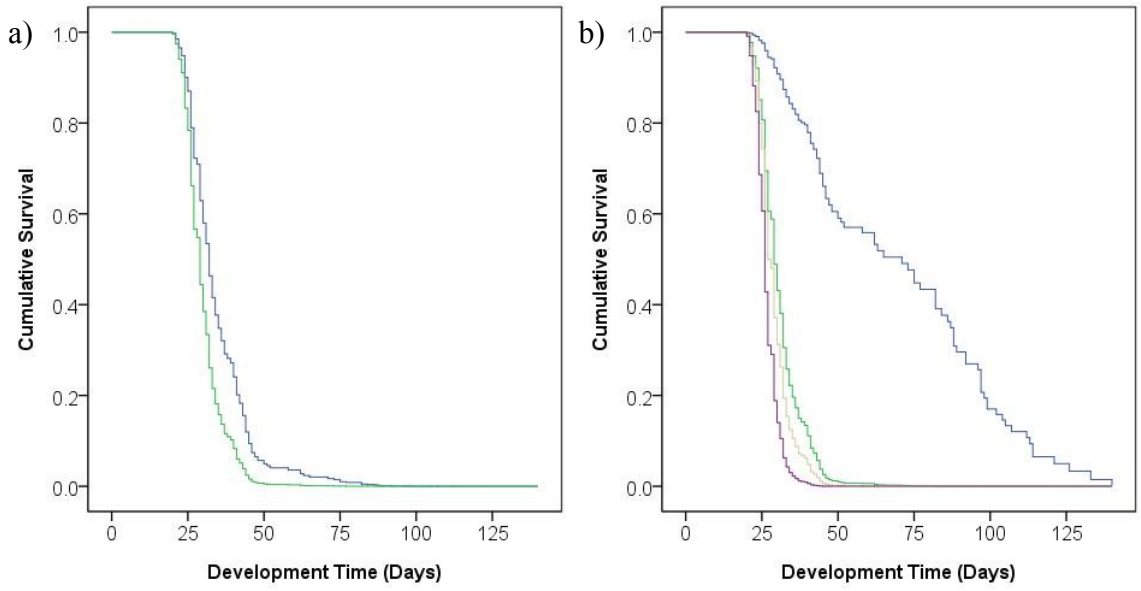


Table 4.4 – Female *P. aegeria* morphological variation in core and margin sites. Values in brackets are Standard Deviation (SD). N = number of individuals. 'Residual thorax mass' and 'Residual abdomen mass' refer to the residual value from the regression of $\log_{10}$ thorax or $\log_{10}$ abdomen mass against $\log_{10}$ total dry mass.

Region	**Variable**	**Temperature**			
		17°C	**20°C**	**23°C**	**26°C**
Core	N	20	12	20	15
	Total Dry Mass (mg)	18.672 (±4.707)	15.675 (±3.329)	15.555 (±3.159)	17.067 (±4.102)
	Abdomen Mass (mg)	7.932 (±2.733)	7.236 (±2.170)	6.805 (±2.107)	8.174 (±2.905)
	Thorax Mass (mg)	5.523 (±1.390)	4.420 (±0.717)	4.649 (±0.866)	4.778 (±1.098)
	Residual Abdomen Mass	-0.036 (±0.013)	0.038 (±0.012)	0.011 (±0.018)	0.031 (±0.015)
	Residual Thorax Mass	0.010 (±0.013)	-0.025 (±0.012)	-0.003 (±0.009)	-0.022 (±0.016)
	Development Time (Days)	81 (±20)	39 (±7)	34 (±6)	31 (±4)
Margin	N	24	30	45	26
	Total Dry Mass (mg)	18.292 (±4.733)	16.041 (±3.825)	17.455 (±5.760)	17.868 (±4.180)
	Abdomen Mass (mg)	8.273 (±3.179)	6.878 (±2.974)	8.123 (±4.324)	8.335 (±2.892)
	Thorax Mass (mg)	5.262 (±1.138)	4.874 (±0.972)	5.181 (±1.743)	5.177 (±1.072)
	Residual Abdomen Mass	-0.013 (±0.013)	-0.011 (±0.011)	-0.001 (±0.008)	0.010 (±0.008)
	Residual Thorax Mass	0.000 (±0.008)	0.008 (±0.010)	0.006 (±0.011)	-0.000 (±0.010)
	Development Time (Days)	68 (±34)	31 (±4)	28 (±5)	25 (±3)

4.4.2 Adult flight morphology

Summary data for means (and SD) of adult morphological variables are shown for females (Table 4.4) and males (Table 4.5). Mean total mass of females among temperature treatments ranged from 15.9mg to 18.5mg, and in males from 10.4mg and 12.1mg. Females were consistently heavier than male butterflies, based on a 3-Way-ANOVA (with region, sex and temperature treatment as fixed factors; sex effect, $F_{1,407} = 339.855$, $p > 0.001$). Therefore males and females are analysed separately in subsequent analyses.

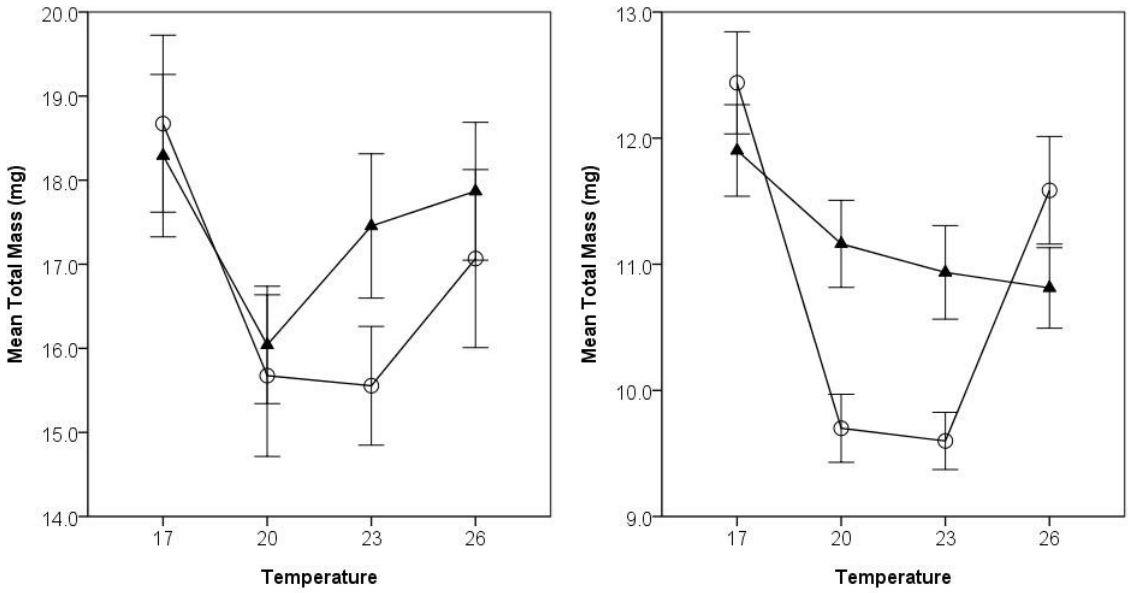
Table 4.5 – Male *P. aegeria* morphological variation in core and margin sites. Values in brackets are Standard Deviation (SD). N = number of individuals. 'Residual thorax mass'/'Residual abdomen mass' refers to the residual value from the regression of $\log_{10}$ thorax/$\log_{10}$ abdomen mass against $\log_{10}$ total dry mass.

Region	**Variable**	**Temperature**			
		17°C	**20°C**	**23°C**	**26°C**
Core	N	15	23	24	26
	Total Dry Mass (mg)	12.439 (±1.569)	9.699 (±1.295)	9.599 (±1.112)	11.586 (±2.175)
	Abdomen Mass (mg)	2.969 (±1.137)	2.018 (±0.434)	1.804 (±0.502)	2.704 (±1.040)
	Thorax Mass (mg)	4.809 (±0.839)	4.086 (±0.572)	4.263 (±0.526)	4.834 (±0.759)
	Residual Abdomen Mass	-0.011 (±0.028)	0.024 (±0.015)	-0.025 (±0.015)	0.003 (±0.012)
	Residual Thorax Mass	-0.026 (±0.022)	-0.006 (±0.005)	0.017 (±0.006)	0.007 (±0.005)
	Development Time (Days)	74 (±31)	34 (±5)	30 (±7)	27 (±2)
Margin	N	32	38	37	36
	Total Dry Mass (mg)	11.902 (±2.050)	11.162 (±2.128)	10.935 (±2.256)	10.812 (±1.915)
	Abdomen Mass (mg)	2.710 (±0.926)	2.640 (±1.214)	2.341 (±1.014)	2.497 (±0.964)
	Thorax Mass (mg)	4.785 (±0.875)	4.572 (±0.804)	4.621 (±0.849)	4.587 (±0.780)
	Residual Abdomen Mass	-0.010 (±0.018)	0.009 (±0.014)	-0.019 (±0.016)	0.022 (±0.018)
	Residual Thorax Mass	-0.010 (±0.009)	-0.005 (±0.006)	0.006 (±0.008)	0.006 (±0.008)
	Development Time (Days)	66 (±28)	29 (±3)	27 (±4)	25 (±7)

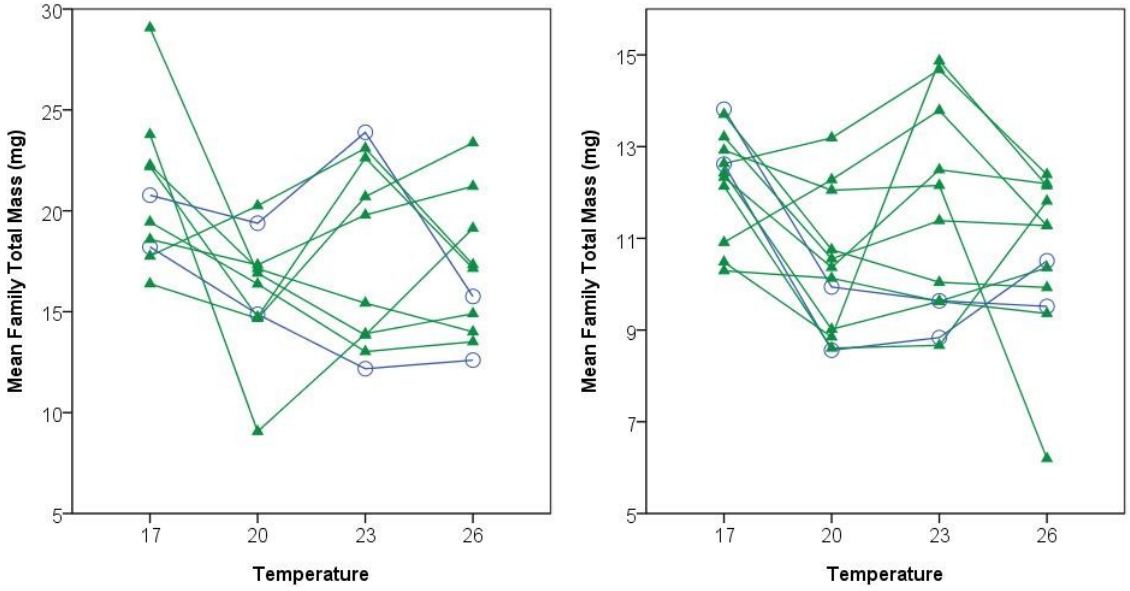
Females from margin sites tended to be heavier than core females in all treatments, except at 17°C (Figure 4.5a; Table 4.4) although differences were not significant. There was also no significant difference between the regions in male butterflies. However, there was a significant interaction effect in males (interaction effect; $F_{3,67} = 2.956$, $p = 0.039$) which is evident from the drop in mass at 20°C and 23°C among the core butterflies compared to 17°C and 26°C (Figure 4.5b; Table 4.5). Margin butterflies had consistent mass in all the temperature treatments. No significant differences in size were detected in relation to temperature, although the effects of site were nearly significant in both sexes

Figure 4.5 – Plot of total mass against rearing temperature; a) female, b) male. Family mean total mass reaction norms (only families with at least one individual in each temperature category are plotted); c) female, d) male. Error bars represent standard error. Triangles represent margin, and circles core insects.

a) b)



c) d)



(site nested in region; female, p = 0.052; male, p = 0.082). This suggests large differences within regions with limited homogeneity between sites within the regions. The family reaction norms suggest little genetic control of mass in response to temperature for either males or females from both regions (Figure 4.5c & d).

Averaged across all temperature treatments, margin females had heavier thoraxes than core females (mean margin = 5.12 mg; mean core = 4.85 mg; Table 4.4) which is consistent with their greater mean total mass. Margin females also appear to have a greater relative investment in thorax mass (Figure 4.6a) at all temperatures except 17°C, though the 2-way ANCOVA found no significant difference between regions ($F_{1,1.98} = 0.069$, $p = 0.817$). This is in contrast to results in Chapter 3 showing increased investment in the thorax at margin sites. There was also no evidence of any difference in relative thorax mass between regions in male butterflies (Figure 4.6b; ANCOVA result). There were no effects of temperature on thorax mass (or interaction effects) for either sex. There was however, a significant difference among sites in females ($F_{2,63} = 3.739$, $p = 0.029$), suggesting much of the apparent difference indicated in figure 4.6b is due to variation between sites in the regions, rather than between regions. As was the case with total mass there is no indication of strong genetic control of thorax mass in response to temperature for males or females from either region (Figure 4.6c & d).

Female butterflies from margin sites had lower relative investment in abdomen mass than core butterflies at 20°C, 23°C and 26°C (Figure 4.7), but there were no differences between the regions ($F_{1,1.98} = 0.006$, $p = 0.944$). By contrast with measures of thorax, there were significant temperature ($F_{3,63} = 4.713$, $p = 0.005$) and site effects ($F_{2,63} = 3.640$, $p = 0.032$) due to lower relative abdomen mass at lower temperatures (17°C; Figure 4.7). There was no difference in relative abdomen mass of males in relation to region, temperature, site or any interaction effects ($P > 0.073$ in all cases). The reaction norms do indicate a consistent pattern of increased investment in abdomen mass at higher temperatures for butterflies from both regions (Figure 4.7b). Though there is no indication of a change in the relative impact of environment vs. genetic control at any of the temperatures investigated.

Based on nested 2-way ANOVA, there was no significant effects for females or males in wing loading in relation to region, temperature, or site. The only significant effect for male aspect ratio was between sites ($F_{2,41} = 4.778$, $p = 0.014$). In contrast, females did exhibit a significant difference in aspect ratio due to region ($F_{1,2.388} = 26.5$, $p = 0.024$) but no other factor was significant (temperature, site, region or interaction effects). This was due to greater wing aspect ratio in margin individuals (core mean = 9.489, SD± 0.243; margin mean = 9.688, SD± 0.317). Higher aspect ratio means the butterflies have longer thinner wings and has been linked to greater acceleration capacity (Berwaerts *et al.* 2002).

Figure 4.6 – Plot of unstandardised residual thorax mass by rearing temperature; a) female, b) male. Family mean residual thorax mass reaction norms (only families with at least one individual in each temperature category are plotted); c) female, d) male. Error bars represent standard error. Triangles represent margin, and circles core insects. 'Residual thorax mass' refers to the residual value from the regression of $\log_{10}$ thorax mass against $\log_{10}$ total dry mass.

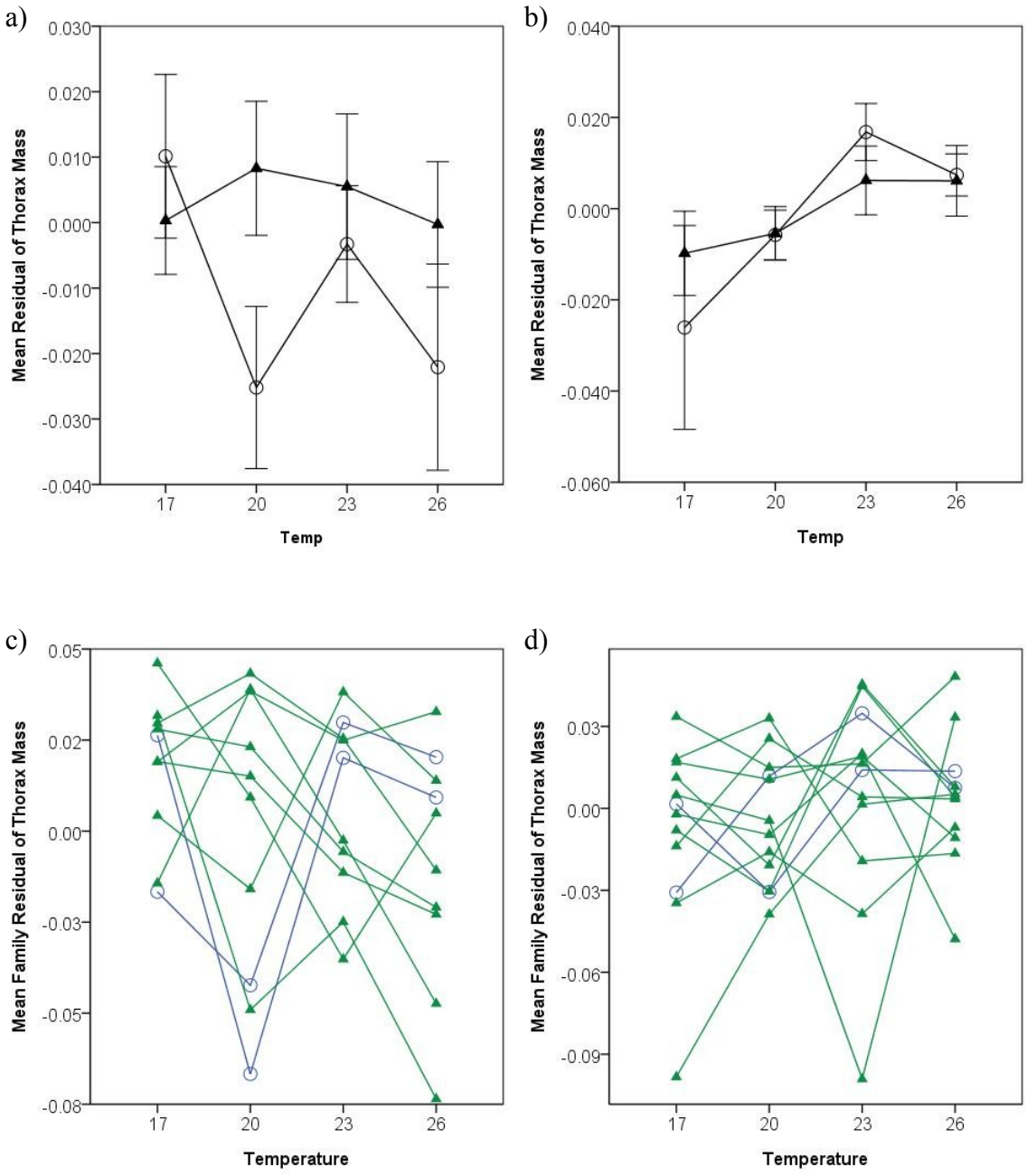
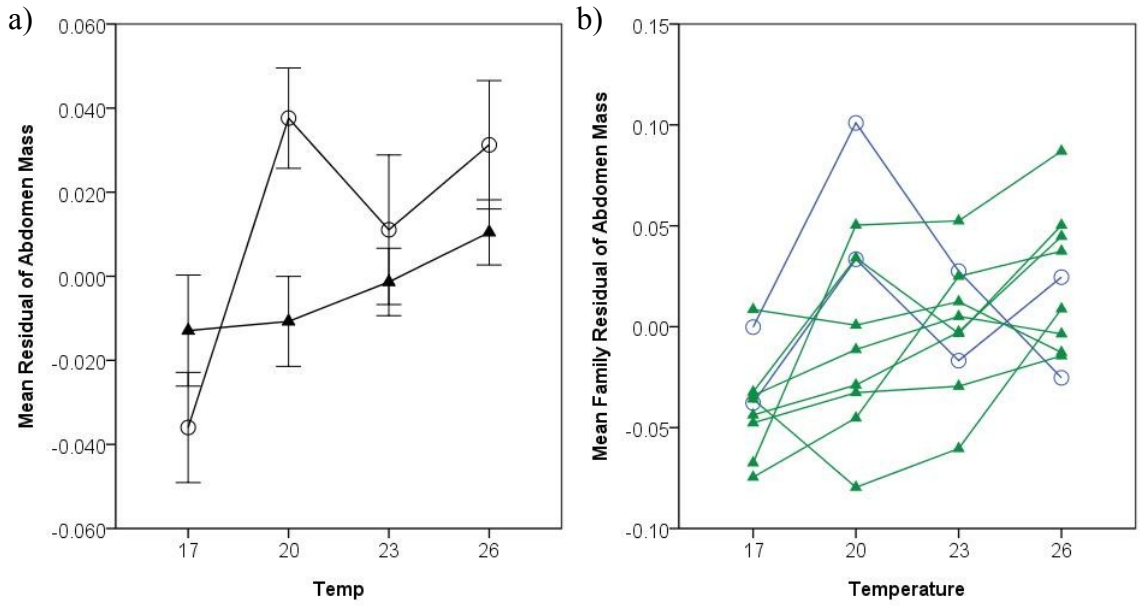


Figure 4.7 – a) Plot of female mean unstandardised residual abdomen mass against rearing temperature. b) Family mean residual abdomen mass reaction norms (only families with at least one individual in each temperature category are plotted). Error bars represent standard error. Triangles represent margin, and circles core insects. 'Residual abdomen mass' refers to the residual value from the regression of $\log_{10}$ abdomen mass against $\log_{10}$ total dry mass.



4.5 Discussion

Some 920 F2 larvae were used in this experiment of which 428 survived to adulthood across four temperature treatments, which originated from four different sites two in each of the regions of interest. Use of F2 butterflies limits the impact of maternal effects which have been demonstrated to have impacts across multiple generations in mites (Benton *et al.* 2005). Therefore our results should be robust to differences brought about by environmental variation between sites from which wild butterflies were collected and reflect genetic differences between the sites. The analyses in this study are limited by the low number of independent data points (5 - 12 family means per factor combination; Table 4.2). Therefore the data only provide weak evidence for differences, or lack of differences, in temperature adaptation between the regions. Low sample sizes also mean analyses lack power to resolve small differences between the regions or to assess traits with high variability between sites. This is the result of the low survival of butterflies from the core and a shortfall in food plants which limited the number of F2 families that could be reared.

4.5.1 Survival and development

We had hypothesised that survival would be greater in F2 families originating from the core particularly at higher temperatures. This was because of the greater genetic diversity at the core theoretically enhancing fitness and the presumed greater use of open habitats at the core selecting for higher optimum developmental temperatures. This prediction was contradicted by the analysis in this study as survival was lower among core families ($p < 0.001$). The significantly lower survival among the core families (core family mean survival 40%; margin mean family survival 60%) was consistent across all temperature treatments, although the cause of this result is unclear. The greatest survival was at 23^{o}C for both the core and margin (in terms of total numbers of adults; Table 4.2), which does not suggest that core butterflies perform better at higher temperatures. Ideally a Cox regression or other survival analysis would be conducted but sufficient data on the time of death was lacking so this analysis could not be performed. Core individuals were more genetically diverse than those from the margin (Table 4.1) and F1 females were mated with males from different mothers to avoid inbreeding. Therefore mortality due to greater expression of deleterious alleles because of inbreeding is unlikely in core individuals; but the ancestry of the wild caught females is unknown and those from the core sites could have been more closely related than margin sites. The core F2 families were transferred to their temperature treatments up to 30 days after the majority of the margin families. This was due to the delay in collecting the wild females from the two core sites and slow development times of the F1 generation. The knock on impact of this was that core F2 butterflies were fed grass grown later in the year which suffers from higher rates of mildew infestation. Powdery mildew on larval host plants can reduce over winter survival of the Glanville fritillary butterfly (*Melitaea cinxia*) by 26% (Laine 2004). This effect has not been directly observed in *P. aegeria* but could be a factor in the observed reduction in survival rate. Additionally the core families were fed a greater amount of *Dactylis glomerata*, although this was principally used to as larval food for the 17^{o}C treatment (core and margin) and would therefore only have a limited impact on the other treatments. The developmental consequence of this change in diet is unknown, though the grass has been used as a food plant in other breeding studies of *P. aegeria* (Sibly *et al.* 1997). As the *Dactylis glomerata* was obtained from wild populations its use could have introduced additional pathogens or predators.

Rearing temperature had a significant effect on development time for both male ($p < 0.001$) and female ($p < 0.001$) *P. aegeria*. This was principally due to slower

development at 17°C, as observed in both groups, and is consistent with previous studies (Sibly *et al.* 1997; Hughes 2004; Gibbs *et al.* 2010). It had been hypothesised that butterflies from the core would develop faster but that margin butterflies would perform better at cooler temperatures. The results did not fully support this as development was slower for butterflies from the core compared to the margin, as demonstrated by the Cox regression (Figure 4.4). Though it does suggest improved development speed among margin butterflies at the coldest temperature which is in line with expectations. Hughes (2004) had previously found shorter development times among core butterflies, although those butterflies came from sites further west in the species core, near the Forest of Dean. The results from Chapter 2 suggest strong genetic differentiation between these parts of *P. aegeria's* core distribution; equivalent to the genetic differentiation observed between the core and margin sites. This suggests scope for genetic differences in response to temperature between the sampling locations used in the two studies. Above 17°C the mean development times in this study differ by no more than 8 days (max SD±7) within a treatment (Table 4.4 & 4.5). Assuming the difference between the regions is not an artefact, the contradictory results of this study and Hughes (2004) would imply that development time is highly variable between sites within regions. Experimental manipulation of three butterfly species using semi natural rearing conditions (Bryant *et al.* 2002) suggests that microhabitat selection (shaded versus exposed sites) affects development rate. Therefore habitat structure at each of our sites (and sites colonised during *P. aegeria's* range expansion) could have greater impacts on larval developmental conditions than differences between the regions. Similarly *Plebejus argus* is known to alter its habitat use based on local weather conditions (Dennis & Sparks 2006), and such behaviour could buffer against selection for altered development speed if laying sites are altered due to local weather patterns. Plastic behavioural responses to habitat structure or local weather patterns could both act to maintain existing developmental rates, and the observed differences between sites in this study and Hughes (2004) may reflect local adaptation or chance differences due to the genetic legacy of a populations initial founders. There was an indication of genetic control of development rate at the warmer temperatures (20°C, 23°C and 26°C) in this study (Figure 4.3c & d), with the core populations shifted towards longer developmental phenotypes. The strength of the genetic control was greatly reduced at 17°C, which would be consistent with ensuring rapid development during the peak of the summer.

4.5.2 Differences in adult morphology

The hypothesis that there would be significant differences in response to temperature between core and margin populations was not supported by the morphological data. Relative thorax mass, relative abdomen mass and wing loading showed no effects due to region, or interaction effects between region and temperature, and total mass also showed no significant effect of region. Excluding site effects only two significant differences were detected; some 30 P values were produced for the morphological variables, which would mean 1 – 2 significant terms would be expected by chance at the 5% significance level.

Female aspect ratio was the only trait that exhibited a significant difference between the regions. Higher aspect ratio is linked to increased acceleration capacity in male *P. aegeria* (Berwaerts *et al.* 2002) but not females. Therefore any selection during range expansion would only be expected to act on males, limiting the potential for differences to develop between the regions. This variable is also known to show inconsistent responses in different experiments comparing core and margin *P. aegeria* in the UK (Hughes 2004). The weak support for consistent differences in aspect ratio supports the notion that the significant result is due to chance differences between sites, rather than being representative of differences between the regions.

Analyses of dispersal measures within butterfly species have also indicated that dispersal ability commonly exhibits considerable variation between populations (Stevens *et al.* 2010a; Stevens *et al.* 2010b). Previous work comparing core and margin populations of *P. aegeria* had demonstrated a trade off in the relative investment in thorax and abdomen mass (Hughes *et al.* 2003); such that female margin butterflies invested more heavily in dispersal. This study did not find any significant difference in either of these traits between the regions, but visual inspection of the data (figures 4.5a and 4.6) does indicate the same pattern of greater investment in thorax mass and lower abdomen mass among margin butterflies at all temperatures except 17^{o}C. The inference that relative investment in abdomen mass or thorax mass is a good measure of reproductive investment is debated (Hanski *et al.* 2006). It has been assumed that greater allocation to thoraxes results in greater flight muscle (which would then not be as readily available for egg production in female butterflies) and therefore improved dispersal ability but this has not been experimentally demonstrated.

Due to the sample size limitations already discussed the analysis is this study lacks power to distinguish small differences therefore the non-significant result does not contradict Hughes *et al.* (2003) but suggests the differences between the regions may be small relative to differences between sites. This suggestion is supported by the significant site effect found for both relative thorax and abdomen mass among female butterflies (site effect for relative; thorax mass $p = 0.029$; abdomen mass $p = 0.032$). Differences between margin sites could be enhanced if selection against individuals with greater investment in thorax mass occurs following colonisation, such that increasing age since colonisation correlates with a reduction in relative thorax size. Adaptation during range expansion in bush crickets leads to higher proportions macroptery in recently-colonised sites, these adaptations are absent within 5 to 10 years following colonisation (Simmons & Thomas 2004). Therefore it is possible that older margin sites (such as M1; Table 4.1) will have lost their adaptations to dispersal due selection for greater fecundity following establishment; the oldest margin site is 16 years old or approximately 24 generations, assuming 1.5 generations per year.

Pararge aegeria's current UK distribution is believed to be lagging behind the suitable climate (Hill *et al.* 1999b) which suggests that margin populations may not be thermally limited. Therefore selection for adaptations to cold temperatures may be weak at the current distribution margin. There are known phenotypic and life history differences between continental populations of *P. aegeria* originating from agricultural and woodland landscapes (Karlsson & Van Dyck 2005; Merckx & Van Dyck 2006). The different landscapes have different thermal characteristics such that woodlands are cooler with more extreme minimum temperatures. The result is selective differentiation in thermal reaction norms between populations originating from the two environments. Development in agricultural landscapes leads to heavier butterflies with greater relative thorax mass (Merckx & Van Dyck 2006). Also butterflies from woodland have greater lifetime egg number and daily fecundity at low temperatures compared to butterflies from agricultural landscapes, whilst performance is reversed at higher temperatures (Karlsson & Van Dyck 2005). Therefore landscape structure has an important impact on thermal adaptation in *P. aegeria*, and woodland comprises a very small proportion of the available habitat in northern England. *Pararge aegeria* is found in gardens and parkland as far north as Yorkshire which indicates use of more open habitats in the margin region which could result in less selection pressure for cold environments, hence the lack of adaptation observed in this study. Analysis of environmental factors that limit butterfly species niche breadth at their range margins suggests that *P. aegeria* is principally limited by water

availability at its range margin (Oliver *et al.* 2009); wetter conditions reduce habitat specificity. Therefore wet conditions may allow the species to overcome the commonly cited restriction to woodland habitats at the range margin, thus allowing the use of warmer open habitats removing selection pressure for cold conditions.

4.5.3 Impact of genetic diversity

It was intended that a comparison of the amount of morphological variation within the regions to genetic diversity would be conducted. Unfortunately this was not possible due to the paucity of the data set and the similarity of the estimates of genetic diversity within the regions (Table 4.1). Visual comparison of the standard deviations (Table 4.4 and 4.5) does not suggest any limitation on the variability observed at the range margin where genetic diversity is lowest. Similarly if the coefficient of variation is plotted against genetic diversity there was no indication of reduced morphological variation in any of the traits measured. Quantitative genetic variation is poorly correlated with measures of genetic diversity, or heritability, according to meta analyses of other studies which include measures of both (Reed & Frankham 2001). As the morphological traits in this study are polygenic (multiple genes regulating the trait) a simple correlation between trait variability and genetic diversity was unlikely. Genetic diversity (heterozygosity) is known to be related to fitness (Reed & Frankham 2003) but in this case the least genetically diverse populations (M1 and M2; Table 4.1) exhibited the greatest survival. Therefore the margin populations do not appear limited by the observed reduction in genetic diversity.

Reduced egg hatching success has been observed in inbred populations of *Melitaea cinxia* (Nieminen *et al.* 2001) and *Bicyclus anynana* (Saccheri *et al.* 1996). Only larva that successfully hatched were used for the rearing experiment, which would mask mortality due to the expression of deleterious alleles. Therefore egg hatching would be of interest for further investigation as a more sensitive measure of the impact of reduced genetic diversity between core and margin populations. The impacts of reduced genetic diversity on populations of *P. aegeria* could be investigated through a comparison of artificially inbred core populations and outbred margin populations.

4.5.4 Conclusions

This study indicated little evidence for morphological variation between core and margin populations of *P. aegeria*, even for traits which had previously been observed to vary

between the regions. It also produced results for development speed and survival contrary to expectations. This indicates that reduced genetic diversity does not limit larval survival rates at *P. aegeria's* range margin. The contrary results for development rate suggest this trait is highly variable between sites and may reflect site specific adaptations.

Chapter 5 – Examining genetic diversity at trailing-edge range margins in the Scotch Argus butterfly (*Erebia aethiops*) during climate driven range retractions.

5.1 Abstract

Range contractions polewards and/or uphill have been documented in northern and montane species at their trailing-edge (warm) range margins during recent climatic warming. This study examined changes in genetic diversity associated with range contraction at trailing-edge margins. Loss of genetic diversity could occur if distribution shifts lead to increased isolation among margin populations or if genetically distinct populations become extinct and such findings would be important for the long term management of range-retracting species. Genetic diversity was investigated in the northern butterfly *Erebia aethiops* (Satyrinae) from four sites at its UK southern range margin and four sites in the core of its UK distribution (137 individuals in total, core and margin sites were ~ 115 km apart). Genetic diversity was compared using AFLPs which revealed little difference in the proportion of polymorphic loci (PPL) or heterozygosity (He) between the core (PPL = 63.6%; He = 2.00) and margin sites (PPL = 61.8%; He = 1.86). Nonetheless, there was significant genetic differentiation between core and margin sites (between region average pairwise F_{ST} = 0.179). This finding was supported by Bayesian clustering, which indicated two principal groups of individuals corresponding to the core/margin region they were collected from. The lack of any reduction in genetic diversity may reflect the relatively recent retraction of *E. aethiops* distribution, within the past 30 yrs. This finding at a retracting range margin contrasts with range expanding species; where reduced genetic diversity has been recorded at leading-edge range margins over similar time periods (Chapter 2). Evidence that genetic diversity is maintained at range margins of range-retracting species implies that genetic factors may not contribute to the extinction of local populations during range retractions. However, continued range contraction could lead to the loss of genetically differentiated margin populations, and the genetic diversity and localized adaptations they support.

5.2 Introduction

Distribution changes due to climate have been a feature of many species evolutionary history during the Quaternary ice ages (Hewitt 2000). Current climatic changes are also driving changes in species distributions (Parmesan *et al.* 2000; Warren *et al.* 2001; Walther *et al.* 2002; Parmesan & Yohe 2003; Walther 2004; Parmesan 2006). Expansions at cool, leading-edge high-latitude/high-elevation range margins are relatively well studied (Hill *et al.* 2001; Karban & Strauss 2004; Hickling *et al.* 2005) and have been demonstrated in a wide range of animal groups (Hickling *et al.* 2006). Studies at species warm, trailing-edge low-elevation and low-latitude range margins also show range changes up-hill and/or northwards, tracking changes in climate (Parmesan *et al.* 1999; Wilson *et al.* 2005; Franco *et al.* 2006). Compared with leading-edge margins, relatively little information is available on trailing edge populations. There have been suggestions that species low-latitude ranges boundaries are more limited by biotic factors than climate (Thomas *et al.* 2006), and also may be more stable than leading edge margins (Parmesan *et al.* 1999; Hampe & Petit 2005). There is evidence that trailing-edge margins are limited by climate (Franco *et al.* 2006), and the apparent stability of trailing edge margins may be due to time lags in climate-induced extinction of local populations, and failure to monitor species at sufficient temporal and spatial resolution to detect local extinctions (Thomas *et al.* 2006).

Species that are shifting their ranges will suffer from a process of local extinction and population fragmentation at their trailing edge. One of the impacts of this process could be reduction of genetic diversity if increased isolation, smaller population sizes and reduced habitat quality occur as a consequence of deteriorating climate conditions, resulting in more frequent population bottlenecks, lower gene flow and greater genetic drift in margin populations (Frankham *et al.* 2002). Reduced genetic diversity has been demonstrated as a result of population fragmentation (Berwaerts *et al.* 1998; Jump & Penuelas 2006; Butcher *et al.* 2009; Collier *et al.* 2010), and the extent of any reductions in genetic diversity will be related to the severity and duration of population bottlenecks or genetic isolation (Frankham *et al.* 2002). Broad scale losses of genetic diversity are apparent in species that have suffered range contractions (Leonard *et al.* 2005; Freeland *et al.* 2007; Anderson *et al.* 2008), although these studies are of species in decline across their range and compare historic and extant levels of genetic diversity, and do not compare margin and core populations.

Low genetic diversity and high rates of inbreeding are known to negatively impact population persistence and the survival and life history traits of individuals (Saccheri *et al.* 1998; Ehlers *et al.* 2008; Vandewoestijne *et al.* 2008; Markert *et al.* 2010). Reduced genetic diversity is also common feature of threatened taxa (Spielman *et al.* 2004) and is believed to be a driver of species extinctions. If range retractions affect genetic diversity then this could impact on the rate of distribution change and the stability of populations. However, studies are lacking on patterns of genetic diversity at contracting trailing edge margins.

To examine genetic diversity in retracting trailing edge range margin populations, we sampled populations of *Erebia aethiops*, a satyrine butterfly with a contracting distribution in the UK (Franco *et al.* 2006). We use AFLPs (Amplified Fragment Length Polymorphisms) to investigate population structure and to compare genetic diversity of *Erebia aethiops* at core and range margin sites and test the hypothesis that genetic diversity is lower at range margin sites.

5.3 Methods

5.3.1 Study species and sample collection

The species occurs in wet, acidic or neutral grasslands, woodland clearings and bogs at up to 500 meters above sea level (Asher *et al.* 2001). The butterfly is not found in heavily grazed areas (Asher *et al.* 2001). Adults occur as a single brood, flying between late July and early September (Asher *et al.* 2001), larvae make use of a range of host plants including Purple Moor-grass (*Molinia caerulea*), Tufted Hair-grass (*Deschampsia cespitosa*), Wavy Hair-grass (*D. flexuosa*), Sheep's-fescue (*Festuca ovina*), Common Bent (*Agrostis capillaris*) and Sweet Vernal-grass (*Anthoxanthum odoratum*) (Fox *et al.* 2006). *Erebia aethiops* is found in mountainous areas throughout central and eastern Europe, from France to Romania, though the species is absent from Scandinavia (Asher *et al.* 2001; Settele *et al.* 2008). Within the UK, *E. aethiops* occurs throughout much of western and central Scotland but is restricted two isolated sites in northern England (Asher *et al.* 2001) (Figure 5.1). This species is one of just three butterfly species in the UK that have a northerly distribution. Historically it had a more widespread distribution including sites in Lancashire, Yorkshire and Northumberland (Asher *et al.* 2001). There has been a decline

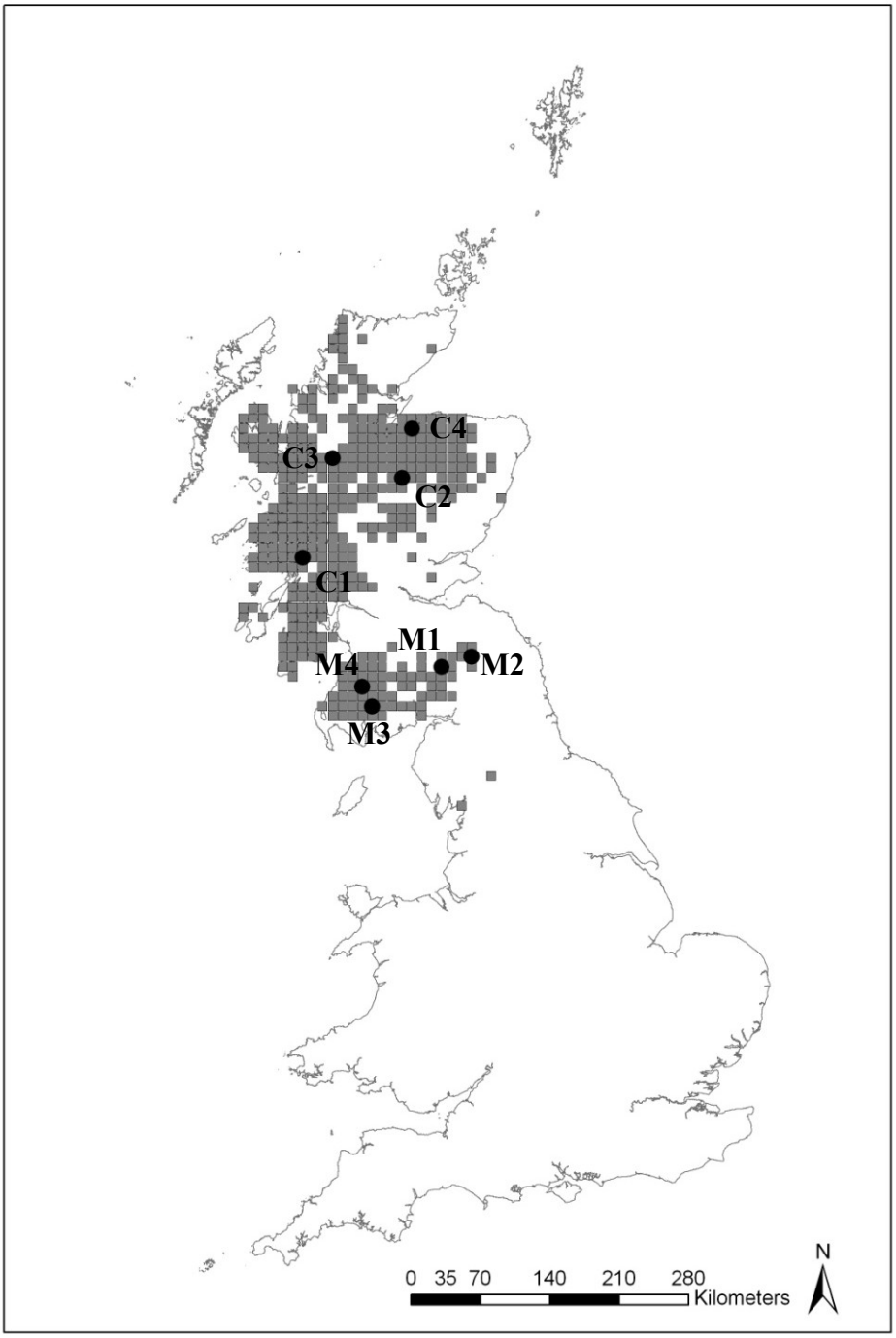


Figure 5.1 – Distribution of *E. aethiops* in the UK at a 10km grid cell resolution. Grey squares show sites occupied between 1983 and 2004. Black circles represent sampling locations, site names are indicated. Ordnance survey grid locations; C1 = NM82; C2 = NH80; C3 = NH12; C4 = NH95; M1 = NT21; M2 = NT52; M3 = NX57; M4 = NX49.

in the species distribution of ~ 10% (as measured by number of occupied 10 km grid cells), between 1982 and 2004, in the UK (Fox *et al.* 2006). A survey of historically occupied sites showed that the species has contracted at its southern range margin (Franco *et al.* 2006), which has shifted northwards by 80 - 90km over ~ 19 years, as a response to

climate warming. These declines have been mirrored by some severe retractions in continental Europe (Asher *et al.* 2001). The declining distribution is contrasted by increasing abundance at monitored sites in the UK (collated index of abundance + 98% between 1979 and 2009) (Botham *et al.* 2009).

In this study, adult butterflies were collected from two regions, one at the range margin and the other in the core of the UK distribution of *E. aethiops*. A total of eight sites were sampled, four margin and four core sites. Each site comprised a single 10-km Ordnance Survey grid cell (Figure 5.1), separated from other sites by at least 10 km. The core and margin regions were > 115 km apart. Margin sites were within the 25% of the most southerly occupied 10 km grid cells (recorded between 1983 - 2004). A total of 20 adult male *E. aethiops* were collected from each site during August of 2007 and 2008. Specimens were killed and stored at -80^{0}C prior to dissection and extraction of DNA.

5.3.2 AFLP protocol and analysis

DNA was extracted from approximately one third of the thorax of each individual using an ammonium acetate based ethanol precipitation method (see Appendix 1). The concentration of the extracted DNA was determined using a Nanodrop Spectrophotometer and then diluted to 10ng/µl prior to AFLP fingerprinting. The AFLP protocol was modified from Vos *et al.* (1995) as described in Whitlock *et al.* (2008a). In addition, the ligation of the adaptor sequences was conducted at 8^{o}C overnight, and a total reaction volume of 10µl was used during the pre-selective PCR, which did not include any formamide. Two separate pairs of pre-selective PCR primers were used per sample (*Eco*RI primer (A) 5'-GACTGCGTACCAATTCT-3' & *Mse*I primer 5'-GATGAGTCCTGAGTAAC-3'; *Eco*RI primer (B) 5'-GACTGCGTACCAATTCA-3' & *Mse*I primer 5'-GATGAGTCCTGAGTAAC-3'). The template DNA was then diluted 1 part in 50 before the selective PCR. The selective PCR was also conducted in a total volume of 10µl without formamide. Three primer pairs were used to generate AFLP markers for *E. aethiops* during the selective PCR's (*Eco*RI-TCT and *Mse*I-CAA, *Eco*RI-TGA and *Mse*I-CTG, *Eco*RI-ATC and *Mse*I-CTG). Positive and negative controls were included with all batches of samples.

The selective *Eco*RI primers were labelled with 5' fluorescent dyes (Applied BioSystems – 6FAM, LIZ and PET) to allow AFLP fingerprints to be produced by capillary electrophoresis using an ABI 3130 Genetic Analyser and the LIZ600 size

standard (Applied BioSystems). Profiles were then visualized using GeneMapper v4.0, and peak height tables were generated. The R script AFLPscore v1.3 (Whitlock *et al.* 2008b) was used to convert the peak height profiles into binary presence/absence genotypes. The script allows the minimization of error rates and removes the subjectivity of manually editing AFLP loci (Whitlock *et al.* 2008b). After the removal of obvious shoulder peaks a peak height table is exported from GeneMapper then a set of user defined thresholds (loci selection and allele calling) are applied and the combination with the lowest error rate can be selected (Whitlock *et al.* 2008b). Twenty samples were replicated to allow the estimation of the mismatch error rate (1.55%), between genotypes of the same individual.

5.3.3 Data analysis

In order to examine genetic differences among sites and regions, the proportion of polymorphic loci at the 95% level (excludes loci where the presence allele is found in < 5% or > 95% of the total sample) and the expected heterozygosity at sites (He) were determined using AFLP-SURV v1.0 (Vekemans 2002). ANOVAs were used to determine if genetic diversity differed between the regions; with region as a fixed factor and the proportion of polymorphic loci or heterozygosity at a site as the dependant variable (using the statistical package PASW v18). Proportions of polymorphic loci were arcsin square root transformed prior to ANOVA analysis. Regression was used to determine if there was a latitudinal cline in genetic diversity (using PASW v18), with the expectation of lower diversity at southern sites. Genetic divergence among sites was investigated using nested Analysis of Molecular Variance (AMOVA) as implemented in Arlequin v3.1 (Excoffier *et al.* 2005), with individuals nested within sites, and sites nested within regions (core/margin). Pairwise estimates of F_{ST} and significance tests for population differentiation were also conducted in Arlequin, in order to discover if the sites were genetically distinctive or homogenous. Due to the limitations of dispersal in *E. aethiops* direct gene flow between more distant sites is unlikely, therefore a pattern of isolation by distance would be expected. This was investigated in Arlequin with a Mantel test which compared pairwise F_{ST} estimates between sites to geographic distance between sites (km). Population structure was further investigated with the Bayesian clustering method used by STRUCTURE v2.3 (Pritchard *et al.* 2000). This approach assigns individuals probabilistically to clusters based on similarities between their genotypes, therefore allowing inferences to be made about the relationships between individuals. This version of the program specifically treats dominant genetic data such as obtained by AFLP (Falush *et al.* 2007). The admixture based ancestry model was used with a burn in length of 20000

and 20000 simulations. The true value of k, number of clusters sampled, was estimated using the method of Evanno *et al.* (2005), based on the second order rate of change (Δk). Twenty runs for each K value, from 1 to 12, were used to determine the modal value of Δk; which indicates the true number of clusters (k) in the data set.

5.4 Results

5.4.1 Genetic diversity at the core and margin

AFLP genotypes were produced for a total of 137 *E. aethiops* individuals from eight study sites. These individuals were represented by 319 loci (90.3% segregating fragments) ranging from 50 to 480bp in length. In terms of genetic diversity, there were few differences between core and margin sites (Table 5.1). Among the core sites, the percentage of polymorphic loci ranged from 59.6% to 66.1% and expected heterozygosity ranged from 0.189 to 0.212. Margin sites had similar values; the percentage of

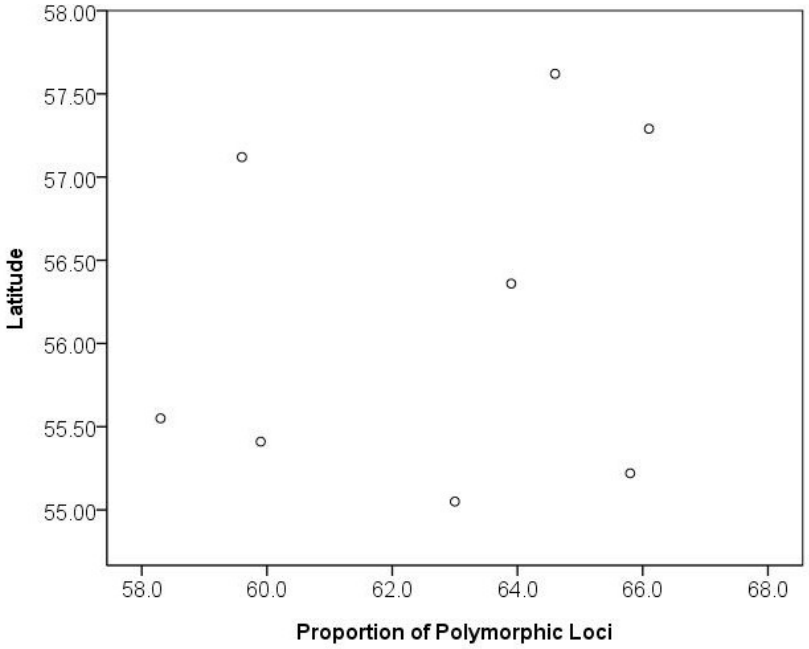
Table 5.1 – Genetic diversity in populations of *E. aethiops* based on AFLP genotypes. 'Core' sites lie within the main distributions of the species in northern Scotland. 'Margin' sites are locations within the most southerly 25% of occupied 10 km OS grid cells.

Region	**Site code**	**Ordnance survey grid reference**	**N**	**Proportion of polymorphic loci**	**Expected heterozygosity (He)**	**SE (He)**
Core	C1	NM82	16	63.9	0.200	0.0096
	C2	NH80	19	59.6	0.189	0.0101
	C3	NH12	18	66.1	0.212	0.0095
	C4	NH95	20	64.6	0.198	0.0097
	Mean			63.6	0.200	0.0097
Margin	M1	NT21	19	59.9	0.171	0.0094
	M2	NT52	17	58.3	0.167	0.0096
	M3	NX57	18	63.0	0.199	0.0099
	M4	NX49	9	65.8	0.209	0.0098
	Mean			61.8	0.186	0.0097

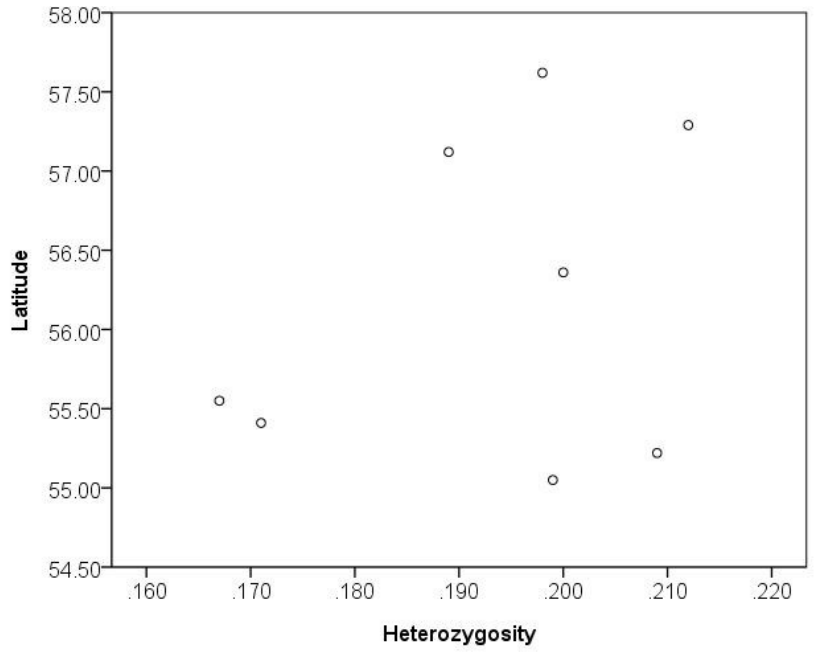
polymorphic loci ranged from 59.9% to 65.8%, and heterozygosity varied from 0.167 to 0.209. There was no apparent trend between genetic diversity and latitude (Percentage of polymorphic loci, $r^2 = 0.063$, $p = 0.55$; Heterozygosity, $r^2 = 0.096$, $p = 0.455$; Figures 5.2 a & b). No significant difference between the core and margin was detected by AMOVA for

Figure 5.2 – Genetic diversity against latitude: a) Proportion of polymorphic loci plotted against latitude; b) Heterozygosity plotted against latitude.

a)



b)



either the proportion of polymorphic loci ($F_{1,6}$ = 0.687, p = 0.439) or heterozygosity ($F_{1,6}$ = 1.358, p = 0.288).

5.4.2 Genetic divergence among sites

There were significant differences in genetic divergence between sites and between regions, (AMOVA; site effect p < 0.001, region effect p = 0.029; Table 5.2). The global F_{ST} estimate was 0.18 and most population pairwise F_{ST} estimates were significantly more differentiated than a random assemblage of individuals (Table 5.3; only the M3/M4 site comparison was not significant in this analysis). The greatest levels of differentiation were recorded between pairs of sites from different regions (average between site F_{ST} = 0.179), and pairwise comparisons of sites within the same region exhibited less differentiation (core region, average between site F_{ST} = 0.099; margin region, average between site F_{ST} =

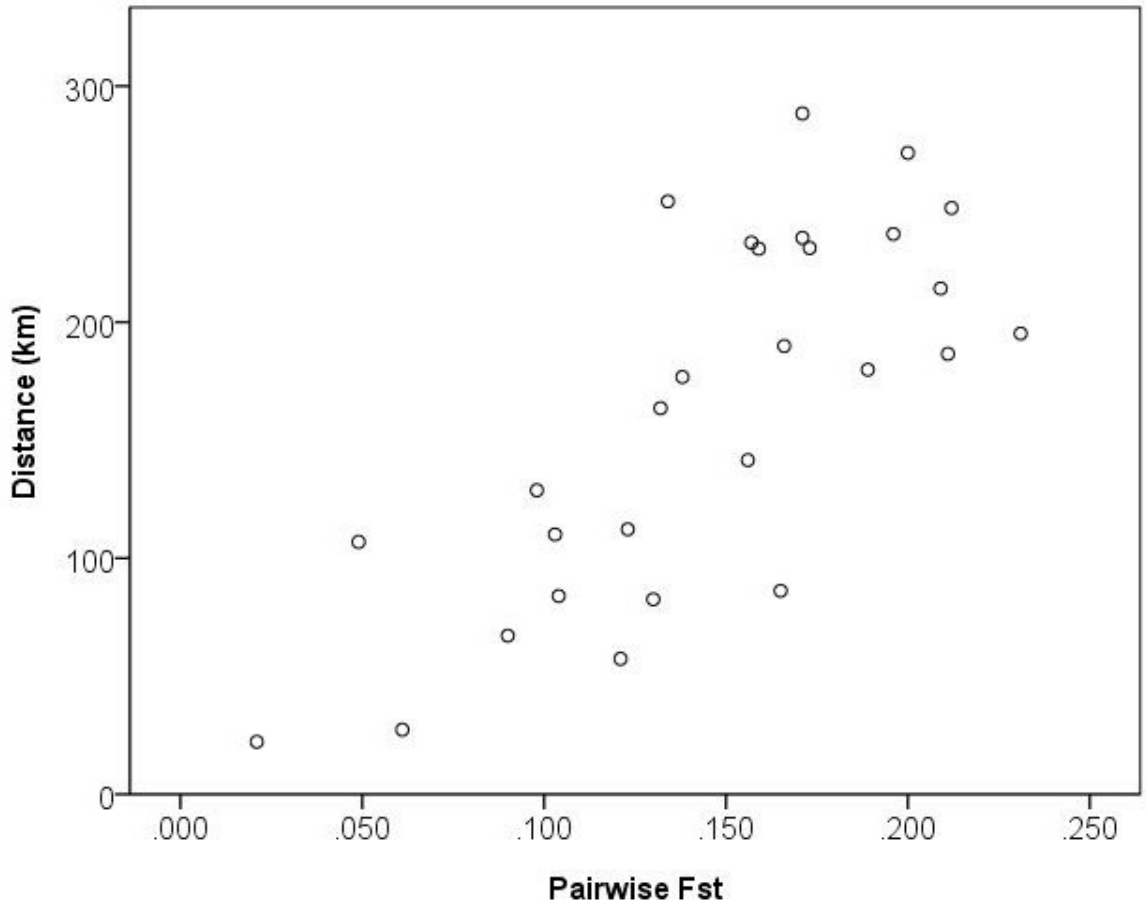
Table 5.2 – AMOVA results for *E. aethiops*. Sources of genetic variation are nested; within sites, between sites in regions and between regions.

Source of Variation	d.f.	Sum of squares	Variance components	Proportion of variation	P value
Among regions	1	260.05	2.73	8.80	0.029
Among sites within regions	6	441.43	2.86	9.23	< 0.001
Within sites	128	3254.13	25.42	81.97	< 0.001

Table 5.3 – Population pairwise F_{ST} estimates of *E. aethiops*. Site names are given in the top row and first column. Values below the diagonal are F_{ST} estimates; stars above the diagonal indicate pairs of sites significantly more differentiated than by chance (at the 5% level) and x indicates non-significantly differentiated pairs of sites.

	C1	C2	C3	C4	M1	M2	M3	M4
C1	-	*	*	*	*	*	*	*
C2	0.098	-	*	*	*	*	*	*
C3	0.049	0.088	-	*	*	*	*	*
C4	0.138	0.121	0.104	-	*	*	*	*
M1	0.189	0.231	0.171	0.212	-	*	*	*
M2	0.166	0.211	0.157	0.196	0.061	-	*	*
M3	0.132	0.173	0.134	0.171	0.130	0.103	-	X
M4	0.156	0.210	0.159	0.200	0.165	0.123	0.021	-

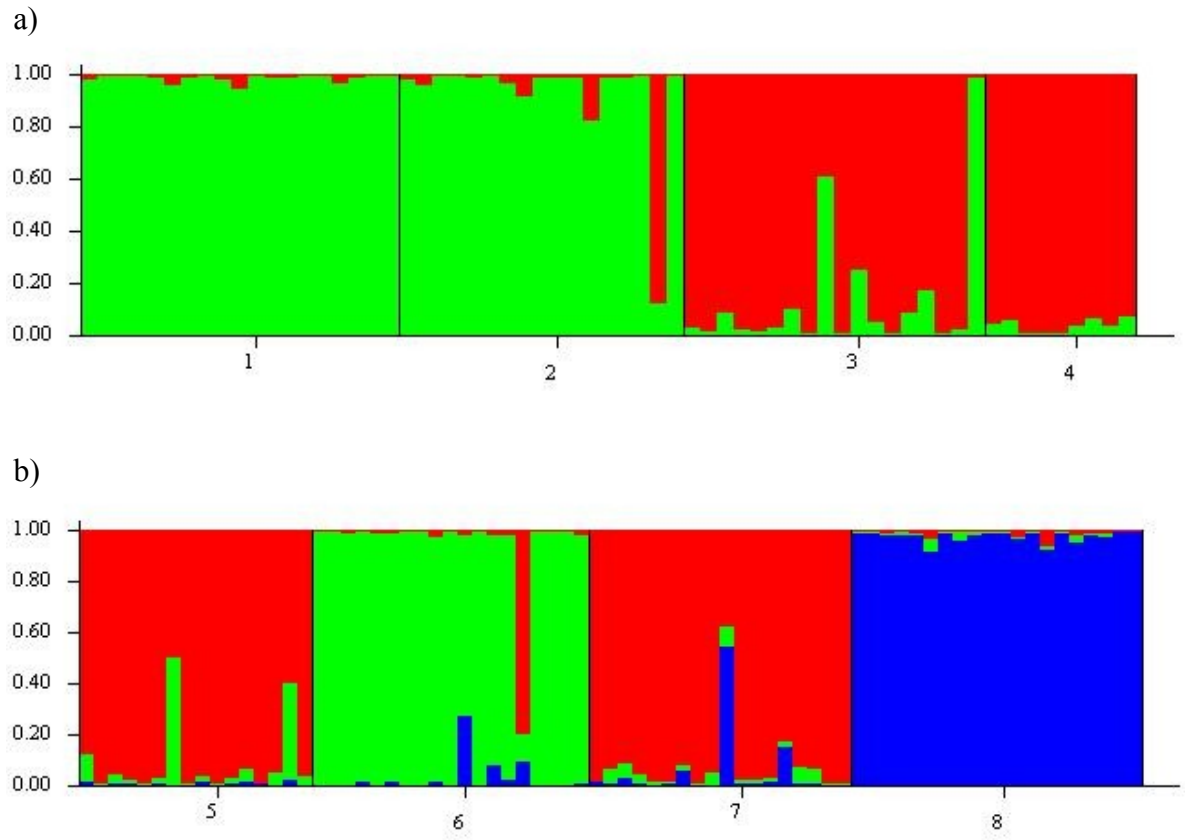
Figure 5.3 – Effect of geographic distance on genetic differentiation between pairs of populations. Distance is the straight line distance (km) between sites, and genetic differentiation is represented by pairwise F_{ST} estimates.



0.100). These F_{ST} estimates also showed a significant isolation-by-distance relationship (Mantel test, r = 0.756, P = 0.003; Figure 5.3).

Output from the Bayesian clustering analysis indicated two clusters (based on the modal Δk), which corresponded with the core and margin regions. This implies that the two regions are highly differentiated; as indicated by the AMOVA, Mantel test and pairwise F_{ST} results. Additional population structure was implied by the F_{ST} estimates and Evanno *et al.* (2005) showed that in situations of isolation by distance only the most differentiated groups are identified, and differentiation in the contact zone is not detected. Therefore the samples from each region were re-analysed as two separate groups (Figure 5.4 a & b). This indicated two clusters within the margin region (M1 + M2 and M3 + M4), and three clusters amongst the core sites (C1 and C3 formed one site). These three pairs of sites correspond to the least differentiated pairwise F_{ST} estimates (pairwise F_{ST}; M1 + M2 =

Figure 5.4 – Probabilities of assignment of *Erebia aethiops* individuals to population clusters; a) individuals from margin sites only (k = 2 clusters), b) individuals from core sites only (k = 3 clusters). Sampling locations are represented as follows; M1 = 1, M2 = 2, M3 = 3, M4 = 4; C1 = 5, C2 = 6, C3 = 7, C4 = 8. Colours indicate different clusters and the proportion of each colour in a column indicates the probability of assignment of an individual to that population. Colours are not consistent between images and so do not infer relationships between the populations in the two images. Black lines separate each of the clusters.



0.061, M3 + M4 = 0.021, C1 + C3 = 0.049; Table 5.3). This indicates there is relatively high gene flow between these pairs of sites.

5.5 Discussion

The expectation that genetic diversity would be reduced at *E. aethiops* southern range margin was not supported by the data from this study. There were only minor differences between the core and margin populations in terms of the proportion of polymorphic loci

observed and expected heterozygosity. Therefore, widespread loss of genetic diversity at the range margin has not occurred. Overall, the proportion of polymorphic loci recorded at sites and expected heterozygosity were high, and similar to those observed at core sites of the southerly distributed expanding species *Pararge aegeria* (PPL = 56.8%, He = 0.203; see Chapter 2). The lack of any significant differences in genetic diversity between core and margin sites is similar to the findings for the non-expanding control species *Maniola jurtina* (see Chapter 2) which also exhibited no significant differences between study regions.

Although there were no significant differences between margin and core sites, the two sites with the lowest genetic diversity were at the range margin (M1 and M2). These sites occurred within an area of managed forest and arable farmland and were the sites at which butterflies were hardest to locate (personal observation), possibly indicating low population sizes. The relatively low expected heterozygosity at these sites (He = 0.167 and 0.171) is indicative of small, isolated populations. Reductions in heterozygosity are associated with reduced population fitness (Reed & Frankham 2003), and greater rates of inbreeding can reduce the ability to adapt to novel environmental stress (Reed *et al.* 2003). In contrast the other two margin sites (M3 and M4), that had greater genetic diversity, were located within the Dumfries and Galloway National Park, a large area of continuous semi-natural habitat. These higher levels of genetic diversity are probably due to the ameliorating effects of larger total population sizes, greater availability of habitat and greater connectivity between habitat patches (Frankham *et al.* 2002). Southern Scottish populations of *Erebia aethiops* have been declining in extent over the last ~ 23 years (Franco *et al.* 2006) and the national distribution extent has contracted over > 30 years (Fox *et al.* 2006). In contrast, abundance has increased over the same time period (Botham *et al.* 2009) which could provide a buffer to losses of genetic diversity. However, these distribution contractions and associated population extinctions have not demonstrably reduced neutral genetic diversity, relative to core populations. Assessment of the current pattern of genetic diversity is limited as no prior information is available on historic genetic diversity. Studies of interglacial patterns of genetic diversity often indicate the greatest genetic diversity in refugal populations (Hewitt 2000), and a cline of reduced genetic diversity with increasing distance from the refugia. *Erebia aethiops* would have had to recolonise the UK following the last glacial period from populations in continental Europe. The post glacial re-expansion into the UK from Europe could have resulted in a similar cline of genetic diversity, with lower genetic diversity at higher latitudes (increased distance from the glacial refugia). Therefore the current southern margin in the UK may

have had greater genetic diversity than the core as a legacy of post glacial colonisation. If genetic diversity at the southern margin exceeded that in the core prior to the current distribution decline losses could be masked. It is also possible that the relatively short time period over which range changes have occurred are not sufficient for the effects of isolation and genetic drift to have become apparent (particularly in more abundant habitat patches which support large populations). For example, studies modelling impacts of forest fragmentation on a tropical satyrine butterfly (Benedick *et al.* 2007) have suggested that time periods of ≥100 yrs are required for genetic erosion to be evident, implying that populations can go extinction from other factors before they suffer any detrimental effects of genetic erosion.

This study focused on broad scale losses of genetic diversity across the range margin, an investigation of peripheral and isolated populations may identify consistent reductions in genetic diversity. Though the data from this study suggests only small reductions should be expected. It would be of interest to sample from the European distribution from areas predicted to be unaffected by future climate changes (Settele *et al.* 2008), for comparison with marginal populations from areas with sharp population declines and those distribution margins that currently appear stable (Asher *et al.* 2001). It would be of interest to extend this work by comparing multiple species responses to range contractions and determine if there are differing patterns of genetic diversity related to habitat availability, as has been done with range expanding butterflies (Hill *et al.* 2006).

Whilst there was little variation in genetic diversity between the regions there was evidence of significant genetic differentiation among sites. AMOVA analysis indicated clear differentiation between sites ($p < 0.001$) and between the two regions ($p = 0.029$). The proportion of variation due to differences between the regions (8.8%) was very similar to that between sites (9.2%), indicating strong differentiation between the regions. Genetic differentiation between sites and regions was supported by the pairwise F_{ST} estimates and population assignment using Structure. Estimates of pairwise F_{ST} values within regions were lower than those between regions (mean within region 0.100; between regions 0.179). Analysis using Structure also indicated two clusters; one populated by margin individuals the other core individuals. All three lines of evidence indicate a clear differentiation between the two regions in this study. The southerly Scottish margin sites are separated from the main distribution by the Firth of Clyde, a distance of ~ 20 km which would constitute a considerable barrier to gene flow (overland the distance is even greater). Reported estimates of dispersal distance in the related satyrine butterfly *Maniola jurtina*

are up to ~ 600 m (Schneider 2003), based on mark recapture results, therefore dispersal between the regions would probably be rare facilitating the observed genetic differentiation. This differentiation could be due to postglacial re-colonisation of the UK (Hewitt 2000), but as the land mass that separates the two regions includes Glasgow urbanization and associated agricultural development seem more plausible. Continued habitat loss and degradation during the 20th century would have served to enhance and maintain the geographic separation of the two regions. This separation may have maintained genetically distinctive range margin sites and continued range contraction could lead to their extinction. Loss of genetically distinct populations has been highlighted as an issue of concern in an era of rapid biodiversity decline (MEA 2005) and climate change has been highlighted as a possible factor leading to these losses (Hampe & Petit 2005). If *E. aethiops* maintains its rate of contraction, at ~ 4.7 km yr^{-1}, its distribution will shift ~ 90 km in the next 20 years (Franco *et al.* 2006) resulting in the loss of the margin sites. However, this rate of decline is less than that which would lead to the species being listed on the UK red list of butterflies. Therefore losses of distinct genetic populations would occur before targeted conservation action is likely to be implemented. This highlights the need for long term planning and conservation management if maintenance of genetic diversity is deemed important, especially if populations in these margin sites also contain unique adaptations to their local environment.

Reductions in genetic diversity were not detected by this study at *E. aethiops* warm-edge range margin, but clear differentiation of margin and core sites was observed. Therefore whilst range contraction has no apparent impact on genetic diversity, continued range contraction will result in the loss of distinct populations of butterflies. This serves as a reminder that if genetic diversity is to be maintained; action, probably if the form of translocations, would be required before range contractions result in concerted conservation action.

Chapter 6 – General Discussion

6.1 Project aims and Outcomes

The aims of this project were to investigate the impacts of distribution shifts on genetic diversity and selection related to climate induced distribution shifts. More specifically the aims were to investigate changing patterns of genetic diversity during distribution expansions and contractions. The project also investigated the extent of local adaptation to temperature and dispersal among populations at a species expanding distribution. The main findings of the previous chapters in this thesis are summarised below.

6.1.1 Summary of Chapter 2

In Chapter two, AFLPs were used to investigate genetic diversity at the expanding range margin of *P. aegeria*. Genetic diversity and differentiation were compared between core and margin populations of both *P. aegeria* and a non-expanding control species *M. jurtina*. The principal hypothesis was that reduced genetic diversity at the range margin compared to the core would be observed in *P. aegeria*, due to repeated founder events. No difference was expected between the core and margin of the control species (*M. jurtina*). As hypothesised significantly lower genetic diversity was observed at the range margin of *P. aegeria* relative to the distribution core. No differences were observed in the control species. *Pararge aegeria* also exhibited high levels of genetic differentiation between core and margin sites. Genetic differentiation between sites within a region was most pronounced at the distribution core, relatively limited differentiation occurred between margin sites. These findings suggest the species range expansion is the result of relatively few long distance dispersal events, resulting in a reduction in genetic diversity and a common ancestry of the margin sites.

6.1.2 Summary of Chapter 3

The relative impacts of genetic and environmental forces on morphological differences between *P. aegeria's* core and margin were also of interest to this study. It had previously been established by laboratory based studies that range margin populations of *P. aegeria* exhibit increased expression of traits associated with greater dispersal ability in

comparison to core populations. The extent to which these data were representative of wild phenotypes was unknown. Therefore wild butterflies were collected from core and margin sites to determine if morphological differentiation between the regions was also apparent in wild butterflies. There were no significant differences between wild butterflies from the two regions (core/margin), although the mean thorax mass was greater at the range margin as was the case among the laboratory reared butterflies. The data did indicate a smaller range of phenotypes among wild butterflies suggesting selection may be acting to limit or remove extremely large or small butterflies. These data indicate that developmental forces in natural populations limit the impact of genetic differences between the regions, and limit the potential impact of these morphological traits on the species ability to track the changing climate.

6.1.3 Summary of Chapter 4

The aim of Chapter four was to investigate the evidence for local adaptation to temperature as a result of climate induced distribution shifts. Expanding populations at the range margin could become adapted to lower temperatures as they exist in the coldest parts of the realised distribution of the original population. Therefore butterflies were collected from core and margin populations of *P. aegeria's* English distribution. Their offspring were then reared under controlled conditions so differences in the development and morphology of F2 butterflies could be compared between the two regions under a range of temperatures (17^{o}C, 20^{o}C, 23^{o}C and 26^{o}C). Contrary to expectation core populations exhibited lower survival and development speed. No consistent differences were detected between the regions in terms of their morphology. The results indicate little to no adaptation to colder optimum temperatures at the range margin. These findings also suggest previously observed morphological differences between the regions may be highly variable between sites, and strongly influenced by location specific factors such as habitat structure.

6.1.4 Summary of Chapter 5

Whilst Chapter two investigated the effect of range expansion on genetic diversity chapter five aimed to study the impact of range contraction on genetic diversity. *Erebia aethiops* was used as the study organism in this instance and AFLPs were used to compare genetic diversity between the core and margin of the species current distribution. Reduced genetic diversity was expected at the contracting range margin due to isolation and smaller population sizes. No significant difference in the amount of genetic diversity was detected

between the range margin and core, though significant genetic differentiation was detected. Therefore range contraction has not resulted in a reduction of genetic diversity within margin populations of *E. Aethiops*. The results do suggest continued range contraction would result in the loss of genetically differentiated populations and any local adaptations they represent.

6.2 Impacts of distribution change on genetic diversity

This work provides clear evidence for the loss of genetic diversity during current range expansion in *P. aegeria*. This is similar to the pattern commonly observed due to postglacial expansions, reduced genetic diversity with increasing distance from glacial refuga; as expected due to the essential similarity between current and post glacial range expansion. Recent work examining microsatellites and allozymes have shown declines of genetic diversity in *P. aegeria* consistent with post glacial expansion in continental Europe (Vandewoestijne & Van Dyck 2010). The degree to which other species will exhibit the same pattern of reduced genetic diversity at expanding range margins will be determined by the balance between habitat availability and species dispersal ability as indicated by Hill *et al.* (2006). The principal difference between current and post glacial range changes is habitat availability, which is widely acknowledged as a major force affecting patterns of global biodiversity (MA 2005). The reduction and fragmentation of habitat, particularly in Europe and North America due to agricultural intensification, will increase the probability of losses of genetic diversity even among more dispersive species. Small and/or fragmented populations, which occur due to habitat loss, are at greater risk from environmental perturbations, the consequences of population bottlenecks and reduced genetic diversity. Though there is some evidence that at local scales changes in weather patterns associated with climate change may alter behaviours in some ectothermic species leading to increased dispersal rates. Cormont *et al.* (2011) demonstrated increased flight duration and colonisation rates in four butterfly species. Behavioural adaptations like these may buffer against losses of genetic diversity and localised population extinctions due to more movement between local habitat patches. There is also evidence that some insects will benefit from less severe winters (Bale & Hayward 2010; Takeda *et al.* 2010). The stink bug, *Nezara viridula*, exhibits greater overwinter survival and reach a reproductive state earlier in the year under simulated climate warming conditions (Takeda *et al.* 2010). These advantages could offset the negative impacts of reduced genetic diversity and help

maintain larger populations limiting the impact of genetic drift. Other than lost genetic diversity, dispersal through highly fragmented habitat is likely to lead to more genetically homogeneous populations at species expanding distribution margins, due to the increased incidence of long distance dispersal events (Bialozyt *et al.* 2006). This pattern was evident in Chapter two where the lowest average genetic divergence between populations was observed at the range margin. The scenario of increased genetic homogeneity due to range expansions would mean that populations at the core of a species' distribution would become more valuable from a genetic perspective. This is because margin populations only represent a limited subset of the genetic diversity retained in the core populations.

The long term fate of genetic diversity within margin populations will be governed by mutation and gene flow from populations at the core. The continued existence of post glacial clines in genetic diversity and genetic differentiation strongly suggests these patterns are resilient to change. Post glacial range change is believed to be the main determinant of patterns of genetic diversity and differentiation in the North American butternut tree (*Juglans cinerea*) (Hoban *et al.* 2010) even after accounting for dramatic population declines in the 20th century. Therefore it appears that reductions in genetic diversity caused by current distribution shifts may remain a feature of margin populations for a very long time. Barriers to gene flow that could maintain low genetic diversity include competitive advantage due to local adaptation of residents, such localised resistance to parasites as has been demonstrated in translocation experiments with sticklebacks (MacColl & Chapman 2010). There are also costs to dispersal which can limit a migrant's performance, as seen in martins, marine bryozoans and fish (Johnson *et al.* 2009; Shima & Swearer 2010; Burgess & Marshall 2011); particularly if dispersing into established populations, further limiting gene flow. Further investigation into the long term stability of population genetic diversity would be interesting and valuable to determine the long term impact of lost genetic diversity. This could be achieved by re-sampling the same sites used in this study after a known time interval and comparing the change in genetic diversity over time.

In contrast to the reduction in genetic diversity at expanding range margins the work presented in Chapter five does not indicate any loss of genetic diversity at species contracting range margins. The study of *E. aethiops* did not demonstrate a reduction of genetic diversity at the southern range margin relative to the core, though this finding only holds true if the assumption that genetic diversity at the range margin was not greater than the core prior to the distribution contraction. This could be the case if the species expanded

from a central European glacial refuge (Hewitt 2000; Schmitt & Hewitt 2004). Therefore it is important to further investigate whether this assumption is representative of reality, and test how applicable these findings are to other species at contracting warm edge range margins. Comparisons of genetic diversity in museum samples of garden tiger moths with extant populations have revealed losses of genetic diversity associated with the species dramatic distribution contraction (Anderson *et al.* 2008). Which shows that over sufficient periods of time or with large range contractions losses of genetic diversity may occur. The analysis did highlight genetic differentiation between the core and margin of *E. aethiops* distribution. This suggests that continued range contractions will result in the loss of genetically differentiated populations. Where genetic differentiation coincides with morphological or physiological adaptation to local environmental conditions such losses would be most damaging to a species. As indicated in Chapter five the southern populations of *E. aethiops* will be lost before the species is listed as a priority for conservation in the UK. This highlights that if genetic diversity is to be conserved in species with contracting distributions action will be required sooner rather than later, to counter this impact of climate change. Some efforts are being made to address this issue; for example the genetic diversity of the endangered aquatic mayfly, *Ameletus inopinatus*, was mapped and compared to the projected distribution based on the expected climate in 2080 (Taubmann *et al.* 2011). The projections indicate a contraction of the species distribution but this work allows conservation efforts to be concentrated on areas with high genetic diversity and sufficient suitable habitat, thus minimising the impact of the distribution decline and associated losses of genetic diversity.

6.2.1 Impacts of changes in genetic diversity

Whilst there is clear evidence that some species will lose genetic diversity as a result of climate change, the impact of these changes on population fitness and adaptability remain uncertain. An investigation into the link between genetic diversity and demographic trends on butterflies in Europe (Schmitt & Hewitt 2004) has suggested a correlation between low genetic diversity and declining populations. This suggests that declines in genetic diversity make species more vulnerable to environmental disturbances. Though the analysis conducted by Schmitt & Hewitt (2004) suffers from the limitation that it did not account for differing degrees of habitat disturbance across Europe. Direct assessment of the effect of genetic diversity on population performance in *P. aegeria* was not possible due to the issues outlined in Chapter four. The evidence from both Chapters three and four indicate that both core and margin populations of *P. aegeria* exhibit a wide range of phenotypes.

This does not suggest that there have been any morphological limitations placed on margin populations. Therefore future climate change is unlikely to more adversely affect either region due to selection for particular morphological traits. It has been suggested that for Roesel's bush cricket (*Metrioptera roeselii*) limited gene flow and isolation increase the rate of adaptation to local optima along a latitudinal size cline (Cassel-Lundhagen *et al.* 2011). Conversely high gene flow limits adaptation in this case. If this were also true for *P. aegeria* then margin populations which exist in a more fragmented landscape with lower gene flow may benefit in the short term from quicker adaptation to local optima. Due to enhanced persistence of populations at the range margin, as a result of their adaptation to local conditions. The greater survival of margin butterflies reported in Chapter four indicates that they will suffer no greater mortality as the climate warms compared to the distribution core. All the morphological traits examined in this thesis are polygenic traits which respond to the interaction of many genes with the environment and are therefore buffered against selection (Frankham *et al.* 2002). Limited selection enhances the probability that much of the functional genetic diversity can be retained at the range margin. Therefore the populations could retain an equivalent genetic potential to adapt to temperature changes, despite losses of neutral genetic diversity. The impact of lost neutral genetic diversity is of greater concern for long term adaptability to novel environmental conditions, acting on genes that are currently selectively neutral.

It had originally been intended that Chapter four would also investigate indicators of both very early mortality (egg hatching rates) and reproductive performance (egg fertility, female laying rate and lifetime fecundity). Temperature has been strongly linked to changes in ovarian dynamics (Gibbs *et al.* 2010) and may have been fruitful avenue of investigation. This was not possible due to the issues addressed in Chapter four, and is unfortunate as these are traits also linked to reduced performance in association with losses of genetic diversity (Saccheri *et al.* 1998). The use of successfully hatched larvae, as occurred in Chapter four, actively selects for a pool of relatively healthy individuals. Individuals that suffer from significantly deleterious combinations of alleles are more likely to suffer infant mortality, a scenario that occurs more frequently in populations with low heterozygosity and high inbreeding (Frankham *et al.* 2002). It would be highly valuable to examine differences in these traits to more fully understand the impact of lost genetic diversity on population performance. It is also the case that performance was only tested under a range of temperatures which is only one feature of the potential future climate. As it has been suggested that *P. aegeria* is principally limited by moisture at its

range margin (Schweiger *et al.* 2006; Oliver *et al.* 2009); so a comparison of responses to water stress may highlight some impacts of lost genetic diversity.

6.3 Impacts of temporal changes on selection

Selection pressure is not constant through time. Adaptations can lose their selective advantages due to changing environmental pressures. Butterflies from older *Melitaea cinxia* populations, which exists as a meta-population, are less dispersive than newly founded populations (Hanski *et al.* 2002). Some species of cricket have exhibited greater proportions of long winged individuals in new populations, these adaptations are lost within 5-10 years reverting to proportions of long winged individuals found in well established populations (Simmons & Thomas 2004). These cases suggest selection has favoured improved dispersal among individuals founding new populations but following colonisation the selective advantage is lost; presumably due to a trade off between reproduction and dispersal. The result would be a decline in adaptations linked to dispersal. This phenomenon has not been directly observed in *P. aegeria* but the results presented in Chapter 3 suggest this may be the case. Wild butterflies from margin sites did not show a significant difference from the core in terms of thorax mass; in contrast to previous laboratory based work (Hughes *et al.* 2003). One of the reasons for this is the smaller thorax masses observed at the oldest margin site. The individuals from which were most similar to butterflies from the distribution core as opposed to the other margin site. If this is due to a reduction in selection for improvements in dispersal ability after populations become establishment then a cline of reduced investment in thorax mass would be expected with increasing site age. Determining if this hypothesis is fulfilled would provide an interesting example of the dynamic nature of selection and could account for the potentially confounding effect of site age on the results presented in Chapter 3.

6.4 Morphology and metabolism related to dispersal

A growing body of empirical work examining trees (Cwynar & Macdonald 1987), frogs (Phillips *et al.* 2010), crickets and butterflies (Simmons & Thomas 2004; Hughes *et al.* 2007) supports the hypothesis that distribution change selects for traits which facilitate

dispersal. Some of these cases infer dispersal ability from variations in morphological characters. The third and fourth Chapters of this work rely, in part, on the inference that morphological characters are related to dispersal ability in *P. aegeria*. Whilst there is clear evidence they are related to flight performance (Berwaerts *et al.* 2002; Berwaerts *et al.* 2008) there is no direct evidence for their use as correlates of dispersal ability and the ability to found new populations. If dispersal relies on a sequence of relatively short flights to move between distant habitat patches, perhaps aided by the use of poor quality habitat, then flight ability seems likely to be a significant factor in dispersal. Alternatively if dispersal involves chance long distance movements or requires undirected movement through uninhabitable areas correlating flight ability to dispersal ability becomes conceptually less satisfying. In this scenario metabolic factors may have a greater influence; perhaps the ability to maintain flight for longer periods or reduce energy requirements during flight would enhance the probability of successfully dispersing long distances. Recent work has illustrated that variation in dispersal ability is associated with metabolism and different alleles of the enzyme phosphoglucose isomerase in some butterflies (Niitepold *et al.* 2009; Mitikka & Hanski 2010). Differences in flight metabolic activity have been detected between alleles of this enzyme which explain differences in dispersal performance. It has also been indicated that there is little impact on the resting metabolic rate for these allelic variants in the Glanville fritillary butterfly (*Melitaea cinxia*) (Niitepold 2010). If the selective cost of maintaining these genotypes in non-dispersive butterflies is low they could act as drivers of dispersal at range margins through changes in the frequencies of the alleles in the population as it expands into new locations. The same study by Niitepold (2010) also indicates that the performance of the PGI alleles is correlated with temperature. Heterozygotes perform best at low temperatures while homozygotes perform better at high temperatures. In a scenario of increasing temperatures there could be a push towards the fixation of this allele, resulting in a more dispersive population during periods of high temperature. There are also sex specific differences on the impact of PGI genotype of flight metabolism in the Glanville fritillary (Niitepold *et al.* 2011). Females have a positive relationship between peak flight metabolic rate and flight duration; the relationship is negative in males. The implication is that female butterflies are better at between population dispersal, improving population establishment rates and increasing the reproductive value of migrants. The influence of enzyme variations on dispersal and flight remains unexplored in *P. aegeria* and it could prove a valuable avenue of research. If there were differences in the metabolic performance of margin populations

of *P. aegeria* it could partially explain the inconsistent differences in morphological traits observed in this study.

6.5 Implications and Conclusions

The results presented in this thesis supports the hypothesis that genetic diversity can be lost due to current distribution shifts, and importantly indicates that the losses can be large (~ 50% reduced genetic diversity at *P. aegeria's* expanding distribution margin). This result is likely to be applicable to species with relatively restricted habitat requirements coupled with a limited propensity for dispersal. Highly dispersive species or habitat generalist species are likely to maintain greater genetic diversity due to greater gene follow during distribution shifts. Either due to greater dispersal between isolated populations or less restrictive habitat use maintaining larger and better connected populations. No evidence was found for reduced performance at the range margin in terms of survival associated with this loss of genetic diversity. Therefore the loss of genetic diversity is unlikely to negatively affect range margin sites in the short term and should not be considered a cause of concern regarding the ability of *P. aegeria* to adapt to climate change; though further investigation would be valuable. The long term impacts of the losses of genetic diversity in range margin populations are much less clear and will depend on the interaction of gene flow, demographic processes, stochastic events and the nature of their future environment.

This study found no support for localised adaptation to temperature at *P. aegeria's* cool-high-latitude range margin. Therefore future climate change is unlikely to differentially affect populations at the current core and margin of the species distribution. Unless specific adaptations to extreme environmental conditions not assessed in this work have been lost or occur at very low frequencies in margin populations. Adaptation to current and future changes in climate could be achieved through phenotypic plasticity and behavioural adaptation, rather than evolutionary change, which would further buffer the potential impacts on different populations. Morphology related to dispersal ability was similar in core and margin populations of wild caught *P. aegeria*, in contrast to differences observed between laboratory reared butterflies from the same regions. This suggests environmental factors during development are a major influence on the morphological characters investigated, limiting the impact of the genetic differences suggested by the

laboratory reared populations. It is therefore arguable that morphological features are poor correlates of dispersal ability during range expansion.

Reduced genetic diversity was not apparent at the contracting range margin of *E. aethiops*. This indicates that genetic diversity is not affected by range contraction in this species, and therefore will not act as a factor driving local extinctions as the distribution contracts due to climate change. Though additional work is required to confirm that this is the case, and determine how applicable the result is to other species. If this result holds true for other species that are declining due to climate change then loss of genetic diversity will have very limited impacts on rates of population extinction or range contraction. Genetic diversity may become a factor for species that find their available habitat more fragmented as their distributions contract but are able to persist over long time periods. This could result in an erosion of genetic diversity due to drift over time compounded by the reduction in gene flow that is associated with habitat fragmentation. The work did highlight the potential to lose genetically distinct populations during range contractions and the need for advanced planning if these losses are to be averted.

Appendix 1 – DNA extraction and AFLP protocol

Described below is the complete protocol used for DNA extraction and AFLP analysis for all samples in this study:

Dissection & DNA Extraction

DNA was extracted from the thorax tissue of each male butterfly. An ammonium acetate methodology was used for DNA extraction. This method was used as the DNA in the tissues was of good quality and available in large quantities, hence other more expensive techniques were not necessary. Approximately one third of each individual's thorax was dissected and placed in a 1.5 ml eppendorf tube; the remaining tissue was retained in storage at -70^{o}C in case further extractions were required. Digsol buffer solution (250 µL, pH 8.0) and Proteinase K (10 µL at 10 mgml^{-1}) was added to the thorax tissue. These were then vortexed to mix the solution and placed in a water bath at 55^{o}C for three hours. Once digested (straw coloured solution) 300 µL of 4M ammonium acetate (pH 7.5) solution is added to each tube. The samples are then vortexed several times for at least 15mins at room temperature. After this time the samples are centrifuged at approximately 11,337 g (or 13,000 rpm in a centrifuge with a 6 cm radius) for 10 min to force the protein and other tissues down to the base of the tubes. The supernatant is then transferred into clean labelled eppendorfs and 1 ml of 100% ethanol is added to each. The samples are then inverted several times to help precipitate the DNA out of solution, at which point they are then centrifuged again for a further 10 min at ~11,337 g to force the DNA to form a pellet at the base of the tube. The remaining ethanol is then poured off without losing the pellet of DNA. 500 µL of 70% ethanol is added and the tubes inverted to wash the pellets, they are then centrifuged for a final time at ~11,337 g for 5 min to ensure the pellet has not dislodged. The ethanol is then poured off and the samples left to dry. Once dry 50 µL of 10 mM Tris, 0.1 mM EDTA, pH 8.0 (Low TE) is added, the tubes are flicked to dislodge the pellet, then placed in a water bath at 65^{o}C for 30 min to dissolve the DNA.

The concentration of extracted DNA is determined using a Nanodrop Spectrophotometer and each sample is then diluted to a 10 ng/ml solution using Low TE. This is then ready for use in the AFLP procedure.

AFLP Protocol

Initially all the samples are digested with a pair of restriction endonucleases, EcoRI and MseI; 2 µL of DNA was added to 1.1 µL of 10x TA buffer, 0.1 µL of BSA (30 $mgml^{-1}$), and 0.1 µL of each of EcoRI and MseI (10 $U\mu L^{-1}$). This solution was maintained at 37^{o}C for 3 hr, to allow complete restriction of the sample DNA. Next 5.5 µL of ligation master mix was added to each digestion and kept at 4^{o}C for at least 12hrs. The master mix consisted of 3 µL of autoclaved distilled water, 1 µL of 5x T4 ligase buffer, 0.5 µL of T4 DNA ligase (1 $U\mu L^{-1}$), 0.5µl of both EcoRI and MseI adaptors (50 µM). The adaptors consist of equal volumes of each of the forward and reverse adaptors corresponding to the same endonuclease; which had been heated to 95^{o}C for 3mins and allowed to cool prior to use. This was to allow the two complimentary sequences to anneal together. After 12hrs had passed the ligation mix was diluted with 50 µL of autoclaved distilled water and was then ready for the pre-selective and selective PCR's.

For the pre-selective PCR 2 µL of ligation mix were added to 3.65 µL of autoclaved distilled water, 1 µL of 10x PCR buffer, 0.3 µL of $MgCl_2$ (50 mM), 1 µL of dNTP (2 mM), 1 µL of each of the two primers (5 µM) and 0.05 µl of Taq DNA polymerase (5 $U\mu l^{-1}$). The PCR conditions were as follows; 72^{o}C for 2 min; twenty cycles of 94^{o}C for 20 sec, 56^{o}C for 30 sec, 72^{o}C for 2 min; then 72^{o}C for 10 min. The PCR product was diluted 1 in 50. From the diluted PCR product 1 µL is added to 9 µL of selective PCR master mix. The master mix consists of 4.65 µL of autoclaved distilled water, 1 µL of 10x PCR buffer, 0.4 µL of $MgCl_2$ (50 mM), 1 µL of dNTP (2 mM), 1 µL of each of the two primers (5 µM) and 0.05 µL of Taq DNA polymerase (5 $U\mu l^{-1}$). The PCR conditions for the selective PCR were; 94^{o}C for 2 min; ten cycles of 94^{o}C for 20 s, 66^{o}C for 30 s and 72^{o}C for 2 min, with a decrease in temperature of 1^{o}C per cycle; 25 cycles of 94^{o}C for 30 s, 56^{o}C for 30 s and 72^{o}C for 3 min; then 72^{o}C for 10 min. For the *P. aegeria* samples two selective bases on the *MseI* primer were used as they produced a suitable number of peaks on the AFLP profile; while three selective bases were used for the *E. aethiops* & *M. jurtina* samples as they produced a greater number of peaks during the screening of potential primer pair combinations. The product from the selective PCR's was diluted 1 in 4 prior to plate set up for fragment analysis.

Plates for fragment analysis were run on an ABI 3130 Genetic Analyser with 0.5 µL of diluted PCR product in each well with 9.5 µL of size standard mixed with formamide. The ABI LIZ 600 size standard was used for the analysis, with 10 µL of size

standard per 1 ml formamide; enough for one plate of 96 samples. Each plate was heated to 95^{o}C for 3 mins then quenched on ice prior to fragment analysis.

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