CLIMATE CHANGE AND HABITAT ASSOCIATIONS AT SPECIES' RANGE BOUNDARIES

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ABSTRACT

Species are more restricted in their habitat associations at their leading-edge range margins where climatic conditions are marginal. Hence they are predicted to broaden their associations in these locations as the climate warms, potentially increasing habitat availability and rates of range expansion. I analysed long-term distribution records (collected by volunteers) and abundance data (UK Butterfly Monitoring Scheme transect data) to investigate how the habitat and host plant associations of two butterfly species that reach their leading-edge range margins in Britain have changed over 40 years of climate warming. The speckled wood (*Pararge aegeria*) is primarily associated with woodland but its habitat associations vary spatially and temporally. I found that this species has a weaker association with woodland in warmer parts of Britain, particularly in regions with warm and wet summers. Over time, its occurrence outside of woodland has increased most where summer and winter temperatures and summer rainfall have increased the most. Field experiments showed that larval performance is poorer in open (grassland) than closed (woodland) habitats, associated with microclimatic differences between habitats. Thus I conclude that slower population growth rates outside woodland play an important role in driving the observed variation in habitat associations. The brown argus (*Aricia agestis*) was previously restricted to using rockrose (*Helianthemum nummularium*) as its larval host plant in Britain, which grows in locations with warm microclimates. I have shown that warmer summers have allowed it to increase its use of Geraniaceae host species, which occur in cooler locations. Geraniaceae species are widespread and so habitat availability has increased substantially for the butterfly, leading to extremely rapid range expansion in this species. Species are broadening their habitat and host plant associations at their leading-edge range margins in response to climate change, resulting in substantial increases in rates of range expansion.

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DECLARATION

I declare that this thesis is my own work and was written by myself, except where specific references have been given to the work of others.

Chapter 5 is based on the published paper:

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CHAPTER 1

GENERAL INTRODUCTION

1.1 CONTEMPORARY CLIMATE CHANGE

The Intergovernmental Panel on Climate Change (IPCC) stated in its most recent assessment report that “warming of the climate system is unequivocal, as is now evident from observations of increases in global average air and ocean temperatures, widespread melting of snow and ice and rising global average sea level” (IPCC 2007). They also stated that current warming is “very likely due to the observed increase in anthropogenic greenhouse gas concentrations” (IPCC 2007).

Mean annual temperatures are estimated to have increased globally by 0.74 °C in the hundred year period from 1906 to 2005, with the rate of increase rising over time and the majority of change occurring after the 1960s (IPCC 2007) (Fig. 1.1). Although the rate of warming has declined in the last decade, after 1998 the next nine warmest years on records were all in the decade 2001-2010, with 2008 being the thirteenth warmest and 2011 the twelfth warmest on record (Brohan et al. 2006, Jones 2012). There is, however, regional variation in observed climate change, with temperature increases being greatest at high latitudes and over continents. Changes in precipitation are also inconsistent among regions, with some, in particular at high latitudes, seeing an increase in rainfall, while others, such as the mid-tropics, experiencing a decrease in rainfall (IPCC 2007). The frequency of extreme events, such as heat waves and heavy precipitation events, have also increased over time in some regions (IPCC 2012).

My thesis concentrates on the biological impacts of climate change in Britain where mean summer temperatures have increased at a rate of 0.28 °C per decade from 1960 to 2010 and mean winters temperatures at a rate of 0.23 °C per decade (MetOffice 2012). Total annual precipitation has also increased throughout Britain during this period (MetOffice 2012).

It is projected that temperature and rainfall patterns will continue to alter over the next century following similar regional patterns to those observed over the past several decades (IPCC 2007). In Britain, it is projected that mean annual temperatures will increase by around 3 °C in the south and 2.5 °C further north by 2100 compared to the 1960-1990 baseline (MetOffice 2012). Annual rainfall is projected to increase by up to 10 % in the north but perhaps decrease in the south of Britain (MetOffice 2012). Uncertainties in projections arise from model inaccuracies and, while there is a commitment to warming for at least the rest of the 21st century due to greenhouse gases already released into the atmosphere and to lags in the global system from thermal inertia in the oceans (Meehl et al. 2005, Wigley 2005), the magnitude of changes will also depend on levels of future greenhouse gas emissions (IPCC 2007, Washington et al. 2009).

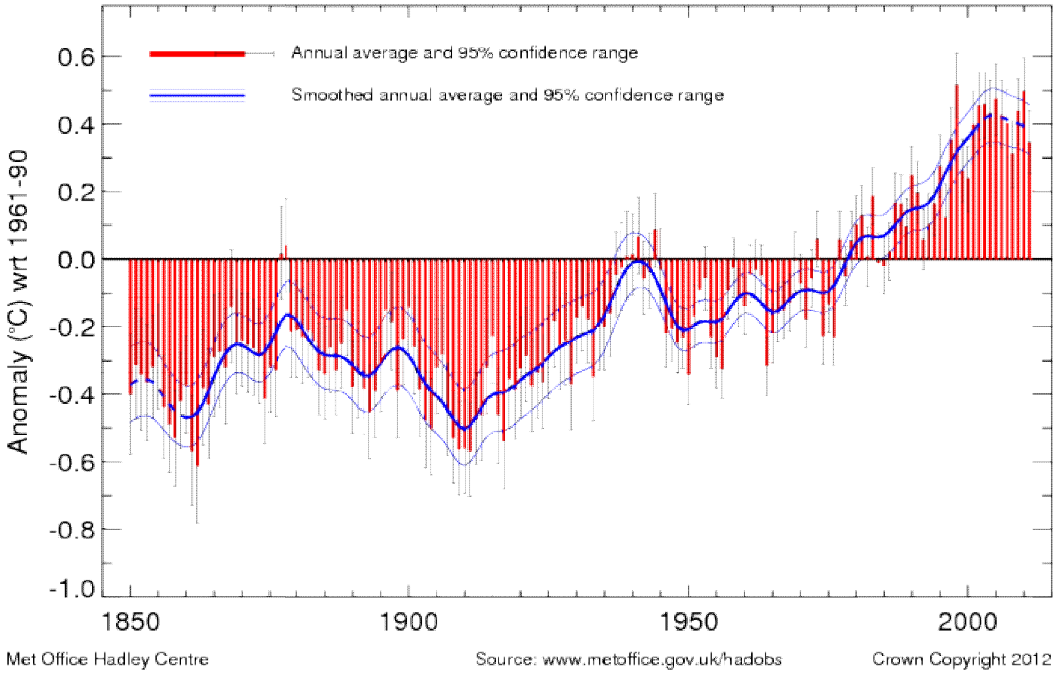

Fig. 1.1 Global annual average temperature anomaly, relative to mean 1961-1990 conditions, based on the HadCRUT3 method of calculation (Brohan et al. 2006).

1.2 ECOLOGICAL IMPACTS OF CLIMATE CHANGE

The effects of climate change on biological systems are now evident (Hughes 2000, Walther et al. 2002, Parmesan and Yohe 2003, Root et al. 2003, Parmesan 2006, Rosenzweig et al. 2007). The most commonly reported responses are shifts in species' distributions to higher latitudes and altitudes and changes in the timing of events. However, species are responding at different rates leading to changes in biotic interactions (Visser and Both 2005, Memmott et al. 2007, Van der Putten et al. 2010). Changes in the abundance, physiology, behaviour and morphology of species in response to climate change may also disrupt biotic interactions (Gilman et al. 2010, Traill et al. 2010), leading to novel communities being created (Berg et al. 2010, Lavergne et al. 2010), with potential consequences for ecosystem functioning (Montoya and Raffaelli 2010, Walther 2010). My thesis concentrates on species' range shifts in response to climate change. In this introduction I discuss how climate limits species' distributions and hence why species are shifting their distributions in response to climate change. I also consider why species are shifting their distributions at different rates before discussing how changes in species' habitat associations might affect rates of range expansion at species' leading-edge range margins.

1.2.1 Climate as a factor limiting species' distributions

A species' distribution is limited by its ecological niche, a concept formalised by Hutchinson (1957) who described a species' fundamental niche as an "*n*-dimensional hypervolume", every point of which corresponds to a state of the environment which would permit a species to persist. The dimensions of a species' fundamental niche are the abiotic and biotic factors required by the species for positive growth. A species can only persist within certain limits of each of these factors e.g. within certain temperature limits, and so this represents one dimension of the species’ niche. Other abiotic factors that may define dimensions of a species’ niche include pH, light, CO_2, and salinity; and biotic factors include host and prey species. In combination these factors define the locations in which a species can occur.

Species may not, however, be at equilibrium with their fundamental niche. The locations in which a species actually occurs are termed its “realised” niche. Populations may, for example, occur in locations outside their fundamental niche in “sinks” where $r<0$ and populations can only persist by immigration from “source” populations (Pulliam 1988). Conversely, locations within a species’ fundamental niche may not be occupied because of dispersal barriers, as is apparent from the success of species introduced to locations beyond their natural range boundary. Within species’ range boundaries, limitations to dispersal may also result in species being absent from patches of suitable habitat (Thomas et al. 1992). Interactions with other species, such as interspecific competition, predation and parasitism, may also limit species’ distributions, as is evident from enemy or competitive release e.g. in invasive species (Keane and Crawley 2002).

It is generally accepted that climate is an important determinant of the range boundaries of the majority of terrestrial animal species (Thomas 2010). Evidence comes from relationships between a species’ distribution and geographic variation in climatic conditions (known as distribution, climate envelope or niche modelling) (Pearson and Dawson 2003), and shifts in geographic ranges as the climate changes (Parmesan et al. 1999). Such approaches have gained criticism because correlation between different climatic variables, and with non-climatic variables, makes it difficult to disentangle causal factors (Gaston 2003, Parmesan et al. 2005, Beale et al. 2008). Other evidence comes from the observation that many species become restricted to particularly warm habitats at cool leading-edge range boundaries or to cool habitats at warm trailing-edge range boundaries, suggesting thermal constraints (Thomas et al. 1999). Laboratory and translocation experiments have also been used to demonstrate directly that individuals are unable to survive the climatic conditions beyond their range boundary (Baskauf and McCauley 2001, Crozier 2003).

Climate can directly limit species' ranges (Hodkinson 1999), for example due to direct effects of extreme cold (Iversen 1944, Baskauf and McCauley 2001, Crozier 2003) or desiccation (van Herrewege and David 1997) or because thermal availability for successful life cycle completion is insufficient (Bryant et al. 1997). However, species are also limited through the indirect effects of climate on biotic interactions. For example, climate may limit the distribution of a species which is an essential resource for another species, and hence limit its distribution also (Hellmann et al. 2008). A species' ability to compete for resources, or to withstand attack from predators or natural enemies, may also be altered by climatic conditions and hence limit its distribution (Davis et al. 1998b).

1.2.2 Range shifts in response to climate change

As the climate changes, most evidence suggests that species do not adapt to new climatic conditions *in situ* but instead shift their distributions to track their climatic niche or go extinct. Evidence from fossils and palynological data reveal that species shifted their ranges under past climatic changes (Davis and Shaw 2001, Huntley 2005). Over the past few decades evidence has shown species from a wide range of taxa, including arthropods, mammals, birds, fish and plants are shifting their ranges to higher latitudes and altitudes (Parmesan and Yohe 2003, Hickling et al. 2006, Chen et al. 2011a). Meta-analyses by Parmesan and Yohe (2003) and more recently by Chen et al.

(2011a) found that the vast majority of species studied that have shifted their ranges have done so in the direction expected from climate change, with this response being termed a "fingerprint" of climate change (Walther et al. 2005). The more recent meta-analysis by Chen et al. (2011a) also increased estimates of the average rate of range expansion from 6.1 km per decade to higher latitudes and 6.1 m per decade to higher altitudes (Parmesan and Yohe 2003) to 16.9 km per decade and 11.0 m per decade, respectively. Moreover, this study showed that distribution shifts have been most rapid in regions where climatic warming has been greatest (Chen et al. 2011a), further supporting the notion that climate change is driving these shifts.

Increasingly, the mechanisms of these shifts are being identified, adding to the evidence that climate change is driving shifts in distribution. Changes in abundance and distribution are ultimately driven by the effect of environmental conditions on the physiology of individuals themselves, or on individuals with which they interact (Helmuth et al. 2005). In some cases, warming climates have had a direct effect on survival, such as increased overwinter survival of individuals at cool range boundaries (Crozier 2004, Battisti et al. 2005). In other cases, warmer climates have facilitated population establishment in new areas which meet thermal thresholds for oviposition (Davies et al. 2006). At trailing edge range boundaries, increased risk of desiccation has been identified as an important factor in driving extinctions and range retractions (Merrill et al. 2008). Trailing-edge extinctions may also arise from changes in biotic interactions. For example, extinctions of populations of the Edith's checkerspot butterfly (*Euphydryas editha*) at its warm trailing-edge boundary in Mexico are related to increased asynchrony between the butterfly and its host plant, leading to the starvation of larvae (Parmesan 1996, McLaughlin et al. 2002, Parmesan 2005).

Evidence for climate-driven expansions at leading-edge range margins far outweighs that for retractions at trailing-edge range margins (Chen et al. 2011a, Sunday et al. 2012), but some trailing-edge extinctions have been attributed to climate warming (Wilson et al. 2005, Franco et al. 2006, Thomas et al. 2006). Differences in rates of detection of climate effects at trailing- and leading-edge range margins may arise from analysing coarse-scale data where extinctions may be more difficult to detect than colonisations because many local population extinctions may be required before a grid cell is empty (Thomas et al. 2006). However, retractions at trailing-edge range margins may actually be proceeding more slowly than expansions at leading-edge range margins (Chen et al. 2011b, Sunday et al. 2012). This may occur because individuals are able to persist by exploiting small-scale environmental heterogeneity at their warm range margin (Gillingham et al. 2012). Alternatively, abiotic factors other than temperature or biotic factors may be more important in limiting trailing-edge range margins and so if species' thermal tolerances are not at equilibrium with their range margins they will be less sensitive to climatic warming and range retractions will not be triggered (Sunday et al. 2012).

1.2.3 Risks to biodiversity from climate change

Climate change is predicted to result in high rates of extinction if species are unable to shift their distributions to track suitable climatic conditions (Peterson et al. 2002, Thomas et al. 2004, Thuiller et al. 2005). Species may be able to ameliorate some of the effects of climate change through physiological acclimatisation (Botkin et al. 2007) or behaviourally through the exploitation of different microclimates in areas with complex topography or habitat structure (Kearney et al. 2009, Gillingham et al. 2012). Micro-evolutionary changes may also aid species' adaptation. In *Drosophila subobscura* populations, increases in the frequency of genotypes with higher heat tolerances have been observed (Rodríguez-Trelles et al. 1996, Rodríguez-Trelles and Rodríguez 1998).

However, such changes have acted on existing variation within species and there is little evidence to suggest that species can evolve physiological tolerance to temperatures above the current limits of the species (Bradshaw and Holzapfel 2006, Parmesan 2006). Climatic changes are likely to exceed species’ physiological tolerances within parts of their range and species will lose range extent or become extinct if they are unable to shift their distributions or do so slowly and “lag” behind climate change. High latitude and montane species and those at the edges of continents are most at risk because they are unlikely to be able to track suitable climatic conditions (Midgley et al. 2002, Raxworthy et al. 2008, Forero-Medina et al. 2011). Small range species with narrow thermal tolerances (climate specialists) are also likely to be particularly at risk (Sandel et al. 2011, Bonebrake and Deutsch 2012). Furthermore, species in the tropics tend to be closer to their upper thermal limits and have poorer acclimatory capacities, giving them less potential to adjust to climatic changes. Thus species in the tropics may be at higher risk of extinction from climate change than temperate species (Addo-Bediako et al. 2000, Calosi et al. 2008, Deutsch et al. 2008, Calosi et al. 2010).

Modelling and translocation studies suggest that many species are lagging behind climate (Hill et al. 2002, Willis et al. 2009, Devictor et al. 2012), as is species richness (Menéndez et al. 2006). In their meta-analysis, Chen et al. (2011a) revealed large variation in the rate at which species have shifted their distributions, consistent with species responding individualistically, as detected in historical data (Huntley 1991). They also showed that many species are lagging behind climate change and that some species are retracting where they might be expected to expand. For latitudinal shifts, Chen et al. (2011a) found that around half of species are lagging behind climate change and for elevational shifts, that over 90 % are lagging behind climate change (surprising given the shorter distances required to keep pace with climate change along elevational gradients) (Chen et al. 2011a).

For conservationists, identifying factors that generate inter- and intra-specific variation in rates of range expansion at leading-edge range margins is important in order to identify those species most at risk from climate change (Williams et al. 2008) and to inform conservation measures that will help species to move through the landscape (Heller and Zavaleta 2009). Some of the factors identified as being important determinants of rates of leading-edge range expansions are discussed below.

1.2.4 Variation in rates of leading-edge range margin expansion

1.2.4.1 Physiological tolerances

Some of the variation in observed rates of range expansion may result from the fact that the climate is not changing evenly around the globe (IPCC 2007). Some variation will also result from the fact that different species are limited in their distributions by different aspects of the climate (e.g. overwinter cold, thermal availability for life cycle completion) and different aspects of the climate are changing at different rates.

1.2.4.2 Reproductive rates

Species with higher reproductive outputs are likely to be able to shift their distributions more rapidly because life history characteristics such as early reproduction, frequent reproduction and high fecundity are likely to increase propagule pressure and hence increase colonisation opportunity (Angert et al. 2011). For example, Perry et al. (2005) found that fish species that have shifted their ranges are those with faster life histories.

1.2.4.3 Habitat availability and dispersal ability

The abundance and spatial arrangement of suitable habitat beyond species' current range margins is also a key factor in determining rates of range expansion. Intraspecific variation in rates of range expansion has been explained by differences in habitat availability between regions, with range expansions being slower in areas where suitable habitat is more fragmented (Hill et al. 2001, Wilson et al. 2009). Specialist and sedentary species also have slower rates of range expansion than generalist and dispersive species because areas of suitable habitat are more difficult to reach (Warren et al. 2001, Pöyry et al. 2009, Mattila et al. 2011). Anthropogenic habitat fragmentation has restricted the ability of many species to respond to climate change (Travis 2003). Analysis of southerly distributed butterfly species in Britain, all of which might be expected to have expanded their ranges northwards as the climate warmed, showed that 34/46 of these species had declined in distribution extent (Warren et al. 2001). Moreover, specialists fared much worse than generalists (26/28 specialist species declined compared with 9/18 generalists). Changes in species richness also lagged behind climate change. Only one third of the predicted increase in richness of the British butterfly fauna has been observed (Menéndez et al. 2006), and assemblages have become increasingly dominated by generalist species (Menéndez et al. 2006).

1.2.4.4 Evolution at range margins

Rates of expansion may increase if species evolve greater dispersal ability at their leading-edge range margins (Hill et al. 2011). For example, wing-dimorphic bush crickets have increased frequency of long-winged forms in populations at leading-edge range margins (Simmons and Thomas 2004); and adult speckled wood butterflies in newly colonised areas have larger thoraxes and greater flight capacity than those in established populations (Hill et al. 1999b). Greater investment in dispersal ability, however, usually involves a trade-off with reproductive output and so dispersal is predicted to decline following colonisation (Simmons and Thomas 2004).

1.2.4.5 Biotic interactions

Changes to biotic interactions will also affect the ability of species to shift their distributions in response to climate change (Hellmann et al. 2012), for example if response rates differ between species groups or trophic levels (Berg et al. 2010). If a target species shares its current range boundary with an obligate host species with a slower rate of range expansion than the target species, then the potential future distribution of the target species will also be limited. Several butterfly species, for example, are limited by the distribution of their host plants, which may limit butterfly range shifts if their host plants fail to shift, or shift more slowly (Gutiérrez and Thomas 2000, Hellmann et al. 2008, Merrill et al. 2008).

Recent modelling studies have assessed the effects of biotic interactions on the potential future distribution of species. Schweiger et al. (2008) developed ecological niche models for Titania's fritillary butterfly (*Boloria titania*) and its host plant common bistort (*Polygonum bistorta*) to predict future available niche space under different climate change scenarios, and then used the overlap between the two distributions to better predict the potential future distribution of the butterfly. Under a scenario of unlimited dispersal for both species, a large area of overlap arises, but it is in the far north of the current distribution of the butterfly and would require long-distance dispersal to colonise. In a scenario of no dispersal, there was only a very small area where both species were predicted to overlap.

In this way, biotic interactions are often viewed as being limiting factors for range shifts in response to climate change; but alterations in interspecific interactions could lead to more rapid than expected range shift. Species may experience "ecological release" if specialist parasitoids are not present or are at reduced abundance in newly colonised areas (Menéndez et al. 2008), or if herbivores, predators or competitors do not shift their ranges with interacting species. Without these interactions, population growth rates may be higher, resulting in faster rates of expansion.

1.3 THE EFFECT OF CLIMATE ON HABITAT ASSOCIATIONS

Habitat availability is a key determinant of the rate at which species are expanding their distributions at their leading-edge range margins. However, an important consideration that has largely been ignored in the literature is that species' habitat associations may change in response to climate change (Oliver et al. 2009), altering habitat availability at leading-edge range margins and consequently affecting rates of range shift (Wilson et al. 2010). My thesis investigates changes in habitat associations at species' leading-edge range margins and this section outlines the conditions under which such a response to climate change might be observed.

1.3.1 Spatial variation in species' habitat associations with climate

Many species are more restricted in their habitat associations at their leading-edge range margins compared with their range core (Lennon et al. 2002, Oliver et al. 2009, Schofield et al. 2009). Many species' distributions are limited by climate, and such changes in habitat associations may be driven by the effects of increasingly marginal climatic conditions as species approach their range edges. In a study of butterfly species that reach their leading-edge range margin in Britain, Oliver et al. (2009) found that species occupied fewer habitats in less climatically favourable regions. There are several possible explanations as to why species become more restricted in their habitat use at their leading-edge range margins, as discussed in the following sections.

1.3.1.1 Microclimatic restriction at the range edge

One hypothesis to explain these patterns is that species become restricted to habitats or micro-habitats with particular microclimates when climatic conditions are marginal for them. For example, if conditions at leading-edge range margins are close to the limit of a species' physiological tolerances to extreme cold temperatures, they may become restricted to particular habitats that provide refuge from these extremes and thus support populations while the rest of the landscape is unsuitable (Strathdee and Bale 1995). Similarly, species have thermal requirements for growth and reproduction and so populations at leading-edge range margins may become restricted to habitats with particularly warm microclimates that meet these requirements. Many ectotherms that reach their cool, leading-edge range margins in Britain, for example, are restricted to southerly-facing slopes and/or low-growing vegetation often with large amounts of bare ground (Thomas 1983, Cherrill and Brown 1992, Thomas 1993, Thomas et al. 1999, Bourn and Thomas 2002) which provide the warmest microclimates in the landscape (Rorison et al. 1986, Weiss et al. 1988, Thomas et al. 1999, Suggitt et al. 2011). This compares with core parts of species' distributions where climatic conditions are less limiting and so populations can occupy habitats with a wider range of microclimates. In central Europe, where spring and summer temperatures are warmer than in Britain, these species occupy sites across a wider range of aspects and with a wide range of vegetation heights (Thomas 1993).

In addition to latitudinal gradients, studies have also demonstrated changes in the microclimatic distribution of individuals along altitudinal gradients. Ashton et al. (2009) demonstrated changes in microclimatic associations of larvae of the butterfly *Parnassius apollo*, from sheltered microhabitats at low elevations to open areas with bare ground and reduced vegetation height and scrub cover at high elevations. Similar patterns were found in dung beetle habitat associations on mountains in northern Spain;

at low altitudes species were generally associated with closed, woodland habitats but as altitude increased they became increasingly associated with open pastures (Menéndez and Gutiérrez 2004). Merrill et al. (2008) examined the location of eggs laid by the black-veined white butterfly (*Aporia crataegi*) along an elevational gradient and found that eggs were located on the north side of plants (corresponding to cooler microclimates) at low elevations whereas they were located on the south side of plants (corresponding to hotter microclimates) at higher elevations.

At species' leading-edge range margins, populations are less stable than those at the range core (Thomas et al. 1994, Curnutt et al. 1996), temporal fluctuations are more strongly synchronised than at the range core (Powney et al. 2010) and population turnover rates are higher than at the range core (Doherty Jr et al. 2003), supporting the role of climatic factors. These abundance patterns may arise because additional habitats become microclimatically suitable in years with particularly favourable climatic conditions, increasing carrying capacity and population size. By contrast, there is no opportunity for individuals to move to warmer microhabitats in particularly cool years because they are already limited to the warmest microhabitats in the landscape, and so all populations at the range edge crash. Thus, relatively modest changes in climatic conditions can lead to wide, synchronous fluctuations in population size and high turnover rates (Powney et al. 2010, Oliver et al. 2012a). Populations at the range core, however, are unlikely to be constrained in their habitat associations by climate and may have more capacity for microclimatic buffering and so population size may not fluctuate as much as at the range margin, and changes in habitat availability may not be as dramatic.

1.3.1.2 Changes in density from range core to edge

Narrowing of habitat associations from range core to leading-edge range margin could alternatively arise from a reduction in population density. Theory predicts that populations decline in density from their range core to their range edge as conditions become increasingly unfavourable (Brown 1984). It might be expected, therefore, that at high densities at the range core individuals are more likely to experience density-dependent pressures from competition for resources or territories or aggregation of natural enemies and hence emigrate from their preferred habitat type into subordinate ‘sink’ habitats (Pulliam 1988, Sutherland et al. 2002). At the range core, therefore, species may display a greater breadth of habitat associations compared with their range edges where population densities are low and individuals can remain in their favoured habitat type.

Evidence for declines in density as species approach their range edges (the “abundant centre hypothesis”) is, however, equivocal. It has been recognised that the pattern of population abundances across species’ ranges can take a number of different forms (Lawton 1993, Gaston 2003). Whilst a decrease in abundance from range core to range edge has been confirmed in some species (Hengeveld and Haeck 1982, Svensson 1992, Brown et al. 1995), other species have been shown to have high population abundances at their range edge (Prince et al. 1985, Curnutt et al. 1996). Sagarin and Gaines (2002) cautioned against adopting this as a general biogeographic rule. Local population density is likely to be determined by many different factors and so smooth clines in population density from range core to edge may not be universally observed (Thomas et al. 1998a). Thus it is unclear whether variation in density within species’ ranges will be an important factor determining variation in habitat associations.

1.3.1.3 Resource quality

Species may be limited to habitats which provide high quality resources at their leading-edge range margins if these are the only sites that support positive population growth when the climate in the region is marginal, and density-independent factors result in general increases in mortality or reduced natality (Lennon et al. 2002). Bumblebee species that reach their leading range edge in Britain, for example, are associated with areas of species-rich vegetation which may be because these are the only habitats where individuals can collect sufficient nectar for colonies to persist when thermoregulatory costs are high (Williams 1988).

1.3.1.4 Biotic interactions

Increased habitat restriction at range boundaries could also be driven by changes in biotic interactions from range edge to core. The desert orange tip butterfly (*Colotis evagore*), for example, is limited to certain coastal sites at its northern range boundary in the south of Spain (Jordano et al. 1991). This butterfly does not enter diapause and so requires larval host plants on which to feed all year round. The locations in which the butterfly persists are in a narrow frost-free coastal zone where the butterfly's host plant *Crataegus spinosa* is able to provide a continuous food supply for larvae. The warm winter microclimate of these sites is also likely to meet the butterfly's thermal threshold for feeding.

Some insect herbivores at their leading range edges feed on only a subset of the host plants they utilise in their range core. They may become restricted to those plant species on which development is fastest (Nylin et al. 2009), or with which they have greatest phenological synchrony because time for development is limited and these are the only host species on which they can successfully complete their life cycle (Hodkinson 1997, Scriber 2002).

1.3.2 Changes in species' habitat associations in response to climate change

Due to relationships between geographic variation in climate and habitat use, it is predicted that species will broaden their habitat associations at their leading-edge range margins as the climate warms. This may arise because more habitats become microclimatically suitable as the climate improves for species. Such patterns have been observed on shorter temporal scales; for example, the Adonis blue butterfly (*Polyommatus bellargus*) becomes restricted to a narrower range of microhabitats for oviposition during the cool autumn generation compared with the warm late spring generation, leading to greater carrying capacity and population size in late spring (Roy and Thomas 2003). Broadening of habitat associations at leading-edge range margins may also arise if population densities increase as the climate becomes more favourable leading to spillover into sink habitats; or because a reduction in climate-related mortality or costs allows colonisation of inferior habitats. Changes in biotic interactions may also be observed, for example if climatic changes improve phenology synchrony between a herbivore and alternative hosts these may be incorporated into its diet.

Changes in habitat use have been observed at the leading-edge range margin of the silver-spotted skipper butterfly (*Hesperia comma*). This species had become very rare in Britain by the 1980s (Thomas et al. 1986) due to loss of sites with its host plant growing in the specific microclimatic conditions required to meet its thermal threshold for oviposition (locations surrounded by bare ground and in sheltered sun spots on southerly-facing slopes). However, the butterfly has expanded its distribution recently as habitat availability has increased due to improvement in grazing regimes and recovery of rabbit populations (Davies et al. 2005) and because climatic warming has resulted in more locations meeting the thermal conditions required by ovipositing females (Thomas et al. 2001). The butterfly now occupies sites on a wider range of different aspects, and has less demand for bare ground, which has increased the number of locations with host plants available for egg-laying (Davies et al. 2006).

Changes in habitat associations at species' leading-edge range margins have consequences for rates of range shifts. Fewer locations in the landscape meet species' requirements at their leading-edge range margins, and so populations are more sparsely distributed in the landscape compared with the range core (Svensson 1992, Thomas et al. 1998a). As the climate changes and habitat associations are less restricted, habitat availability will increase. Thomas et al. (1999) used information on the habitat associations of four heathland species at the core of their range in central France to predict how habitat availability might change at the edge of their range in Britain as the climate warms. For the silver-studded blue butterfly (*Plebejus argus*), they estimated that under a 2-3 °C warmer climate, the current heathland landscape could provide 4-20 times more habitat patches within the metapopulation, each patch 2-4 times the size of existing patches and one-half to one-eighth the distance apart. This increase in habitat availability and decrease in habitat fragmentation would be likely to increase rates of range shift.

Wilson et al. (2010) used metapopulation models to estimate rates of range shift in the silver-spotted skipper butterfly with and without the changes in habitat use that have been observed in this species during recent climate warming. They found that increased habitat availability arising from increased availability of suitable microclimates in the landscape increased population sizes and colonisation rates of the butterfly, which in turn resulted in faster predicted range expansion rates.

1.4 THESIS AIMS

Thus expansion of habitat associations at species' leading-edge range margins has the potential to enhance the ability of species to expand their distributions and hence could reduce the threat from climate change. Identification of alterations in species' habitat requirements as the climate changes is also important as this may require habitat management prescriptions for focal species to be adapted to ensure long-term conservation of species. Such patterns have, however, so far only been described from one species (silver-spotted skipper butterfly; Davies et al. 2006) and so it is important to establish whether such changes are occurring in other species to determine whether this might be a widespread pattern. The mechanisms driving the relationship between climate and species' habitat associations have also rarely been investigated and information is required to determine what these might be. Furthermore, the magnitude of the effect of broadening habitat associations at species' leading-edge range margins on rates of range expansion is largely unknown and information is required on this to assess whether this will be an important factor in driving rates of range expansion. The main aims of my thesis are, therefore:

- To establish the effects of climate on the spatial and temporal patterns of species' habitat associations at leading-edge range margins.
- To evaluate the mechanisms driving the relationship between climate and species' habitat associations.
- To assess the effect of changes in habitat associations on rates of leading-edge range margin expansion.

Chapter 2

My thesis focuses on two butterfly species that reach their leading-edge range margins in Britain: speckled wood (*Pararge aegeria*) and brown argus (*Aricia agestis*). In Chapter 2, I provide background information on their distributions and ecologies in Britain.

Chapter 3

In Chapter 3, I examine relationships between climate and the habitat associations of the speckled wood butterfly. I examine how the butterfly's habitat associations have changed through time and which climatic factors are important in affecting these associations.

Chapter 4

The mechanisms driving relationships between climate and habitat associations remain largely unknown. In Chapter 4 I investigate whether microclimatic differences between woodland and open habitats in winter and summer drive the spatial and temporal variation in the habitat associations of the speckled wood butterfly, via direct effects of climate on butterfly survival and performance.

Chapter 5

In Chapters 3 and 4 I examine changes in the use of broad habitat types in response to climate change. Species also become restricted in their host plant use towards their leading-edge range margins but it is unclear whether they will be able to incorporate other species into their diet as the climate changes. In Chapter 5 I investigate whether climate warming has led to an increase in the number of host plant species used by the brown argus butterfly at its expanding range margin in Britain. This butterfly has shown an unexpectedly rapid rate of expansion in Britain, and I examine the effect that changes in host plant availability have had on range expansion over the past 30 years. I also examine possible mechanisms that have driven changes in host plant use.

Chapter 6

I summarise the main findings from Chapters 3-5 and discuss my findings in relation to my original aims. I discuss the wider implications of my findings, in particular the consequences for conservation management under climate change and priorities for research in the future.

CHAPTER 2

INTRODUCTION TO STUDY SPECIES

2.1 BUTTERFLIES AS STUDY SPECIES

Butterflies have played a key role in the study of the biological impacts of climate change (Menéndez 2007). This is partly because, as short-lived ectotherms, they are likely to respond rapidly to changes in environmental conditions (Bale et al. 2002). In addition, thanks largely to their attractive nature and ease of sampling in the field, butterflies have been the centre of ecological investigation for many decades. Thus there is a wealth of information available regarding individual species' ecologies. Also available in Britain is information relating to the distribution of individual species dating back to the 19th century (Heath et al. 1984, Asher et al. 2001, Fox et al. 2006, Fox et al. 2011) and records of population abundances from transects starting in 1976 (Pollard and Yates 1993). Such long-term datasets are invaluable when studying the impacts of global change and I have used both extensively throughout my research. My study of the effects of climate on habitat associations at range boundaries has focussed on two species, the brown argus (*Aricia agestis*) and speckled wood (*Pararge aegeria*) butterflies, for which background information is given in the following sections. These species were selected because they have been reported to show spatial and temporal variation in their habitat associations, but the drivers of these changes are unknown.

2.2 SPECKLED WOOD

2.2.1 Speckled wood distribution and ecology

2.2.1.1 Distribution

The speckled wood butterfly (*Pararge aegeria*) (Linnaeus, 1758) (Fig. 2.1) belongs to the subfamily Satyrinae within the family Nymphalidae and is widely distributed throughout Europe, North Africa and east to the Urals (Asher et al. 2001). The distribution of speckled wood in Britain has altered over time (section 2.2.2).

Fig. 2.1 Speckled wood butterfly (photo credit: sannse).

2.2.1.2 Life cycle

Voltinism of the speckled wood butterfly varies throughout its range in relation to temperature, with continuous generations in northern Africa and southern Spain through to one generation per year in central Sweden (Nylin et al. 1995). In Britain, the butterfly usually has two flight periods per year (Fig. 2.2), and is unusual amongst butterflies in that in Britain and southern Sweden it can overwinter in both larval and pupal life stages. Overwintering larvae do not enter a true diapause in Britain, but individuals can resume feeding and continue to develop when temperatures rise above their development threshold of around 6°C (Blakeley 1996), unlike those in southern Sweden where conditions are colder and larvae enter a “true” diapause (Wiklund and Friberg

2011). Variation in over wintering development stages of individuals and the ability of larvae to resume feeding when conditions are suitable over winter, results in a protracted emergence of adults in spring and through into early summer. The earliest spring-emerging adults (which over-wintered as pupae) lay eggs which commonly develop through to generate another adult generation in late summer or early autumn; these individuals will then lay eggs and give rise to over-wintering larvae. In contrast, the early summer adults (which over-wintered as larvae) give rise to progeny that will commonly overwinter as pupae. In particularly warm years there is a small third adult emergence in the autumn (Shreeve 1986b). Developmental pathway (direct development, larval diapause or pupal diapause) is determined by photoperiod and temperature cues (Shreeve 1986b, Nylin et al. 1989, 1995, Wiklund and Friberg 2011). This life history means that adults can be seen on the wing anytime between March and October in south and central Britain (Asher et al. 2001). Further north in Scotland, where the climate is cooler, the length of the flight period is reduced (Asher et al. 2001), with the species showing both temporal and geographic variation in patterns of emergence (Hodgson et al. 2011).

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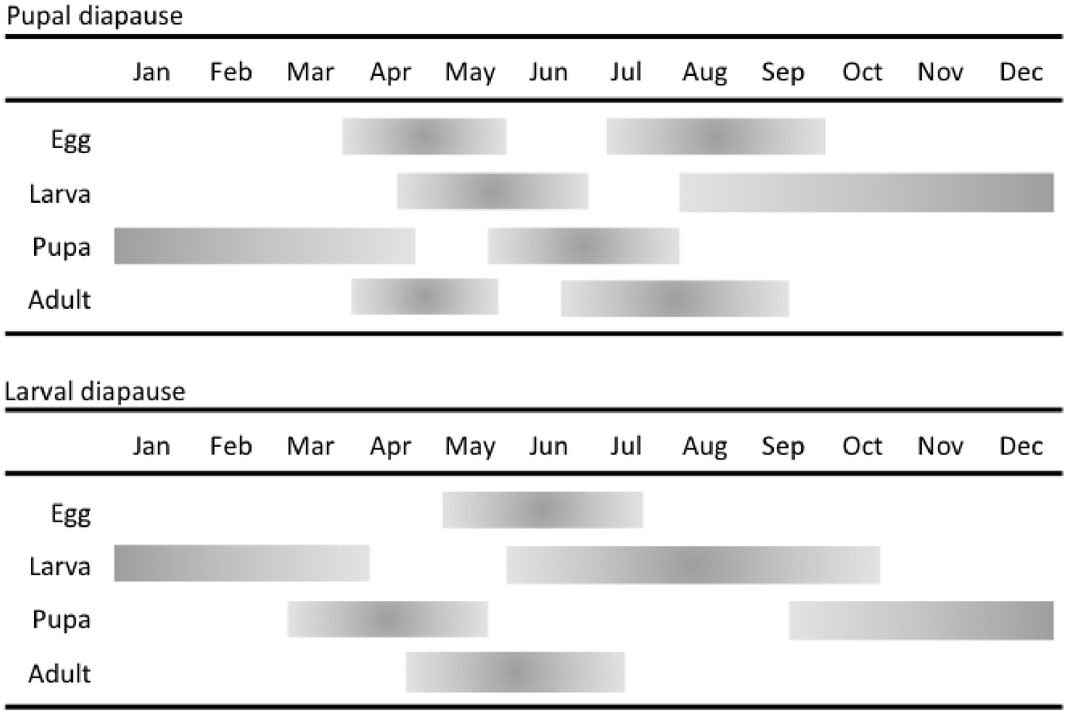

Fig. 2.2 Life cycle of the speckled wood butterfly in Britain. Typical developmental pathways of individuals that overwinter as larvae and pupae and their offspring are displayed separately. Differences in development times due to microclimatic variation mean that some individuals may follow different pathways, however. For example, the fastest developing individuals that overwintered as larvae may produce rapidly-developing individuals that are able to complete a full summer generation with adults that emerge in late summer. In particularly warm years there may also be a small third generation in September and October. Overlap between emergences mean that speckled wood can be seen on the wing from April through to early autumn.

Females usually mate once soon after emergence (Wickman and Wiklund 1983). Males adopt one of two strategies for locating females: they either perch in large sunlit patches waiting for females to pass, or they patrol between sun patches searching for females. Males can switch between strategies; generally perching is favoured in cooler conditions because patrolling results in greater heat loss (Shreeve 1984). Males, therefore, commonly adopt a perching strategy earlier in the year, when conditions are cooler (Wickman and Wiklund 1983) and spring-emerging adults are morphologically adapted for this strategy (Van Dyck and Wiklund 2002). Perching males are highly territorial and fiercely defend large sunspots, which are in short supply, against competing males (Davies 1978). Large sunspots may provide an advantage because males can more easily visually detect passing females (Bergman et al. 2007, Bergman and Wiklund 2009) and because they provide a thermal advantage so males have a higher thoracic temperature and can more readily pursue females (Velde et al. 2011).

Females lay eggs singly on a range of grass species including cocksfoot (*Dactylis glomerata*), false brome (*Brachypodium sylvaticum*), Yorkshire fog (*Holcus lanatus*) and couch grass (*Elytrigia repens*) (Asher et al. 2001). These grasses are all common and widespread species throughout Britain and so host plant availability is unlikely to be a limiting factor for speckled wood's distribution (Fig. 2.3). Females select specific microclimatic conditions for egg-laying. In spring and autumn the hottest available locations are used for egg-laying, whereas in summer both the coolest and hottest locations are rejected, and thus females maintain laying temperatures within the range of 21-30 °C throughout the year (Shreeve 1986a). Females also favour wet areas for egg-laying (Wiklund and Persson 1983) which is thought to be to reduce the risk of egg or host plant desiccation. Larvae rest under grass blades and feed on leaf edges and pupation occurs on the host plant or on vegetation nearby (Asher et al. 2001).

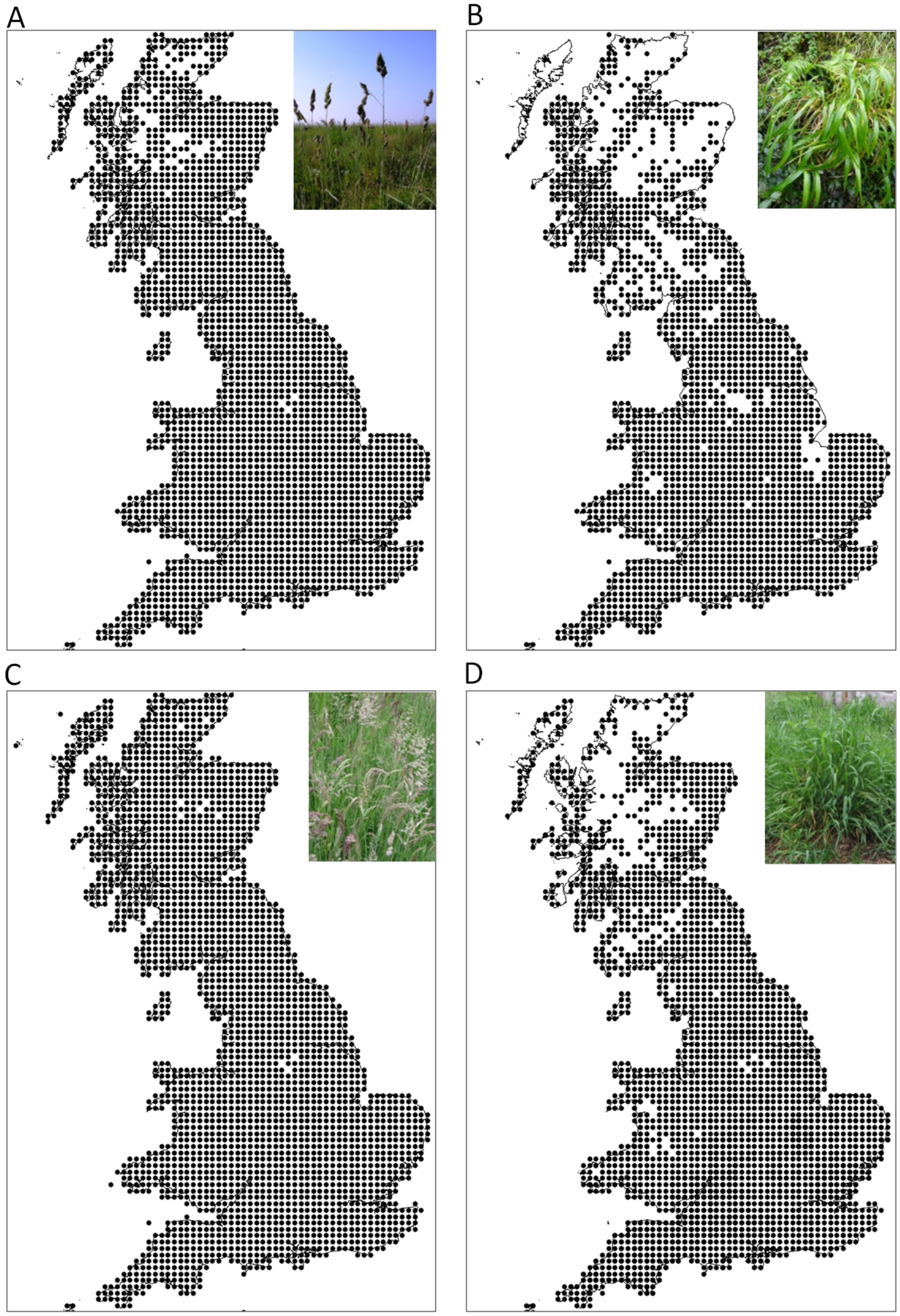

Fig. 2.3 Distribution of four of the speckled wood butterfly’s host plants in Britain (10 km × 10 km resolution records submitted to the Botanical Society of the British Isles from 1987 onwards). (A) Cocksfoot (photo credit: Sebastian Bieber), (B) false brome (photo credit: Pere prilpz), (C) Yorkshire fog (photo credit: Robin Stott) and (D) couch grass (photo credit: Rasbak).

2.2.1.3 Population dynamics

Speckled wood butterflies form large populations of up to several hundred adults in woodland or along wooded tracks (Asher et al. 2001). Hill et al. (2003) estimated the maximum dispersal rate of speckled wood butterflies to be 1.03-2.7 km per year from rates of climate-related range shifts. However, the average dispersal distance of individuals within the range is likely to be reduced compared with the invasion front where individuals have evolved increased dispersal abilities, and where expansion rates may be determined by the movements of the most vagile individuals (Hill et al. 1999b, Hughes et al. 2003). Data from mark-release-recapture of individuals in Belgium showed that many individuals fly less than 300 m and only a few more than 1000 m (Berwaerts et al. 1998). However, speckled wood has been able to colonise the island of Madeira, whose nearest landmass is 540 km away, perhaps carried on a strong air current, although there is the possibility of accidental or deliberate human introduction (Owen et al. 1986). Speckled wood populations decline in years following hot, dry summers (Pollard 1988, Roy et al. 2001, Morecroft et al. 2002), again suggesting that this species is sensitive to host plant desiccation.

2.2.2 Recent range expansion

In Britain, the speckled wood butterfly has undergone marked range shifts over the past 200 years (Asher et al. 2001). For most of the nineteenth century it was widely distributed throughout Britain, reaching as far north as central Scotland, but at the end of the nineteenth century and beginning of the twentieth century its range contracted and it essentially became restricted to south-west England and Wales as well as to a localised area of western Scotland (Downes 1948, Asher et al. 2001, Hill et al. 2001). In the 1920s the butterfly’s range began to re-expand (Asher et al. 2001) and this has continued into the 21st century (Fox et al. 2006) (Fig. 2.4). In England, its distribution has expanded as far north as Northumberland and the butterfly has also expanded from its Scottish refugia, but it has not yet recolonised all of its previous distribution (Fox et al. 2006). The butterfly's range has also extended northwards elsewhere in Europe, including in Denmark, the Netherlands and Sweden (Parmesan et al. 1999).

These range shifts are associated with changes in climatic conditions. From the 1930s onwards the climate has generally been warming and the ranges of many butterfly species have expanded northwards (Parmesan et al. 1999, Asher et al. 2001, Fox et al. 2006), but with slower or no expansion during cooler periods. However, it appears that its leading-edge range margin expansion is lagging behind climate change (Hill et al. 1999b) which is likely to be due to the fragmentation of suitable habitat reducing colonisation success (Hill et al. 2001).

Population densities within the speckled wood's range have also generally increased over the twentieth century (Fox et al. 2006). As well as changes in climatic conditions, as a shade-loving species, speckled wood may also have benefitted from the decline in coppicing during the 20th century (Asher et al. 2001).

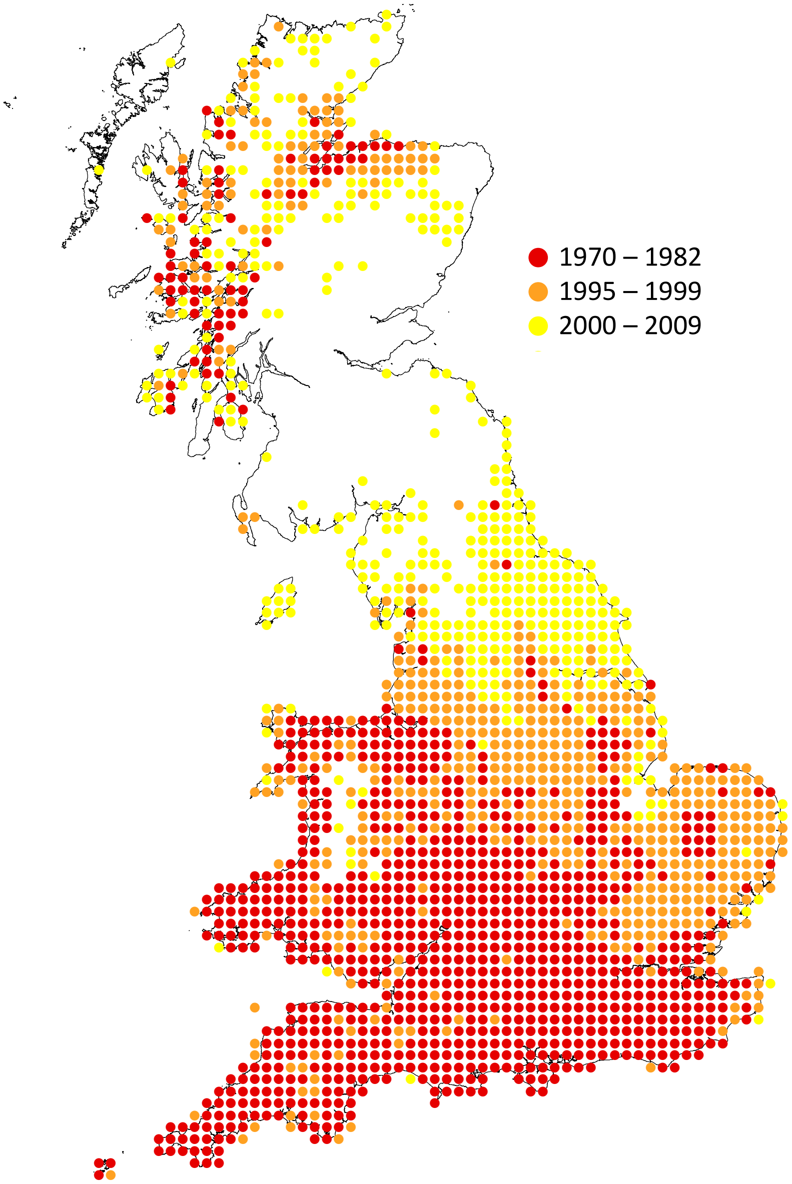

Fig. 2.4 Recent range expansion of the speckled wood butterfly. Symbols represent the time period in which speckled wood butterfly was first recorded in10 km × 10 km grid squares. Time periods correspond with intensive recording periods coordinated by Butterfly Conservation and associated with the publication of national atlases in 1970-1982 (Heath et al. 1984), 1995-1999 (Asher et al. 2001); and 2000-2004 (Fox et al. 2006) and 2005-2009 (Fox et al. 2011) combined.

2.2.3 Habitat associations

Speckled wood is primarily a butterfly of woodland throughout its range, but in some regions it also appears to occur in more open landscapes, such as parks and gardens and hedgerows in agricultural landscapes (Asher et al. 2001, Merckx et al. 2003). “Woodland” and “agricultural” populations of the speckled wood butterfly in northwest Europe have been the subject of much study, primarily in terms of understanding the impacts of habitat fragmentation on individual life history, morphology and behaviour.

Microclimatic conditions differ between woodland and agricultural habitats (Chen et al. 1993, Morecroft et al. 1998, Suggitt et al. 2011) and there is some evidence that this has effects on the morphology and behaviour of adults. For example, at low temperatures females originating from woodland have a higher maximum daily fecundity and lifetime number of eggs than those from agricultural landscapes; and this pattern is reversed at higher temperatures (Karlsson and Van Dyck 2005). Females from agricultural populations also have furrier thoraces than those from woodland (Merckx et al. 2008), and males from agricultural landscapes have darker wings than those from woodland (Berwaerts et al. 1998, Merckx and Van Dyck 2006). These adaptations may improve heat absorption and insulation in less sheltered agricultural habitats where butterflies experience greater convective cooling (Merckx et al. 2008).

In addition to microclimatic differences, increased fragmentation of resources in agricultural habitat has led to morphological and behavioural changes. For example, individuals in agricultural landscapes have greater relative thorax mass than those in woodland (Berwaerts et al. 1998, Merckx and Van Dyck 2006). Larger thoraces have been related to greater investment in flight ability (Dempster et al. 1976), and so larger thoraces in agricultural landscapes probably reflect a requirement for greater foraging distances as resources are more fragmented. Individuals from agricultural landscapes also have better habitat-finding abilities than those from woodland, to aid location of habitats from a greater distance (Merckx and Van Dyck 2007).

Woodland is the preferred habitat of individuals originating from both woodland and agricultural landscapes (Merckx et al. 2003) and agricultural habitats apparently provide poorer quality habitat for speckled wood than does woodland, at least within the climatic regions studied. For example, juvenile mortality rates are higher in agricultural habitats than woodland (Merckx and Van Dyck 2006). Females tend to lay smaller eggs under high temperatures and when they have to fly more (Gibbs et al. 2010c, Gibbs et al. 2010b, Gibbs and Van Dyck 2010), which are conditions more typical of agricultural environments. Smaller eggs have longer development times, lower hatching success and reduced desiccation resistance, and give rise to larvae with longer development times and higher risk of infection (Gibbs et al. 2010c, Gibbs et al. 2010b, Gibbs et al. 2010a) supporting the notion of lower survival in agricultural habitats. Reduced desiccation resistance of eggs in agricultural landscapes is likely to be particularly detrimental in these hotter, less humid environments. Host plant desiccation also appears to have more severe effects on offspring of individuals from agricultural landscapes; on drought stressed plants, larvae from agricultural habitats have longer development times and produce smaller adults which lay eggs with lower hatching success compared with individuals from woodland habitats (Gibbs et al. 2011b).

2.2.4 Thesis aims for speckled wood

The degree to which speckled wood occurs outside woodland varies throughout its range. It appears to be more restricted to woodland in parts of its range with cold winters, such as the north of Britain and Sweden, suggesting a role of temperature (Asher et al. 2001, Gibbs et al. 2011c). However, it is also more restricted to woodland in Catalunya, Spain, than it is in Britain, which may be related to summers being hotter and drier in Catalunya (Suggitt et al. 2012). It is also thought that the butterfly has relaxed its association with woodland in northwest Europe over recent decades (Merckx et al. 2003).

Despite anecdotal evidence of spatial and temporal variation in the habitat associations of the speckled wood butterfly, such patterns have not been tested empirically and the mechanisms driving this variation are unknown. My aims are, therefore:

- To establish whether there is spatial variation in the association of speckled wood with woodland and whether this association is related to spatial variation in climatic conditions.
- To establish whether habitat associations have changed over time and whether changes are related to changes in the climate.
- To investigate the mechanism(s) driving variation in habitat associations, specifically whether microclimatic differences between woodland and non-woodland habitats affect the survival and performance of speckled wood larvae.

This work is presented in Chapters 3 and 4.

2.3 BROWN ARGUS

2.3.1 Brown argus distribution and ecology

2.3.1.1 Distribution

The brown argus butterfly (*Aricia agestis*) (Dennis & Schiffermüller, 1775) (Fig. 2.5) is a member of the family Lycaenidae. It is distributed throughout Europe, North Africa, the Middle East and Siberia. It reaches its northern leading-edge range boundary in Denmark, southern Sweden and southern Britain. In the north of Britain a closely related species, the northern brown argus (*Aricia artaxerxes*), is present. Morphologically the two species are very similar, although in Scotland the northern brown argus has a medial white spot on the forewing (Fig 2.5). Analysis of mitochondrial DNA shows the two species belong to two distinct genetic lineages (Aagaard et al. 2002, Mallet et al. 2011) but analysis of nuclear DNA sequence data has revealed a 150-200 km band of *Aricia* populations in northern England and Wales which show evidence of hybridisation between the two taxa (Fig. 2.5) (Mallet et al. 2011). Hybridisation may have occurred as long ago as during rapid warming following the last post glacial period 11500 yrs ago or during more recent range shifts in the past few hundred years (Mallet et al. 2011). However, the distribution data we have from these species indicates that hybridization would not have been active during the 20th century. The southern boundary of this hybrid zone also represents the transition between bivoltine *Aricia* populations in the warmer south and univoltine populations in the north, where time for life cycle completion is more limited (Burke et al. 2005) (Fig. 2.5).

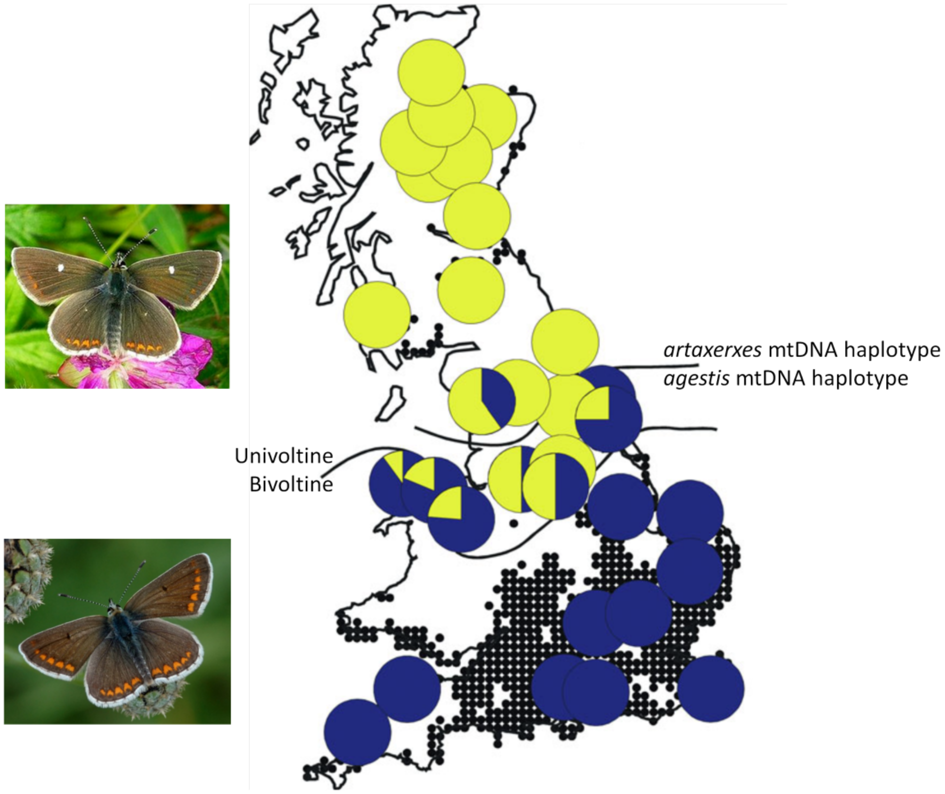

Fig. 2.5 Distribution of *Aricia* populations in Britain adapted from Mallet et al. (2011). Pie charts show proportions of individuals from different populations with *artaxerxes*-type (yellow) and *agestis*-type (blue) nuclear DNA identified by Mallet et al. (2011). The northern of the two lines shows the transition from *artaxerxes*-type mtDNA in the north to *agestis*-type mtDNA in the south. The southern line shows the transition in generation number; univoltine to the north of the line and bivoltine to the south. Small black circles represent 10 km × 10 km Ordnance Survey grid squares with *Aricia* records.

2.3.1.2 Life cycle

In my research I have considered only “pure” *Aricia agestis* populations south of the zone of hybridisation. In all of these populations, adults have two flight periods each year, one from early May to the end of June and another from mid-July to mid-September (Fig. 2.6). Adult females lay eggs singly on the leaves of their host plants, common rockrose (Cistaceae; *Helianthemum nummularium* (L.) Mill.) and species of the Geraniaceae family. Eggs are usually laid on the underside of leaves, although some are laid on the upper surface, especially on Geraniaceae plant species. Females laying on common rockrose select plants growing in sheltered locations, and on large, lush leaves, with thick mesophylls and high nitrogen content (Bourn and Thomas 1993). These leaves may have greater nutritional value, in particular when soil moisture content is low. Larvae feed by day on the underside of leaves and are attended by ants, although the benefit gained from this is unclear (Asher et al. 2001). Those larvae from eggs laid by females in the spring flight period develop quickly before pupating and emerging as adults in late summer. Larvae from eggs laid during the late summer flight period develop more slowly and in the autumn hibernate close to the ground. They pupate the following spring before emerging as adults during the spring flight period.

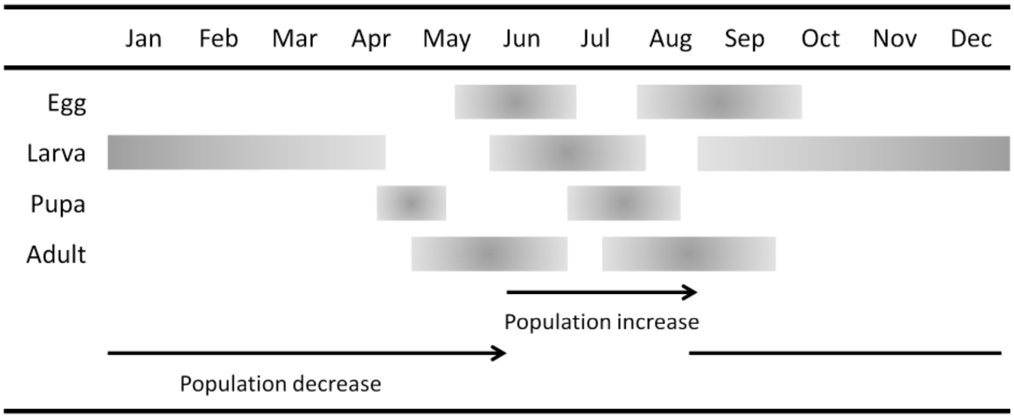

Fig. 2.6 Life cycle of the brown argus butterfly in Britain.

2.3.1.3 Population dynamics

Historically, brown argus was a fairly localised species, with its occurrence largely limited by the highly fragmented distribution of its main host plant, common rockrose (Heath et al. 1984); although this has changed in recent years (see below and Chapter 5).

Brown argus appears to have fairly high levels of transfer of individuals between nearby populations (Bourn and Thomas 1993, Lai and Pullin 2005, Buckley et al. 2012). Mark-release-recapture in pure brown argus populations revealed mean dispersal distances of 114 and 89 m for females and males, respectively, with 12.5 % of females moving 320 m or further (equivalent to <1 % moving 1 km) (Bourn and Thomas 1993). However, using mark-release-recapture data from a brown argus metapopulation in north Wales (in the hybrid zone between pure southern and pure northern brown argus), it was estimated that only 3.24 % of the population travel further than 1 km and 1.27×10^{-13} % of the population would be expected to travel further than 10 km (Wilson and Thomas 2002).

Population densities fluctuate greatly between generations, with numbers generally being much higher in the second flight period of the year than the first. Annual fluctuations in population densities are also wide (Bourn and Thomas 1993) and are correlated to some extent with climatic conditions, with densities being higher in years with warm summers, in particular June to August temperatures (Roy et al. 2001). This is probably due to the limited time available for completion of the “fast” generation between the spring and summer flight periods.

Brown argus population densities in rockrose sites are low compared with other butterfly species (Bourn and Thomas 1993), especially given the high abundances that rockrose can achieve (see below and Chapter 5). This may be due to the effects of parasitoids, which seem to attack brown argus larvae at a high rate (e.g. 54 % of caterpillars collected from sites with a long established population of brown argus were parastised) (Menéndez et al. 2008). Alternatively, the abundance of the specific types of leaves selected by adult females for egg-laying may be a limiting factor (Bourn and Thomas 1993). These types of leaves may receive a high egg load which may lead to very localised intraspecific competition on individual leaves and plants during the larval stages, despite the overall density of larvae on the host plant population being low.

2.3.1.4 Host plant ecology

Common rockrose is a long-lived, evergreen perennial, flowering in June and July and shedding seeds in July and August (Grime et al. 1988). It is low-growing (usually <200 mm) and can form large mats of cover. It is strongly associated with dry, species-rich calcareous grasslands with short swards. Its distribution in Britain is, therefore, limited to regions with calcareous soils and, within these, to areas that have not undergone agricultural intensification (Fig. 2.7A).

The most commonly used species of the Geraniaceae family in Britain are common storksbill (*Erodium cicutarium* (L.) L'Her. ex Ait.) and dove's-foot cranesbill (*Geranium molle* (L.)). Brown argus has also been found to be capable of feeding on other Geraniaceae species under laboratory conditions, namely cut-leaved cranesbill (*G. dissectum*), small-flowered cranesbill (*G. pusillum*) and round-leaved cranesbill (*G. rotundifolium*) (Greatorex-Davies and Pollard 1997). In the wild, eggs have been found on mountain cranesbill (*G. pyrenaicum*) and meadow cranesbill (*G. pratense*) (Greatorex-Davies and Pollard 1997). The extent to which these species are used in the wild is unknown.

Common storksbill is an annual which flowers from mid- to late-summer. It occurs throughout Britain (Fig. 2.7B) but is restricted to well-drained sandy and rocky places, sand dunes, summer-parched grasslands and heaths (Preston et al. 2002). Dove's-foot cranesbill is a winter annual, overwintering as a rosette; it can flower between April and September and set seed between June and October (Grime et al. 1988). It is associated with disturbed habitats such as field margins, road verges and small-scale disturbances in semi-natural pastures. This plant is widespread and common in England and Wales, but has a more restricted distribution in Scotland (Fig. 2.7C).

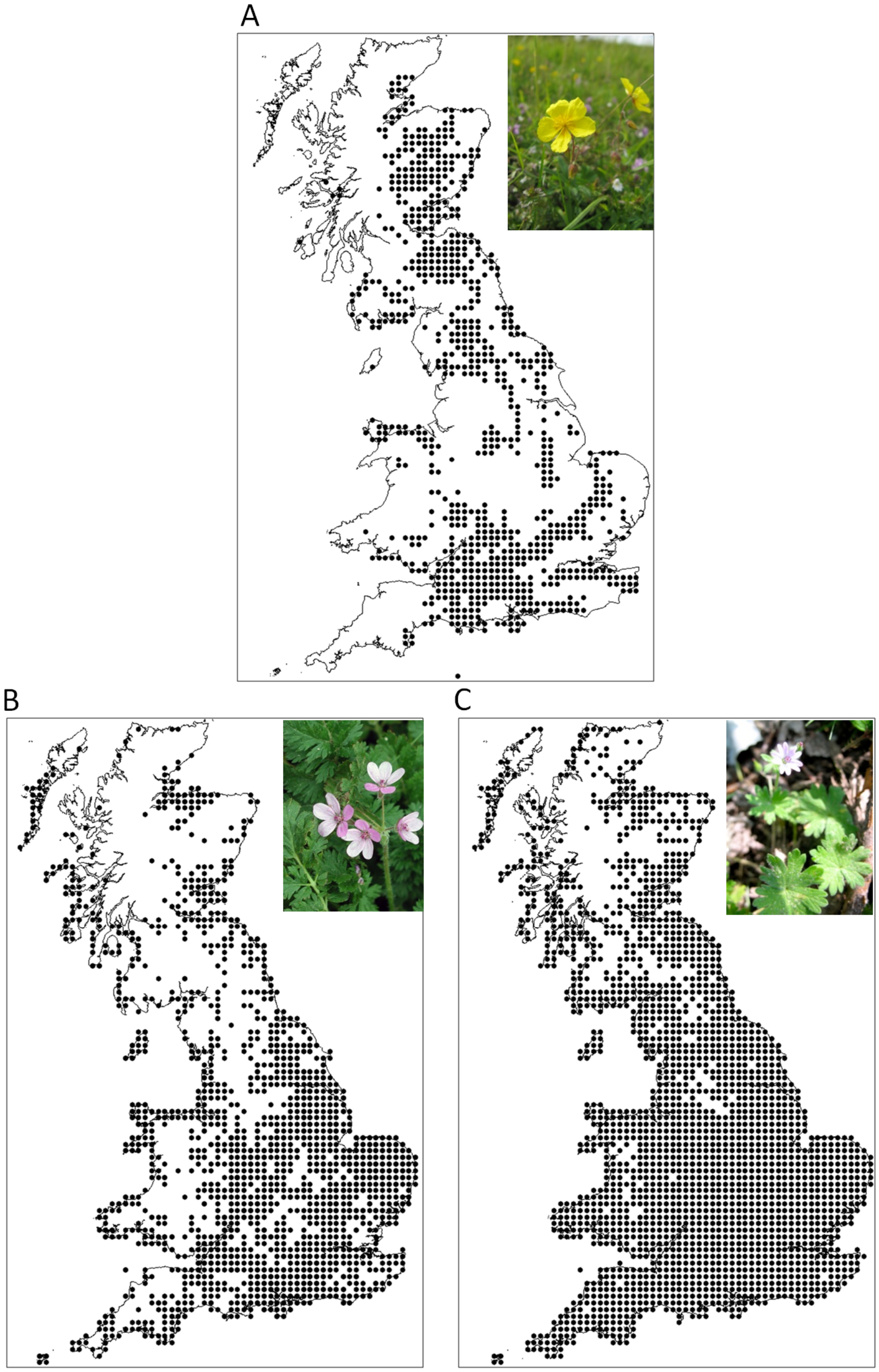

Fig. 2.7 Distribution of host plants of brown argus (10 km × 10 km resolution records submitted to the Botanical Society of the British Isles from 1987 onwards). (A) Common rockrose (photo: Rachel Pateman), (B) common storksbill (photo: Peter Llewellyn) and (C) dove’s-foot cranesbill (photo: Alison Jukes).

2.3.2 Recent range expansion

2.3.2.1 Patterns of expansion

In the 1980s in Britain, the brown argus butterfly was considered a rare and declining species with an estimated 40 % of former populations having disappeared (Bourn and Thomas 1993). However, since this time brown argus has expanded its range in Britain (Fig. 2.8) in association with climate change. Range expansion in this species has been rapid, spreading northwards in Britain by 79 km in 20 years (Chapter 5), compared with the average expansion rate documented for species globally of 16.9 km per decade (Chen et al. 2011a). Rapid range expansion was surprising in this species because it was historically considered fairly sedentary and specialist; species with these characteristics have generally not expanded their ranges during recent climate warming, unlike generalist mobile species (Warren et al. 2001).

Unexpectedly rapid range expansion could be partly due to escape from natural enemies. Menendez et al. (2008) found the rate of parasitism of brown argus larvae in newly colonised sites to be approximately half that in areas with long-established populations. Rates of range expansion are highly dependent upon the size of new populations and their intrinsic rate of increase, as this affects the likelihood of population establishment and the number of subsequent dispersers. Reduced parasitism may increase population growth rates and hence lead to higher than expected expansion rates.

However, there appears to be another factor that has contributed to rapid range expansion in this butterfly, and that is a change in the types of host plant used by this species, as discussed below.

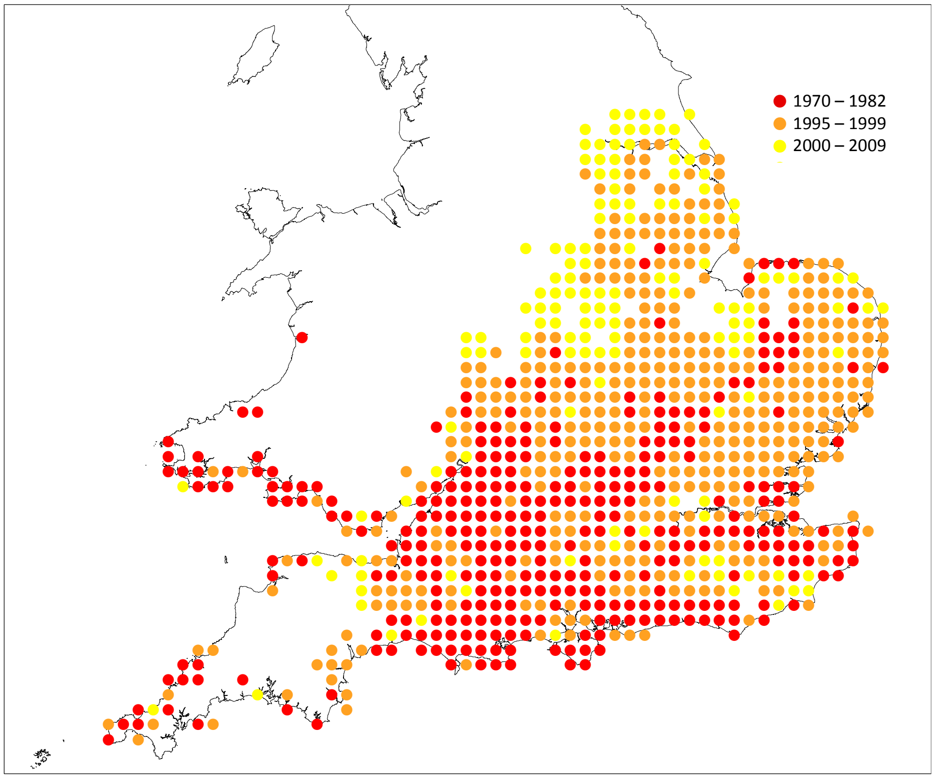

Fig. 2.8 Recent range expansion in the brown argus butterfly. Symbols represent the time period in which the brown argus butterfly was first recorded in10 km × 10 km grid squares. Time periods correspond with intensive recording periods coordinated by Butterfly Conservation and associated with the publication of national atlases in 1970-1982 (Heath et al. 1984), 1995-1999 (Asher et al. 2001), 2000-2004 (Fox et al. 2006) and 2005-2009 (Fox et al. 2011).

2.3.2.2 Use of alternative host plants

In more southerly parts of its range in Europe, brown argus uses a wide range of host plants, including common rockrose and several members of the Geraniaceae family, including *Geranium dissectum*, *G. molle*, *G. rotundifolium*, *G. purpureum*, *G. tuberosum*, *G. asphodeloides*, *G. sanguineum*, *Erodium cicutarium*, *E. acaule*, *E. chium* and *E. ciconium* (Tolman 1997, Lafranchis 2000). In contrast, in Britain, brown argus was historically largely restricted to chalk and limestone downs where it used common rockrose as its host plant. Red circles in Fig. 2.8 show the distribution of brown argus in

Britain in the period 1970-1982 and Fig. 2.7A shows the distribution of common rockrose in Britain. The two distributions correspond well, suggesting that during this period brown argus was largely restricted to rockrose-containing areas. There were also some populations of brown argus in areas where common rockrose does not occur and in these locations it is likely that brown argus was using Geraniaceae species such as common storksbill and dove’s-foot cranesbill which have very widespread occurrence in Britain (Fig. 2.7, B and C). Brown argus populations using Geraniaceae species were typically observed in coastal sand dune habitats, but also sometimes in heaths and very occasionally on clay soils, for example in woodland (Heath et al. 1984). Fig. 2.8 shows how the distribution of brown argus has expanded in Britain over the past 30 years. The butterfly is no longer restricted to areas where common rockrose occurs; its distribution has spread into areas where the only host plants available are Geraniaceae species. Why brown argus was not previously using Geraniaceae species and what has triggered an increase in their use, is unclear, and is the basis of Chapter 5. This work particularly concentrates on the role of dove’s-foot cranesbill in the recent range expansion of *A. agestis*, but the role of other host plants, especially common storksbill and cut-leaved cranesbill, warrants further investigation.

2.3.2.3 Preference and performance

Patterns of host species use by insect herbivores are traditionally viewed in terms of preference-performance relationships. Brown argus butterflies might not have historically used Geraniaceae species as hosts either because females would not lay eggs on these plant species (preference), or because their larvae did not have the physiological ability to develop on these plants (performance), or both. Therefore, it is important to assess whether there are physiological or behavioural constraints that might have prevented the brown argus from colonizing Geraniaceae hosts.

Previously-published experiments (Thomas et al. 2001), in which potted rockrose and three Geraniaceae species were placed out in the field at sites with brown argus populations, revealed that female butterflies would lay some eggs on Geraniaceae, even in populations where rockrose was the traditional host and where it was the *only* naturally-available host plant. Populations in regions of rapid northwards expansion showed a particularly strong egg-laying affinity for Geraniaceae, especially favouring dove's-foot cranesbill, the commonest host plant in those regions; this trait was retained after captive breeding, and hence was likely to have a genetic basis. A recent study has also found evidence of evolutionary change in brown argus associated with range shift and the authors suggest selection for Geraniaceae-preferring genotypes has allowed brown argus to spread rapidly through the landscape (Hanski 2011, Buckley et al. 2012). However, even colonizing butterflies have retained the capacity to lay on rockrose when they colonized previously unoccupied rockrose sites (Thomas et al. 2001). Overall, brown argus populations analyzed to date have sufficiently flexible oviposition preferences that they can lay on, and hence potentially colonize, any rockrose or Geraniaceae site encountered. This flexibility implies that egg-laying preferences were not likely to have confined the butterfly to rockrose prior to the 1980s.

Larval performance is also unlikely to have acted as a constraint on colonization success because larval performance is usually better on Geraniaceae than on rockrose. Previous laboratory experiments showed that larvae develop faster and produce heavier pupae when fed dove's-foot cranesbill compared with rockrose (average 1.2 times faster development and 1.1 times heavier pupae when reared on dove's-foot cranesbill than rockrose). This is true regardless of larval origin (i.e., whether larvae come from a population where they naturally feed on Geraniaceae or rockrose), or the temperature at which they were reared, suggesting that the increased use of Geraniaceae has not occurred because these plants are physiologically better hosts at warmer temperatures (Musche 2001, Bodsworth 2002).

2.3.3 Thesis aims for brown argus

It is, therefore, unclear why brown argus was previously restricted to common rockrose in Britain and what has led to a recent increase in its use of Geraniaceae species and hence range expansion. My aims are, therefore:

- To establish whether recent changes in climatic conditions are related to an increased use of Geraniaceae hosts by brown argus.
- To establish the mechanisms driving increase in the use of Geraniaceae by brown argus.
- To assess the effect of increase in use of Geraniaceae on habitat availability in the landscape and the consequences of this for the rate of range expansion of the butterfly.

This work is presented in Chapter 5.

CHAPTER 3

EFFECTS OF CLIMATE ON TEMPORAL AND SPATIAL VARIATION IN HABITAT ASSOCIATIONS OF THE SPECKLED WOOD BUTTERFLY (*PARARGE AEGERIA*)

3.1 ABSTRACT

Many species are more restricted in their habitat associations at their leading-edge range margins where climatic conditions are marginal compared with their range core. Habitat associations are, therefore, predicted to broaden over time in these regions in response to climate warming as conditions become more favourable and climatic constraints relax for these species. However, thus far, evidence is only available from one species. In this Chapter, I examined spatial and temporal patterns of habitat associations of the speckled wood butterfly *Pararge aegeria*, which traditionally favoured woodland habitats in Britain. Analysis of spatial patterns of habitat use over 1000 km of latitude revealed that the butterfly was less restricted to woodland in regions with warm winters and warm summers, and that the association with woodland was weaker in regions with warm and wet summers than in regions with warm and dry summers. Analyses of temporal changes in habitat associations over 40 years of climate change also showed that the butterfly's association with woodland has weakened most in locations where temperature and summer rainfall have increased most. These patterns of habitat associations could be driven by direct effects of microclimate in "open" (i.e. non-woodland) habitats adversely affecting speckled wood growth and survival, or by climate boosting population densities in woodland and resulting in density-dependent dispersal into suboptimal 'open' habitats. Regardless of the mechanism causing these changes, an increase in the use of open habitats by the butterfly could increase its rate of range expansion.

3.2 INTRODUCTION

The climate is changing (IPCC 2007) and a suite of biological responses have been observed, including changes in species' phenologies (Roy and Sparks 2000) and distributions (Chen et al. 2011a). Evolutionary responses (Bradshaw and Holzapfel 2006) and changes in biotic interactions (Berg et al. 2010, Traill et al. 2010) have also been observed and all of these responses may lead to changes in community composition and ecosystem functioning (Montoya and Raffaelli 2010, Walther 2010). One response to climate change that has received little attention, however, is changes in species' habitat associations. Habitat associations of species are important because they determine the fraction of a landscape that can be occupied and hence the dispersal and metapopulation dynamics of species in patchy landscapes. Habitat associations also have implications for how land management could affect the distribution of species, and hence the impacts of conservation management decisions.

Many species show spatial variation in their habitat associations in relation to geographic variation in climate (Anthes et al. 2008, Ashton et al. 2009). In particular, species often become restricted to a narrower set of habitat types where climatic conditions are marginal for the species (Thomas et al. 1999, Lennon et al. 2002, Oliver et al. 2009). This might arise because species are restricted to certain habitats which provide microclimatic conditions that allow individuals to survive or complete their life cycles in regions where climatic conditions are generally unsuitable. For example, many species are restricted to the warmest habitats at their leading-edge range margins (Cherrill and Brown 1992, Thomas 1993, Thomas et al. 1999). Variation in species' habitat associations may also arise indirectly through the effects of climate on interacting species. For example, spatial variation in rainfall may affect host plant quality for herbivores and so in areas with low rainfall herbivores may become restricted to host plants growing in more humid habitats (Anthes et al. 2008). Variation in habitat associations may also be mediated through the effects of climate on species' population density. Where climatic conditions are favourable for a species, population densities are also likely to be high and so density-dependent dispersal of individuals away from the primary habitat may increase the range of habitats occupied.

Species' habitat associations may not only vary with climatic conditions in space, but also in time. For example species can shift their associations to habitats with more favourable microclimates as conditions change during the seasons (Shreeve 1984) and can utilise a wider range of habitats during climatically more favourable seasons (Roy and Thomas 2003). It is expected, therefore, that habitat associations will also change as a consequence of anthropogenic climate warming. In particular it is expected that at leading-edge range margins, species will be able to occupy a wider range of habitats as climatic constraints relax (Thomas et al. 1999). Changes in habitat associations over time have been observed at the leading-edge range margin of the silver-spotted skipper butterfly (*Hesperia comma*), which has relaxed its association with south-facing slopes over the past 30 years as sites on other aspects have become thermally suitable (Thomas et al. 2001, Davies et al. 2006). However, this represents a shift only in the locations used within the butterfly's favoured habitat type (calcareous grasslands) and so it is unclear whether shifts to different broad habitat types will be a widespread or substantial response to climate change (Suggitt et al. 2012). Such changes in habitat use are important because habitat availability affects rates of range expansion at leading edges (Hill et al. 2001, Wilson et al. 2009). If habitat associations relax, habitat availability will increase resulting in larger population sizes and a decrease in distance between habitat patches and hence more rapid rates of range expansion (Wilson et al. 2010).

In this Chapter I investigate the speckled wood butterfly (*Pararge aegeria*) which reaches its leading-edge range margin in Britain, and examine whether climate affects (1) spatial variation in habitat associations over 1000 km of latitude and (2) temporal variation in habitat associations over four decades of regional warming. The speckled wood butterfly is widely distributed throughout Europe, North Africa, and east to the Urals (Asher et al. 2001). Throughout its range it is primarily associated with woodland but can also occur in other habitat types such as parks, gardens and hedgerows in agricultural landscapes (Asher et al. 2001, Merckx et al. 2003). Anecdotal evidence is that the butterfly is more restricted to woodland in cooler parts of its range in Britain (Hill et al. 1999a, Asher et al. 2001) and Europe (Gibbs et al. 2011c). This contrasts with patterns seen in many ectotherms that become restricted to more open, warmer habitats in the coolest parts of their ranges (Thomas 1993), but may arise if woodlands provide protection from extreme cold temperatures over winter compared with more open habitat types (Suggitt et al. 2011). In more southerly parts of its range in Catalunya, Spain, where summers are hot and dry and winters are mild, speckled wood is more strongly associated with woodland than it is in Britain (Suggitt et al. 2012). This may be due to negative effects of host plant desiccation which are likely to be more severe in open habitats compared with woodland. Individual fitness (Talloen et al. 2004, Gibbs et al. 2011a) and population densities (Pollard 1988, Roy et al. 2001, Morecroft et al. 2002) are negatively affected by larval host plant desiccation and previous studies have shown that speckled wood become more restricted to woodland in drought years (Schweiger et al. 2006).

I examined the relationship between summer and winter temperature and rainfall and spatial variation in the butterfly's habitat associations in Britain and tested the hypotheses that:

- Speckled wood is more restricted to woodland in regions with colder winters.

- Speckled wood is more restricted to woodland in regions with dry summers.

Speckled wood has undergone rapid range expansion in Britain in response to recent climate warming (Hill et al. 1999a) and so I examined whether its habitat associations have also altered over time in response to changing climatic conditions. I tested the hypotheses that:

- Speckled wood's association with woodland has relaxed most in places where winter temperatures have increased most.
- Speckled wood's association with woodland has relaxed more in places that have become wetter in the summer.

3.3 MATERIALS AND METHODS

3.3.1 Quantifying the association of speckled wood with woodland

In order to examine spatial and temporal variation in the habitat associations of the speckled wood butterfly I used butterfly distribution data from a database of butterfly records submitted by volunteers and collated by Butterfly Conservation and the Centre for Ecology and Hydrology (Chapter 2). These data allowed me to investigate spatial patterns in the strength of the butterfly's association with woodland over a 1000 km gradient of latitude, and temporal changes in this association over 40 years.

For the spatial analyses, I used butterfly distribution data to compute an "index of association" of the butterfly with woodland for each 100 km × 100 km UK Ordnance Survey grid square (hereafter termed "100 km grid square") in Britain (Fig 3.1). I used fine scale 100 m × 100 m occurrence records (hereafter termed "100 m records") of speckled wood for the period 1970 to 2009. I assigned 100 m records as being ‘woodland’ or ‘non-woodland’ records using habitat information derived from remotely sensed satellite data (Land Cover Map 2000; Fuller et al. 2002). Land cover data classifies the landscape into 26 habitat categories at a 25 m × 25 m resolution and I included both broadleaved and coniferous woodland habitat categories as ‘woodland’. I classified 100 m speckled wood records as being ‘woodland’ records if there was any woodland within the 100 m × 100 m grid square the record came from, and ‘non-woodland’ records if there was no woodland in the 100 m × 100 m square.

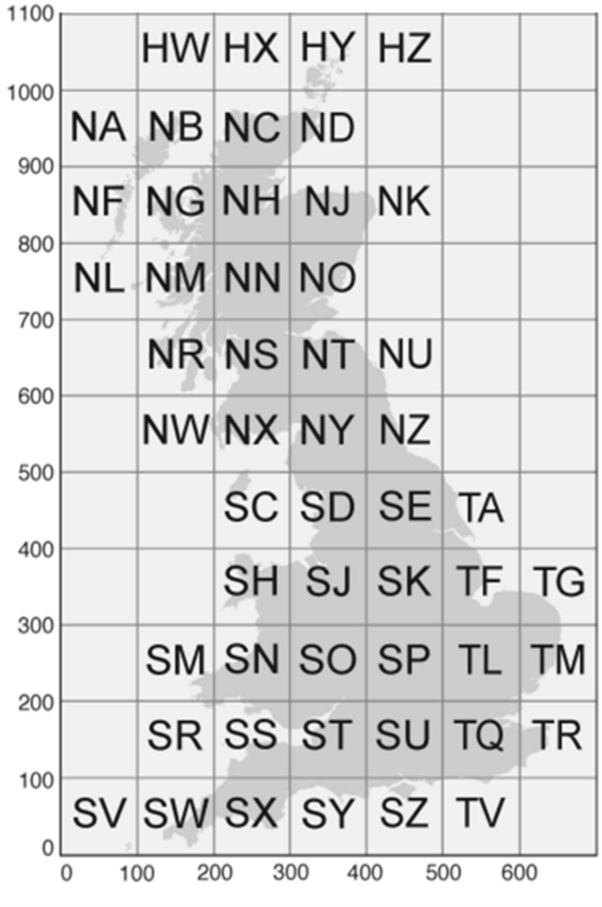

Fig. 3.1 100 km × 100 km UK Ordnance Survey grid squares used in the analysis of speckled wood’s habitat associations. Some squares only contain small areas of land or are outside the range of speckled wood so habitat association values could not be calculated.

To assess strength of association of the butterfly with woodland in each 100 km grid square I calculated the proportion of all 100 m speckled wood records that were woodland records. However, recorder effort in woodland versus non-woodland varies between 100 km grid squares which could lead to error in such a measure. To control for this variation in recorder effort between habitats I used records of other butterfly species as evidence of locations that had been surveyed. All 100 m records of any butterfly species were assigned as being either woodland or non-woodland records using the same methodology as for speckled wood records. I then calculated the fraction of woodland recorded squares with speckled wood records and the fraction of non-woodland recorded squares with speckled wood records. These fractions were used to compute the proportion of speckled wood records in woodland (*W*), after accounting for differences in the number of recorded squares in woodland and non-woodland habitats, following Eqn. 1. The fractions were calculated using only “unique” records i.e. if speckled wood had been recorded in the same 100 m × 100 m square multiple times the square was only counted once and if multiple species were observed in a 100 m × 100 m square the square was only counted once. Thus *W* in Eqn.1 represents the expected proportion of speckled wood recorded locations that were in woodland if woodland and non-woodland had been equally well recorded.

$$W = \frac{\frac{n}{a}}{\frac{n}{a}+\frac{m}{b}} \qquad [1]$$

W = proportional relative occurrence of speckled wood in woodland

n = total number of speckled wood woodland records in a 100 km grid square

m = total number of speckled wood non-woodland records in a 100 km grid square

a = total number of woodland recorded squares in a 100 km grid square

b = total number of non-woodland recorded squares in a 100 km grid square

In Eqn. 1, $W = 1$ would mean all speckled wood individuals were in woodland, $W = 0$ would mean all individuals were in non-woodland habitats and a value of $W = 0.5$ would mean speckled wood individuals were equally distributed between woodland and non-woodland (correcting for any differences in recording effort between woodland and non-woodland habitat types). A W_i value was calculated for each 100 km grid square i.

For temporal analyses, an index of association of speckled wood with woodland was calculated using the same method but instead of pooling data across all years a W_{ij} value was calculated for each 100 km square i in each year j (1970 to 2009).

3.3.2 Climate variables

Climate variables were derived for the period 1970 to 2006 from historical monthly 5 km × 5 km grid square resolution mean temperature (°C) and total rainfall (mm) data ('UKCP09' data, MetOffice 2009). I calculated four climate variables likely to be important for butterfly growth and survival: mean winter temperature (December to February), mean summer temperature (June to August), total winter rainfall (December to February) and total summer rainfall (June to August). These 5 km × 5 km values were averaged by 100 km squares across all years and for each year separately to provide estimates of climatic conditions for spatial and temporal analyses, respectively.

3.3.3 Statistical analysis

3.3.3.1 Spatial analysis

Spatial variation in the strength of speckled wood's association with woodland in relation to climate was analysed using logistic regression. For these analyses the response variable was W_i, the association of speckled wood with woodland from 1970-

2009 in each 100 km grid square, and I analysed four explanatory climate variables, winter and summer temperature and rainfall. Only 100 km grid squares with ≥ 20 unique 100 m records of speckled wood were included in the analysis to ensure a robust calculation of association with woodland (average number of speckled wood records per 100 km grid square = 2055; number of 100 km grid squares = 34). Analyses were undertaken using the programme R (R Development Core Team 2007). If models were overdispersed, as indicated by the residual standard error exceeding the degrees of freedom, "quasibinomial" errors were specified (Crawley 2007). Temperature variables are strongly correlated with each other (Pearson's correlation coefficient of mean summer temperature and mean winter temperature was 0.76), as are rainfall variables (Pearson's correlation coefficient of total summer rainfall and total winter rainfall was 0.93), and so I ran separate logistic regression models of W_i for each of the four climate variables. I also ran a logistic regression model with all four climate variables and their interactions included together as explanatory variables. Model reduction was performed with non-significant terms removed in turn, beginning with the highest order interactions and working down to main effects. Main effects were retained if they were not significant but were present in a significant interaction term. For all models I used Moran's I to test for spatial autocorrelation of residuals.

3.3.3.2 Temporal analysis

I examined whether the change over time in speckled wood's association with woodland in a 100 km grid square was related to the change in climate over time in that region. First I ran separate logistic regressions for each 100 km grid square with W_{ij} (association of speckled wood with woodland in each 100 km grid square in each year) as the response variable and year as the explanatory variable. The slope coefficients from each of the year on W_{ij} models were used to assess changes in habitat associations in each 100 km grid square over time and used in subsequent analyses. Negative slopes imply a weakening of the butterfly's association with woodland over time and positive slopes imply the butterfly is becoming more restricted to woodland over time. Steeper slopes imply a more rapid change in association over time. Only 100 km grid squares with $\geq$ 20 speckled wood records in $\geq$ 10 years were included in the analysis (n = 20 100 km grid squares). I ran linear regressions for each of the four climate variables using data from 1970 to 2006. The slope coefficients from the year on climate models examined how the climate had changed over time in each 100 km grid square and were then used as explanatory variables in the subsequent analysis.

In order to assess changes in habitat associations in relation to changes in climate I regressed the rate of change in climate over time (slope coefficients from year on climate models for each 100 km grid square) on rate of change in strength of association with woodland over time (slope coefficients from year on W_{ij} models for each 100 km grid square). Negative slopes imply the butterfly is weakening its habitat association with woodland more rapidly in places where the climate variables (temperature or rainfall) are increasing more rapidly. Positive slopes imply the butterfly is strengthening its association with woodland more rapidly in places where the climate variables (temperature or rainfall) are increasing more rapidly. Separate models were performed for each climate variable separately as well as a model of the same structure that included all those climate variables that best explained spatial variation in association with woodland. I used Moran's I to test for spatial autocorrelation of residuals, for all models.

3.4 RESULTS

3.4.1 Spatial variation in association with woodland

Estimates of W_i varied between 0.56 and 0.81showing the butterfly is more strongly associated with woodland than open habitats throughout its British range, but varies in the strength of this association (Fig. 3.2). Separate logistic regressions of each of the four climate variables on the strength of speckled wood's association with woodland showed that the butterfly was more strongly associated with woodland in places with cooler winters, cooler summers, wetter winters and wetter summers (Table 3.1). However, there was significant spatial autocorrelation of residuals for all four models (Moran's I: all $P < 0.05$), suggesting additional factors were important in explaining spatial variation in the strength of speckled wood's association with woodland.

When all four climate variables and their interactions were included in the same model and model reduction performed, the terms that remained in the minimal adequate model were summer temperature, summer rainfall and the interaction between these main effects (Table 3.1). In places with cool summers, there is no relationship between the strength of the butterfly's association with woodland and summer rainfall (logistic regression: slope coefficient = 0.002, $t_{1,13} = 1.44$, $P = 0.173$) (Fig. 3.3A). In places with warm summers, however, there was a significant negative relationship showing that the butterfly is more strongly associated with woodland in warm and dry places than warm and wet places (logistic regression: slope coefficient = -0.002, $t_{1,17} = -2.16$, $P = 0.045$) (Fig. 3.3B). Thus in warm places the butterfly occurs in a wider range of habitats than in cool places, but in places with warm and dry summers it is more restricted to woodland than in places with warm and wet summers. This model predicts observed variation in the strength of association with woodland well (R^2 observed versus fitted values = 0.74;

Fig. 3.2: (A) observed strength of association of speckled wood with woodland and (B) fitted values from the minimal adequate model). There was no significant spatial autocorrelation in the residuals (Moran's I: SD = 0.027, *P* = 0.381).

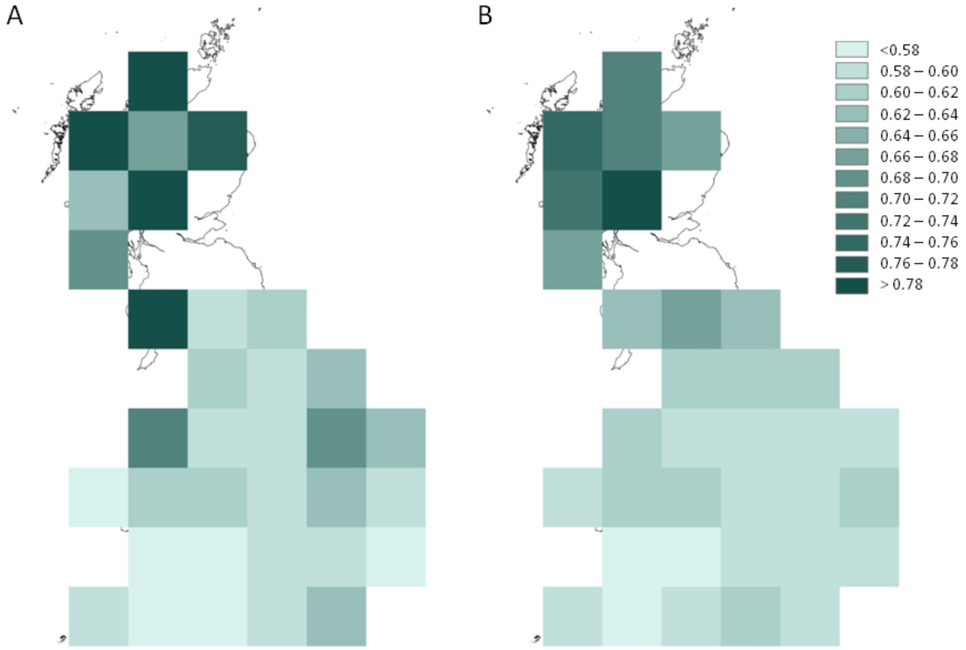

Fig. 3.2 Strength of speckled wood's association with woodland (W_i) in 100 km grid squares throughout Britain. A value of $W_i = 1$ indicates that all individuals were observed in woodland, a value of $W_i = 0$ means all individuals were observed outside of woodland, and a value of $W_i = 0.5$ means individuals were equally likely to be found in woodland and non-woodland habitats (controlling for recording effort). Observed values ranged between 0.56 and 0.81 showing that the butterfly is primarily associated with woodland but varies in its strength of association with woodland. (A) Shows observed values; and (B) fitted values from the minimal adequate model (see details in text). 100 km grid squares without values are those outside of the range of speckled wood (Fig. 2.4) or with insufficient speckled wood records to calculate a W_i value.

Table 3.1 Summary of results from analyses of the relationships between climate and spatial variation in speckled wood's associations with woodland.

Independent variable(s) in model		**d.f.**	**Intercept**	**Slope**	**Slope S.E.**	**t value**	**P value**	**Moran's I**
Summer temperature		32	1.633	-0.080	0.029	-2.770	0.009	Sig
Winter temperature		32	0.795	-0.089	0.032	-2.776	0.009	Sig
Summer rainfall		32	0.202	0.001	0.0007	1.582	0.123	Sig
Winter rainfall		32	0.345	0.0002	0.0003	0.677	0.503	Sig
Summer temperature, summer rainfall and their interaction	**Summer temperature**	30	-1.572	0.156	0.098	1.599	0.120	Non-sig
	Summer rainfall			0.021	0.007	3.076	0.004	
	Interaction			-0.002	0.0005	-3.320	0.002	

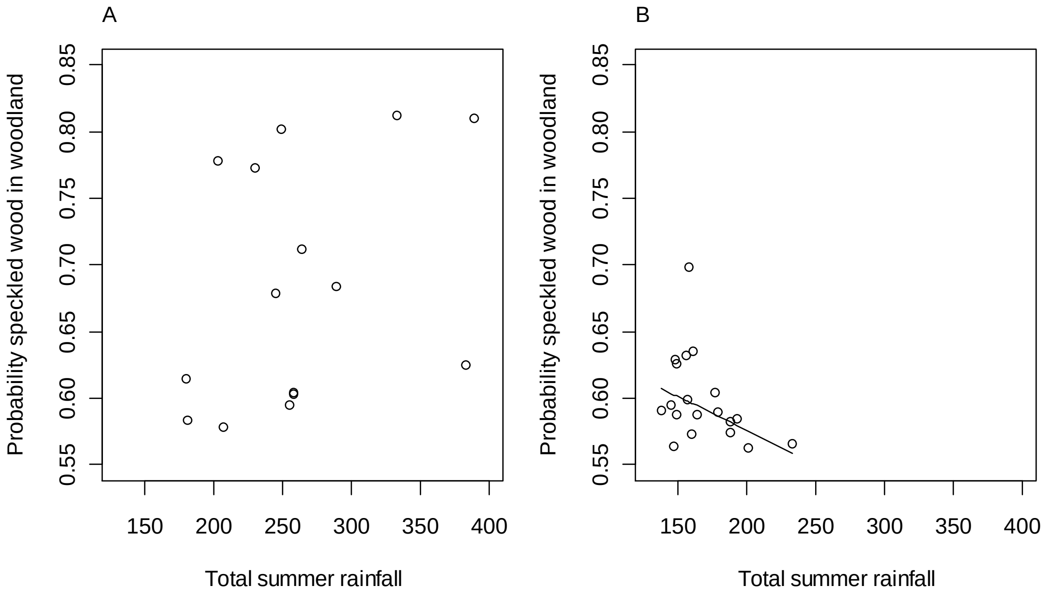

Fig. 3.3 Interaction effect between summer temperature and summer rainfall on spatial variation in the strength of speckled wood's association with woodland. Graphs show the relationship between strength of speckled wood's association with woodland (W_i) and rainfall in locations with (A) lower than average mean summer temperature; and (B) greater than average mean summer temperature. The butterfly does not vary in its association with woodland in cool areas but in warm areas is more strongly associated with woodland in areas that are dry than wet.

3.4.2 Temporal variation in association with woodland

Speckled wood's association with woodland has declined in more 100 km grid squares than those in which it has increased (decrease in 13/20 100 km grid squares) and the rate of change in W_{ij} varied over time from an increase of 0.02 per year to a decrease of 0.06 per year (Fig. 3.4). There was no evidence of spatial autocorrelation of residuals in any of the temporal models (Moran's I: all $P > 0.05$). Speckled wood's association with woodland has declined most rapidly in those 100 km grid squares where average winter temperature has increased most, where summer temperature has increased most and where summer rainfall has increased most. However, there was no relationship with change in winter rainfall (Table 3.2; Fig. 3.5). Thus I conclude that the relationships between climate and habitat associations are similar in both space and time. When change in summer temperature and change in summer rainfall were included in the same model, the interaction term was non-significant and so removed and both of the main effects were approaching significance.

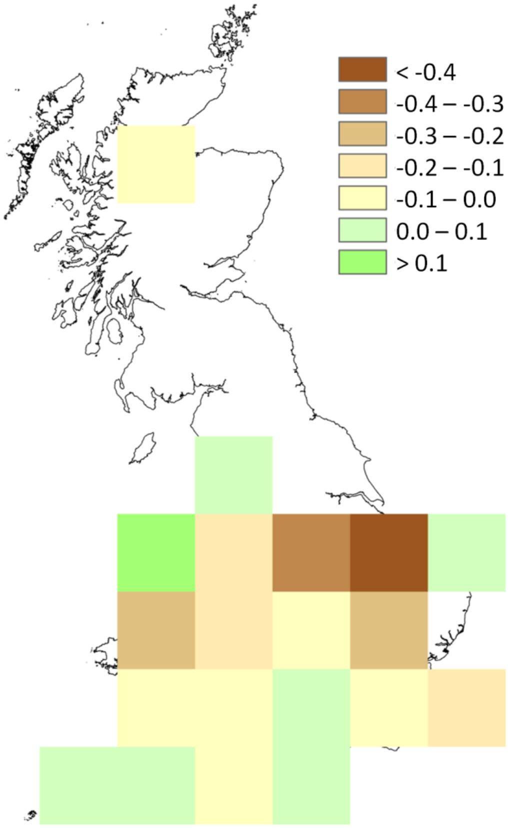

Fig. 3.4 Change in speckled wood’s association with woodland over time. Values are the slope coefficient from regressions of year on W_{ij}, the index of association of speckled wood with woodland in each year. Negative slope values indicate the butterfly has weakened its association with woodland over time and positive slopes indicate it has become more restricted to woodland over time. Steeper slopes indicate a faster rate of change in habitat associations.

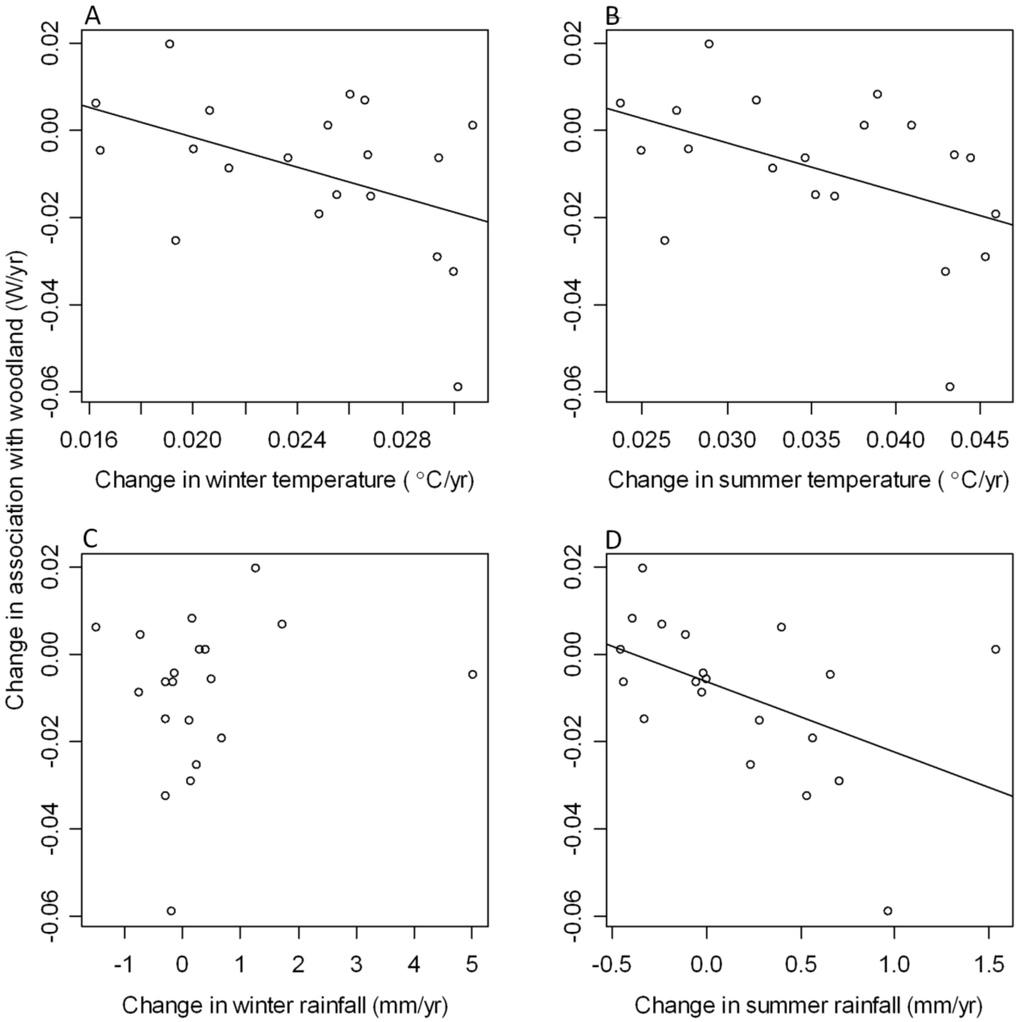

Fig. 3.5 Relationship between change in the strength of speckled wood's association with woodland over time (W_{ij}) and change over time in climate. (A) Mean winter temperature, (B) mean summer temperature, (C) total winter rainfall, and (D) total summer rainfall. The butterfly's association with woodland has weakened most rapidly in places where winter and summer temperatures and summer rainfall have increased most.

Table 3.2 Summary of results from analyses of the relationships between change in speckled wood's associations with woodland over time and change in climatic conditions over time

Independent variables(s) in model		**d.f.**	**Intercept**	**Slope**	**Slope S.E.**	**F ratio**	**P value**	**Moran's I**
Change in summer temperature		18	0.030	-1.108	0.450	4.496	0.048	Non-sig
Change in winter temperature		18	0.033	-1.729	0.816	4.949	0.039	Non-sig
Change in summer rainfall		18	-0.006	-0.016	0.006	5.587	0.030	Non-sig
Change in winter rainfall		18	-0.010	0.002	0.003	0.441	0.515	Non-sig
Change in summer temperature and change in summer rainfall	**Summer temperature**	17	0.025	-0.896	0.470	5.016	0.074	Non-sig
	Summer rainfall			-0.013	0.007		0.056	

3.5 DISCUSSION

I have shown that the speckled wood butterfly varies in its use of habitat in space and over time. The data confirm that the butterfly is primarily associated with woodland in Britain (all $W_i > 0.5$), but that it varies in the strength of its association with woodland throughout its range. An observed W_i of 0.81 in 100 km grid square NG (Figs. 3.1 and 3.2) means that in this region 81 % of locations in which speckled wood has been recorded are in woodland (controlling for variation in recorder effort between woodland and non-woodland); compared with 56 % in 100 km grid square SX (Figs. 3.1 and 3.2). This demonstrates a marked difference in the relative distribution of individuals between woodland and non-woodland habitats throughout the species' British range.

Variation in the butterfly's habitat associations were related to climatic conditions. The butterfly is more strongly associated with woodland in places with cooler winter and summers. However, this relationship is modified by rainfall as the butterfly is more strongly associated with woodland in warm and dry places than in warm and wet places. Over time, the butterfly has weakened its association with woodland most in areas where winter and summer temperature and summer rainfall have increased most. These results suggest that similar climate factors are affecting speckled wood's association with woodland over space and time.

Correlation of climate variables, however, makes it difficult to disentangle the relative importance of these different factors. Furthermore, difficulties in interpretation could arise from the fact that data were analysed at 100 km grid square resolution. Thus the climate variables used in the analysis might not reflect those in the specific locations in which the butterfly occurs, particularly in regions with high topographic heterogeneity. Some error in measurement may also arise from the fact that the map of woodland cover was a snapshot using data collected between 1999 and 2001 (Fuller et al. 2002). Woodland cover has changed over time, for example, increasing slightly during the period covered by this analysis (Smith and Gilbert 2003). Thus some butterfly records may have been incorrectly assigned as being woodland or non-woodland records. Despite this, patterns of habitat association varied with climate as predicted. Here I discuss possible mechanisms driving the relationship between spatial and temporal variation in the butterfly's habitat associations with climate and consequences of changes in habitat associations for rates of range shift.

3.5.1 Mechanisms driving observed patterns

I believe there are two primary mechanisms that could explain the relationships I have observed between speckled wood's habitat associations and climate. Firstly, microclimatic differences between woodland and open habitats could have a negative effect on the survival and performance of individuals outside of woodland and the magnitude of this effect could vary with macroclimatic conditions. Alternatively, climate may affect the density of speckled wood populations which could have an indirect effect on habitat associations through density-dependent dispersal into alternative non-woodland habitats under climatically favourable conditions. These mechanisms are not necessarily mutually exclusive and may act in concert, or different factors may be more important in different parts of the butterfly's range.

3.5.1.1 The role of microclimate over winter

Speckled wood is more restricted to woodland in places with colder winters. Woodland provides a buffered microclimate, with narrower diurnal fluctuations in temperature and less extreme minimum temperatures compared with open habitats (Morecroft et al. 1998, Suggitt et al. 2011). All organisms have minimum temperatures beyond which they cannot survive (Bale 2002) and above this cold exposure can result in chill injuries which negatively affect fitness (Hutchinson and Bale 1994). Increased restriction of speckled wood to woodland in regions with cold winters could arise because conditions outside of woodland lead to high mortality rates and reduced fitness of survivors and hence smaller more vulnerable populations, whereas the buffering effect of the woodland means individuals can survive within these habitats. In regions with milder winters, however, microclimate conditions outside of woodland may have less of an effect on the survival and fecundity of individuals in open habitats resulting in larger populations outside of woodland.

The hypothesis that winter cold might be a limiting factor for speckled wood is supported by evidence from Sweden where winters are colder than in Britain and the butterfly is thought to be entirely restricted to woodland (Gibbs et al. 2011c). In the south of Sweden, speckled wood can overwinter in the larval or pupal life stages, as in Britain, but larvae enter a true diapause (Wiklund and Friberg 2011) whereas in Britain larvae can resume feeding when temperatures reach their development threshold (Blakeley 1996). Furthermore, in central Sweden where winter temperatures are even lower, it is thought that speckled wood are only able to over winter as pupae which are thought to be more cold tolerant than larvae (Nylin et al. 1995).

3.5.1.2 The role of microclimate over summer

The increased restriction of speckled wood to woodland in areas with warm and dry summers could also be due to physiological constraints on the butterfly’s occurrence outside of woodland in these climatic conditions. Low rainfall leads to desiccation of host plants which cause mortality through starvation or negatively affect the fitness of insect herbivores in less extreme conditions (Scriber 1977). The speckled wood butterfly is thought to be particularly sensitive to host plant desiccation. Female adults select host plants for egg-laying that are in particularly humid locations (Wiklund and Persson 1983) which suggests they avoid locations where eggs or host plants will desiccate. Evidence that populations decline in years following dry summers supports this idea (Roy et al. 2001, Morecroft et al. 2002). Laboratory studies show that individuals reared on drought-stressed plants take longer to develop and achieve lower adult body mass (Talloen et al. 2004), which is supported by field evidence that shows individuals are smaller (and thus are likely to have lower fecundity) in drier years (Gibbs et al. 2011a). Levels of humidity and soil moisture are higher in woodland than open habitats (Chen et al. 1993) thus host plant desiccation is likely to be greater in open habitats. In regions where temperatures are hot and rainfall is low, individuals may be unable to tolerate host plant desiccation in open habitats but in regions where rainfall is high conditions may be suitable outside of woodland. Furthermore, physiological differences between individuals originating from woodland and open habitats means those from open habitats are more susceptible to egg desiccation (Gibbs et al. 2010c, Gibbs et al. 2010b, Gibbs et al. 2010a) and suffer more from host plant desiccation (Gibbs et al. 2011b). Host plant desiccation may, therefore, drive a stronger association of speckled wood with woodland in regions where summers are relatively hot and dry such as the south east of England and Catalunya (Suggitt et al. 2012). Previous studies have shown that speckled wood becomes more restricted to woodland in drought years (Schweiger et al. 2006), supporting the role of host plant desiccation in affecting patterns of habitat use.

3.5.1.3 Microclimate and temporal changes in habitat associations

Association of the butterfly with woodland has weakened most over time at the butterfly’s leading-edge range margin, and particularly in the east of England (Fig. 3.4) where both winter temperatures and summer rainfall have increased. Thus changes in climatic conditions in these regions could have reduced lethal or sublethal effects of cold or host plant desiccation in “open” habitats, allowing speckled wood to occupy these habitats. Relaxation of these physiological constraints over time could have, therefore, led to an increase in numbers of the butterfly outside woodland habitats. In contrast, the butterfly’s association with woodland does not appear to have changed much in the south-west of England over time, where its association with woodland was historically comparatively weak and has remained weak. This is a region of England in which winters are mild and summers are warm and wet and so conditions may have been suitable for the butterfly to occur outside of woodland prior to recent climate change.

3.5.1.4 The role of density

A second, not necessarily mutually exclusive hypothesis is that variation in speckled wood’s association with woodland is an indirect result of the effect of climate on population density. It would be expected that speckled wood densities are lower in regions where climatic conditions are less favourable. Woodland is the preferred habitat of the speckled wood butterfly (Merckx et al. 2003) and at low densities they may remain in woodland, but when densities are high, density-dependent pressures could result in emigration of individuals from woodland into less favoured habitat types (Baguette and Schtickzelle 2006, Nowicki et al. 2009). Speckled wood males are known to be territorial and defend sunspots in which they wait for passing females to mate with (Davies 1978) and so high densities could force males to move to subordinate habitats in search of territories. The same climatic variables as discussed above (winter cold and summer rainfall) could drive variation in population density. The butterfly’s habitat associations are also related to summer temperature which could also be an important driver of density. For example, thermal availability for development is greater when summers are warmer, leading to completion of more generations per year (Shreeve 1986b). Warm summer conditions also increase thermal availability for egg-laying perhaps resulting in greater realised fecundity. Both of these factors could result in higher population growth rates and lead to density-dependent dispersal into other non-woodland habitats.

The butterfly's association with woodland has weakened most over time in areas where the butterfly has expanded its distribution in the east of England (Figs. 2.4 and 3.4). As climate has changed over time and conditions become more suitable, the butterfly has expanded its distribution (Hill et al. 1999a). Its rate of range expansion is higher in areas with greater woodland cover (Hill et al. 2001) and so appears to colonise woodland first. Following colonisation numbers build up in woodland and then stabilise (Pollard et al. 1996). This could be the point at which the butterfly begins to experience density-dependent pressures and moves out into alternative habitats. Weaker patterns of change over time in other parts of the butterfly's range, such as the south-west of England could be because this area has long-established populations of speckled wood which had already undergone density-dependent spillover into alternative habitats prior to the beginning of the analysis in the 1970s.

3.5.2 Consequences for range expansion

Rates of range expansion are determined by habitat availability at species' leading-edges, with rates of expansion being faster where there is more suitable habitat, and where habitats are less fragmented (Wilson et al. 2009). Woodland cover is highly fragmented in Britain and covers just 11.6 % of the land surface (Fig. 3.6) thus long dispersal distances may be required to reach new habitat beyond the butterfly's current range margin. The butterfly has expanded its range more rapidly in regions where woodland cover is greater, suggesting that ability to disperse to new woodland patches is a limiting factor.

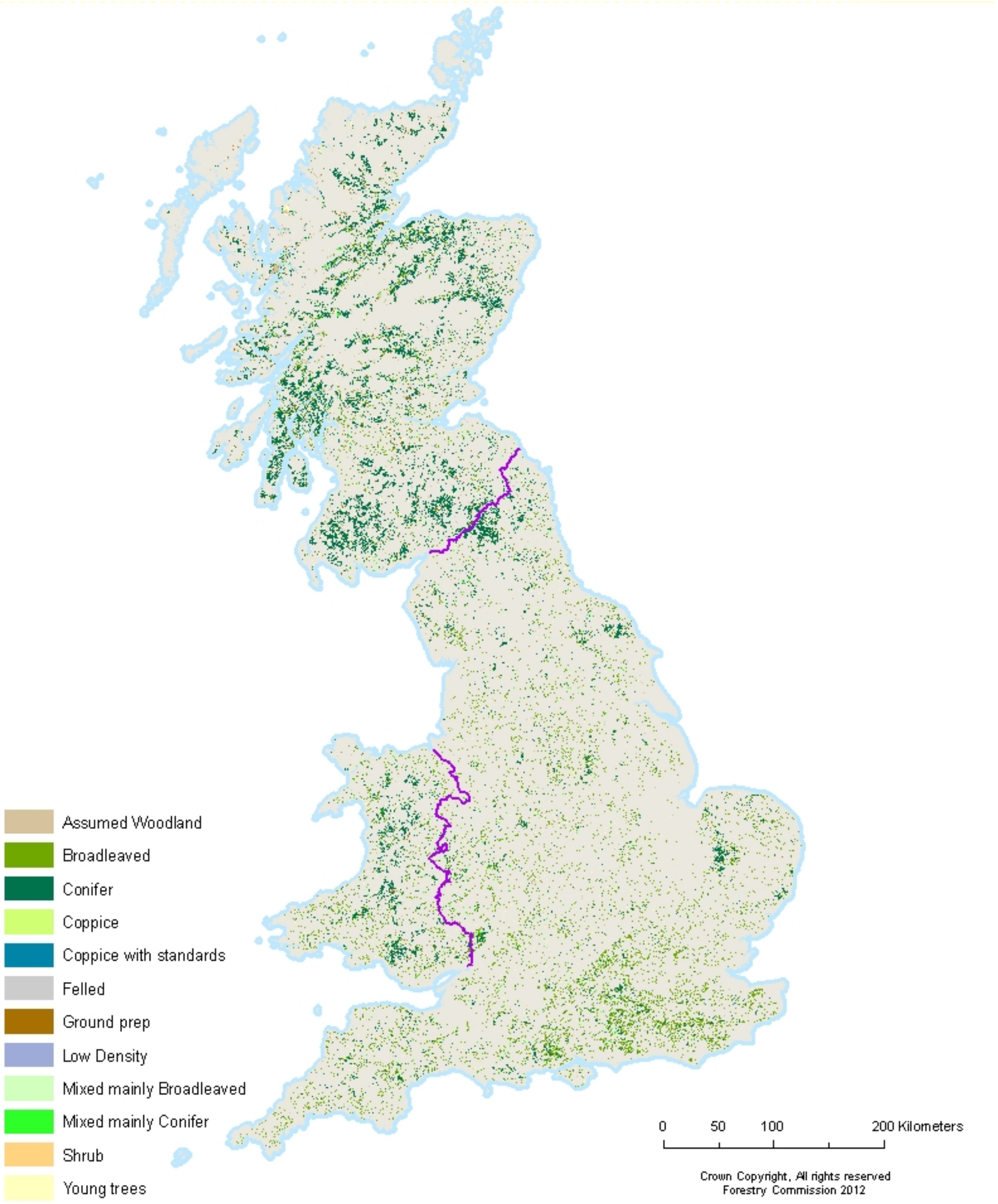

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Fig. 3.6 Woodland cover in Britain over 0.5 ha based on the Forest Commission's National Forest Inventory, updated in 2011.

Speckled wood's association with woodland has weakened over time, in particular at its expanding range margin. For example, in 100 km grid square SE (Fig. 3.1), the butterfly has gone from 77 % of occurrences being recorded in woodland in the period 1980-1989 to 60 % in the period 2000-2009 (controlling for differences in recorder effort between habitats). An increase in the ability of the butterfly to use open habitats could, therefore, reduce the dispersal distance required for individuals to reach new habitats, as well as increasing the sources of colonists. Thus relaxation of the butterfly's constraint to woodland at its expanding range margin could increase rates of range expansion compared with a situation where the butterfly remains restricted to woodland (Wilson et al. 2010). However, in other parts of its range, if summers become warmer and drier in future, as is projected for some parts of Britain (MetOffice 2012), conditions outside woodland may become less suitable and speckled wood may become more restricted to woodland.

3.5.3 Summary

I have demonstrated that the speckled wood butterfly shows variation in its habitat associations throughout its range in Britain in relation to climatic conditions. I have also shown that the butterfly has weakened its association with woodland in areas where climatic conditions have become more favourable for speckled wood. Broadening of habitat associations at the butterfly's leading-edge range margin could lead to faster rates of range expansion than if habitat associations had remained static. However, correlations among climatic variables make it difficult to determine the specific mechanisms driving these patterns of habitat association. This is explored further in Chapter 4.

CHAPTER 4

INTERACTIONS BETWEEN HABITAT AND MICROCLIMATE AFFECT LARVAL PERFORMANCE

4.1 ABSTRACT

Many species have more restricted habitat associations at their leading-edge range margins where climatic conditions are marginal, and thus species are predicted to broaden their habitat associations in these locations as the climate warms. However, the mechanisms underpinning the effect of climate on species' habitat associations have rarely been tested. In this Chapter, I focus on the speckled wood butterfly and investigate whether variation in its use of woodland *versus* more open grassland habitats is related to local microclimates in these different habitats, and the effect of microclimate on larval survival and performance. I reared speckled wood larvae in woodland and grassland habitats over winter 2008-2009 and summer 2009. The butterfly is more restricted to woodland in parts of its range with colder winters (Chapter 3), but despite the coldest temperature being 3.8 °C lower in grassland than in woodland during the experiment, I found no difference in mortality between habitats. The butterfly is also more restricted to woodland in places with hot and dry summers, suggesting an effect of host plant desiccation, but I again found no difference in mortality between individuals reared in woodland and grassland over summer. However, growth rates were slower in grassland than woodland in winter and summer and, in winter, pupae were smaller in grassland compared with woodland. The role of winter microclimate was supported by laboratory experiments which showed that larval development time increased with severity of cold exposure, thus providing an explanation for slower larval development times in grassland. These results suggest that variation in local microclimates could affect population growth rates in different habitats, and thus play an important role in driving variation in observed patterns of species' habitat associations.

4.2 INTRODUCTION

Spatial and temporal variation in species' habitat associations are related to variation in climatic conditions. Species become restricted to a narrower set of habitats at their leading-edge range margins where climatic conditions are marginal (Oliver et al. 2009, Chapter 3). Thus, as the climate changes and conditions improve at species' leading-edge range margins, species may be able to occupy a wider range of habitats (Thomas et al. 1999, Davies et al. 2006, Chapter 3), with potential consequences for increasing rates of range shift (Wilson et al. 2010). The majority of studies examining the relationship between climate and habitat associations are correlative and the underlying mechanisms driving these relationships remain largely unexplored (but see Davies et al. 2006). One possible mechanism is that, at their range margins, species are at the limit of their climatic tolerances or requirements, and so populations become restricted to habitats which provide the microclimatic conditions required for survival or life cycle completion.

Topography and habitat structure are significant modifiers of local microclimates. Slope and aspect modify microclimate by altering the amount of solar radiation received by a surface. At high- and mid-latitudes, equator-facing slopes receive more direct radiation and so in sunny conditions achieve higher maximum temperatures and consequently higher mean temperatures than slopes of other aspects (Rorison et al. 1986, Suggitt et al. 2011). This effect can occur at a variety of scales from sides of mountains to sides of ploughed furrows or anthills in a field (Weiss et al. 1988).

Vegetation structure can also modify climatic conditions (Geiger 1957). In woodland ("closed") habitats, compared with grassland ("open") habitats, the presence of a canopy reduces wind speed and solar radiation, resulting in higher humidity and soil moisture and a dampening of temperature extremes (i.e. maximum temperatures are lower and minimum temperatures are higher in woodland compared with open habitats) (Chen et al. 1993, Morecroft et al. 1998, Suggitt et al. 2011). Within open habitats, there is also small spatial scale variation in microclimate. Within a grassland sward or heathland vegetation or a sphagnum hummock, for example, temperatures are generally more stable (extremes are dampened) and wind speed slower close to the ground compared with the top of the vegetation (van der Molen and Wijmstra 1994, Turlure et al. 2011). This effect is reduced as vegetation becomes shorter and sparser. Thomas et al. (1999), for example, found that temperatures at ground level were 3-8 °C warmer under 1-3 cm tall vegetation than under vegetation taller than 10 cm.

Insects can be directly limited by climate at their leading-edge range margins if conditions are too cold for them to survive (Crozier 2003, Bale and Hayward 2010) or because thermal availability for development (Bryant et al. 1997) or egg-laying (Davies et al. 2006) is insufficient. In such cases, therefore, populations may become restricted to locations with microclimatic conditions that meet their requirements, even if the surrounding landscape is unsuitable. For example, many insects at their leading-edge range margins are restricted to habitats that provide particularly warm microclimates in the summer, suggesting that they are limited by thermal requirements for development or egg-laying (Cherrill and Brown 1992, Thomas 1993, Thomas et al. 1999).

Here I investigate the mechanisms affecting variation in the habitat associations of the speckled wood butterfly (*Pararge aegeria*). Speckled wood is primarily a butterfly of woodland (Asher et al. 2001, Merckx et al. 2003) but in Chapter 3 I showed that the extent to which it is able to use more open habitat types varies with climatic conditions. In this Chapter I investigate whether differences in microclimate between closed and open habitats affect larval survival and performance of speckled wood, and could therefore play a role in driving the observed patterns in the butterfly's habitat associations.

In Chapter 3, I showed that speckled wood is more restricted to woodland in regions with colder winters. This could arise because woodland provides a buffer against extreme temperatures and so winter temperatures fall lower in open habitats (Morecroft et al. 1998, Suggitt et al. 2011). In insects, severity and duration of cold exposure can result in injury which is lethal if the intensity or duration exceed a certain threshold (Sinclair and Roberts 2005, MacMillan and Sinclair 2011). In parts of the species' range with cool winters, temperatures in woodland may exceed the cold tolerance of larvae and so the butterfly would become restricted to woodland habitats in which temperatures are less extreme. Duration and severity of cold exposure can also have sublethal effects on individuals. Chill injuries can result in longer development times (Turnock et al. 1985) which could reduce the number of generations completed per year and increase the risk of mortality during development (Pollard 1979). Chill injury can also reduce adult fecundity (McDonald et al. 1997). These sublethal effects could, therefore, have additional consequences for the viability of populations outside woodland. In addition to severity and duration of exposure, diurnal fluctuations in temperature tend to be more extreme in grassland than in woodland as maximum temperatures as well as minimum temperatures are dampened in woodland (Suggitt et al. 2011). Thus individuals may be chilled at a faster rate in grassland and may be more likely to have food in their gut when they are chilled, both of which can reduce cold tolerance (Kelty and Lee 1999, Woodman 2010).

I carried out field experiments in winter to examine differences in microclimate and larval survival and performance between woodland and grassland habitats. Specifically I tested the hypotheses that:

- Severity and duration of cold exposure are greater in grassland than woodland.

- Diurnal temperature fluctuations are greater in grassland than woodland.
- Over winter larval survival is lower in grassland than woodland.
- Development time is longer, growth rate is slower and pupal mass is lower in grassland than woodland.

Another trend in patterns of the speckled wood's habitat associations found in Chapter 3 was that speckled wood is more strongly associated with woodland in warm and dry areas than warm and wet areas. This pattern could be driven by the effects of host plant desiccation on survival and individual performance and hence the viability of populations. Humidity and soil moisture are lower in grassland than woodland (Chen et al. 1993) and so host plant desiccation is likely to be greater in grassland and this effect is likely to be greater in regions where summers are hotter and drier. Speckled wood populations decline in years following low rainfall which is thought to be due to the effects of host plant desiccation and larval starvation (Pollard 1988, Roy et al. 2001, Morecroft et al. 2002). Larvae reared on drought-stressed plants in lab studies take longer to develop and achieve lower adult mass (Talloen et al. 2004) and in dry years wild individuals tend to be smaller (Gibbs et al. 2011a). I, therefore, carried out a field experiment in summer to test the hypotheses that:

- Host plant desiccation is greater in grassland than woodland.
- Larval survival is lower in grassland than woodland.
- Development time is longer, growth rate is slower and pupal mass is lower in grassland than woodland.

As factors other than temperature could affect survival, development time and pupal mass in woodland *versus* grassland in the field (e.g. host plant quality), I also carried out a laboratory experiment to test the effect of severity and duration of cold exposure on speckled wood larvae in a controlled setting. I chose severity treatments because they approximate to the minimum temperatures experienced in woodland (-5 °C) and grassland (-10 °C) in the field experiment (Table 5.1). Durations were chosen because they allow comparison with accumulated degrees below zero in the field experiment (Tables 5.1 and 5.2). I tested the hypotheses that:

- Larval survival decreases with duration of exposure and this effect is greater at -10 °C than at -5 °C.
- Development time is longer, growth rates are slower and pupal mass is lower with increased duration of exposure and this effect is greater at -10 °C than at -5 °C.

4.3 METHODS

4.3.1 Field experiments

4.3.1.1 Experimental design

Larvae were reared in woodland and grassland over winter 2008-2009 and summer 2009 to examine survival rates and larval performance in different habitats. Adult female speckled wood butterflies were collected from Bishop Wood, near Selby, North Yorkshire (UK Ordnance Survey Grid Reference SE5533) in August 2008 and 2009. Wild-collected adults were kept separately on pots of *Poa pratensis* plants for egg-laying in greenhouses at the University of York. Grass seed was sourced from Emorsgate Seeds (Kings Lynn, UK) and grown in Levington F2 compost with sand. Pots were watered daily. Second instar larvae were transferred onto new plants grown in the same conditions. and then transferred to field sites. Pots were covered with netting to prevent grazing of grass plants and predation and parasitism of larvae (Fig. 4.1). Pots were placed in woodland and grassland sites in the Vale of York (Fig. 4.2) which is close to the northern range boundary of the species in England (Fig. 2.4).

Fig. 4.1 Enclosures used for rearing speckled wood larvae in woodland and grassland sites. Pots with host plants were dug into the ground and covered with netting.

(1) Winter experiment

To examine effects of winter temperature, larvae were placed at a woodland site (Bishop Wood; Fig. 4.2 site W1; OS grid ref SE5533), and a grassland site (Wistow; Fig. 4.2 site G1; OS grid ref SE6035). At each site, pots were placed 1.5 m apart along 2 transects (20 pots per transect), and dug in so that the plant pot was flush with the surface of the soil. Pots were set up in September 2008, when all plants were of similar age and size. Plants were replaced during the experiment when there was no edible material left. There was a split-brood design, and larvae from each female (n = 40 females) were split evenly between pots placed in woodland and grassland. Pots contained 5 to 15 larvae, depending on the number of eggs laid by females.

(2) Summer experiment

To investigate the effects of summer drought conditions, experiments were set up in three woodland (Fig.4.2; Bishop Wood, site W1; the Grange, W2, OS grid ref SE6957; Skipwith Common, W3, OS grid ref SE6637) and three grassland sites (Fig.4.2; Wistow, site G1; University of York campus, G2, OS grid ref SE6150; Skipwith Common, G3, OS grid ref SE6637). Pots (seven per site) were placed 1.5 m apart along one transect per site (n = 42 pots in total). Insufficient females laid enough eggs for a split brood design and so eggs from each female (n = 19 females) were split evenly between treatments but assigned randomly to pots within treatments, with all pots receiving 5 larvae. Plants were then left to desiccate naturally in the field.

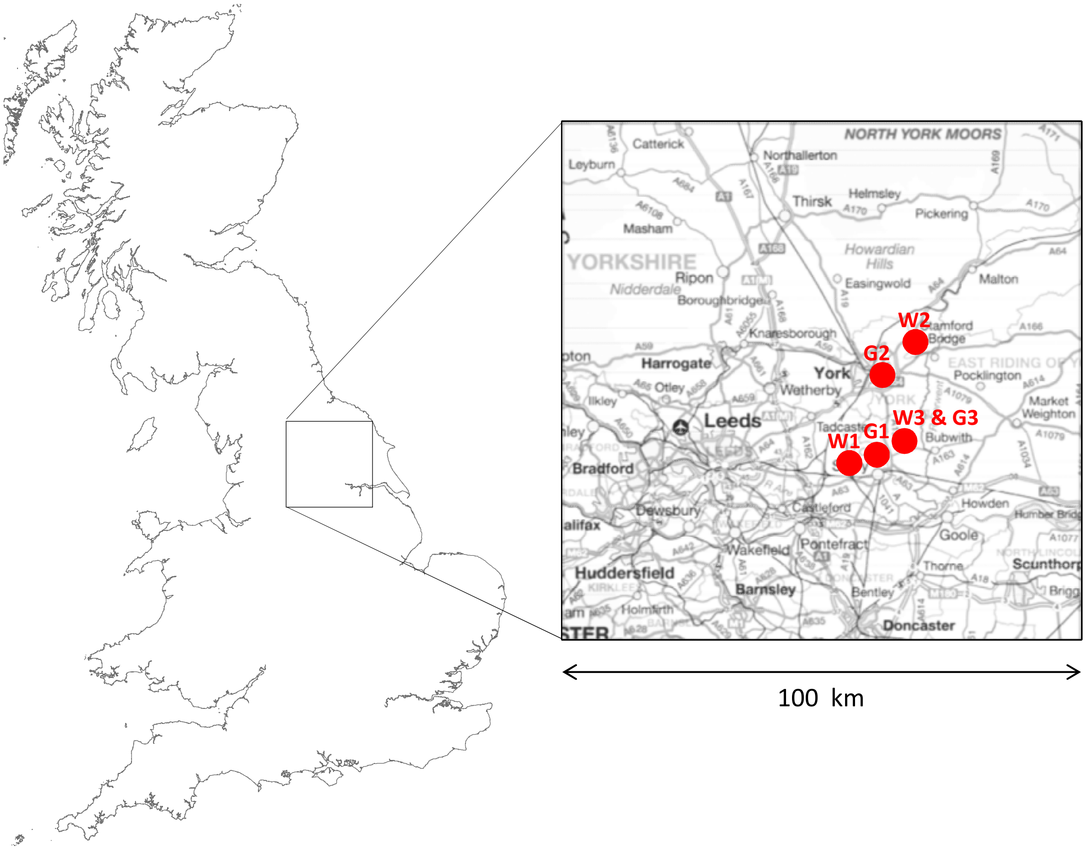

Fig. 4.2 Location of field sites for winter and summer field experiments. W1-3 are woodland sites and G1-3 are grassland sites. Contains Ordnance Survey data © Crown copyright and database right (2012).

4.3.1.2 Microclimate measurements

Eight pots in each site had temperature data loggers suspended at 30cm above the ground, within the netting, to take hourly temperature readings. Loggers were wrapped in foil to minimise the effect of direct solar insolation. In winter, these were iButton data loggers (model DS1922L) and in summer, were HOBO pendant data loggers (model UA-001-08) (both accurate to 0.4 °C within the temperature ranges experienced in the experiments).

Over winter, six climate variables were computed, chosen because they are known to affect survival and performance in insects: absolute minimum temperature and average daily minimum temperature were calculated as measures of severity of cold exposure. Hours spent below 0 °C was calculated as a measure of duration of exposure and freezing degree days below 0 °C (FDD0, calculated by summing all hourly temperature readings below 0 °C) as a combination of severity and duration of exposure. Mean diurnal range (daily maximum temperature – daily minimum temperature) was calculated as a measure of temperature fluctuation. Growing degree days above 5 °C (GDD5, calculated by summing all hourly temperature readings above 5 °C) was calculated as a measure of thermal availability for development; a higher GDD5 should result in faster development time. Loggers were placed out in the field from September 23rd 2008 to June 16th 2009. Over summer, only GDD5 was calculated. Loggers were placed out in the field from August 7th 2009 to September 14th 2009.

4.3.1.3 Host plant water content

A sample of grass (n = 38 due to loss of some pots) measuring 3 cm in diameter in total was cut, flush with the soil surface, from each of the potted plants at the end of the experiment. Samples of grass (n = 28 in each of the 6 sites) were also taken from the vicinity of the experiment in order to determine whether patterns seen in the potted plants were replicated in the wild. Samples were taken from grass growing closest to the point 2 m and 4 m perpendicular to each pot on both sides of the transect. Samples of grass measuring 3 cm in diameter in total were taken and were cut flush with the soil surface. Samples were weighed, dried in an oven at 60 °C for 24 hours, and then weighed again to compute water content.

4.3.1.4 Measures of insect performance

For the winter experiment, larval survival was calculated for each pot as the proportion of larvae that pupated. For the summer experiment, survival was the proportion of individuals that pupated, or were still alive as larvae at the end of the experimental period.

For the winter experiment, pots were checked and pupae collected weekly and so development time was calculated as the number of weeks from placing larvae into the field to pupation. As development was faster in summer, pots were checked every other day during the summer experiment, and development time was calculated as the number of days from translocation of larvae into the field to pupation. Upon pupation, individuals were collected and fresh mass measured on the same day using a Sartorius balance accurate to 0.1 mg. Pupal mass was used to compare potential fecundity in individuals reared in woodland and grassland. In Lepidoptera, pupal mass has been found to be positively correlated with potential and realised fecundity (e.g.Tammaru et al. 2002). For individuals from the winter experiment, I also measured adult dry mass. Adult butterflies were frozen within 24 hours of eclosion, then dried in an oven at 60 °C for 24 hours, and weighed on a Sartorius balance accurate to 0.1 mg. Adult dry mass was strongly correlated with pupal mass ($n = 41$, $R^2 = 0.73$) and is known to be positively correlated with fecundity in speckled wood (Karlsson and Wickman 1990, but see Hughes et al. 2003). Therefore, pupal mass was used as a measure of potential fecundity. Growth rates (mg per week for the winter experiment and mg per day for the summer experiment) were calculated as pupal fresh mass divided by development time.

4.3.1.5 Data analysis

Due to failure or loss of many of the data loggers over winter, data from only 2 loggers in woodland and 4 in grassland were available, and summary data (average values from successful loggers) are presented in Table 4.2. Over summer, data from loggers from all woodland sites and all grassland sites were pooled and a Mann-Whitney U test used to compare GDD5 between habitats.

In the summer experiment, water content of potted host plants was compared between woodland and grassland using Mann-Whitney U-tests due to violation of the assumption of homogeneity of variance. Water content of wild grass samples in woodland and grassland was compared using a t-test.

I tested for a difference in larval performance between pots in woodland and grassland. Due to low rates of pupation in the summer experiment, and therefore low numbers of pots with development time and pupal weight data available, I pooled data across sites and compared all pots in woodland with all pots in grassland. For comparing survival rates, proportion data for each pot were arcsine square root transformed and woodland and grassland pots compared using t-tests. For other performance variables mean values per pot were always used to avoid pseudoreplication. Grassland and woodland pots were compared using t-tests.

4.3.2 Laboratory experiment

4.3.2.1 Experimental design

I investigated the lethal and sublethal effects of severity and duration of cold exposure on speckled wood larvae in the lab. 15 adult female speckled wood were collected from Bishop Wood (Fig. 4.2, site W1), and placed in cages with *P. pratensis* to lay eggs. Larvae were reared in controlled cabinets at 13 °C and 12:12 photoperiod on *P. pratensis*. Speckled wood has three potential developmental pathways: direct development, pupal diapause and third instar larval diapause. The photoperiod and temperature conditions chosen have previously been shown to induce larval diapause in speckled wood from Britain (Nylin et al. 1989).

Immediately prior to being exposed to experimental treatments, 3rd instar larvae were transferred to cabinets at 5 °C without food for 3 days to minimise the amount of food in the gut because presence of nucleators in the gut can increase the risk of injury from cold (Sinclair et al. 2003). Larvae from the same female were split evenly between treatments. Groups of 10 larvae were placed in flat-bottomed glass tubes in a tray of antifreeze (to maintain a stable temperature), and transferred into incubators where the temperature was reduced to -5 °C or -10 °C at a rate of 1 °C per minute. A total of three tubes was then removed from the -5 °C incubator after 2, 4, 6 and 8 days and from the -10 °C incubator after 1, 2, 3 and 4 days. The maximum duration of exposure for each treatment results in freezing degree days similar to the maximum experienced in the field experiment (Tables 4.1 and 4.2).

Table 4.1 Duration of below 0 °C larval exposure and accumulated degrees below 0 °C in experimental treatments in laboratory experiment.

Duration of exposure (days)	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Hours spent below 0 °C	24	48	72	96	120	144	168	192
Freezing degrees days below 0 °C at -5 °C	-	240	-	480	-	720	-	960
Freezing degree days below 0 °C at -10 °C	240	480	720	960	-	-	-	-

Upon removal from cold exposure, mortality was not assessed immediately because individuals could be in chill coma. Larvae were transferred to a 5 °C controlled cabinet for two days, and larval survival was then determined by movement in response to mechanical stimulus. Live larvae were then transferred to *P. pratensis* (larvae from one treatment group on each plant, maximum n = 10) for 18 days at 15 °C, and then transferred to 20 °C. Pots were checked every day for newly pupated individuals, which were weighed 24 hours after pupation. Development time was calculated as the number of days from removal from experimental treatment to pupation. Survival rates to pupation were recorded. Pupae were then kept in pots with moistened filter paper to prevent desiccation and rates of successful eclosion were calculated.

A “control” group of 10 larvae that were not chilled, was also kept at 5 °C for 8 days, the duration of the longest cold exposure treatment, and then treated as per the experimental larvae. Performance variables from these larvae were compared with experimental larvae.

4.3.2.2 Data analysis

Larval survival, development time, pupal mass and growth rates were analysed using analysis of covariance (ANCOVA), with temperature as a fixed factor and duration of exposure as a covariate. Survival was calculated relative to survival of the control group as proportion of larvae alive in treatment group/proportion alive in control group. Mean values for each variable were calculated per pot.

4.4 RESULTS

4.4.1 Winter field experiment

Temperature data showed that severity of cold was greater in grassland than woodland: the lowest temperature recorded in grassland was 3.8 °C lower than that in woodland and the mean daily minimum temperature was 4.7 °C lower in grassland than woodland. A similar length of time was spent below 0 °C in woodland and grassland (444 hours and 453 hours respectively), but because temperatures were colder in grassland, accumulated degrees below zero was greater in grassland than in woodland (614 freezing day degrees in woodland and 911 freezing day degrees in grassland). The mean diurnal range of temperatures was greater in grassland than woodland (13.9 °C in grassland compared with 7.9 °C in woodland), and GDD5 was greater in grassland (963 growing day degrees) than in woodland (803 growing day degrees).

Table 4.2 Microclimate data from data loggers placed 30 cm above the ground in woodland and grassland sites (Fig. 4.2) which collected data every hour from 23rd September 2008 to 16th June 2009.

Climate variable	**Woodland**	**Grassland**
Absolute minimum temperature (°C)	-5.1	-8.9
Mean daily minimum temperature (°C)	-0.2	-4.9
Hours spent below 0 °C	444	453
Freezing day degrees below 0 °C	614	911
Mean diurnal range (°C)	7.9	13.9
Growing degree days above 5 °C	803	963

There was no significant difference in larval survival between woodland and grassland sites (Fig. 4.3A) (Table 4.3), although there was high variance among pots (range 0 to 0.86 proportion surviving). Development time was significantly shorter in woodland than grassland (Fig. 4.3B) and pupae were heavier in woodland than grassland (Fig. 4.3C), resulting in significantly faster larval growth rates in woodland compared with grassland (Fig. 4.3D) (Table 4.3).

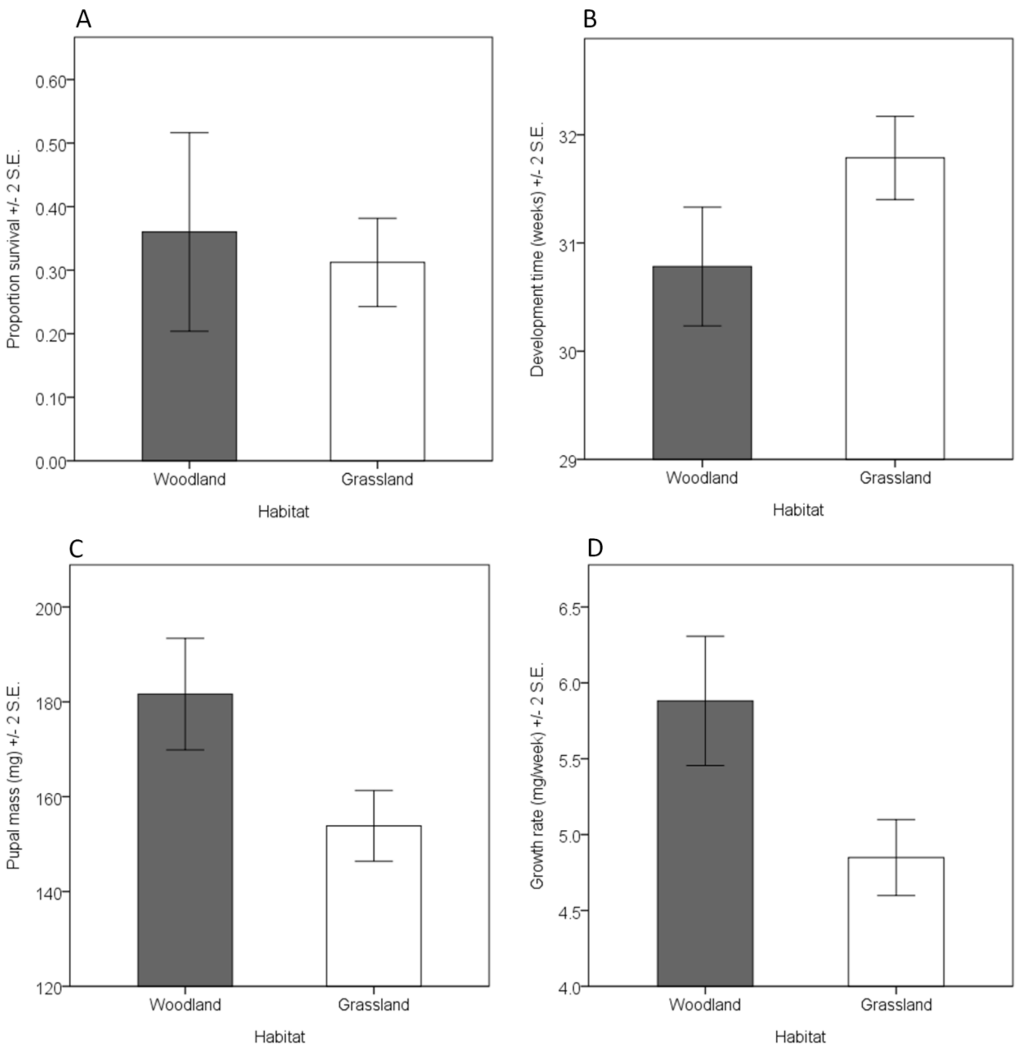

Fig. 4.3 Measures of speckled wood larval performance in woodland and grassland habitats over winter 2008-2009. (A) Mortality before pupation; (B) development time to pupation (weeks); (C) pupal mass (mg); and (D) growth rate (mg per week). Means and +/- 2 SEs are plotted.

4.4.2 Summer field experiment

There was no significant difference in water content of potted plants in grassland (mean = 34.3 % water) compared with woodland (mean = 45.9 %; Mann-Whitney U test: Z = -1.257, n = 38, *P* = 0.217), however water content in wild grass was significantly lower in grassland (mean = 50.5 %) than woodland (mean = 66.7 %; t-test: t = -10.25, d.f. =

163 $P < 0.0001$). Analysis of logger data showed that GDD5 was significantly higher in grassland (mean = 621 day degrees) than it was in woodland (mean = 506; Mann-Whitney U test: $Z = -3.703$, $n = 20$, $P < 0.0001$).

There was no difference in larval survival rates between habitats (Fig. 4.4A) (Table 4.3). However, there was high variance in survival rates among pots (range, 0 to 1 proportion surviving). Development time was shorter in woodland than grassland (Fig. 4.4B), and pupae were heavier in woodland (Fig. 4.4C), with differences being marginally non-significant (Table 4.3). Larval growth rates were significantly higher in woodland than grassland (Fig. 4.4D) (Table 4.3).

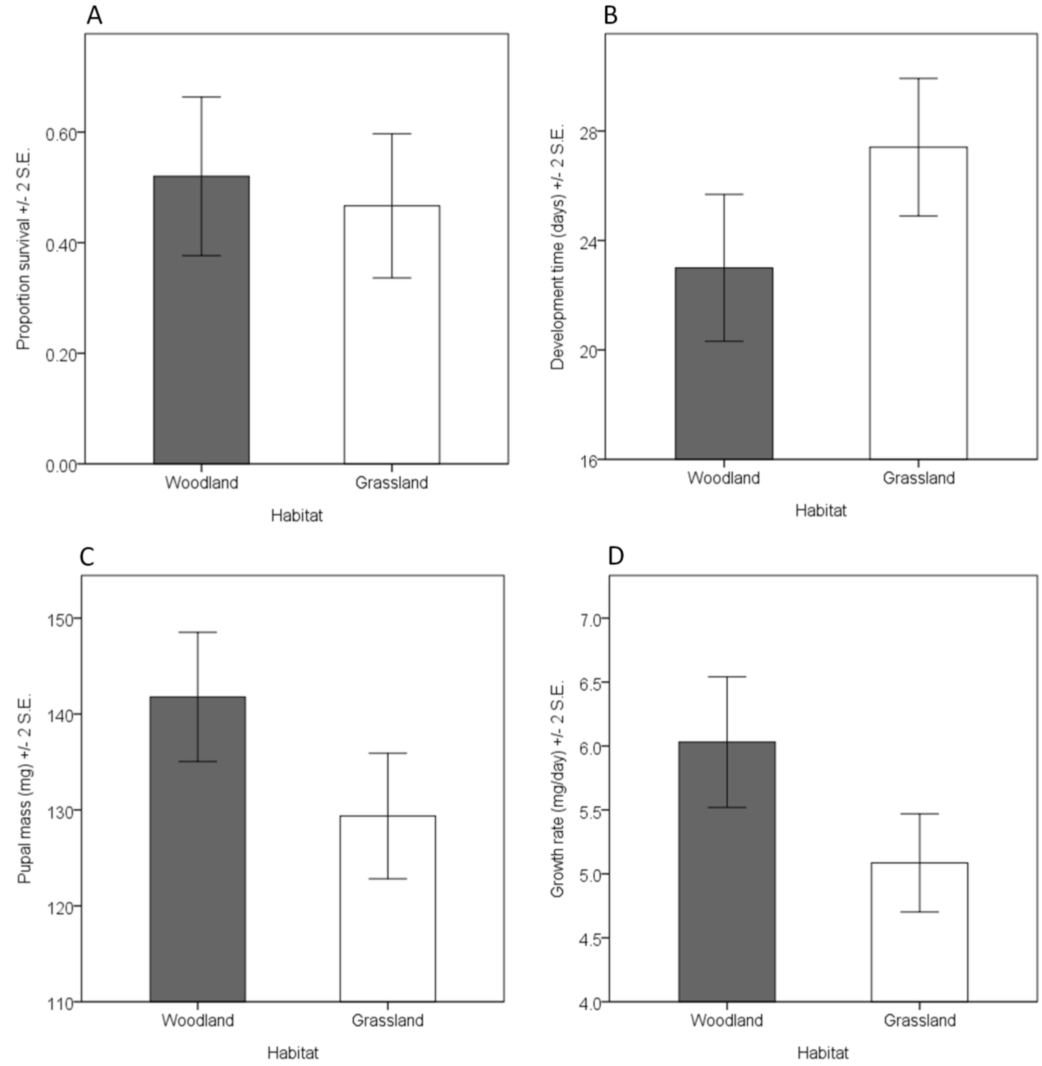

Fig. 4.4 Measures of speckled wood larval performance in woodland and grassland over summer 2009. (A) Mortality before pupation; (B) development time to pupation (days); (C) pupal mass (mg); and (D) growth rate (mg per week). Data for mean values and +/- 2 SEs are plotted.

Table 4.3 Summary of differences in larval performance in woodland and grassland in winter and summer field experiments and results of statistical tests for differences between habitats.

Season	**Insect performance variable**	**Mean woodland**	**Mean grassland**	**t-value**	**d.f.**	**P-value**
Winter	**Survival (proportion)**	0.36	0.31	-0.866	45	0.391
	Development time (weeks)	30.8	31.8	2.880	43	0.006
	Pupal mass (mg)	181.6	153.8	3.919	42	<0.001
	Growth rate (mg/week)	5.88	4.85	-4.262	42	<0.001
Summer	**Survival (proportion)**	0.52	0.47	-0.503	39	0.618
	Development time (days)	23.0	27.4	-2.114	14	0.053
	Pupal mass (mg)	142.6	126.8	1.917	14	0.076
	Growth rate (mg/day)	6.30	4.73	3.031	14	0.009

4.4.3 Lab experiment

In the lab, duration of exposure to cold but not severity of cold exposure decreased larval survival two days after exposure (ANCOVA: exposure temperature: $F_{1,4} = 3.855$, $P = 0.121$; duration of exposure: $F_{1,4} = 31.105$; $P = 0.005$) (Fig. 4.5A). However, there was a significant interaction between exposure temperature and duration of exposure ($F_{1,4} = 26.876$; $P = 0.007$), which is likely to be due to the greater decrease in survival with duration of exposure at -10 °C than at -5 °C. Similarly, duration of chilling but not severity decreased survival to pupation (ANCOVA: exposure temperature: $F_{1,4} = 2.356$, $P = 0.200$; duration: $F_{1,4} = 50.947$; $P = 0.002$; interaction: $F_{1,4} = 27.650$, $P = 0.006$) (Fig. 4.5B) and eclosion success (ANCOVA: exposure temperature: $F_{1,4} = 0.923$, $P = 0.391$; duration: $F_{1,4} = 138.185$; $P < 0.001$; interaction: $F_{1,4} = 67.390$, $P < 0.001$) (Fig. 4.5C).

By contrast, both increasing severity and duration of exposure increased the development time of larvae (ANCOVA: exposure temperature: $F_{1,14} = 7.066$, $P = 0.019$; duration of exposure: $F_{1,14} = 18.588$; $P < 0.001$). The significant interaction term between exposure temperature and duration ($F_{1,14} = 24.265$, $P < 0.001$), is probably because the increase in development time with duration of exposure only applied to individuals exposed to -10 °C and not to -5 °C (Fig. 4.5D). There was no significant effect of severity or duration of exposure on pupal mass (ANCOVA: exposure temperature: $F_{1,14} = 0.075$, $P = 0.789$; duration of exposure: $F_{1,14} = 0.624$; $P = 0.443$; interaction: $F_{1,14} = 0.806$, $P = 0.385$) (Fig. 4.5E), or growth rate (ANCOVA: exposure temperature: $F_{1,14} = 2.611$, $P = 0.128$; duration of exposure: $F_{1,14} = 4.345$; $P = 0.056$), although this was approaching significance. There was also a significant interaction term ($F_{1,14} = 7.190$, $P = 0.018$) (Fig. 4.5F) because growth rates declined with duration of exposure at -10 °C but did not at -5 °C.

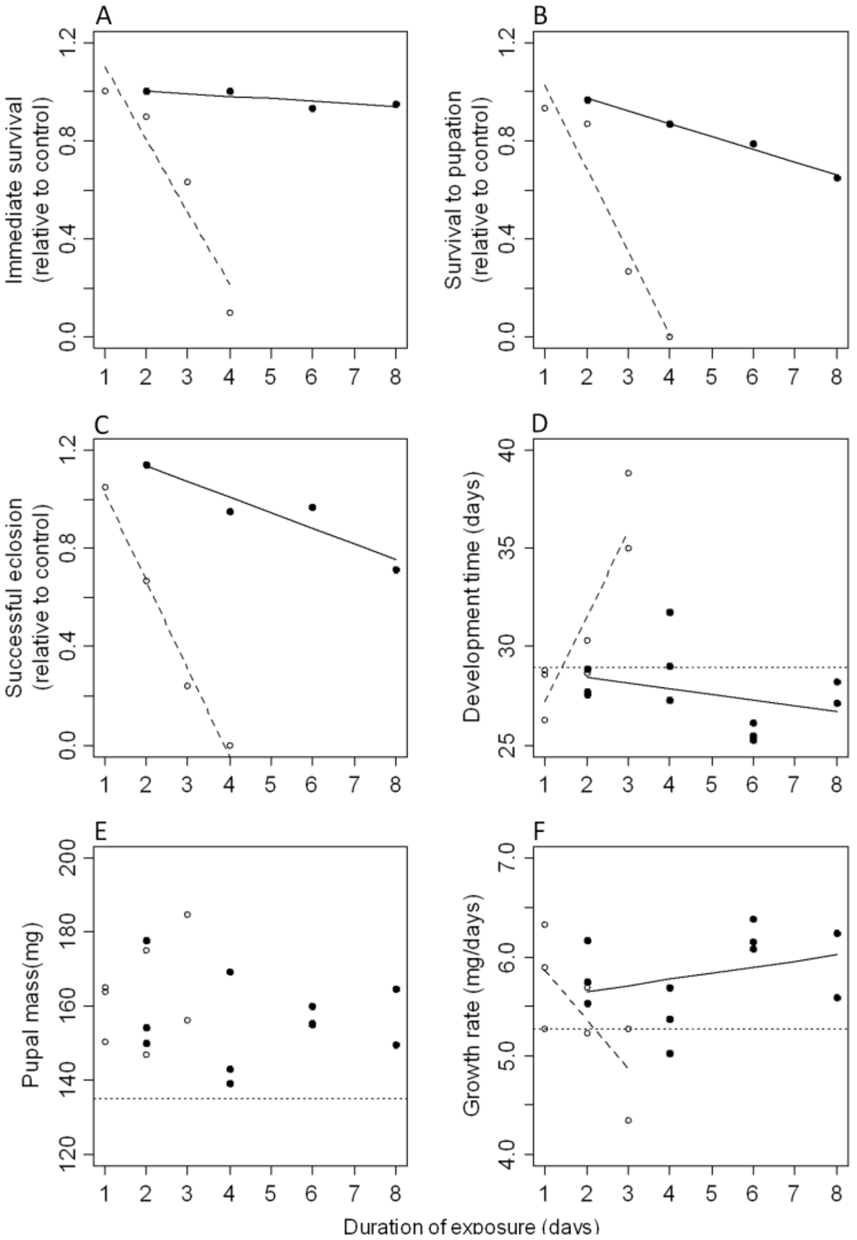

Fig. 4.5 Lethal and sublethal effects of cold exposure on speckled wood larvae in laboratory experiment. Solid lines and closed symbols are larvae exposed to -5 °C and long dashed lines and open symbols are larvae exposed to -10 °C. For (A-C), survival rates are calculated as a proportion of survival rates of control groups thus values are >1 if survival of treatment groups exceed that of control groups. For (D-F) short dashed lines are average values for control groups. (A) Survival two days after exposure; (B) survival to pupation; (C) successful eclosion (D) development time to pupation (days); (E) pupal mass (mg); and (F) growth rate (mg per week).

4.5 DISCUSSION

In line with previous studies (e.g. Suggitt et al. 2011), minimum temperatures recorded in grassland over winter in the field experiment were colder than in woodland. However, contrary to my prediction, I did not find a significant decrease in larval survival rates in grassland compared with woodland. I did, however, find that larval growth rates were slower in grassland, development times were longer in grassland, and pupal mass was lower in grassland over winter. During the summer, although mean water content of host plants was lower in grassland than woodland this difference was not significant. Again, I did not find a difference in survival of larvae between woodland and grassland. However, larval growth rates were again slower in grassland than woodland and there was a trend for longer development times and lower pupal mass in grassland than woodland. Poorer larval performance could result in slower population growth rates and smaller populations in grassland, which may be sufficient to drive the variation in speckled wood's habitat associations observed in Chapter 3.

4.5.1 Effect of winter cold on speckled wood larval survival and performance

There was no significant difference in survival rates between woodland and grassland, contrary to my expectations. The experiment was only undertaken in one year, which if particularly mild could have reduced the expected effect of habitat on mortality. However, the winter of 2008-09 was cold, with an average daily minimum temperature of 1.8 °C compared with an average of 2.3 °C for the period 1900-2010 (Central England Temperature series, Parker et al. 1992). Even in a particularly cold year, therefore, differences in cold experienced between habitats appear to have been insufficient to generate any difference in mortality. The absolute minimum temperature experienced in grassland was considerably lower than that in woodland (-8.9 °C in grassland compared with -5.1 °C in woodland). In the lab experiment, however, individuals exposed to -10 °C for 24 hours did not have higher mortality rates than the control group, and thus the difference in absolute minimum temperature experienced in the field experiments of -8.9 °C *versus* -5.1 °C in grassland and woodland habitats might not be expected to be sufficient to generate differences in mortality rates between habitats.

However, mortality from cold exposure does not necessarily only occur in response to temperatures exceeding an organism's absolute tolerance. Chill injury is cumulative and mortality increases with both severity and duration of exposure (Nedvěd et al. 1998, Turnock and Fields 2005). This effect was observed in the lab experiment where immediate survival of speckled wood larvae and subsequent survival to pupation and eclosion all decreased with duration of cold exposure, and this effect was greater at -10 °C than -5 °C. Mean daily minimum temperatures were lower in grassland than woodland (-4.9 °C in grassland compared with -0.2 °C in woodland) and so although individuals in woodland and grassland experienced similar amounts of time below 0 °C (444 hours in woodland and 453 hours in grassland), lower minimum temperatures in grassland meant individuals also experienced greater accumulated degrees below zero in grassland compared with woodland (614 freezing degree days in woodland and 911 freezing degree days in grassland). Thus accumulation of chill injury would be expected to be greater in grassland than woodland. However, this difference does not appear to have generated a difference in larval mortality between habitats. Fluctuating temperatures experienced in the wild can be beneficial if temporary exposure to higher temperatures allows repair of chill injuries (Renault et al. 2004) and this effect may have been sufficient to prevent mortality from cold temperatures in grassland.

While there were no differences in larval mortality between woodland and grassland, I did find differences in larval performance: development times were longer in grassland than woodland (woodland, mean = 30.8 weeks, grassland, mean = 31.8 weeks) and pupal mass was lower in grassland (woodland, mean = 181.6 mg, grassland, mean = 153.8 mg) resulting in lower growth rates in grassland than woodland (woodland, mean = 5.88 mg per week, grassland, mean = 4.85 mg per week). Chill injury can have sublethal effects on insects which increase with severity and duration of cold exposure. Chill injury can result in increased development times (Turnock et al. 1985), reduced fecundity (McDonald et al. 1997) and reduced survival rates of offspring (Hutchinson and Bale 1994), perhaps due to the increased metabolic and time costs of repairing a greater accumulation of cold-induced injuries (Lalouette et al. 2007). In the lab experiment, development time increased with duration and severity of cold exposure, suggesting that longer development times observed in grassland could be due to more severe cold exposure in this open habitat.

In the lab experiment, however, I did not find an effect of severity or duration of cold exposure on pupal mass. Furthermore, both groups appeared to have greater pupal mass than the control group. Lower pupal mass in grassland than woodland in the field experiment could, therefore, be due other aspects of the microclimate that were not investigated in the lab experiment, such as diurnal fluctuations in temperature. Not only are daily minimum temperatures lower in grassland than woodland, but daily maximum temperatures are also higher in grassland. A greater diurnal temperature range in grassland means that temperatures decline to sub zero temperatures at a faster rate than in woodland, which in some insects has been shown to decrease cold tolerance ability (Kelty and Lee 1999, Woodman 2010). This could be because at faster rates of cooling, there is a reduced opportunity for rapid cold hardening (RCH), a process whereby individuals can increase their protection from chill injury induced by brief exposure to moderately low temperatures (Lee et al. 1987). Thus individuals in grassland not only experience lower temperatures but could be physiologically less well protected from the cold. Alternatively, because temperature fluctuations are greater in grassland, there may be a greater need for RCH and so more resources may be devoted to this process which may carry a physiological cost (Overgaard et al. 2007).

Furthermore, it is thought that speckled wood larvae in Britain do not enter a true diapause, but that if winter temperatures rise above their development threshold of between 5 °C and 6 °C, larvae resume feeding (Blakeley 1996). In grassland, therefore, there is a greater chance of individuals resuming feeding because temperatures exceed the development threshold, which may be followed by freezing temperatures and larvae suffering damage due to the presence of food in the gut which can act as ice nucleators (Woodman 2010). There is also a greater chance of temperatures falling below freezing in autumn and spring in grassland than in woodland, when larvae are certain to be feeding during the day, leading to increased risk of chill injury from presence of food in the gut.

Only one grassland and one woodland site were used for the winter experiment making it difficult to conclude whether the observations of larval survival and performance I observed are robust. However, some of results from the field experiment were supported by those from the lab experiment, suggesting that the effects seen were due to differences in cold exposure of butterflies between habitats. Furthermore, microclimatic differences between the habitat types were consistent with those previously observed (Chen et al. 1993, Morecroft et al. 1998, Suggitt et al. 2011) suggesting the differences are a general pattern between woodland and grassland.

4.5.2 Summer larval performance

There was no difference in water content of plants larvae were reared on in grassland (mean 34.3 % water content) or in woodland (mean 45.9 % water content). I found no difference in larval mortality between woodland and grassland, which may have been due to the lack of any difference in host plant desiccation between habitats. I did, however, find that wild grass samples had significantly lower water content in grassland (mean 50.5 %) than woodland (mean 66.7 %), supporting the idea that host plant desiccation is higher in grassland. Water content of wild grass samples was higher than in the experimental pots, implying that all experimental pots were dry, leading to similar larval mortality rates in both habitats. However, development time for larvae was on average 4.4 days faster and pupae were 1.1 times heavier in woodland compared with grassland, and growth rates were significantly higher in woodland than grassland. The trend for lower water content of host plants in grassland could have been a contributory factor to poorer larval performance in grassland but these remains unsubstantiated and the mechanisms driving this pattern require further investigation.

4.5.3 Consequences of microclimate differences for speckled wood’s habitat associations

Differences in the performance of populations of speckled wood in woodland and grassland could be achieved by differences in survival, and/or by variation in population productivity as mediated by differences in growth rates and fecundities. My original prediction was that microclimatic differences between woodland and grassland would lead to elevated mortality levels in grassland both over winter and summer and that these would explain observed patterns in the butterfly’s habitat associations. However, survival was not significantly different between the habitats. In contrast, increased larval growth rates, reduced development time and greater pupal weights in woodland all suggest that differences in developmental performance between the habitats could underlie the species' habitat associations. In combination, these performance metrics are likely to lead to higher population growth rates in woodland than in grassland, under the conditions studied.

Higher pupal mass in woodland is likely to lead to increased adult fecundity and hence maximum potential population growth rates. I found that pupae in woodland were 1.12 (summer) to 1.18 (winter) times heavier than in grassland, potentially resulting in 1.5-1.8 times more eggs laid in woodland (Karlsson and Wickman 1990). However, this interpretation is open to question because realised fecundity is affected by adult female longevity as much as, or more than, by size (Leather 1984).

Faster development times in woodland could result in an increase in population growth rates for at least two reasons. First, the overall risk of mortality prior to adulthood might be expected to be reduced in rapidly-developing larvae, if the daily mortality risk is constant (Pollard 1979). Secondly, the speckled wood has a flexible number of generations it can complete in a year, with up to three generations in climatically favourable years (Shreeve 1986b). Differences in generation times between grassland and woodland were relatively small (1 week in winter and 4 days in summer), but they could be sufficient to enable some individuals to complete an additional generation (at least in some years) in woodland but not in grassland, increasing the overall population growth rate in woodland.

In combination, these factors are likely to affect the relative population growth rates in woodland *versus* grassland, although they do not represent absolute barriers to the survival of speckled wood outside woodland. This is consistent with the observation in Chapter 3 that speckled wood was not completely restricted to woodland in the coolest parts of its range or in the hottest and driest parts of its range. Rather, there are quantitative differences in performance, which may be sufficient to increasingly confine the speckled wood to its most favoured habitat in climatically marginal regions.

4.5.4 Summary

Chapters 3 showed that the speckled wood butterfly shows spatial and temporal variation in its habitat associations that are related to variation in climatic conditions. This Chapter provides some support for the hypothesis that microclimate affects these patterns. Chapters 3 and 4 considered variation in species' habitat, but insect herbivores have also been shown to vary the host plants species they use with climate (Scriber 2002, Nylin et al. 2009). Chapter 5 investigates species' use of different larval host plants, and whether changes in climatic conditions have resulted in changes in the interactions between the brown argus butterfly and its host plants.

CHAPTER 5

TEMPERATURE-DEPENDENT ALTERATIONS IN HOST USE DRIVE RAPID RANGE EXPANSION IN A BUTTERFLY

5.1 ABSTRACT

Responses of species to climate change are extremely variable, perhaps because of climate-related changes to interactions among species. We show that temperature-related changes in the dependence of the butterfly *Aricia agestis* on different larval host plants have facilitated rapid range expansion. Historically, the butterfly was largely restricted to a single plant species, *Helianthemum nummularium*, but recent warmer conditions have enabled the butterfly to increasingly use the more widespread plant species *Geranium molle*. This has resulted in a substantial increase in available habitat and rapid range expansion by the butterfly (79 kilometers northwards in Britain in 20 years). Interactions among species are often seen as constraints on species' responses to climate change, but we show that temperature-dependent changes to interspecific interactions can also facilitate change.

5.2 INTRODUCTION

Many species are altering their ranges in response to climate warming (Hickling et al. 2006), but patterns of expansion vary greatly among species (Warren et al. 2001, Chen et al. 2011a). Some species have retreated where they might have been expected to expand, whereas others have expanded considerably faster than expected based on the rate of climate change (Chen et al. 2011a). There are many potential explanations for variation in patterns of range change, including habitat availability (Hill et al. 2001), land use change (which can cause retractions), and dispersal ability (Warren et al. 2001). Laboratory and modelling studies also suggest that altered interspecific interactions could represent a major source of variation in determining range changes (Park 1954, Davis et al. 1998b, Davis et al. 1998a, Luoto and Heikkinen 2008, Schweiger et al. 2008, Van der Putten et al. 2010). Specifically, such interactions with other species are most commonly regarded as additional constraints because they may limit species to a narrower set of physical conditions (and hence narrower geographic ranges) than their fundamental, climatic niches might otherwise allow. Here, we provide field evidence of responses within individual populations and across geographic ranges to show that changing biotic interactions can accelerate rather than constrain distribution change.

Our study species is the brown argus butterfly (*Aricia agestis*). The butterfly reaches its northern range boundary in Britain, where it was scarce and declining in the 1980s (Bourn and Thomas 1993) and largely restricted to using rockrose (Cistaceae, *Helianthemum nummularium*) as its larval host plant, in calcareous grasslands (Heath et al. 1984). Plant species in the Geraniaceae family are used by the butterfly in continental Europe (Tolman 1997) but were rarely used historically in Britain (Heath et al. 1984), despite them being widespread. Since the 1980s, the butterfly has rapidly extended its distribution northwards (Fig. 5.1A) (Asher et al. 2001, Thomas et al. 2001), which is unusual for a species previously considered to be relatively specialized and sedentary (Warren et al. 2001), and colonized large areas where rockrose is absent. We report here how temperature-dependent changes in the butterfly’s association with larval host plants of the Geraniaceae family underpin this unexpectedly rapid range expansion.

Using distributional data we show that over the past 35 years the butterfly has increased its occurrence in areas where the only available hosts are Geraniaceae species and that this has been associated with an increase in summer temperatures. We also show that the butterfly is likely to have benefitted from an increase in the frequency of warm summers as population growth rates of the butterfly on both hosts are higher in years with warmer summers. Using these relationships between summer temperature and distributional and population dynamic changes in the butterfly we hindcast patterns over the past two centuries using historical climate data and show that only in the past two decades would the climatic conditions have been such that population growth of the butterfly is predicted to be greater on Geraniaceae than rockrose and a net shift in distribution into Geraniaceae sites is predicted. We then examine possible mechanisms that could make Geraniaceae species suitable hosts for the brown argus butterfly in Britain under warm but not cool climatic conditions. We show that rockrose achieves high local abundances and grows in places with warm microclimates compared with Geraniceae species and thus is likely to be the only host to support populations of the butterfly when climatic conditions are marginal. Finally, we compare the distribution of rockrose and Geraniaceae species in the landscape and show that Geraniaceae species are much more widespread, allowing rapid expansion of the butterfly when these species are available for use as larval host plants.

5.3 MATERIALS AND METHODS

5.3.1 Distributional changes of brown argus

5.3.1.1 Rate of northern range boundary expansion

First we wanted to calculate the rate of range shift of the brown argus butterfly in the UK over the past four decades to compare with other species. To do this we used 10 km × 10 km resolution UK Ordnance Survey grid square records (hereafter termed “10 km records”) of the butterfly for the period 1970 to 2009. These records were derived from a database of butterfly records submitted by volunteers and collated by Butterfly Conservation and the Centre for Ecology and Hydrology. The average latitudes of the 10 northernmost 10 km records of brown argus for the periods 1970-1987 (prior to the substantial increase in use of Geraniaceae, Fig. 5.1B) and 1988-2009 were calculated.

The rate of range expansion was calculated as the difference in latitude between the midpoints for these periods, giving a km per decade rate.

5.3.1.2 Change in use of Geraniaceae by brown argus over time

To examine whether the use of Geraniaceae species as larval host plants by the brown argus butterfly has changed over time we calculated from 1975 to 2009 the annual fraction of records of brown argus that were in locations where the only available host was a Geraniaceae species. 10 km records of common rockrose were extracted from a database of plant records submitted by volunteers and managed by the Botanical Society for the British Isles (accessed May 2010). Brown argus 10 km records for each year were assigned as being from rockrose sites if the 10 km grid square had a rockrose record, or as being from Geraniaceae sites if the grid square had no rockrose record. Geraniaceae species used for egg-laying and as larval food plants by brown argus (*Geranium molle*, *G. dissectum* and *Erodium cicutarium*) have almost complete coverage in Britain at 10 km grid square resolution and so can be assumed to be available as hosts outside the range of rockrose. Relative occurrence of brown argus on Geraniaceae sites for each year was calculated as the fraction of brown argus records in 10 km squares without a rockrose record. We tested for a change over time using a Spearman rank correlation (due to the non-linear nature of the relationship) between year and fraction of brown argus records in 10 km squares without a rockrose record. We only used data after 1975 because after this date there were more than 50 brown argus records each year, sufficient to provide an estimate of the division of records between rockrose and Geraniaceae sites.

We used 10 km × 10 km resolution data for this analysis because plants are very well recorded at this resolution, and finer resolution data are very patchy and would produce unreliable estimates. However, because Geraniaceae also occur in rockrose-containing 10 km squares, the reported distributional increase in use of Geraniaceae may have been conservative if the butterfly also expands over time onto Geraniaceae within 10 km squares that contain rockrose, as well as colonizing new 10 km squares where rockrose is absent. Likewise, it may have been an overestimate if brown argus was using Geraniaceae within rockrose-containing squares prior to its range expansion. We therefore also analysed finer resolution plant and butterfly data from a well-recorded region, the English county of Bedfordshire. Detailed botanical studies have been undertaken in this region and so we were able to assign brown argus records more reliably to locations with rockrose or dove's-foot cranesbill (*Geranium molle*) (the main host species facilitating the expansion of brown argus; see section 5.3.4). We extracted 2 km × 2 km resolution records of these plant species from the BSBI central database and 2 km × 2 km brown argus records from the Butterfly Conservation database. For each year, the number of brown argus records in 2 km squares with a dove's-foot cranesbill record and the number in 2 km squares with a rockrose record were calculated. A logistic regression of year *versus* the proportion of brown argus records in grid squares with a dove's-foot cranesbill record was used to test for a change in the relative occurrence of brown argus in locations with this Geraniaceae species over time. Logistic regression was used for this analysis due to the small numbers of brown argus records in some years.

5.3.1.3 Relationship between climate and occurrence of brown argus in Geraniaceae sites

To test for an effect of temperature on the occurrence of brown argus in Geraniaceae sites, we ran separate linear regressions of mean summer temperature (June, July, August) (MST), mean winter temperature (December, January and February) (MWT) and mean annual temperature (MAT) on the change in the fraction of records in

Geraniaceae-only 10 km squares from the previous year (fraction in current year minus fraction in previous year, used to de-trend the data). Temperature data were derived from the Central England Temperature (CET) dataset (Parker et al. 1992), which provides monthly temperature values for central England.

All of the analyses reported in this paper were conducted with MST, MWT and MAT. However, there was no significant effect of MAT in any of the analyses, and MWT was only marginally significant in one model (P = 0.038, negative effect on annual population growth, with no statistical effect on distribution change). Therefore, we only include analyses for summer temperature, which were consistently significant.

5.3.2 Population dynamics of brown argus in rockrose and Geraniaceae sites

5.3.2.1 Data and density calculation

To establish the population-dynamic basis for the distributional change in host plant use, we compared the dynamics of and analyzed the effect of climate on brown argus populations associated with different larval host plants, on the basis of count data by volunteers from over 200 fixed transects that form part of the UK Butterfly Monitoring Scheme (Pollard and Yates 1993). The scheme compiles observations of butterflies made by volunteers who regularly walk a network of transects between April and September within set criteria for weather and time-of-day. An index of abundance is calculated for each species on each transect for each year and, for species where it is appropriate, for separate flight periods within a year (Rothery and Roy 2001).

Data were downloaded for the 531 transects where brown argus had ever been recorded. These were split into transects where rockrose or Geraniaceae species were the main larval host plant, based on host plant distribution data, habitat descriptions given by transect recorders and geological data (rockrose is associated with chalk and limestone grassland). Host plant use was clear for 380 transects but we excluded the remainder, where host plant use was uncertain.

Separate first and second flight period counts and total annual abundance indices for the butterfly were extracted from the UK BMS database for these transects. Abundances were converted into densities (count per km) using transect lengths (all transects are 5 m wide). For each year from 1976 onwards, mean butterfly densities were calculated across rockrose and Geraniaceae sites separately. Mean total annual densities were calculated, as well as mean densities for first and second flight periods separately. Transects with fewer than 4 years with positive counts of brown argus were excluded, leaving data from a total of 207 transects for the analysis. Transects were also excluded from calculations of density for periods when the butterfly was absent (pre-colonization, or following extinction); with absence defined as four consecutive years with zero counts.

5.3.2.2 Population density and variability

To examine whether population dynamics of the brown argus butterfly differ between sites where it uses rockrose and sites where it uses Geraniaceae species we first compared population density and variability. A difference in log mean annual population density in rockrose and Geraniaceae sites was tested for using a t-test with values paired by year (data points in Fig. 5.2A). We then used mean generation densities of brown argus in rockrose and Geraniaceae sites to examine whether the variability of populations differs on the two different hosts. We used a Levene’s test to test for a difference.

5.3.2.3 Analysis of effect of climate on annual population growth rates

We were also interested in the effect of climate on the population dynamics of the butterfly in sites with different host species. We tested for an effect of climate on relative change in population density from one year to the next (*ln* annual density minus *ln* density in previous year). We ran separate ANCOVA models for each of the three climate variables described above (summer, winter, annual) with population growth rate as the response variable and climate and host plant as explanatory variables. For these analyses we used data from 1990 onwards, the period when population density counts were available from more than 5 transects with each host plant in each year (data from an average of 50 transect sites per host plant per year).

5.3.2.4 Analysis of effect of climate on generation-to-generation changes in population density

We also tested for an effect of climate on the relative change in population density between successive generations (*ln* generation density minus *ln* density in previous generation) in rockrose and Geraniaceae sites using ANCOVA, with climate and host plant as explanatory variables. Separate models were run for changes in population density from first to second flight period and for second to first flight period. Change from the first flight period (May and June) to the second flight period (July and August) represents the increase in butterfly numbers over the summer and so we included mean summer temperature as the climatic explanatory variable in models. Change from the second flight period in one year to the first flight period in the following year represents the over-winter change (usually decline) in population size, and so the model was run with mean winter temperature as the explanatory variable. Data from 1995 onwards were used in the analysis, the period for which population density counts were available for each generation from more than 5 transects on each host plant in each year (slightly fewer transects in the UK BMS database had separate flight period abundance data than annual abundance data and so a mean of 42 transect sites per host plant per year were used in this analysis).

5.3.2.5 Change in brown argus population density over time

Finally, we used separate Spearman rank correlations between annual populations density and year to test whether brown argus population densities in sites with rockrose and Geraniaceae species had changed over time.

5.3.3 Historic projections in host plant use by brown argus

We then used the relationships we calculated above using recent data between (1) the occurrence of brown argus in Geraniaceae-only areas (from distributional data) and summer temperature; and (2) population growth rates of brown argus in rockrose and Geraniaceae transect sites and summer temperature to estimate these parameters under past climatic conditions.

5.3.3.1 Estimation of past distribution changes towards or away from Geraniaceae sites

For this, we used the relationship between the change in fraction of brown argus records at Geraniaceae sites from the previous year and mean summer temperature (Eqn.1 in section 5.4.1; Fig. 5.1D; methods in section 5.3.1.3). Using mean summer temperatures calculated from historical CET data we estimated the change in fraction of occurrences of brown argus in Geraniaceae sites for all years back to 1800, when fine-scale historical climate data became available. Changes for all years within a decade were then summed to give an estimate of the expected net relative increase or decrease in occurrence in Geraniaceae-only areas, for each decade.

To assess the accuracy of these estimates we used historical records of brown argus derived from the same database as the modern records we used previously. We assigned records as being from rockrose areas or Geraniaceae-only areas using the same methodology described in section 5.3.1.2. Because there are few records available for this period we lumped data to calculate the fraction of records in Geraniaceae-only areas for different climatic periods. There were too few records prior to 1900 to accurately assess the fraction of records in Geraniaceae-only areas.

5.3.3.2 Calculation of estimated growth rates

For this, we used the relationships between annual population growth rate and mean summer temperature at rockrose and Geraniaceae sites (Eqns. 2 and 3 in section 5.4.2; Fig. 5.2C; methods in section 5.3.2.3). We used mean summer temperatures calculated from historical CET data to estimate population growth rates of brown argus on rockrose and Geraniaceae under past climatic conditions. These annual estimates were then averaged across all years within decades from 1800-1809 onwards and the average growth rate on Geraniaceae subtracted from that for rockrose to determine the relative performance of brown argus populations in Geraniaceae sites compared with rockrose sites.

5.3.3.3 Issues with this approach

There are some additional issues to consider when estimating these parameters under past conditions based on recent data. Firstly, some of the mean summer temperatures in the 19th century are lower than those experienced during the period on which relationships are based and we cannot be sure that a linear relationship between the relative performance on different host plants and temperature extends outside the range of data we have. In addition, estimates are based only on mean summer temperature and other climatic and biotic constraints may also contribute to past performance, such as winter temperatures and density-dependent factors such as parasitism (section 2.3.1.3).

The abundance of the hosts may have changed through time and this could affect population growth rates, although it is unlikely that Geraniaceae populations would have been rarer in the past (section 5.5.2). We also know that evolutionary changes have occurred in the brown argus butterfly which have affected its propensity to lay eggs on Geraniaceae as hosts (Thomas et al. 2001). Notwithstanding these *caveats*, the historical reconstruction of past performance is consistent with observations for at least the first half of the 20th century, for which some empirical distribution data are available.

5.3.4 Factors affecting brown argus population dynamics in rockrose and Geraniaceae sites

We then examined factors that might have led to the observed increase in use of Geraniaceae over time. To do so this we focused primarily on the Geraniaceae host plant dove's-foot cranesbill, because this is apparently the main species facilitating the range expansion of brown argus (Thomas et al. 2001). The two other main Geraniaceae hosts are: cut-leaved cranesbill (*G. dissectum*), which is suitable for larval development in the laboratory but appears less suitable in the wild because it desiccates in late summer, such that many larvae feeding on it would starve (personal observations); and common storksbill (*Erodium cicutarium*), which is an important host on coastal sand dune systems and in other localized habitats, but its more restricted distribution means that it cannot be responsible for the broad-scale, inland range expansion of the brown argus.

We examined three factors which could affect the use of different host species under different climatic conditions. First, we examined whether hosts differ in their local abundance because host abundance affects butterfly population size and larger populations are likely to be more robust to extinction under marginal climatic conditions. Second, we examined whether the microclimatic conditions in which the two hosts grow differs as this could affect their suitability for the butterfly under different climatic conditions. Finally, we looked at the performance of brown argus larvae feeding on different hosts. A poor quality host could also result in lower population growth rates and smaller population sizes, making the butterfly more vulnerable to extinction, particularly under marginal climatic conditions.

5.3.4.1 Resource abundance

We estimated the extent of host plant availability for brown argus through site visits. Sites with rockrose or dove's-foot cranesbill to be visited were identified using 100 m × 100 m resolution UK Ordnance Survey grid square records from the BSBI database (hereafter termed "100 m grid square"). A total of 30 rockrose sites across well recorded counties (Bedfordshire, Cambridgeshire and Suffolk) were selected. In order to compare density of the two host plant groups, a dove's-foot cranesbill site was then selected at random (when more than one record was available) from all those within a 10 km radius of each of the 30 rockrose sites. Rockrose records were rejected if they were within 300 m of a rockrose site from which data had already been collected.

A GPS unit was used to locate each 100 m grid square record. The 100 m grid square was then searched to establish the presence of the plant. Absence of the plant from the grid square may have been due to the grid reference being recorded incorrectly by the original recorder, and so the surrounding eight 100 m grid squares were also searched, in random order, until either the plant was found or all squares had been searched and the plant was not found. If the host plant was found then the study was carried out in that 100 m grid square; if it was not found then the site was abandoned and another selected.

Two 100 m long transects were walked (running east-west) in each 100 m grid square, one transect 25 m north and the other 75 m north of the southern edge of the 100 m square. Where part of the transect route was inaccessible (e.g. private land or impenetrable), transect routes totalling 200 m were walked in accessible parts of the square (transects at least 20 m apart). We rejected 100 m squares with less than 200 m of accessible transects. Host plant frequency was measured as the percentage of 2 m × 2 m quadrats along transects in which the plant was recorded. To provide unbiased estimates for the entire 100 m square (including inaccessible locations), frequencies of occurrence in the 2 m quadrats within each habitat type present were weighted by the proportion of each habitat type in the 100 m square.

Host plant cover was recorded in up to five 2 m quadrats in which the plant had been recorded along transects, selected at random after the transect was walked (all quadrats were measured if five or fewer quadrats contained the host plant). The average percent cover value of all quadrats was taken as the value for each site visited.

5.3.4.2 Aspect of sites with alternative host species

To compare the aspect of sites occupied by rockrose and Geraniaceae species, we used fine resolution data on the location of these species collected from three different sources:

1) 100 m grid square resolution records for rockrose and dove’s-foot cranesbill obtained from the BSBI database for four English counties that are particularly well recorded at this resolution: Bedfordshire, Dorset, Isle of Wight and Suffolk.
2) Locations of UK BMS butterfly transects that had contributed to our butterfly density calculations (100 m resolution grid square records provided by UK BMS).

3) Thirty 100 m resolution rockrose and 30 dove’s-foot cranesbill records selected at random from BSBI records across Bedfordshire, Cambridgeshire and Suffolk in which presence of the species was confirmed in the field (section 5.3.4.1).

The slope and aspect of these 100 m grid square plant records were derived from Digital Elevation Models (derived from the NERC Earth Observation Data Centre). For all plant data sets, sites were split into southerly-facing (slopes >5° and aspect 90°-270°) and other sites. Chi-squared tests were used to determine whether rockrose and dove’s-foot cranesbill sites were equally distributed between southerly-facing slopes and other sites.

5.3.4.3 Performance of brown argus larvae on alternative hosts

As outlined in Chapter 2, brown argus butterflies might not have historically used Geraniaceae species as hosts if females would not lay eggs on these species and/or because larvae did not have the physiological ability to develop on these plants. Evidence suggests that females have flexible oviposition preferences and have retained the ability to lay on Geraniaceae plant species even in locations where the only available host is rockrose (Thomas et al. 2001). Egg-laying preferences are, therefore, unlikely to have historically restricted the butterfly to rockrose.

Previous laboratory experiments suggest that larval performance is also unlikely to have acted as a constraint on the ability of brown argus to colonise Geraniaceae as larval performance is usually better on Geraniaceae than rockrose (average 1.2 times faster development and 1.1 times heavier pupae when reared on dove’s-foot cranesbill than rockrose) (Musche 2001, Bodsworth 2002). We complemented this laboratory-based work with a field experiment. Mated first generation female brown argus butterflies were collected from sites along a chalk escarpment in southern England (Chiltern Hills). Females were kept in separate cages in a greenhouse, with potted rockrose plants for egg laying. Upon hatching, larvae were transferred onto potted rockrose and dove's-foot cranesbill plants for translocation into the field. Larvae from each adult were split equally between host plant species to account for possible family effects. Eight translocation sites in the Chilterns and surrounding areas with known brown argus populations were used. Five rockrose and five dove's-foot cranesbill plants were dug into the ground at each site, with five larvae on each plant. Plants were covered with fine netting so larvae remained on the plants, were watered regularly in the field to prevent desiccation, and were replaced as required. Plants remained in place throughout the summer, corresponding with the normal period of development of larvae from eggs laid by first generation adults in this area. Plants were checked every day for pupae, which were collected. Development time was calculated as the number of days from translocation of larvae into the field to pupation. Pupal mass was measured within 48 hours of pupae being collected. Mean values were calculated for each pot and data were analysed using ANOVA with host plant as a fixed factor and site as a random factor.

5.3.5 Distribution of rockrose and dove's-foot cranesbill in the landscape

We compared the relative distribution of rockrose and dove's-foot cranesbill in the landscapes colonized to assess the potential effect of being able to use dove's-foot cranesbill on range expansion. We used data from two counties (Bedfordshire and Suffolk) that were well recorded for plants during the most recent BSBI intensive recording period of 1987-1999, in regions where the brown argus had expanded its distribution. We identified all 100 m grid squares in these counties where rockrose and/or dove's-foot cranesbill had been recorded. We used the number of separate 100 m resolution rockrose and dove's-foot cranesbill records to estimate the relative occurrence of the two plant species in each county. It is likely that dove's-foot cranesbill is under-recorded at this detailed spatial resolution (100 m) in comparison to rockrose (dove's-foot cranesbill is widespread yet localized within 100 m squares; rockrose occurs in species-rich grasslands that are heavily visited by botanists), and hence dove's-foot cranesbill is likely to be even more widespread in the landscape than Fig. 5.7 suggests.

5.4 RESULTS

5.4.1 Distributional changes of brown argus

The brown argus butterfly has spread northwards in Great Britain by ~79 km in 20 years (Fig. 5.1A). This is 2.3 times faster than both the average expansion rate documented for species globally (median 16.9 km per decade) and for butterflies in the UK (mean 17.5 km per decade) (Chen et al. 2011a).

There has been an increase over time in the fraction of all occurrences of the butterfly in locations where the only available host plants were in the family Geraniaceae (Spearman's rank correlation between year and fraction of 10 km × 10 km resolution observation records in Geraniaceae-only areas: $r_s = 0.82$, n = 35 years, $P < 0.001$) (Fig. 5.1B). A significant increase in use of Geraniaceae over time was also found using finer resolution (2 km × 2 km) data from Bedfordshire (logistic regression of proportion of brown argus records in 2 km × 2 km squares with Geraniaceae host plants versus year: $z_{1,23} = 2.13$, $P = 0.033$; 42 % brown argus records in sites with Geraniaceae host plants during the period 1970-1987 compared with 70 % during the period 1988-2009) (Fig. 5.1C). Thus, the conclusion that brown argus has disproportionately expanded its distribution into areas that contain only Geraniaceae host plants is robust to the scale of analysis. This has been achieved through an increase in occurrence in Geraniaceae-only areas because occurrence on rockrose does not appear to have declined.

Increases in the occurrence of brown argus in Geraniaceae-only areas were associated with warm summers [linear regression of the between-year change in the fraction of brown argus records in Geraniaceae-only grid squares *vs.* mean summer temperature (MST): Eqn. 1, change = 0.03(MST)–0.47; Pearson $r = 0.45$, $F_{1,32} = 8.35$, $P = 0.007$] (Fig. 5.1D).

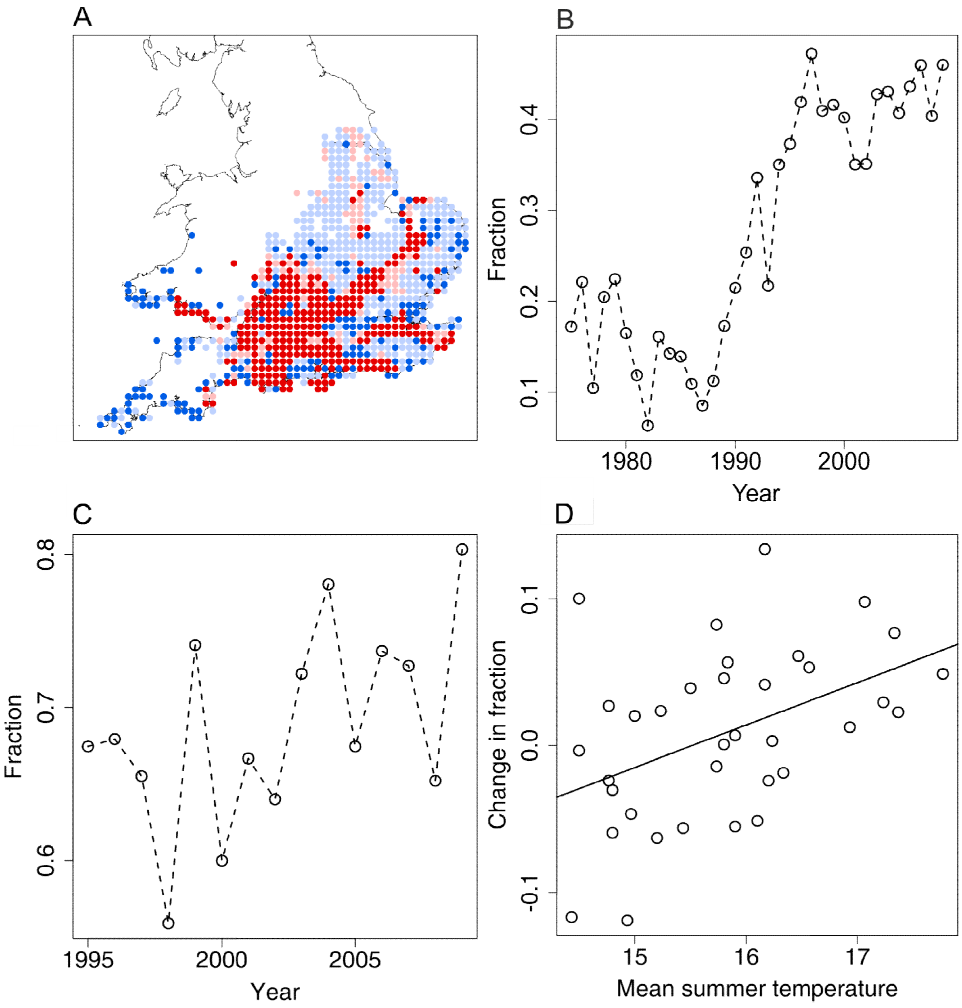

Fig. 5.1 Distribution changes of the brown argus butterfly. (A) Occurrence of brown argus in 10 km × 10 km grid squares that contain rockrose (red) and Geraniaceae only (blue). Records from the period 1970–1987 [before the increase in use of Geraniaceae (B)] are in dark shades; new 10 km × 10 km squares colonized in the period 1988–2009 are in light shades. (B) Increase in the fraction of brown argus distributional records from 10 km × 10 km grid squares across Britain where only Geraniaceae hosts are present. (C) Increase in the fraction of brown argus distributional records from 2 km × 2 km grid squares in Bedfordshire where dove's-foot cranesbill has been recorded as being present. (D) Relationship between the change from previous year in the fraction of brown argus distributional records from 10 km × 10 km grid squares where only Geraniaceae hosts are present and MST.

5.4.2 Density of brown argus in rockrose and Geraniaceae sites

Rockrose sites have a significantly higher density of brown argus than Geraniaceae sites (paired t-test on annual density: t = -14.85, d.f. = 20, $P < 0.001$) (Fig. 5.2A) and support significantly more stable populations of brown argus than Geraniaceae sites (Levene's test for equality of variances: $W = 10.97$, n = 2 30-generation sequences, $P = 0.002$) (Fig. 5.2B).

Warmer summers result in higher brown argus population densities on both rockrose and Geraniaceae [analysis of covariance, relationship between annual population growth rate and MST, with host as categorical variable: $F_{1,35} = 6.85$, $P = 0.013$; Eqn. 2, population growth on rockrose = 0.21(MST)–3.30; Eqn. 3, population growth on Geraniaceae = 0.33(MST)–5.18] (Fig. 5.2C), with no effect of mean annual temperature, and little or no effect of winter temperature.

No relationship was found between the over-winter change in abundance and mean winter temperature, but relative population growth from first to second flight period was significantly positively related with summer temperature at rockrose and Geraniaceae sites [ANCOVA, relationship between population growth rate and MST, with host as categorical variable: $F_{1,27} = 5.45$, $P = 0.027$; population growth on rockrose = 0.23(MST)–3.32, population growth on Geraniaceae = 0.44(MST)–5.67] (Fig. 5.2D).

There has been a 5.3-fold increase in brown argus population density in Geraniaceae sites between 1976-1985 and 2000-2009 (Spearman rank correlation between year and density on Geraniaceae: $r_s = 0.76$, n = 34 years, $P < 0.001$) (Fig. 5.2A). In contrast, no increase in overall population density occurred at rockrose sites (Spearman's rank correlation between year and density on rockrose: $r_s = 0.25$, n = 34 years, $P = 0.162$; 1.1-fold density increase from 1976-1985 to 2000-2009) (Fig. 5.2A), even though butterfly abundance increased temporarily during warm summers.

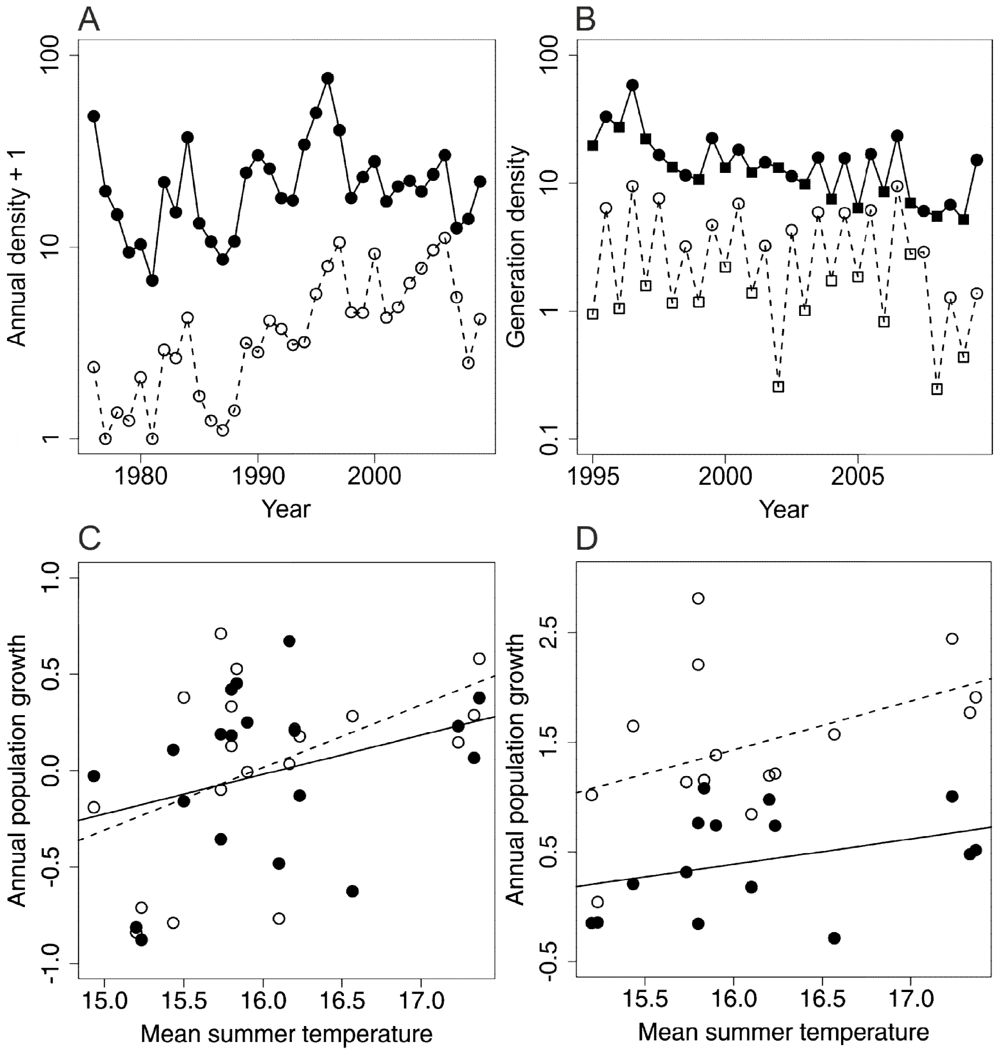

Fig. 5.2 Population dynamics of brown argus in rockrose and Geraniaceae sites. (A) Mean annual density (count per kilometre) of brown argus in rockrose (solid line, solid symbols) and Geraniaceae (dashed line, open symbols) sites from 1976 to 2009. (B) Same as for (A), but separating 1995 to 2009 population counts into numbers for first (squares) and second (circles) adult flight periods each year. Relationship between (C) brown argus annual population growth and (D) brown argus summer population growth and mean summer temperature in rockrose (solid line, solid symbols) and Geraniaceae (dashed line, open symbols) sites.

5.4.3 Historic projections in host plant use by brown argus

The climate over most of 19th and 20th centuries was cooler than at present, and during this period our models predict a retraction in the distribution of brown argus away from Geraniaceae in most decades and lower relative population growth rates on Geraniaceae than rockrose (Fig. 5.3). Thus both the distribution and population growth rate analyses indicate that the rockrose host plant was more favourable than Geraniaceae under these cooler conditions. Historical records of brown argus support this prediction, with only 19 % of occurrences of brown argus coming from Geraniaceae-only areas during the cool beginning of the 20th century (1900-1929), increasing to 30 % during the warmer 1930s and 1940s and dropping back down to 16 % during the cooler period from 1950 to 1989 (insufficient records are available from the 19th century to assess the relative occurrence in Geraniaceae-only areas). In the past two decades, as the frequency of warm summers has increased our models (Eqns. 1 to 3) lead to the expectation that brown argus will have experienced higher population performance on Geraniaceae than on rockrose and have expanded in its distribution onto Geraniaceae (Fig. 5.3), as has been observed (Fig. 5.1).

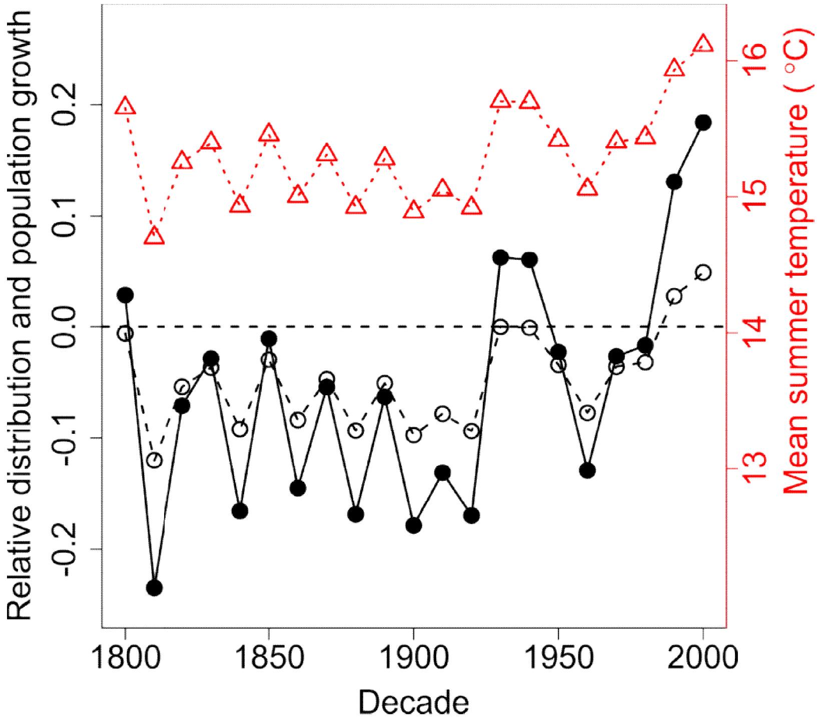

Fig. 5.3 Estimated decadal net increase (positive) or decrease (negative) in the fraction of all brown argus occurrences associated with Geraniaceae sites in the past (solid line, solid circles). Difference in estimated annual population growth rates, averaged across decades, between Geraniaceae and rockrose sites (mean for Geraniaceae sites minus mean for rockrose sites) (long dashed line, open circles; positive values indicate higher relative population growth on Geraniaceae, and negative values indicate higher performance on rockrose). MST for each decade (short red dashed line and triangles). Dates on scale bar refer to first year of each decade for which estimates have been calculated.

5.4.4 Factors affecting population dynamics at rockrose and Geraniaceae sites

5.4.4.1 Resource abundance

There was a significantly higher percentage of 2 m quadrats occupied by rockrose than dove's-foot cranesbill within 100 m grid squares (Mann-Whitney *U*-test: Z = -2.682, n = 60, *P* = 0.007; mean rockrose = 15.2 %, mean dove's-foot cranesbill = 3.4 %) (Fig. 5.4A) and a significantly higher percentage cover of rockrose than dove's-foot cranesbill within 2 m quadrats that contained the plants (Mann-Whitney *U*-test: Z = -5.393, n = 60, *P* < 0.001; mean rockrose = 12.8 %, mean dove's-foot cranesbill = 2.4 %) (Fig. 5.4B). Over a 100 m grid square, this equates to an estimated 23 times greater cover of rockrose than dove's-foot cranesbill.

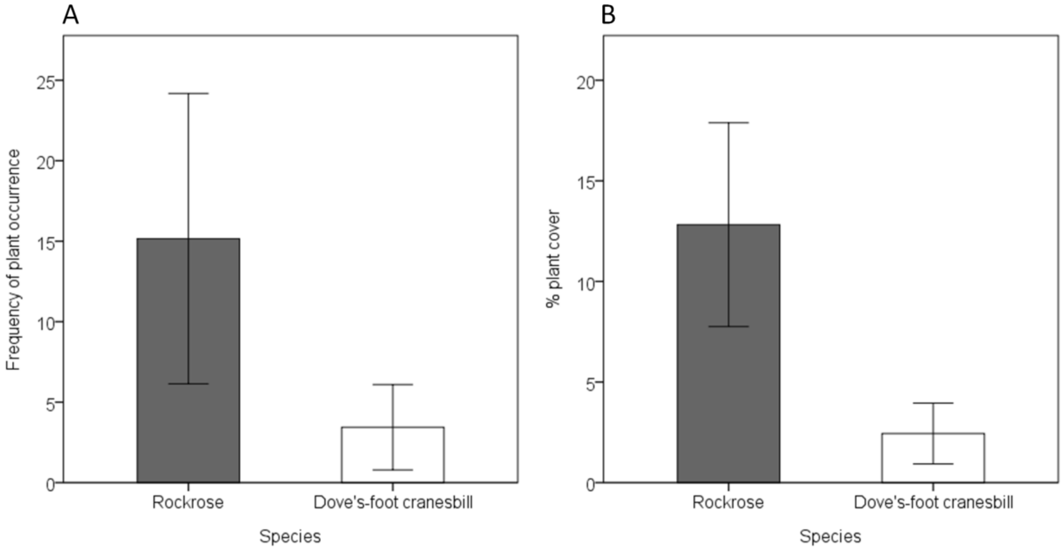

Fig. 5.4 Local abundance of rockrose and dove's-foot cranesbill within 100 m × 100 m grid squares where host plants were present. (A) Mean number (and 95 % CI) of 2 m × 2 m quadrats along 200 m transects in which rockrose (filled bar) and dove's-foot cranesbill (open bar) were recorded. (B) Mean percentage (and 95 % CI) cover of rockrose (filled bar) and dove's-foot cranesbill (open bar) within 2 m × 2 m quadrats where the plants were present.

5.4.4.2 Aspect of sites with alternative host species

There was a significantly greater proportion of rockrose records than dove’s-foot cranesbill records on southerly-facing slopes in all situations considered (Fig. 5.5). Based on 100 m × 100 m resolution records from the BSBI, there was a significantly greater proportion of rockrose records on southerly-facing slopes in (A) Bedfordshire ($\chi^2 = 46.7$, d.f. = 1, $P < 0.001$), (B) Dorset ($\chi^2 = 32.2$, d.f. = 1, $P < 0.001$), (C) Isle of Wight ($\chi^2 = 21.1$, d.f. = 1, $P < 0.001$) and (D) Suffolk ($\chi^2 = 9.0$, d.f. = 1, $P = 0.002$). A significantly greater proportion of butterfly transect locations with rockrose were on southerly-facing slopes than those with Geraniaceae ($\chi^2 = 25.9$, d.f. = 1, $P < 0.001$). Finally, a significantly greater proportion of ground-truthed rockrose records were on southerly-facing slopes than ground-truthed dove’s-foot cranesbill records ($\chi^2 = 13.5$, d.f. = 1, $P < 0.001$).

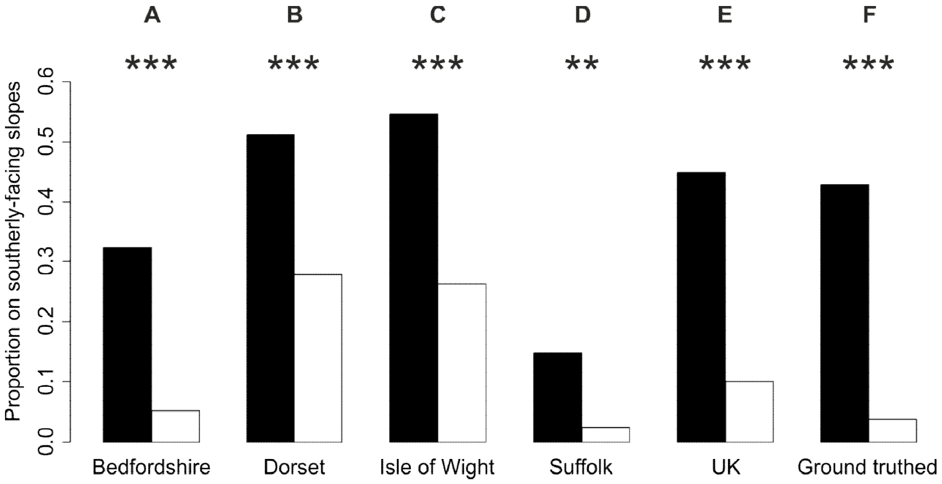

Fig. 5.5 Proportions of sites that have a southerly aspect (90° – 270° and slope >5°) for rockrose, (solid bars) and dove’s-foot cranesbill (open bars), based on 100 m × 100 m resolution records from the Botanical Society of the British Isles (BSBI) database in well recorded regions, namely (A) Bedfordshire, (B) Dorset, (C) Isle of Wight and (D) Suffolk; (E) butterfly transect locations used for the brown argus population dynamics analysis; and (F) a random selection of BSBI records ground-truthed to confirm the presence of the species concerned.

5.4.4.3 Performance of brown argus larvae on alternative hosts

Development time was significantly faster for larvae reared on dove’s-foot cranesbill compared with rockrose (ANOVA: $F_{1,68} = 5.98$, $P = 0.017$, on average 1.1 times faster on dove’s-foot cranesbill than rockrose; Fig. 5.6A), and pupal mass was significantly greater for larvae reared on dove’s-foot cranesbill compared with rockrose (ANOVA: $F_{1,66} = 10.18$, $P = 0.002$, on average 1.1 times heavier on dove’s-foot cranesbill than on rockrose; Fig. 5.6B).

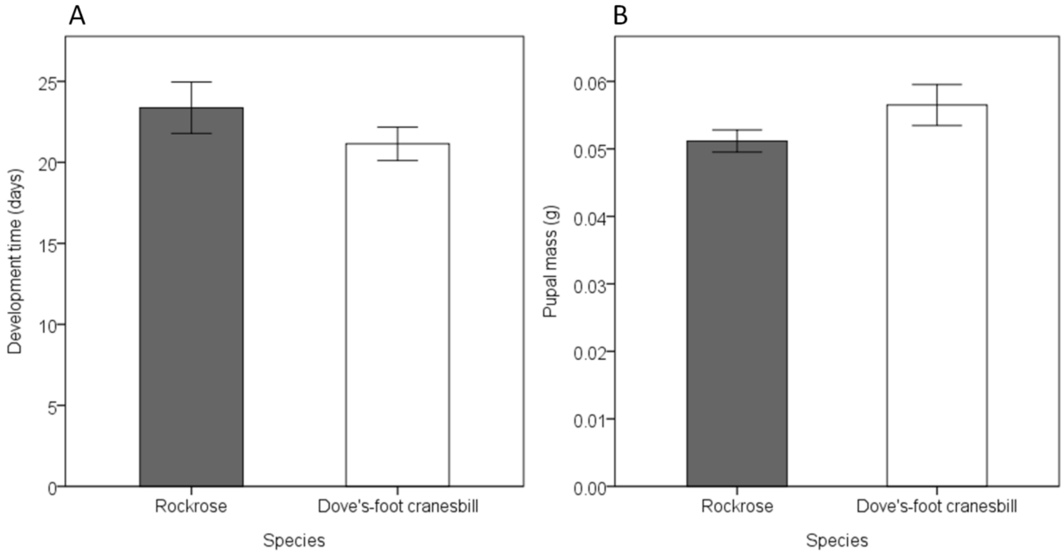

Fig. 5.6 Performance of brown argus reared on different host plants. (A) Mean development time (and 95 % CI) of larvae reared on rockrose (solid bar) and dove’s-foot cranesbill (open bar). (B) Mean pupal mass (and 95 % CI) resulting from larvae reared on rockrose (solid bar) and dove’s-foot cranesbill (open bar).

5.4.5 Distribution of rockrose and dove’s-foot cranesbill in the landscape

Based on 100 m × 100 m grid squares with records of host plants, dove’s-foot cranesbill is 4 to 17 times more widespread than rockrose in counties where rapid expansion has taken place (Fig. 5.7).

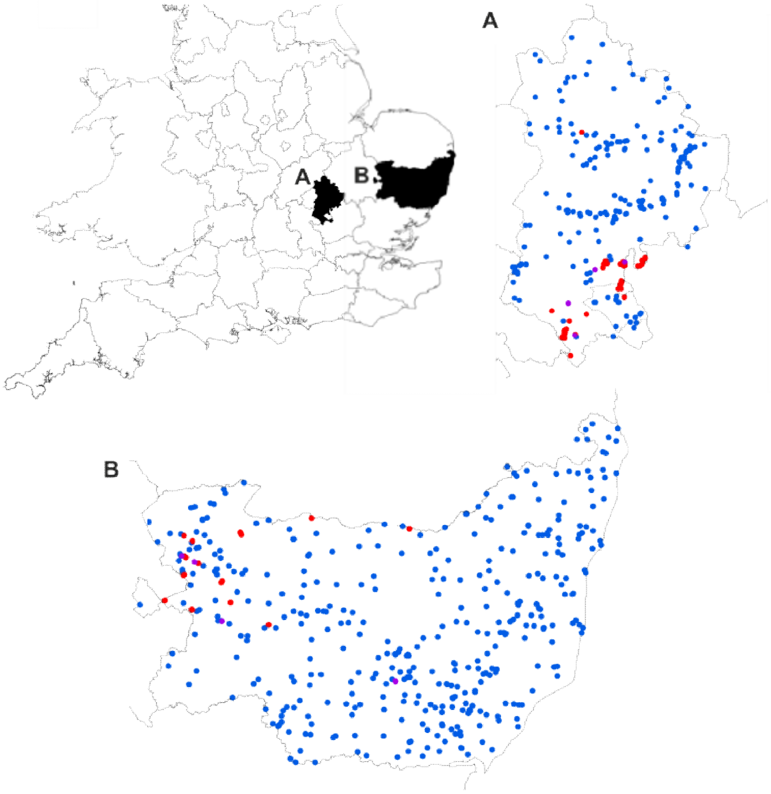

Fig. 5.7 Availability of rockrose and cranesbill in the landscape. 100 m × 100 m grid squares with records of rockrose (red symbols), dove’s-foot cranesbill (blue symbols) or both species (purple symbols) in two well-recorded counties: (A) Bedfordshire and (B) Suffolk. Rapid range expansion took place in Bedfordshire and Suffolk, associated with the increased use of dove’s-foot cranesbill and other Geraniaceae.

5.5 DISCUSSION

We have shown how different niche properties of plants determine their suitability as larval host species for a butterfly under different climatic conditions. We have shown that as climatic conditions change over time, host plant use by the butterfly can change, resulting in an increase in habitat availability and rapid range expansion.

5.5.1 Relationship between climate and host plant use by brown argus

Evidence from distribution data shows that during the 1970s and 1980s brown argus was largely restricted to areas where rockrose was available but that during the 1990s there was a significant increase in the occurrence of brown argus in areas where the only available hosts were Geraniaceae species (Fig. 5.1, A, B and C). Incremental increases in the occurrence of brown argus in Geraniaceae-only areas were associated with warm summers (Fig. 5.1D). Evidence from population dynamic data reveals that brown argus population growth rates are also higher in years with warmer summers (Fig. 5.2, C and D) and that brown argus population densities have increased in sites where they use Geraniaceae as hosts over time (Fig. 5.2A).

Thus it appears that in Britain, rockrose is the only suitable host for brown argus when summers are cool but as the frequency of warm summers increases the butterfly is also able to use Geraniaceae species. Our models of the relationship between summer temperature and the occurrence and population growth of brown argus in Geraniaceae sites predict that during the 19th and much of the 20th centuries there would have been a net shift of the butterfly’s distribution away from Geraniaceae sites and that population growth rates would have been lower in Geraniaceae sites than in rockrose sites (Fig. 5.3). Only in the past two decades have summer temperatures been warm enough that the models predict higher population growth rates on Geraniaceae than rockrose and a shift in distribution towards Geraniaceae, as has been observed.

5.5.2 Mechanisms driving observed patterns

Brown argus is likely to have survived past, cooler periods predominantly as localized populations in warm sites that contain large rockrose populations. Rockrose achieves high local densities compared with dove's-foot cranesbill (Proctor and Griffiths 1956, Grime et al. 1988) (Fig. 5.4). The long-lived perennial rockrose also has more stable populations than the annual and ruderal dove's-foot cranesbill. These differences between the plants enable rockrose to support larger and more stable populations (Fig. 5.2, A and B). Moreover, rockrose frequently grows in areas of short turf on southerly facing slopes (Lakhani and Davis 1982) (Fig. 5.5), which provide warm microclimates [southerly aspects receive greater direct radiation and achieve higher maximum summer temperatures (Hutchins et al. 1976)]. As recently as the early 1980s, the brown argus was mainly associated with rockrose populations on sheltered south-facing slopes (Bourn and Thomas 1993). The few historical records of Geraniaceae-feeding populations from this period were predominantly in sand dunes (Heath et al. 1984), which also provide warm microclimates.

Summer temperatures in Britain from 1990 to 2009 were on average 0.78 °C warmer than between 1800 and 1989 and this is likely to have increased the thermal suitability of sites for brown argus, especially those that are not southerly facing. This would have increased the ability of Geraniaceae-containing sites to support brown argus population growth resulting in the increase in population density observed in Geraniaceae sites (Fig. 5.2A). In contrast, no increase in overall population density occurred at rockrose sites (Fig. 5.2A), even though butterfly abundance increased temporarily during warm summers. This suggests that other factors limit population density on rockrose. High levels of parasitism and availability of hosts with specific leaf types selected for egg-laying may be limiting factors (section 2.3.1.3).

5.5.3 Other potential mechanisms

There are other possible mechanisms which could explain the observed increase in use of Geraniaceae with warm summers which are discussed here. Firstly, this patterns could be due to spillover of individuals from rockrose sites into Geraniaceae sites as population densities increase with the frequency of warm summers. However, dispersal distances estimated for brown argus (section 2.3.1.3) are insufficient for single-generation emigration from rockrose sites (i.e., density-dependent emigration, spillover or source-sink effects) to account for the increase of brown argus observations in Geraniaceae sites many ten of kilometres from the nearest rockrose site (Fig. 5.1A, Fig. 5.7).

Secondly, if the distribution of the host plants had changed over time this could cause distributional changes in the butterfly. Rockrose is strongly associated with dry, species-rich calcareous grasslands with short swards. Large amounts of such habitats were lost from Britain from the mid-20th century onwards due to agricultural intensification or abandonment. This may have led to historical declines in the brown argus butterfly (Bourn and Thomas 1993). Dove’s-foot cranesbill is associated with disturbed habitats such as field margins, road verges and small-scale disturbances in semi-natural pastures. As such, local abundances may have fluctuated over time in response to land use change. For example, the species may have declined in abundance as pastures were improved and as herbicide use increased on field margins. Abundance may have increased, however, with the introduction of certain environmental stewardship schemes. Despite this, the species is known to have been widespread since records became available and thus for the period we are concerned with. It has been widespread in the British countryside throughout the 19th, 20th and early 21st centuries. Fluctuations in the local abundances of both of these hosts over time are likely to have had some impact on the local abundance and distribution of brown argus. However, there has been no widespread change in the abundance of either host over the past 3 decades that can explain the observed expansion of brown argus onto Geraniaceae.

It is also possible that other ecological or evolutionary changes are occurring in the host plants in response to climate change, which might affect their suitability for brown argus, but there is no evidence that such changes have taken place.

Finally, preference and performance experiments undertaken here and previously (Thomas et al. 2001, Bodsworth 2001, Musche 2002) reveal that even populations with a long-established association with rockrose have the capacity to successfully utilize Geraniaceae. Not only will they accept Geraniaceae for egg-laying, but larvae originating from rockrose sites actually perform better on dove’s-foot cranesbill than on the traditional rockrose host (presumably for nutritional reasons), developing faster and producing heavier pupae (which are thereby likely to have higher fecundities). Therefore, we conclude that constraints other than female egg-laying behaviour and larval physiological capacity must have been responsible for the historical restriction to rockrose.

5.5.4 Consequences for range expansion

We have shown that the brown argus butterfly has shifted its distribution northwards in Britain by ~79 km in 20 years, over twice the average rate for butterfly species in Britain. Once the brown argus can establish populations on cranesbill, the high frequency of cranesbill populations in the landscape permit it to spread between populations of this host plant without the need for long-distance dispersal. The butterfly's capacity to use Geraniaceae has been aided by the spread of butterfly phenotypes that readily select Geraniaceae plants for egg-laying (Thomas et al. 2001; Buckley et al. 2012) and by a degree of escape from natural enemies (parasitoids) associated with historical rockrose sites (Menéndez et al. 2008). These processes have come together to generate an unexpectedly rapid transformation in the metapopulation dynamics of the butterfly from a highly localized distribution associated with southerly-facing rockrose-containing calcareous grasslands to widespread use of virtually any grassland with rockrose or Geraniaceae host plants. Ecological and evolutionary adjustments by the butterfly, interacting with alternative host plants that differ in their niches and life-history traits, have resulted in rapid range expansion of this previously rare and declining butterfly. We suggest that altered interactions among species do not necessarily constrain distribution changes but can facilitate expansions.

CHAPTER 6

GENERAL DISCUSSION

Species are shifting their distributions in response to climate change (Parmesan and Yohe 2003) but the rate of expansion at leading-edge range margins varies within (Hill et al. 2001) and between species (Chen et al. 2011a). Many species are lagging behind climate change (Devictor et al. 2012) and this is concerning because they may be at risk of extinction if they are unable to keep pace with climate warming (Thomas et al. 2004, Thuiller et al. 2005). Thus identifying factors that affect rates of leading-edge expansion is important. Habitat availability is one of these factors, with expansion being slower in areas where habitat is more highly fragmented (Hill et al. 2001, Wilson et al. 2009). However, species' habitat associations are predicted to broaden at leading-edge range margins as the climate warms (Thomas et al. 1999), which could, therefore, increase habitat availability and rates of range expansion. Changes in habitat associations have, however, received very little attention (Davies et al. 2006) and identifying the occurrence and drivers of such changes has been the focus of my thesis. In this Chapter I discuss the key findings of my thesis in relation to my original aims (section 6.1) and evaluate my experimental approaches (section 6.2). I then discuss whether these findings are applicable to other species (section 6.3) and whether changes in habitat associations might be expected in other parts of species' ranges (section 6.4). I discuss implications of my findings for conservation (section 6.5) and give suggestions for future work (section 6.6).

6.1 THESIS AIMS AND FINDINGS

Aim 1: To establish the relationship between climate and the spatial and temporal patterns of species' habitat associations

I have shown for two species, the speckled wood butterfly (*Pararge aegeria*) and the brown argus butterfly (*Aricia agestis*) that variation in habitat and host plant associations at leading-edge range margins are related to variation in climatic conditions. In particular, I have shown that species' habitat and host plant associations are narrower under more marginal climatic conditions and that these associations have broadened over time in response to climate change. Speckled wood is primarily a butterfly of woodland but in Chapter 3 I showed that temperature and rainfall affect its strength of association with woodland. The species is less restricted to woodland in warmer parts of Britain and it is less restricted to woodland in places with warm and wet summers than places with warm and dry summers. I also showed that changes in speckled wood's habitat associations over time are related to changes in climatic conditions: it has weakened its association with woodland most in areas that have warmed most and where summer rainfall has increased most.

In Chapter 5 I showed that the brown argus butterfly has altered its host plant associations over time in Britain in response to climate warming. I showed that the butterfly was previously largely restricted to using common rockrose, but over time has increased its occurrence in areas where the only host species available are members of the Geraniaceae family. I demonstrated that the butterfly's increased use of Geraniaceae is linked to an increase in the frequency of warm summers by showing that annual increases in the occurrence of the butterfly in sites where the only available host plants are Geraniaceae species was greater in years with warmer summers.

Thus I have added to the existing body of evidence that species are broadening their habitat associations at their leading-edge range margins in response to climate change. Furthermore, I have shown that shifts can occur in relation to the use of different habitat types as well as in relation to larval host plants used.

Aim 2: To evaluate the mechanisms driving the relationship between climate and species' habitat associations

Analyses to establish relationships between species' habitat associations and climate were correlative, but I have also made progress towards establishing potential mechanisms driving these relationships. In Chapter 4 I used field experiments to show that larval performance was poorer in grassland than woodland both over winter and summer. Using laboratory experiments I showed that longer larval development times in grassland than woodland over winter can be attributed to more severe cold exposure in grassland, but the causes of smaller pupae resulting from larvae that have been reared in grassland over winter and slower larval growth rates in grassland over summer require further investigation. Poorer larval performance in grassland could lead to slower population growth rates and smaller populations in open habitats and thus play an important role in driving patterns of habitat associations in this species.

For microclimatic differences between open and closed habitats to drive the spatial and temporal patterns of habitat associations observed in Chapter 3, performance should be better in woodland than grassland, as has been shown here; and performance should be better in less climatically marginal areas, which needs to be tested. For example, speckled wood is more restricted to woodland in locations with colder winters such as central Scotland and the upland areas of Wales, compared with places with mild winters such as south-west England. If differences in microclimatic conditions were an important factor driving these patterns it would be expected that deleterious effects of overwinter cold on larval performance would be greater in open habitats and in locations with colder winters. Eventually climatic conditions may become sufficiently marginal that only populations in woodland are able to survive. Field experiments or lab experiments that replicate the range of conditions experienced in woodland and grassland across the butterfly’s range in Britain would help to further assess the effects of microclimate on patterns of habitat associations.

In Chapter 5 I identified differences between rockrose and dove’s-foot cranesbill (the main Geraniaceae host used by brown argus) that are likely to have played a role in the restriction of brown argus to rockrose under cooler climatic conditions, and the increase in the use of Geraniaceae species in years with warmer summers. Firstly, using fine-scale occurrence data and new field data I showed that rockrose is more strongly associated with southerly-facing (warm) slopes than dove’s-foot cranes-bill which is generally found on flat (cooler) ground. Thus rockrose is likely to present a microclimatic advantage to brown argus under cooler climatic conditions when time for life cycle completion is limited. Using UK Butterfly Monitoring Scheme (UK BMS) transect data I also showed that brown argus populations are considerably smaller in sites where the main host is a Geraniaceae species compared with those where the host is rockrose, which my field data show is due to Geraniaceae species occurring at lower local abundances than rockrose. Using UK BMS data I showed that brown argus populations on Geraniaceae also fluctuate more widely in size than those on rockrose, which are more stable. Small, unstable populations are more vulnerable to extinction, particularly when climatic conditions are unfavourable. All these factors are likely to have made Geraniaceae species unsuitable hosts when climatic conditions were poor. Using UK BMS data I have shown that brown argus population sizes have increased on Geraniaceae over the past 30 years. This is likely to be because they are now more thermally suitable, and while populations are still smaller than those on rockrose, they are now likely to be less vulnerable to extinction.

Aim 3: To assess the effect of changes in habitat associations on rates of leading-edge range margin expansion

In Chapter 3 I demonstrated that the speckled wood butterfly has weakened its association with woodland over time, particularly at its expanding range margin. Although it still remains primarily a butterfly of woodland, an increase in the ability of the butterfly to use alternative habitats could help it to expand its distribution. Woodland cover is very fragmented in Britain and so an increase in the number of speckled wood populations outside of woodland will reduce dispersal distance between suitable habitat patches, increase the number of dispersers and hence increase the number of colonisation events. Although the butterfly appears to be lagging behind climate change (Hill et al. 2002) due to fragmentation of woodland (Hill et al. 2001), observed rates of expansion may be faster when habitat associations become broader compared with a situation where the butterfly remained restricted to woodland. However, the degree to which range expansion increases following a change from 23 % to 40 % of speckled wood occurrences at the range margin being in non-woodland habitats requires further investigation.

In Chapter 5 I showed that the brown argus butterfly has expanded its distribution at its leading-edge range margin by ~79 km in 20 years, which is 2.3 times faster than the average rate of species globally (median 16.9 km per decade) (Chen et al. 2011a). Rapid range expansion was unexpected in this butterfly given its previously restricted distribution in Britain (Warren et al. 2001). Its recent rapid range expansion is due to the species increased ability to utilise Geraniaceae species which are very widespread in the landscape, hence substantially increasing available habitat and reducing dispersal distance between habitat patches. Rockrose has a restricted distribution whereas Geraniaceae species are very common and widespread (Fig. 2.7), and so an increased ability to use Geraniaceae species has enabled much faster expansion compared with a situation where the butterfly remained restricted to using only rockrose. Evolutionary changes (Thomas et al. 2001, Buckley et al. 2012) and a degree of escape from natural enemies (Menéndez et al. 2008) may also have contributed to increased rates of recent range expansion in this butterfly.

6.2 EXPERIMENTAL APPROACHES

6.2.1 Use of existing datasets

Many of the conclusions from my thesis were arrived at through analysis of large existing datasets comprising records collected by volunteers. Patterns of speckled wood's habitat associations and brown argus' host plant associations were elucidated using butterfly records collated by Butterfly Conservation and the Centre for Ecology and Hydrology. For the brown argus analyses, plant records from a database of records collected by volunteers and collated by the Botanical Society of the British Isles were also used as well as butterfly abundance data from the UK Butterfly Monitoring Scheme. These datasets have been used widely in scientific research, particularly in detecting changes in species' responses to climate change, such as changes in distribution (Parmesan et al. 1999), abundance (Roy et al. 2001) and phenology (Roy and Sparks 2000). These datasets are, however, not without their problems which can limit their use.

6.2.1.1 Distribution datasets

Distribution data are collected non-systematically and volunteers are encouraged to submit records of any butterfly species they have observed, although effort is made to achieve complete geographic coverage at a 10 km × 10 km grid square resolution for the production of published Atlases. Problems arise, therefore, when trying to detect trends in the data because of variation in recorder effort. Spatially, recorder effort is much greater in areas with high human population densities, and recorder effort has increased substantially through time (Fox et al. 2006). The first butterfly atlas (Heath et al. 1984) was based on 185,649 records submitted in the period 1970-82, whereas the second atlas (Asher et al. 2001) was based on 1,710,586 records from the period 1995-99 and the third (Fox et al. 2006) 1,616,620 records submitted in the period 2000-04. Furthermore, the proportion of records submitted at a fine resolution (100 m grid resolution), which are particularly useful when considering species' habitat associations, has also increased over time.

Thus analyses may be biased by variation in recorder effort. My estimation of leading-edge range margin expansion by the brown argus butterfly, for example, could have been exaggerated by an increase in recorder effort. However, the estimation of range change was based on data pooled for each study period (1970-1987 and 1988-2009) which should improve coverage and hence the estimate of the location of the northern range boundary for each period. In addition, because the butterfly was localised in the first period its 10 km grid square resolution distribution is likely to have been well described. Furthermore, I only used records south of the butterfly's zone of hybridisation with the northern brown argus, *Aricia artaxerxes* (Mallet et al. 2011) and thus my estimated rate of expansion will have been an underestimate if 'pure' southern brown argus individuals have expanded into this region.

The plant database also has problems with variation in recorder effort. Fine resolution (100 m) records vary considerably in their abundance between counties, presumably due to variation in the enthusiasm of local recorders. Furthermore, recorder effort also varies between host plant species which presented a problem in Chapter 5 when quantifying the availability of alternative host plants in the landscape for brown argus. Rockrose is a species of unimproved or semi-improved calcareous grasslands which tend to be relatively species-rich and hence attract a lot of recording effort. Dove’s-foot cranesbill, by contrast, is a common species which is less likely to compel people to submit records; and it occurs in habitats that are less likely to be visited by recorders. Thus achieving a robust comparison of the distribution of the two host plant species in the landscape is difficult. In Chapter 5, I tried to account for this issue by analysing data only from two counties that were well recorded, but the coverage of dove’s-foot cranesbill in these counties is still likely to be an underestimate.

6.2.1.2 Transect data

Data from the UK Butterfly Monitoring Scheme are standardised: transects are only walked in certain weather conditions, the same route is repeated each year and the same recording methodology used so that measures of butterfly abundance can be compared between sites and years (Pollard and Yates 1993). However, these data also have certain biases because many of the transects are on “high-quality” sites such as nature reserves and so butterfly trends in these sites may not be representative of the wider landscape (Fox et al. 2006). Transects are also much more numerous in the south of Britain than the north, and those in the north tend to have been added to the scheme more recently. However, this did not cause a problem for the analysis of brown argus data because the butterfly has a southern distribution in Britain. When investigating speckled wood habitat associations I used transect data to test whether the species’ strength of association with woodland was related to butterfly density. Changes in butterfly habitat associations can be quantified by calculating the proportion of individuals seen in different sections (habitats) on the transect (Oliver et al. 2009). However, this analysis did not reveal any clear trends in habitat associations. This may have been due to the low number of transects in northern Britain, which from my analysis in Chapter 3, appears to be where much of the variation in habitat associations occurs. Alternative methods are, therefore, required to investigate the effect of density on the butterfly's habitat associations.

6.2.2 Assessing microclimatic conditions experienced by organisms

Much of my work investigating mechanisms affecting patterns of species' habitat associations has considered the effect of microclimatic conditions on butterfly larval stages. However, assessing the microclimatic conditions experienced by individuals in the wild is problematic. I used translocation experiments in Chapter 4 to assess the effect of microclimatic differences between woodland and open habitats on larval survival and performance. This is a good way of capturing broad microclimatic differences between habitats and their effects on larvae. However, it would be interesting to explore in finer detail the different conditions experienced by individuals in closed and open habitats in the wild. Adult females are selective in the locations in which they lay eggs with respect to microclimate (Shreeve 1986a) and so those locations in which I placed pots may not be locations in which larvae would occur naturally. Furthermore, larvae may be able to move to modify the microclimatic conditions they experience. This has been observed in other species (Turlure et al. 2010) but the extent to which speckled wood might undergo behavioural thermoregulation is unknown, and opportunities for such behaviour were restricted in the experiment by constraining larvae to the potted host plants.

The brown argus butterfly is most likely restricted to using rockrose in cooler years because rockrose occurs on southerly-facing slopes which are warmer than the flat ground where Geraniaceae species tend to occur. Again, it would be interesting to further explore in more detail the differences in microclimatic conditions experienced by brown argus larvae when feeding on rockrose *versus* Geraniaceae species. For example, the structure of the vegetation that rockrose and Geraniaceae species grow in may differ, such as the amount of bare ground surrounding the plant, the vegetation height, and the height of leaves above the ground. The temperature of the leaves of the different plant species may also differ as leaves vary in thickness and colour. Furthermore, more information is required regarding the exact locations and times at which the larvae feed and rest to understand which aspects of the microclimate might be most important.

6.3 GENERALITY OF FINDINGS

6.3.1 Spatial patterns in habitat associations in other species

I have shown that the brown argus and speckled wood butterflies have broader habitat/host plant associations at their leading-edge range margins as the climate has become more favourable for the species. A similar pattern has been reported in the silver-spotted skipper butterfly (*Hesperia comma*) (Thomas et al. 2001, Davies et al. 2006). However, the extent to which this might be a generic response to climate change in other species is largely unknown.

Many species become more restricted in their habitat associations at their leading-edge range margins suggesting that changes over time could be a widespread phenomenon. Much of the evidence comes from insects and these habitat association patterns are consistent with an increased reliance on habitats with favourable microclimates when species are at the limits of their climatic tolerances (Jordano et al. 1991, Cherrill and Brown 1992, Andersen 1993, Thomas 1993, Strathdee and Bale 1995, Bourn and Thomas 2002). There are also a small number of examples from reptiles (Thomas et al. 1999, Schofield et al. 2009). Given the changes in habitat associations currently reported (Davies et al. 2006, Chapter 3, Chapter 5), it would be reasonable to expect species restricted to certain microclimates at their leading-edges to broaden their habitat associations as the climate becomes more favourable. Insect herbivores may also be restricted to certain host species at their leading range margins due to restrictions of phenological synchrony (Hodkinson 1997, Scriber 2002, Nylin et al. 2009). As the climate warms such restrictions would be expected to relax and host plant associations become more similar to those in the core of these species' ranges.

Species from other taxa also become more restricted in their habitat use at their range margins, although patterns may not be due to any physiological restriction to microclimatically suitable habitats. For example, Lennon et al. (2002) found that marginal populations of Alaskan trees were restricted to shallow slopes whereas in the core of their range they occupy sites of any slope type, but prefer shallow slopes. Thus Alaskan trees appear to become restricted to their favoured habitat type at their leading-edge. This could arise through a reduction in density-dependent pressures at the range edge because populations are at low densities in marginal climatic conditions. Thus increasing climatic favourability at the leading-edge as the climate warms might still be expected to lead to an increase in habitat breadth as densities increase.

In contrast, some species, including plants and birds, appear to show the opposite trend and occupy a wider range of habitats at higher latitudes (Diekmann and Lawesson 1999, Fuller et al. 2007), perhaps because species richness declines with latitude leading to a reduction in interspecific competition. If potential competitors shift their distributions to higher latitudes in response to climate change these species might become more restricted in their habitat associations in these locations. Species might also display variation in habitat associations due to differences in habitat availability between regions or due genetic isolation and local adaptation in populations at the range edge. In such cases, species might not change habitat associations in response to climate change.

6.3.2 Rates of range expansion

A major implication of my work is that a relaxation of constraints on habitat use will affect the rate at which species shift their distributions in response to climate change. An increase in the types of habitats, microhabitats or host plants that a species is able to use will increase habitat availability in the landscape and thus reduce the required dispersal distance to colonise new habitat patches (Thomas et al. 1999). Furthermore, a relaxation of constraints may increase the availability of habitat on a very local scale and hence permit an increase in population sizes (e.g. the ability to utilise host plants in a wider range of sward heights). More and larger populations will increase the number of colonising propagules and hence the speed of range shift (Wilson et al. 2010).

However, the effect that any broadening of habitat associations will have on rates of range expansion with depend on how widespread “new” habitats are and on the species’ ability to disperse to reach them. In the case of the brown argus, for example, the incorporation of additional host plant species into the butterfly’s diet had substantial consequences for its rate of range expansion because these species are widespread compared with the host they were previously restricted to and the butterfly had sufficient dispersal ability to reach them. By contrast, increase in habitat breadth in the silver-spotted skipper butterfly has not resulted in such marked changes in rates of range expansion. The butterfly has become less restricted to southerly-facing slopes as summer temperatures have increased (Thomas et al. 2001, Davies et al. 2006), but its only host plant, sheep’s fescue (*Festuca ovina*), has a highly fragmented distribution and so the butterfly has still been unable to colonise many of the habitat patches which have become thermally suitable (Wilson et al. 2009). Wilson et al. (2010) used metapopulation models to simulate rates of range expansion under different scenarios of habitat availability (the range of aspects over which the butterfly can occupy). This showed that while an ability to use sites of all aspects is likely to increase future rates of range expansion compared with a scenario where the butterfly remains restricted to southerly-facing slopes, the butterfly would still lag behind climate change. Similarly, Roy and Thomas (2003) have shown that seasonal shifts in habitat use by the Adonis blue butterfly at its leading-edge range margin in Britain suggest its habitat associations should broaden as the climate warms, but because the butterfly is extremely sedentary and the calcareous grassland habitats where its host plant is found are highly fragmented, the butterfly's ability to exploit these new resources is likely to be limited. Thus many species, in particular specialist species, will fail to experience any increase in habitat availability as a consequence of altered habitat associations sufficient to allow them to expand their distributions through fragmented landscapes.

6.4 PATTERNS IN OTHER PARTS OF SPECIES' RANGES

This study has focussed on changes in species' habitat associations at cool leading-edge range margins in response to climate change. However, changes in habitat associations may occur in other parts of species' ranges. Importantly, if species are restricted to locations with the coolest microclimates at their warm trailing-edge range margins, any increase in temperature is likely to lead to the extinction of these populations. However, whether species' cool and warm range boundaries are limited by different factors is a subject of much debate. It has been suggested that while abiotic factors, such as temperature, are important in limiting species' cool range margins, biotic factors, such as competitive interactions and predation may be more important at species' warm range margins (MacArthur 1972, Brown et al. 1996, Parmesan et al. 2005). This has been supported to some extent by studies that have shown that as the climate has warmed, retractions at species' trailing-edge range margins have proceeded at slower rates than expansions at leading-edge range margins (Chen et al. 2011b, Sunday et al. 2012). However, in some cases extinctions at warm range margins have been attributed to climate change (Wilson et al. 2005, Franco et al. 2006).

If species are not at the limit of physiological tolerances at their warm trailing-edge range boundaries they might not be expected to be more restricted in their habitat associations as they are at their leading-edge range margin. However, there are studies showing that species become restricted in their habitat use at their warm range margins, which suggest species ranges are limited by heat and/or desiccation tolerance. For example at its warm, low elevation range boundary, the black-veined white butterfly (*Aporia crataegi*) lays eggs only on the north side of plants which provide cool microclimates, in contrast to its high elevation boundary where it lays eggs only on the south side of plants (Merrill et al. 2008). Many plants become restricted to particularly humid habitats where climatic conditions are warm and dry (Diekmann and Lawesson 1999, Landi and Angiolini 2008). It has been suggested that species may be able to persist for longer than expected at their warm trailing-edge range margins if they can exploit fine-scale microclimatic heterogeneity and move to more favourable locations (Hampe and Petit 2005). However, if populations are already limited to small and isolated areas of the landscape which provide the coolest/most humid microclimates, any increase in temperature and/or decrease in rainfall could rapidly drive these populations to extinction.

In the core of species' ranges, climatic constraints on habitat use tend to be less severe and so species display a wider range of habitat associations than at their range edges (Bourn and Thomas 2002). However, as the climate changes over time, climatic conditions in these locations may become more marginal and so species may become more restricted in their habitat associations in these locations. In the case of the ant *Myrmica sabuleti*, for example, in regions where it is currently found on a range of aspects and in a variety of sward heights it may become increasingly restricted to north-facing slopes and tall swards as climates warm, as is currently typical at its southern range margin (Thomas et al. 1998b, Bourn and Thomas 2002). In the case of the speckled wood butterfly, my analyses showed that in warm and dry conditions the butterfly is more restricted to woodland. The butterfly is also more restricted to woodland in Catalunya than it is in Britain (Suggitt et al. 2012). Thus while the butterfly currently seems to be benefitting from climate warming in Britain by expanding its distribution and becoming more general in its habitat use, it may be that as temperatures increase it becomes more restricted to woodland, as has been observed in particularly hot years (Schweiger et al. 2006). Decreases in habitat availability may lead to smaller, more fragmented, less stable and more vulnerable populations and resulting in local extinctions.

6.5 CONSERVATION IMPLICATIONS

6.5.1 Habitat management

The need for a more dynamic approach to conservation management as the climate changes has been widely recognised (Heller and Zavaleta 2009). According to my findings, one component of this will be the need to adapt conservation management plans for species as their habitat requirements alter. Appropriate conservation management is important for maintaining large, robust populations. This will increase the opportunity for adaptation to new conditions and increase the number of emigrants which could colonise newly climatically suitable areas and so help species to track climate change (Hodgson et al. 2009, Hole et al. 2011) .

Continued monitoring of species' habitat requirements could be undertaken to ensure that management prescriptions continue to be appropriate. I showed that spatial and temporal patterns of habitat associations are affected by similar climatic variables, supporting the idea that these climate variables affect species' habitat associations and could be used to inform future management prescriptions (although it should be recognised that other non-climatic factors might also be driving spatial variation in habitat associations).

For example, *M. sabuleti* is at its leading range edge in southern England. In order to complete development in relatively cool British summers, this ant species is largely restricted to south-facing slopes and, within these, is further restricted to warmer patches where the sward is short or sparse (Thomas et al. 1998b, Bourn and Thomas 2002). *M. sabuleti* is the obligate host ant of the large blue butterfly (*Maculinea arion*) in Britain and loss of areas of very short turf due to cessation of management, reduced intensity of grazing, and the effects of myxomatosis on the rabbit population, led to the collapse of *M. sabuleti* populations and subsequently the extinction of the large blue butterfly in Britain (Thomas 1980). Recognition of the specific microclimatic requirements of its host ant has been key to the re-establishment of the large blue butterfly in southern England (Thomas et al. 2009). However, for the continued conservation of the large blue butterfly in Britain it has been proposed that sward height is allowed to increase in order to recreate conditions the ant occupies in warmer, more southerly parts of its distribution in Europe (Settele and Kühn 2009).

Detailed studies of habitat requirements, as presented in this thesis, may not be possible or required for all species. Furthermore, the habitat requirements of all species that a site supports may not be the same. Thus, maintenance and/or creation of habitat heterogeneity will maximise the chances of suitable habitat being available for all species (Oliver et al. 2009, Game et al. 2011). Maintenance of habitat diversity within and between patches has been proposed as a strategy to conserve metapopulations because it will reduce the likelihood of correlated extinctions under unfavourable weather conditions (Kindvall 1996). Greater local availability of habitat and topographic heterogeneity has also been shown to promote stability of populations (Oliver et al. 2010). In practical terms, site managers could, for example, introduce grazing or cutting regimes that will create a variety of sward heights and hence a variety of microclimatic conditions. However, this approach will involve a trade-off with aggregation of existing habitat types which may reduce the size of populations of species with specific requirements. Thus a better option would be to expand existing reserves and create new reserves which increase the range of habitats and microclimates available to species (Hole et al. 2011). Ensuring heterogeneity in the landscape may also aid colonisation at species' leading-edge range margins by increasing the chance that suitable habitat is available.

6.5.2 Other drivers of change

In a recent study, Oliver et al. (2012b) examined temporal trends in habitat associations of 27 butterfly species that reach their leading-edge range margin in Britain. These are species that according to my findings would have been expected to broaden their habitat associations in response to climate change. While the study found that species temporarily broadened their habitat associations in years with favourable weather conditions, they found that 74 % of species had shown long-term contractions in their habitat associations over 30 years. Declines in habitat breadth were strongly correlated with declines in population density, probably as a result of declines in habitat quality and/or amount. Increased habitat specialisation could, therefore, be due to a reduction in density-dependent effects as population sizes decline. Oliver et al. (2012b) argue that any benefit of climate warming increasing habitat availability appears to have been outweighed by negative effects of habitat destruction and degradation. Other work has found that species are more restricted in their habitat associations in areas where habitat is more fragmented (Botham et al. 2011). These studies highlight the importance of reducing the other drivers of change such as degradation and fragmentation of habitats.

6.6 FURTHER WORK

6.6.1 Are other species altering their habitat associations?

Evidence that species are changing their habitat associations at their leading-edge range margins in response to climate change is from three species, all of which are butterfly species in Britain. Thus more evidence is required from other species and future studies should extend the taxonomic and geographic breadth of knowledge.

In Britain, biological recording and monitoring is increasing in intensity and “citizen science” schemes where volunteers are encouraged to submit records of species they have observed are increasing in popularity. Improvements in technology (e.g. global positioning system units) also mean that many more records are of a high spatial resolution, which is important for assessing species’ habitat associations. Land cover data are available to distinguish between broad habitat types and digital elevation models can be used to establish the topographic associations of species. Thus, analysis of species’ habitat associations is increasingly possible for a wider range of species in Britain. However, in many parts of the world biological recording schemes of this type do not exist, and land cover data at fine spatial resolution is lacking. Thus assessments of species' habitat associations may continue to be biased towards species with leading-edge range margins in regions where these types of data are available. My work has emphasised the value of such recording schemes and the importance of extending the geographic coverage of such schemes. Availability of data on species' habitat associations throughout their entire distributions would also be useful for predicting future habitat associations under climate warming, based on those currently observed in warmer regions. This would help to adapt conservation management strategies for species, as described above.

6.6.2 Are species restricted in their habitat associations at their trailing-edge range margins?

There is very little evidence to suggest whether or not species become increasingly restricted in their habitat associations at their trailing-edge range margins. If this is the case, local extinctions could occur rapidly if a small increase in temperature leaves species with no cool habitats to move to if they already occupy the coolest parts of the landscape. Thus from a conservation perspective information on these patterns is important to establish the risk to species from climate change. Such information will also inform fundamental ecological debates as to whether species are limited by climatic or biotic factors at their warm range margins.

6.6.3 What are the consequences of changing habitat associations for rates of range shift?

One of the main motivations for studying how habitat associations change in response to climate change is to better understand leading-edge range expansion. I have demonstrated a change in habitat associations in response to climate change for the brown argus and speckled wood butterflies, but I have not established the effect this has on rates of range expansion. Observed rates of range expansion in these species could be compared with a scenario where habitat or host plant associations had remained the same (as in Wilson et al. 2010) to determine the magnitude of the effect.

Conservation resources are limited and so risk assessments are beginning to be developed to identify species most at threat from climate change (Williams et al. 2008, Rowland et al. 2011, Thomas et al. 2011). Part of a species' vulnerability is its ability to shift its distribution to keep pace with climate change. Thus developing methods of predicting whether species will alter their habitat associations and the effect of this on rates of range expansion will help in informing risk assessments of species under climate change.

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