Effects of morphology, habitat and weather on the movement behaviour of range-expanding butterfly species

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Thesis submitted for the degree of Doctor of Philosophy
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September 2013

Content

Abstract

Dispersal capacity is an important trait enabling species to respond to climate change, particularly in fragmented landscapes, where individuals often need to move longer distances to colonise new areas. It is therefore crucial to understand species' movement behaviour and how it is affected by environmental variables to plan effective conservation measures for a wide range of species. This thesis aimed to enhance the current understanding of the role of dispersal in enabling species to respond to global environmental change using seven species of butterflies. I identified that species and individuals with longer move bouts (step length) cross habitat boundaries more frequently. Because step length is relatively easy to measure for butterflies, it could potentially be used as a general proxy of species dispersal capacity at a landscape level. I also found a higher investment in morphological traits that enhance dispersal capacity for one out of three range-expanding species in newly colonised sites at the range margin. Differences in species dispersal capacity and habitat availability could explain why only one species showed increased investment in dispersal at the margin. Also, there was poor evidence of a relationship between movement and an associated morphological trait; wing length was not associated with increased mobility for two out of four species which suggests that increased dispersal ability may not always be linked to morphological changes during range expansion. Finally, an assessment of the relative importance of temperature, habitat quality and structure on the movements of two species within their habitats revealed no contribution of the habitat variables for one of the species, whilst temperature, habitat size, edge ratio and vegetation height significantly affected movements of the second species. Overall, this thesis highlights that responses to global environmental change are highly dependent on species and its interactions with the environment.

Acknowledgments

This research was funded by School of Environmental Sciences, University of East Anglia and Kone Foundation.

I am in great debt to my supervisors Aldina Franco and Paul Dolman for all the guidance and support they have provided throughout the project. This thesis would not have come together without Aldina fighting for me to get the studentship and her unwaning enthusiasm. I would like to also thank my examiners Hans Van Dyck and Jenny Gill for their useful comments on the thesis. This thesis has also benefited greatly from the input of Audrey Zannese, who has patiently helped me particularly with statistics. I would also like to thank all the Strangles people for good advice and suggestions on the project, providing an inspirational atmosphere to work in and for all the laughs. I have also been lucky to share office with a lot of great people who have been of great help and company: Kabello, Joe, Christina, Hugh, Scott and Miranda have been especially important for me.

The Butterfly Conservation butterfly observation data was a foundation for the study site selection. Therefore I am grateful for the thousands of people who have contributed to collecting the data and to Butterfly Conservation and in particular Richard Fox for sharing the data with us. I also received advice from members of Butterfly Conservation that I would like to thank: Andy Brazil, Rob Parker, and Chris and Alan Dawson. Tiago Silva also deserves a thank you for programming the personal computers. I am also thankful to Natural England for the permission to conduct the field work and to several land owners who provided access to their land in particular the Forestry Commission and Neal Armour-Chelu. I would also like to acknowledge that Ann Supatchaya, Dan Reed, Alastair Feather, Audrey Zannese and Aldina Franco provided me with the butterfly pictures used in the thesis.

The field work would not have been possible without the help off many people but I would like to especially thank: Julia, Kirsty, Uzma, Ann Supatchaya, Davi and Pip. I am forever grateful to Julia for making me laugh and feeding me lovehearts when I was tired during field research.

Special thanks go to my friends. I am very fortunate to have met such a lovely people that gave me so much energy. I could not have done without you, Anjali, Viv, Julia, Freydis, Phil, Barnaby, Ben, Olly, Alessia and Iain. Of all the kayaking and climbing friends I would like to mention Paul and Pete who took me out to play.

Kaikista suurin kiitos kuuluu vanhemmilleni ja Riika siskolle. Te olette olleet äärettömän tukevia koko minun opiskeluni ajan ja ymmärtäväisiä vaikka en ole ehtinyt viettää paljoa aikaa teidän kanssanne. Tuhannet kiitokset!

Chapter 1: Introduction

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Climate change

Impacts of the ongoing climate change on the worlds' biota have now been reported across most continents, oceans and taxonomic groups (Root et al. 2003; Parmesan 2006; Rosenzweig et al. 2008; Poloczanska et al. in press). Independent of the possible climate change mitigation that may reduce the future greenhouse gas emissions it has become apparent that the world is likely to be committed to more than a further 2°C increase in average global temperature before the end of the century, with associated changes in precipitation patterns (IPCC 2007). Although species have already responded to environmental changes during their evolutionary history (Huntley 1991; Harris 1993) the fast rate at which the current climate is changing (IPCC 2007) is raising concerns about species ability to respond and adapt (Visser 2008). It is therefore critical to build understanding which species and ecosystems will be able to respond to climate change and how. This enables assessing species vulnerability to climate change and planning of more efficient conservation measures (Thomas et al. 2004; Heikkinen et al. 2010).

Climate change does not work in isolation of other factors, such as habitat destruction and degradation, that are likely to increase the risk of extinctions (Brook et al. 2008) and make it harder to predict species responses (Parmesan et al. 2013). Climate change together with habitat loss have been predicted to be key processes resulting in global biodiversity loss

(Mantyka-Pringle et al. 2012). Climate change has been shown to already affect biological communities (Parmesan 2006) and biodiversity loss is bound to have severe consequences on ecosystem services that humans depend on (Chapin III et al. 2000).

Even the least severe climate change scenarios are likely to cause considerable changes in the world's biodiversity (Lawler et al. 2009). Using species-area relationships, several authors estimated that, due to loss of suitable area caused by climate warming, 9-62% of mammals inhabiting mountain areas would go extinct (McDonald & Brown 1992) while Thomas et al. (2004) estimated that even with mid-range climate change scenario, extinction rate was between 15 to 37 % across several taxa, depending on whether a full dispersal or a no dispersal scenario was used. These predictions of species responses to climate change are often done based on climate envelope models that correlate current distributions with current climate data and then predict climatically suitable areas in the future that have conditions where species are likely to persist. For these predictions to be more accurate they would need to incorporate information about species dispersal abilities (Thuiller et al. 2008) as species dispersal capacity plays a crucial role in their ability to track suitable climate space (Crozier & Dwyer 2006). An understanding of species dispersal capacity will help predict which species are capable of responding to environmental changes and this will help developing adaptation and mitigation strategies for biodiversity conservation (Dawson et al., 2011).

Responses to climate change

Species and populations have three different ways they can response to climate change: 1) If enough variation or plasticity exists they can adapt and stay put. 2) They can move to new suitable habitats. 3) They can go extinct. Evidence from plants on past responses to changes in global temperatures indicates that local adaptations to new conditions seem to be more of an exception and distribution changes or extinctions are more likely outcomes (Huntley 1991).

A variety of ecological responses to climate change have been reported to date from changes in phenology, migration patterns, distributions and species interactions for example as competitors, hosts and predators (Parmesan 2006 and references therein). For example, Parmesan and Yohe (2003) calculated advancement of phenological events by 2.3 days per decade, on average, for 172 species across taxa while some migratory birds have advanced their arrival on breeding grounds (Ahola et al. 2004). Fitter (2002) showed that British plants had advanced their flowering time by 4.5 days during the past decade and a study of British butterflies found that 26 out of 35 species had advanced their first appearance during the last two decades (Roy & Sparks 2000).

One of the best documented ecological responses to climate change is species' range shifts which are now reported across several taxonomic groups (Hickling et al. 2006). Within a 25 year time period in England 12 out of 16 different taxonomic groups had expanded their range northwards and only three species (amphibians and reptiles) had shifted significantly southwards (Hickling et al. 2006). British birds have moved north on average by 18.9 km during 20 years (Thomas & Lennon 1999) and Parmesan and Yohe (2003) calculated that birds, butterflies and alpine herb species have shifted their ranges towards poles on average 6.1 km per decade or 6.1 meters per decade upwards. 64% of the 35 European butterfly species had changed their distributions between 35 to 240 km northwards during the past century (Parmesan et al. 1999) while one of the fastest reported shifts are among Finnish butterflies some for which the rate of change was over 300 km within the study period of 1992-1996 to 2000-2004 (Pöyry et al. 2009).

Most of the changes in species distribution in response to climate change have documented expansion of range towards poles and to higher altitudes possibly while documented range retractions are still few, most likely due to time lags and difficulty of recording local extinctions (Thomas et al. 2006). However, a few studies are now emerging reporting also range retractions at the warm edge of the range and at lower altitudes (Franco et al. 2006; Wilson et al. 2007).

The ability of species to respond to environmental changes can change due to altered ability to disperse, reproduce and changes in the degree of habitat fragmentation and biological interactions. For example, Menendez et al. (2008) showed that *Aricia agestis* butterfly had reduced parasitism at the newly colonised areas. *Polygonia c-album* butterfly has changed is host plant preference at the expanding range margin (Braschler & Hill 2007) which has enabled this species to expand its range at a higher rate. The same is likely to apply also to other generalist species for which the ability to track climate change might be underestimated without knowledge of possible host switches (Braschler and Hill 2007). *Hesperia comma* butterflies are now, due to increasing temperature, able to use a wider range of habitats in terms of slope at the range margin when previously they were restricted to warm south and west facing slopes (Thomas et al. 2001). This increases the amount of habitat available for this species and eases range expansion (Thomas et al. 2001).

Dispersal

Dispersal can be defined as “any movement of individuals or propagules with potential consequences for gene flow across space” (Ronce 2007) and it is a fundamental process in ecology, evolution and conservation (Ronce 2007). Dispersal has recently gained a lot of attention because of its crucial role in the conservation and evolution of species facing major environmental changes such as habitat loss and fragmentation, climate change, and their interactions (Kokko & Lopez-Sepulcre 2006; Ronce 2007). For butterflies, Thomas et al (2011) identified low adult dispersal, together with larval habitat quality, as main drivers of long term population trends. Dispersal also affects the scale at which population sizes fluctuate and this has implications for the management of populations and it determines the scale at which actions should take place to conserve the habitats (Wilson and Thomas 2002).

Factors affecting movement and dispersal

Rates of responses to climate change are highly dependent on taxa and species as well as other environmental variables than climate. How species react to climate change are predicted to depend on their population dynamics, physiology, life-history traits and dispersal abilities (Parmesan 2006; Parmesan and Yohe 2003). For example, *Pararge aegeria* butterfly is likely to be constrained with its range expansion due to habitat fragmentation (Hill et al. 1999b, 2001). Out of British butterflies, generalists and more dispersive species have been able to shift their distributions and maintain their population sizes better than more specialist and sedentary species that have suffered declines because habitat loss has outweighed the positive responses to climate warming (Warren et al. 2001). Similarly, Pöyry and colleagues (2009) found that the Finnish butterflies that are mobile, live on the forest edges, and use woody plants as a hosts have been more successful in shifting their ranges. Thus, dispersal capacity, as well as habitat availability, is playing a crucial role in determining species responses to warming climate (Hill et al. 1999b, 2001; Warren et al. 2001). Butterflies and birds in Europe have been calculated to lag on average 135 km and 212 km behind the climate warming (Devictor et al. 2012). The great variation in the response to climate change within several taxonomic groups highlights the importance of species traits and external drivers, such as land use changes, in the rate of responses to climate change (Hickling et al. 2006; Chen et al. 2011).

To be able to predict which species and populations might be particularly vulnerable to climate change and habitat fragmentation it is important to understand species dispersal abilities and movement.

Scale of movement and behaviour at boundaries

Dispersal can be divided into three stages: emigration, vagrant stage and immigration (Ims & Yoccoz, 1997). Individual decisions to cross a habitat boundary or not affects the first of these stages and therefore the emigration rate. Individuals can move by using two different types of movements; fast and directed moves intended for dispersal, or routine moves that are slower, less directed explorative moves associated with resource use such as finding mates (Van Dyck & Baguette 2005). However, dispersal can also occur as a by-product of these "routine" moves, particularly in fairly continuous landscapes (Van Dyck & Baguette 2005). It is often challenging, impractical or expensive to study dispersal or animal movements through its whole life or home range. Gathering information about species movement at a smaller scale within a habitat is likely to be less labour intensive. Understanding small scale moves within habitat patches can help in understanding dispersal process at the larger scale and dispersal between patches of habitat (Wiens et al. 1993; Ims & Yoccoz 1997; Auckland et al. 2004) through for example knowledge of responses to resources and habitat structures that affect emigration from a habitat (Schultz et al. 2012).

Movement within a habitat relates to movement at a larger scale and is mediated by behaviour at habitat boundaries. The propensity of individuals to cross a habitat boundary is the first stage of the dispersal process and therefore impacts the quantity of emigrants (Stamps et al. 1987). There are ample studies looking at the behaviour at habitat edges because of its importance for understanding how organisms respond to landscape structures (e.g. Merckx et al. 2003; Conradt & Roper 2006; Dennis 2010; Schultz et al. 2012). For example, Ries and Debinski (2001) investigated how butterflies respond to different types of habitat edges and found individuals respond strongly even to, what we would perceive as, subtle boundaries but the responses are modified by the edge structure and local environment. Knowledge of species behaviour within habitat and responses to boundaries has proven beneficial for predicting species movement and designing conservation strategies (Schultz 1998) and providing habitat restoration recommendations based on individual movement data within a habitat (McIntire et al. 2007).

Changes at the range margin

There is now accumulating evidence from theoretical models (Dytham 2009; Burton et al. 2010) as well as empirical evidence, particularly for insects (Thomas et al. 2001; Hughes et al. 2003; Simmons & Thomas 2004; Hill et al. 2011), that colonising individuals are not a random sample of the source population but likely to possess traits that are associated with increased dispersal ability. Therefore dispersal cannot be viewed as static trait of a species but it is expected to be under selection pressure during range expansion. An increased dispersal rate may facilitate species ability to track climatically suitable areas and counteract negative impacts of habitat fragmentation by enabling individuals to move over longer distances (Thomas et al. 2001).

Because of the difficulty of quantifying insect dispersal in the field compared to measuring morphology, dispersal capacity of individuals and species has frequently been inferred indirectly from morphological traits. Individuals from newly colonised areas have been found to have heavier and wider thoraxes, longer wings and larger body sizes (Hill et al. 2011); traits that have been correlated with increased flight ability, such as acceleration capacity, in butterflies (Chai & Srygley 1990; Dudley & Srygley 1994; Berwaerts et al. 2002; Sekar 2012). However, changes in dispersal ability have not always been found linked with changes in morphology (e.g. Hanski et al. 2004; Mitikka & Hanski 2010).

For some species an increase in dispersal ability has been associated with a trade-off with fecundity, measured as a decrease in abdomen mass in butterflies (Hill et al. 1999a; Hughes et al. 2003). However for other species increased dispersal ability was associated with increased fecundity (Hanski et al. 2006). Models have suggested that range expansion might select for increased dispersal ability and fecundity if it comes with a trade-off with competitive ability instead (Burton et al. 2010). Because linking the morphological traits to flight capacity has predominantly been done in laboratory conditions there is no clear knowledge how morphological traits translate into differences in movement in the field.

It is likely that increased dispersal ability will come at a cost and differences in morphology at the margin will disappear with time (Simmons and Thomas 2004). How fast these changes take place depends on the how much it is selected against in the novel environment and plastic responses. As an example, for wing-dimorphic bush crickets the differences were only observed at margins that were recently (within 5-10 years) colonised (Simmons and Thomas 2004) while a study of a *Pararge aegeria* butterfly found differences in the dispersal ability evident even after >30 years since colonisation (Hughes et al. 2003). Furthermore, selection pressure on dispersal capacity during range expansion is likely to vary between sexes (Hill et al. 1999a) and changes in dispersal capacity, as a response to climate change, have more often been documented in female than male butterflies (Hill et al. 1999a, Hughes et al. 2007).

Environmental factors influencing dispersal and movement

It has been acknowledged that dispersal is not a fixed trait that is similar across individuals, populations of species and time (Van Dyck & Baguette 2005; Baguette & Van Dyck 2007; Stevens et al. 2010b). Instead it has been shown to vary, not only due to response to range expansion, but also for example due to habitat structure and fragmentation causing evolutionary changes in species mobility (Merckx et al. 2003; Schtickzelle et al. 2006; Merckx & Van Dyck 2007). Thus, it is not surprising that there is high within-species and between populations variability in dispersal abilities (Stevens et al. 2010a).

For predicting how individuals move there is a need to understand how they react to different variables in their environment. Butterfly emigration rates have been shown to be influenced by habitat quality measured as a quantity and quality of host plants and the amount of nectar sources (e.g. Odendaal et al. 1989; Kuussaari et al. 1996). The importance of shelter as a resource, providing roosting, mate location, resting and basking places, for butterflies has only fairly recently been acknowledged and is likely to have a large impact on butterfly flight (Dennis & Sparks 2006). The influence of habitat patch size and shape on emigration has received a lot of interest (Dover & Settele 2009 and references therein) while there are only few studies looking at butterfly movement within a habitat patch in relation to habitat quality and structure (Fownes & Roland 2002; Auckland et al. 2004; James 2006).

Ectothermic species such as butterflies are highly influenced by weather and temperature in particular. This is evident from the large impact of weather and temperature on population fluctuations and trends (Roy et al. 2001) as well as butterfly flight (e.g. Shreeve 1984; Dennis & Sparks 2006) and habitat use (Dennis & Sparks 2006). Also larval development time depends on temperature (Nylin & Gotthard 1998). Weather influences butterflies also indirectly e.g. through host and nectar plant phenology (Parmesan 2006 and references there in). In temperate environments it is likely that temperature is a limiting resource for butterflies that often are required to increase their body temperature over ambient temperatures for flight and ovipositioning (Kingsolver 1983). Temperature has been shown to be positively correlated with colonising frequency of butterflies and moths (Mitikka et al. 2008; Cormont et al. 2011; Franzen & Nilsson 2012) and therefore changes in climate have been predicted to facilitate range expansion by increasing the time available for flight for some species (Cormont et al. 2011; Franzen & Nilsson 2012). Also, for disentangling the effect of other factors on butterfly flight it is important to take into account the prevailing weather during the flight. Morphological traits, such as body size, can also influence how temperature affects flight of individuals, as shown for butterflies and damselflies (Gilchrist 1990; Samejima & Tsubaki 2010).

Butterflies as model species for movement

Why study butterflies?

Butterflies are known to being sensitive to changes in their habitats and climate (Haddad et al. 2008; van Swaay et al. 2008) and considered a good indicator of change and abundance also for other terrestrial insect groups (Thomas 2005). As heliotherms butterflies are predicted to respond readily to climatic changes particularly because of their short life cycles (Bale et al. 2002). Furthermore, natural history of most butterfly species is well known and there are good historical records of distributions and phenology, particularly in the UK (Dennis 1992; Asher et al. 2001).

Butterflies are an excellent taxonomic group for studying dispersal, movement and behaviour at boundaries because they can be individually marked and followed (Dennis 1992; Asher et al. 2001). Butterflies also represent a group that has suffered population declines: 93% out of habitat specialist and 76% of all butterfly species in the UK have shown declines since the 1970’s (Maclean 2010) while European grassland butterflies have declined almost 50% over two decades (van Swaay et al. 2013) thus urgent conservation measures that take into account climate change and the long-term survival of the species are needed.

Measuring butterfly dispersal and movement

Most butterflies cannot be followed by radio tracking techniques, hence researchers often rely on other methods such as mark release recapture (MRR) studies for determining species dispersal capacity. This provides valuable information about species dispersal, how it is affected by the landscape and how species move between habitat patches (Turchin 1998). MRR studies have, however, been shown to be dependent on the scale at which study is conducted (Schneider 2003) and to underestimate the rare long distance movements that can be crucial for species dispersal (Wilson & Thomas 2002). Harmonic radar has been developed for tracking insects (Riley & Smith 2002) and used successfully with butterflies (Ovaskainen et al. 2008; Niitepõld et al. 2009). This method is, however, expensive and limited to open landscapes where the signal is not obstructed.

Another method used for estimating animal movement is the flag technique, in which individuals are released and followed directly, one at the time. Butterflies can be followed from a distance that is long enough not to disturb the animal's normal behaviour and numbered flags are dropped on regular time intervals or, every time it lands (Schultz 1998; Turchin 1998). The result is a movement path that can be mapped using triangulation or GPS and several movement parameters such as move lengths, turning angles and speed can be calculated (Turchin 1998). The method has been successfully applied on butterflies (Schultz & Crone 2001). The flag method allows gathering of more detailed information about individual movement behaviour, such as behaviour at boundaries and factors affecting it, but can practically only be done at a short time and spatial scale. The benefit of the flag method is that it is not as labour intensive as most MRR studies and therefore it enables study of several species at the same time and in the same habitat thereby producing data that are comparable across species. This method focuses on the local scale, day to day movements and, because long-distance dispersal events of most butterfly species are rare, they can only be detected if a large number of individuals are tracked. However, in this thesis I link these day to day movements with species'/individuals' predisposition to move over longer distances.

Study species

Anthocharis cardamines (orange-tip, Linneaus 1758) from Pieridae family is a common and widespread species that meets is northern distribution limit in Scotland where it has recently expanded its range (Asher et al. 2001; Fox et al. 2006, see Figure 1 in Chapter 4). It is a univoltine species that flies in Britain normally between mid-April and mid-June (Asher et al. 2001). *A. cardamines* is found in a range of grassy and often damp habitats from meadows to road-verges (Asher et al. 2001). It uses several crucifers as its host plants, but *Cardamine pratensis* (cuckoo flower) and *Alliaria petiolata* (garlic mustard) are the main host plants (Asher et al. 2001). *Pieris napi* (green-veined white, Linneaus 1758) from Pieridae family is found throughout most of Britain where it is considered to have a stable range (Asher et al. 2001). It is a common and widespread species that uses a variety of habitats but is often associated with damp and lush vegetation. Like *A. cardamines*, it uses a range of crucifers as hosts but *C. pratensis* and *A. petiolata* are most commonly used (Asher et al. 2001). In Northern Britain this species has two broods per year, the first one being in flight at similar times as *A. cardamines*.

Aricia agestis (brown argus, Denis & Schiffermüller 1775) is a species from the Lycaenidae family that meets it northern limit in England where it has expanded its range north and towards the coast in the east in the last three decades (Asher et al. 2001; Fox et al. 2006). The range expansion has been attributed to climate change as well as shifts in host plant use (Thomas et al. 2001; Pateman et al. 2012); the populations at the northern parts of its range used to be restricted to calcareous and limestone habitats where its host plant *Helianthemum nummularium* (common rock-rose) is found (Warren 1986). However, during the past 20 years the species has been found to increase the use of Geranium and Erodium species (Geraniaceae), *Geranium molle* (dove's-foot crane's-bill) and *Erodium cicutarium* (common stork's-bill) in particular, also further north due to warmer conditions allowing it to use these plants that normally grow in less sheltered and warm locations in comparison to *H. nummularium* (Thomas et al. 2001; Pateman et al. 2012). *Aricia agestis* has two broods a year; the first one flying normally in May and June and the second one fly from mid-July until mid-September in areas of this study (Asher et al. 2001; Brazil et al. 2011). *Polyommatus icarus* (common blue, Rottemburg 1775) is a very common and wide spread Lycaenidae species that is found throughout most of Britain (Asher et al. 2001). It occurs in variety of grassy areas where its host plants are found (Asher et al. 2001). The main host plant is *Lotus corniculatus* (common bird's-foot-trefoil) but also *Medigago lupulina* (black medic) and *Trifolium repens* (white clover) are used (Asher et al. 2001) and found in the study areas (pers. observation). *Polyommatus icarus* can have from one to three broods per year in Britain (Asher et al. 2001) but the study populations had two broods per year with flight times corresponding with those of *A. agestis* (Brazil et al. 2011).

Pyronia tithonus (gatekeeper, Linneaus 1771) is a Nymphalidae species that is expanding its range in England (Asher et al. 2001; Fox et al. 2006). It uses several grass species, such as *Agrostis* spp. and *Festuca* spp., as its host plants and is found in grasslands with tall grasses (Asher et al. 2001). Most of the adults of this single brooded species fly in July and August (Asher et al. 2001). *Maniola jurtina* (meadow brown, Linneaus 1757) from Nymphalidae family occurs throughout Britain. It is found in range of grassland habitats where its host grasses *Festuca* spp. and *Agrostis* spp. and other species are found. This single brooded species flies normally between June and August (Asher et al. 2001). *Coenonympha pamphilus* (small heath, Linneaus 1757) is a Nymphalidae species that is listed as a UK biodiversity action plan (BAP) species due to its decline in distribution and population sizes, despite it being widely distributed species in the UK (Fox et al. 2006). It occurs in grasslands where fine grasses, such as *Festuca* spp. and *Poa* spp. that it uses as a host plants, are found (Asher et al. 2001). This species varies from one to three broods per year depending on the area but within the study area the first brood was in flight from mid-May until mid-June (Brazil et al. 2011, pers. observation).

Objectives of the thesis

The main aims of my thesis were to address these four issues: a) Study species movement within habitat and responses to boundaries and how these are linked. This was done in order to understand if within-habitat movement measures can be used for estimating emigration rate and movement at a larger scale. b) Investigate if there are general patterns in how dispersal-related morphology traits differ at newly colonised areas compared with areas that have been occupied for longer. This can help in estimating if species are likely to evolve morphological traits that enable them to respond to climate change by shifting their ranges at higher rates. c) Explore if there is a link between morphology and movement parameters in the field and thereby evaluate the justification of using morphological traits as proxies for dispersal capacity. d) Lastly, I aimed to determine the influence of different environmental factors (ambient temperature, habitat quality and structure) on butterfly movement.

Thesis structure

This thesis contains 6 chapters. *Chapter 1* consists of the general introduction and setting the context for the thesis. *Chapters 2-5* are written in the form of scientific papers. At the time of submission *Chapter 2* is in press (Kallioniemi et al., in press) and *Chapters 3-5* are presented as manuscripts in preparation.

In *Chapter 2* I studied inter-species differences in movement of seven British butterflies. I linked the butterfly movement within a habitat to likelihood of crossing habitat boundaries across species and within species. In *Chapter 3* I investigated the link between morphology and butterfly movement in the field by comparing wing lengths of individuals of two range-expanding butterfly species from newly colonized areas and from areas where they had existed for longer. I also compared wing lengths of close relatives of the study species that have existed at both areas for long. Furthermore, I investigated how well different movement parameters measured in the field are correlated with wing length that is often used as a proxy for butterfly flight capacity and looked for interactive effects of wing length and temperature on flight. For *Chapter 4* I searched for signs of increased dispersal capacity in three range-expanding butterfly species at the newly colonized margin areas by comparing morphology of individuals sampled from core and margin areas of the species range. Again, I also measured individuals of control species that are close relatives to the range-expanding species and have existed at both areas for long. *Chapter 5* investigates the relative influence of habitat quality, structure and ambient temperature on butterfly flight. *Chapter 6* brings together the key findings of the thesis.

Literature

Ahola, M., T. Laaksonen, K. Sippola, T. Eeva, K. Rainio, and E. Lehikoinen. 2004. Variation in climate warming along the migration route uncouples arrival and breeding dates. Global Change Biology **10**:1610–1617.

Asher, J., M. Warren, R. Fox, P. Harding, G. Jeffcoate, and M. A. Jeffcoate, Stephens. 2001. Millennium Atlas of Butterflies in Britain and Ireland. Oxford University Press, Oxford, United Kingdom.

Auckland, J. N., D. M. Debinski, and W. R. Clark. 2004. Survival, movement, and resource use of the butterfly *Parnassius clodius*. Ecological Entomology **29**:139–149.

Baguette, M., and H. Van Dyck. 2007. Landscape connectivity and animal behavior: functional grain as a key determinant for dispersal. Landscape Ecology **22**:1117–1129.

Bale, J. S., G. J. Masters, I. D. Hodkinson, C. Awmack, T. M. Bezemer, V. K. Brown, J. Butterfield, A. Buse, J. C. Coulson, and J. Farrar. 2002. Herbivory in global climate change research: direct effects of rising temperature on insect herbivores. Global Change Biology **8**:1–16.

Berwaerts, K., H. Van Dyck, and P. Aerts. 2002. Does flight morphology relate to flight performance? An experimental test with the butterfly *Pararge aegeria*. Functional Ecology **16**:484–491.

Braschler, B., and J. K. Hill. 2007. Role of larval host plants in the climate-driven range expansion of the butterfly *Polygonia c-album*. Journal of Animal Ecology **76**:415–423.

Brazil, A., Dawson, A., and C. Dawson. 2011. Thirty years of Norfolk butterflies - An Atlas of distribution. Butterfly Conservation, Norfolk Branch, Wareham, United Kingdom.

Brook, B. W., N. S. Sodhi, and C. J. A. Bradshaw. 2008. Synergies among extinction drivers under global change. Trends in Ecology & Evolution **23**:453–460.

Burton, O. J., B. L. Phillips, and J. M. J. Travis. 2010. Trade-offs and the evolution of life-histories during range expansion. Ecology Letters **13**:1210–1220.

Chai, P., and R. Srygley. 1990. Predation and the flight, morphology, and temperature of neotropical rain-forest butterflies. American Naturalist **135**:748–765.

Chapin III, F. S. et al. 2000. Consequences of changing biodiversity. Nature **405**:234–242.

Chen, I.-C., J. K. Hill, R. Ohlemueller, D. B. Roy, and C. D. Thomas. 2011. Rapid range shifts of species associated with high levels of climate warming. Science **333**:1024–1026.

Conradt, L., and T. J. Roper. 2006. Nonrandom movement behavior at habitat boundaries in two butterfly species: Implications for dispersal. Ecology **87**:125–132.

Cormont, A., A. H. Malinowska, O. Kostenko, V. Radchuk, L. Hemerik, M. F. WallisDeVries, and J. Verboom. 2011. Effect of local weather on butterfly flight behaviour, movement, and colonization: significance for dispersal under climate change. Biodiversity and Conservation **20**:483–503.

Crozier, L., and G. Dwyer. 2006. Combining population-dynamic and ecophysiological models to predict climate-induced insect range shifts. American Naturalist **167**:853–866.

Dawson, T.P., S.T. Jackson, J.I. House, I.C. Prentice, and G.M. Mace. 2011. Beyond predictions: biodiversity conservation in a changing climate. Science **332**:53–58.

Dennis, R. L. H. (Ed.). 1992. The ecology of Butterflies in Britain. OUP Oxford, Oxford, United Kingdom.

Dennis, R. L. H. 2010. A resource-based habitat view for conservation: butterflies in the British landscape. John Wiley and Sons, Oxford, United Kingdom.

Dennis, R. L. H., and T. H. Sparks. 2006. When is a habitat not a habitat? Dramatic resource use changes under differing weather conditions for the butterfly *Plebejus argus*. Biological Conservation **129**:291–301.

Devictor, V. et al. 2012. Differences in the climatic debts of birds and butterflies at a continental scale. Nature Climate Change **2**:121–124.

Dover, J., and J. Settele. 2009. The influences of landscape structure on butterfly distribution and movement: a review. Journal of Insect Conservation **13**:3–27.

Dudley, R., and R. Srygley. 1994. Flight physiology of neotropical butterflies - Allometry of airspeeds during natural free-flight. Journal of Experimental Biology **191**:125–139.

Dytham, C. 2009. Evolved dispersal strategies at range margins. Proceedings of the Royal Society B-Biological Sciences **276**:1407–1413.

Fitter, A. H., and R. S. R. Fitter. 2002. Rapid changes in flowering time in British plants. Science **296**:1689–1691.

Fownes, S., and J. Roland. 2002. Effects of meadow suitability on female behaviour in the alpine butterfly *Parnassius smintheus*. Ecological Entomology **27**:457–466.

Fox, R., J. Asher, T. Brereton, D. Roy, and M. Warren. 2006. State of Butterflies in Britain and Ireland. Pisces Publications, Newbury, United Kingdom.

Franco, A. M. A., J. K. Hill, C. Kitschke, Y. C. Collingham, D. B. Roy, R. Fox, B. Huntley, and C. D. Thomas. 2006. Impacts of climate warming and habitat loss on extinctions at species' low-latitude range boundaries. Global Change Biology **12**:1545–1553.

Franzen, M., and S. G. Nilsson. 2012. Climate-dependent dispersal rates in metapopulations of burnet moths. Journal of Insect Conservation **16**:941–947.

Gilchrist, G. 1990. The consequences of sexual dimorphism in body size for butterfly flight and thermoregulation. Functional Ecology **4**:475–487.

Haddad, N. M., M. Holyoak, T. M. Mata, K. F. Davies, B. A. Melbourne, and K. Preston. 2008. Species' traits predict the effects of disturbance and productivity on diversity. Ecology letters **11**:348–356.

Hanski, I., C. Erälahti, M. Kankare, O. Ovaskainen, and H. Sirén. 2004. Variation in migration propensity among individuals maintained by landscape structure. Ecology Letters **7**:958–966.

Hanski, I., M. Saastamoinen, and O. Ovaskainen. 2006. Dispersal-related life-history trade-offs in a butterfly metapopulation. Journal of Animal Ecology **75**:91–100.

Harris, A. H. 1993. Wisconsinan Pre-Pleniglacial biotic change in South-eastern New Mexico. Quaternary Research **40**:127–133.

Heikkinen, R. K., M. Luoto, N. Leikola, J. Pöyry, J. Settele, O. Kudrna, M. Marmion, S. Fronzek, and W. Thuiller. 2010. Assessing the vulnerability of European butterflies to climate change using multiple criteria. Biodiversity and Conservation **19**:695–723.

Hickling, R., D. B. Roy, J. K. Hill, R. Fox, and C. D. Thomas. 2006. The distributions of a wide range of taxonomic groups are expanding polewards. Global Change Biology **12**:450–455.

Hill, J. K., Y. C. Collingham, C. D. Thomas, D. S. Blakeley, R. Fox, D. Moss, and B. Huntley. 2001. Impacts of landscape structure on butterfly range expansion. Ecology Letters **4**:313–321.

Hill, J. K., H. M. Griffiths, and C. D. Thomas. 2011. Climate change and evolutionary adaptations at species' range margins. Pages 143–159 in M. Berenbaum, R. Carde, and G. Robinson, (eds.). Annual Review of Entomology, Vol 56. Annual Reviews, Palo Alto.

Hill, J. K., C. D. Thomas, and D. S. Blakeley. 1999a. Evolution of flight morphology in a butterfly that has recently expanded its geographic range. Oecologia **121**:165–170.

Hill, J. K., C. D. Thomas, and B. Huntley. 1999b. Climate and habitat availability determine 20th century changes in a butterfly's range margin. Proceedings of the Royal Society of London Series B-Biological Sciences **266**:1197–1206.

Hughes, C. L., C. Dytham, and J. K. Hill. 2007. Modelling and analysing evolution of dispersal in populations at expanding range boundaries. Ecological Entomology **32**:437–445.

Hughes, C. L., J. K. Hill, and C. Dytham. 2003. Evolutionary trade-offs between reproduction and dispersal in populations at expanding range boundaries. Proceedings of the Royal Society of London Series B-Biological Sciences **270**:S147–S150.

Huntley, B. 1991. How plants respond to climate change - migration rates, individualism and the consequences for plant-communities. Annals of Botany **67**:15–22.

Ims, R. A., and N. G. Yoccoz. 1997. Studying transfer processes in metapopulations: emigration, migration, and colonization. In: Hansi I. and Gaggiotti O. (Eds.). *Metapopulation Biology, Ecology, Genetics and Evolution.* Pages 247 – 265. Elsevier Academic Press, Oxford, United Kingdom.

IPCC (2007) Climate Change 2007: Synthesis Report: Contribution of working groups I, II and III to the Fourth Assessment Report of the Intergovernmental Panel of the Climate Change [Core writing team, Pachauri, R. K. and Reisinger, A. (eds.)]. IPCC, Geneva, Switzerland, 104pp.

James, M. 2006. Intra-patch movement in the Sinai Baton Blue butterfly: influence of micro-habitat and individual characteristics. Egyptian Journal of Biology **8**: 51-66.

Kingsolver, J. 1983. Ecological significance of flight activity in Colias butterflies - Implications for reproductive strategy and population-structure. Ecology **64**:546–551.

Kokko, H., and A. Lopez-Sepulcre. 2006. From individual dispersal to species ranges: Perspectives for a changing world. Science **313**:789–791.

Kuussaari, M., M. Nieminen, and I. Hanski. 1996. An experimental study of migration in the Glanville fritillary butterfly *Melitaea cinxia*. Journal of Animal Ecology **65**:791–801.

Lawler, J. J., S. L. Shafer, D. White, P. Kareiva, E. P. Maurer, A. R. Blaustein, and P. J. Bartlein. 2009. Projected climate-induced faunal change in the Western Hemisphere. Ecology **90**:588–597.

Maclean, N. (Ed.). 2010. Silent Summer: The State of Wildlife in Britain and Ireland. Cambridge University Press, Cambridge, United Kingdom.

Mantyka-Pringle, C. S., T. G. Martin, and J. R. Rhodes. 2012. Interactions between climate and habitat loss effects on biodiversity: a systematic review and meta-analysis. Global Change Biology **18**:1239–1252.

McDonald, K. A., and J. H. Brown. 1992. Using montane mammals to model extinctions due to global change. Conservation Biology **6**:409–415.

McIntire, E. J. B., C. B. Schultz, and E. E. Crone. 2007. Designing a network for butterfly habitat restoration: where individuals, populations and landscapes interact. Journal of Applied Ecology **44**:725–736.

Menendez, R., A. Gonzalez-Megias, O. T. Lewis, M. R. Shaw, and C. D. Thomas. 2008. Escape from natural enemies during climate-driven range expansion: a case study. Ecological Entomology **33**:413–421.

Merckx, T., and H. Van Dyck. 2007. Habitat fragmentation affects habitat-finding ability of the speckled wood butterfly, *Pararge aegeria* L. Animal Behaviour **74**:1029–1037.

Merckx, T., H. Van Dyck, B. Karlsson, and O. Leimar. 2003. The evolution of movements and behaviour at boundaries in different landscapes: a common arena experiment with butterflies. Proceedings of the Royal Society of London Series B-Biological Sciences **270**:1815–1821.

Mitikka, V., and I. Hanski. 2010. Pgi genotype influences flight metabolism at the expanding range margin of the European map butterfly. Annales Zoologici Fennici **47**:1–14.

Mitikka, V., R. K. Heikkinen, M. Luoto, M. B. Araujo, K. Saarinen, J. Pöyry, and S. Fronzek. 2008. Predicting range expansion of the map butterfly in Northern Europe using bioclimatic models. Biodiversity and Conservation **17**:623–641.

Niitepõld, K., A. D. Smith, J. L. Osborne, D. R. Reynolds, N. L. Carreck, A. P. Martin, J. H. Marden, O. Ovaskainen, and I. Hanski. 2009. Flight metabolic rate and Pgi genotype influence butterfly dispersal rate in the field. Ecology **90**:2223–2232.

Nylin, S., and K. Gotthard. 1998. Plasticity in life-history traits. Annual review of entomology **43**:63–83.

Odendaal, F. J., P. Turchin, and F. R. Stermitz. 1989. Influence of host-plant density and male harassment on the distribution of female *Euphydryas anicia* (Nymphalidae). Oecologia **78**:283–288.

Ovaskainen, O., A. D. Smith, J. L. Osborne, D. R. Reynolds, N. L. Carreck, A. P. Martin, K. Niitepõ ld, and I. Hanski. 2008. Tracking butterfly movements with harmonic radar reveals an effect of population age on movement distance. Proceedings of the National Academy of Sciences of the United States of America **105**:19090–19095.

Parmesan, C. et al. 1999. Poleward shifts in geographical ranges of butterfly species associated with regional warming. Nature **399**:579–583.

Parmesan, C. 2006. Ecological and evolutionary responses to recent climate change. Annual Review of Ecology Evolution and Systematics **37**:637–669.

Parmesan, C., M. T. Burrows, C. M. Duarte, E. S. Poloczanska, A. J. Richardson, D. S. Schoeman, and M. C. Singer. 2013. Beyond climate change attribution in conservation and ecological research. Ecology letters **16 Suppl 1**:58–71.

Parmesan, C., and G. Yohe. 2003. A globally coherent fingerprint of climate change impacts across natural systems. Nature **421**:37–42.

Pateman, R. M., J. K. Hill, D. B. Roy, R. Fox, and C. D. Thomas. 2012. Temperature-dependent alterations in host use drive rapid range expansion in a butterfly. Science **336**:1028–1030.

Pöyry, J., M. Luoto, R. K. Heikkinen, M. Kuussaari, and K. Saarinen. 2009. Species traits explain recent range shifts of Finnish butterflies. Global Change Biology **15**:732–743.

Ries, L., and D. Debinski. 2001. Butterfly responses to habitat edges in the highly fragmented prairies of Central Iowa. Journal of Animal Ecology **70**:840–852.

Riley, J. R., and A. D. Smith. 2002. Design considerations for an harmonic radar to investigate the flight of insects at low altitude. Computers and Electronics in Agriculture **35**:151–169.

Ronce, O. 2007. How does it feel to be like a rolling stone? Ten questions about dispersal evolution. Annual Review of Ecology, Evolution, and Systematics **38**:231–253.

Root, T. L., J. T. Price, K. R. Hall, S. H. Schneider, C. Rosenzweig, and J. A. Pounds. 2003. Fingerprints of global warming on wild animals and plants. Nature **421**:57–60.

Rosenzweig, C. et al. 2008. Attributing physical and biological impacts to anthropogenic climate change. Nature **453**:353–357.

Roy, D. B., P. Rothery, D. Moss, E. Pollard, and J. A. Thomas. 2001. Butterfly numbers and weather: predicting historical trends in abundance and the future effects of climate change. Journal of Animal Ecology **70**:201–217.

Roy, D. B., and T. H. Sparks. 2000. Phenology of British butterflies and climate change. Global Change Biology **6**:407–416.

Samejima, Y., and Y. Tsubaki. 2010. Body temperature and body size affect flight performance in a damselfly. Behavioral Ecology and Sociobiology **64**:685–692.

Schneider, C. 2003. The influence of spatial scale on quantifying insect dispersal: an analysis of butterfly data. Ecological Entomology **28**:252–256.

Schtickzelle, N., G. Mennechez, and M. Baguette. 2006. Dispersal depression with habitat fragmentation in the bog fritillary butterfly. Ecology **87**:1057–1065.

Schultz, C. B. 1998. Dispersal behavior and its implications for reserve design in a rare Oregon butterfly. Conservation Biology **12**:284–292.

Schultz, C. B., and E. E. Crone. 2001. Edge-mediated dispersal behavior in a prairie butterfly. Ecology **82**:1879–1892.

Schultz, C. B., A. M. A. Franco, and E. E. Crone. 2012. Response of butterflies to structural and resource boundaries. Journal of Animal Ecology **81**:724–734.

Sekar, S. 2012. A meta-analysis of the traits affecting dispersal ability in butterflies: can wingspan be used as a proxy? The Journal of animal ecology **81**.

Shreeve, T. 1984. Habitat selection, mate location, and microclimatic constraints on the activity of the Speckled wood butterfly *Pararge Aegeria*. Oikos **42**:371–377.

Simmons, A. D., and C. D. Thomas. 2004. Changes in dispersal during species' range expansions. American Naturalist **164**:378–395.

Stamps, J., M. Buechner, and V. Krishnan. 1987. The effects of edge permeability and habitat geometry on emigration from patches of habitat. American Naturalist **129**:533–552.

Stevens, V. M., S. Pavoine, and M. Baguette. 2010a. Variation within and between closely related species uncovers high intra-specific variability in dispersal. Plos One **5**.

Stevens, V. M., C. Turlure, and M. Baguette. 2010b. A meta-analysis of dispersal in butterflies. Biological Reviews **85**:625–642.

Thomas, C. D. et al. 2004. Extinction risk from climate change. Nature **427**:145–148.

Thomas, C. D., E. J. Bodsworth, R. J. Wilson, A. D. Simmons, Z. G. Davies, M. Musche, and L. Conradt. 2001. Ecological and evolutionary processes at expanding range margins. Nature **411**:577–581.

Thomas, C. D., A. M. A. Franco, and J. K. Hill. 2006. Range retractions and extinction in the face of climate warming. Trends in Ecology & Evolution **21**:415–416.

Thomas, C. D., and J. J. Lennon. 1999. Birds extend their ranges northwards. Nature **399**:213–213.

Thomas, J. 2005. Monitoring change in the abundance and distribution of insects using butterflies and other indicator groups. Philosophical Transactions of the Royal Society B-Biological Sciences **360**:339–357.

Thomas, J. A., D. J. Simcox, and T. Hovestadt. 2011. Evidence based conservation of butterflies. Journal of Insect Conservation **15**:241–258.

Thuiller, W., C. Albert, M. B. Araújo, P. M. Berry, M. Cabeza, A. Guisan, T. Hickler, G. F. Midgley, J. Paterson, and F. M. Schurr. 2008. Predicting global change impacts on plant species' distributions: future challenges. Perspectives in Plant Ecology, Evolution and Systematics **9**:137–152.

Turchin, P. 1998. Quantitative analysis of movement. Sinauer Associates, Sunderland Massachusetts, USA.

Van Dyck, H., and M. Baguette. 2005. Dispersal behaviour in fragmented landscapes: Routine or special movements? Basic and Applied Ecology **6**:535–545.

Van Swaay, C. A., P. Nowicki, J. Settele, and A. J. van Strien. 2008. Butterfly monitoring in Europe: methods, applications and perspectives. Biodiversity and Conservation **17**:3455–3469.

Van Swaay C. et al. 2013. The European Grassland Butterfly Indicator: 1990-2011. European Environment Agency. Luxembourg.

Visser, M. E. 2008. Keeping up with a warming world; assessing the rate of adaptation to climate change. Proceedings of the Royal Society B: Biological Sciences **275**:649–659.

Warren, M. S. 1986. Notes on habitat selection and the larval host-plants of the Brown argus *Aricia agestis* marsh fritillary *Eurodryas aurinia* and painted lady *Vanessa cardui* in 1985. Entomologist's Gazette **37**:65–67.

Warren, M. S. et al. 2001. Rapid responses of British butterflies to opposing forces of climate and habitat change. Nature **414**:65–69.

Wiens, J. A., N. C. Stenseth, B. V. Horne, and R. A. Ims. 1993. Ecological mechanisms and landscape Ecology. Oikos **66**:369.

Wilson, R. J., D. Gutierrez, J. Gutierrez, and V. J. Monserrat. 2007. An elevational shift in butterfly species richness and composition accompanying recent climate change. Global Change Biology **13**:1873–1887.

Wilson, R., and C. D. Thomas. 2002. Dispersal and spatial dynamics of butterflies (Chapter 13). In: Bullock, J.M., Kenward R.E. and Hails R.S. (Eds.): *Dispersal Ecology*. pp 257-278. Blackwell Science Ltd., Oxford, United Kingdom.

Chapter 2: Inter- and intra-specific differences in butterfly behaviour at boundaries

Published as :

Kallioniemi, E., Zannese, A., Tinker, J. E., Franco, A. M.A. In press. Inter- and intra-specific differences in butterfly behaviour at boundaries. *Insect Conservation and Diversity*

Abstract

1. To plan effective conservation measures and to predict which species will be able to change distribution in response to climate change, there is an increasing need to understand species dispersal abilities and how species move in complex landscapes. Responses to habitat boundaries affect emigration rates from habitat and are therefore important determinants of species dispersal. There are, however, few studies linking dispersal parameters to likelihood of crossing barriers across several species.
2. In this study, mobility and likelihood of crossing boundaries, which are presented here as tall and dense tree plantations, were investigated for seven butterfly species. Effects of adult age and sex on the mobility and behaviour at boundaries were also analysed.
3. Our results demonstrate differences in movements and response to habitat boundaries between species belonging to different butterfly families. Pieridae species were the most likely to cross boundaries and most mobile, whilst with the Lycaenidae species only a small fraction of individuals crossed the tall dense boundary.
4. Individuals and species that moved with longer move bouts (i.e. steps) were more likely to cross boundaries. Therefore, we propose using step length, which is relatively easy to measure, as a proxy for butterfly dispersal at the landscape level.
5. Female butterflies moved less than males within habitat but crossed boundaries more often than males, indicating that dispersal data needs to be collected for the two sexes separately to provide more accurate estimates of species ability to colonise new areas.

Keywords: Animal movement; boundary behaviour, edge behaviour, insect conservation, Lepidoptera, Lycaenidae, Nymphalidae, Pieridae, step length.

Introduction

Climatically suitable areas for many species are moving towards the poles and higher elevations (Huntley et al., 2007; Settele et al., 2008) driving species to either shift their ranges (Hickling et al., 2006) or adapt to new climatic conditions (Parmesan, 2006). Habitat loss and fragmentation may hinder colonisations of new climatically suitable areas (Warren et al., 2001; Fahrig, 2003; Pöyry et al., 2009), particularly for species with limited dispersal ability (Warren et al., 2001; Pöyry et al. 2009; Mattila et al., 2011). Incorporating species-specific dispersal abilities in bioclimatic models can increase the accuracy of predictions of distribution change (Brooker et al., 2007; Buse & Griebeler, 2011; Jaeschke et al., 2013). For most species, such information is, however, not available even though there is increasing recognition of the need for dispersal data (e.g. Pöyry et al., 2009; Stevens et al., 2010a). To plan effective conservation management under climate change, there is a pressing need to quantify species dispersal ability and understand movement behaviour in complex landscapes.

Butterflies are an excellent taxonomic group for studying dispersal and behaviour at boundaries because they can be individually marked and followed and their natural history and distributions are generally well known (Dennis, 1992; Asher et al., 2001). Butterflies are also recognized as useful indicators of environmental change for other terrestrial insect groups (Thomas, 2005). It is important to understand which species might be at risk of losing climatic space, as a large proportion of butterflies are lagging behind the current global environmental change (Devictor et al., 2012) and have suffered population declines (Asher et al., 2001; Fox et al., 2006). A meta-analysis including 30 different butterfly species showed that adult dispersal ability is one of the main drivers of long term butterfly population trends (Thomas et al., 2011).

Dispersal can be defined as “any movement of individuals or propagules with potential consequences for gene flow across space” (Ronce 2007) and has been viewed as a three step process with emigration, travelling through landscape and immigration or colonisation (e.g. Ims & Yoccoz, 1997). During dispersal species respond to landscape structures and habitat boundaries and this affects emigration rates and thereby population dynamics (Ries & Debinski, 2001). The likelihood of crossing a boundary can potentially be a good indicator of species long-distance dispersal ability as it represents an active decision of emigration and therefore represents the first part of the dispersal process. Several studies have found large differences between species behaviour at habitat edges (e.g. Haddad, 1999; Kuefler et al., 2010). For example, Wood and Samways (1991) found that some butterfly species do not appear to cross water, while others do not cross tree plantations. Furthermore, information of species responses to boundaries can be used for estimating the usefulness of physical corridors for species (Haddad, 1999). Few studies, however, have measured the rate of crossing of different boundaries and the impact of boundaries on dispersal and these have focused on only one or two species (Merckx *et al*., 2003; Conradt & Roper, 2006; Dennis, 2010; Schultz et al., 2012). Although Norberg et al. (2002) found that butterfly species that were more mobile within open parts of a population cage had a higher frequency of moving though unsuitable habitat, this was not formally tested. To our knowledge, only Kuefler et al. (2010) have studied the link between movement parameters and the likelihood of entering specific habitats. They found for *Satyrodes appalachia* butterfly a negative relationship between the probability of entering habitat and the rate at which individuals moved through it. By understanding species movement behaviour and responses to boundaries, we can better predict the effects of different landscape management decisions. For example, in England, there are proposals to increase the amount of woodland from 9 to 12% by 2060 (Lawton et al., 2010) which could potentially have negative effects for open habitat species for which tree plantations may act as dispersal barriers, while this is likely to benefit several woodland species. Determining how movement parameters and likelihood of crossing boundaries are linked has implications for modelling movement at a larger scale.

Only a few studies have compared the dispersal abilities of several species in the same context (see Stevens et al*.,* 2010b) even though differences in dispersal among species are widely recognized. A better understanding of differences between related species in their dispersal capacity will clarify if related species data can be used as a proxy when species specific data are not available.

Males and females are likely to experience different selection pressures on their dispersal (Gros et al., 2008, 2009) and several studies have shown variation in dispersal and movement depending on individual sex or age (Gall, 1984; Kuussaari et al*.,* 1996; Schultz & Crone, 2001; Bergman et al., 2011). Understanding these within-species differences helps to accurately account for dispersal in demographic models and therefore the use of more than one value for species traits in modelling has been encouraged (Stevens et al., 2010b).

In this study, the differences among and within butterfly families in their movement parameters and probability of crossing of boundaries were quantified for seven butterfly species in Britain. As forests have previously been found to hinder dispersal of open habitat species (Shreeve, 1981; Kuussaari et al., 1996; Mousson et al., 1999), in this study tall tree plantations and tree line were used as the boundary at which to observe the butterfly behaviour. Here we relate the likelihood of crossing habitat boundaries to dispersal parameters at an individual level and across several butterfly species. We expected species and individuals that show greater mobility to be more likely to cross boundaries. Within-species differences in dispersal and behaviour at boundaries were also studied because this can potentially have a large impact on colonising ability and population dynamics. Females and males can experience different costs of dispersal (Gros et al. 2008). We expected females to cross barriers more frequently because several studies have shown female butterflies to be more dispersive (Kuussaari et al., 1996; Skorka et al., 2013) and have higher emigration rates (Skorka et al., 2013; Rabasa et al., 2007, but see Fric et al., 2010 and Wang et al., 2004) possibly due to benefits of laying eggs in multiple different locations (Gall, 1984; Baguette et al., 1996; Delattre et al., 2010). However, males may benefit from active flight within the habitat by increasing encounter rates with potential mates, and it might be difficult to find unmated females after dispersing (Baguette & Neve, 1994; Baguette et al., 1998; Hirota, 2004).

Materials and methods

Study species and sites

Two Pieridae species; *Pieris napi* (L.,green-veined white) and *Anthocharis cardamines* (L., orange- tip), two Lycaenidae species; *Aricia agestis* (Denis & Schiffermüller, brown argus) and *Polyommatus icarus* (Rottemburg, common blue)*,* three Nymphalidae species; *Maniola jurtina* (L., meadow brown) and *Pyronia tithonus* (L., gatekeeper) and a UK Biodiversity Action Plan priority species *Coenonympha pamphilus* (L., small heath) were included in the study. *Coenonympha pamphilus* has shown >50% long-term population decline and 29% distribution decline in the UK and therefore gathering further knowledge of *C. pamphilus* is recommended for its conservation (Fox et al., 2006). Three of the species, *A. cardamines, A. agestis* and *P. tithonus*, have expanded their ranges in the last 20 years, while *P. napi* and *P. icarus* have shown relatively stable population and distribution trends in the UK (Fox et al., 2006). All seven species studied can be classified as generalist and most occur in a wide range of open habitats (Asher et al., 2001).

From April to September 2010, we recorded 191 individual flight paths of released individuals of five of the species; *A. agestis, P. icarus, P. napi*, *A. cardamines* and *C. pamphilus*. The study was conducted at three sites (size of 8 to 40 ha) at Eastern England (WGS 84: 52.3423, 0.685; 52.5092, 0.6203; and 52.4114, 0.9131); these grass heath habitats were surrounded by dense closed-canopy conifer plantations (of 25-35 years, average height of 10-20m). The tree plantations were considered unsuitable habitat for all the study species due to shadiness and lack of host and nectar plants. In 2011 (from June to August) 128 tracks of *M. jurtina* and *P. tithonus* were collected at two sites (WGS84 53.5917, -1.7634 and 53.5455, -0.9748) in Yorkshire. These were grassland sites (2 to 7 ha) surrounded by dense tree hedges, of approximately 7.5±0.35m and 9.5±0.45m height, where butterflies could not visually perceive habitat behind the hedge without crossing the hedge. Butterflies were released at 2-3 different locations at each study site. To understand if there were differences in the weather conditions between the two years of the study, we compared the temperatures at which the butterflies were released during the overlapping release months (June-August). The temperatures during the release experiments were on average only 0.65 °C warmer in 2011 and therefore unlikely to affect the results of this study.

Butterfly tracking

Butterflies were tracked between 10.00 and 18.00 on sunny days when the temperature was above 15 °C and the wind speed did not exceed five on the Beaufort scale. Butterflies were collected using sweep nets. Similar numbers of individuals from different species and sexes were tracked each day at study sites that were alternated every day to reduce the effect of release conditions and release site. Because of inherently different thermal tolerance, optimum flight temperatures and differences in flight seasons, the species included in this study were likely to experience different thermal conditions even when released in similar weather conditions. By releasing the butterflies throughout their entire flight seasons and at suitable conditions for flight we captured the range of conditions the butterflies were exposed to and the characteristics of their movement behaviour. Immediately after being caught, butterflies were cooled down in a cool box at 7-15 °C for at least 15 minutes and marked individually on their hind wing. The sex was determined for five of the species but sexes could not be determined for *C. pamphilus* or *P. napil* thus, sexes were pooled in some analyses. Age of the butterfly was recorded as one for recently emerged (young) butterflies and two for individuals with wing wear (old). After marking, the butterflies were cooled again for >30 minutes and were subsequently released and tracked individually.

Each butterfly was released close to the boundary in open grassland habitat with host plant and nectar sources available. Release locations were sites with host plants, nectar sources and other individuals of the same species, but were at least 500m away from where butterflies were caught thus, all butterflies were released in unfamiliar locations. All individuals were released within a distance to a boundary from which they were able to reach the boundary with three average step lengths (minimum duration of the tracks included in the analysis). Each individual was released at slightly different locations depending on the host and nectar plants and individuals released further than three species-average step lengths from the boundary were later removed from the analysis. Thus, the families have different average release distances from the boundary (mean distance ± SD from release point to the boundary: 16±7.6 m, 9.1±4.3 m, 9.5±3.5 m for Pieridae, Lycaenidae and Nymphalidae respectively). Butterfly flight tracks were recorded using a flag technique (described in detail in Schultz, 1998) in which numbered flags are dropped on the butterfly flight path every 20 s or when a butterfly lands. Individuals were tracked for 15 flags or until lost (See Figure 1). Flag locations were recorded with differential GPS with 10 cm accuracy (Magellan ProMark III GPS). Each butterfly path was assigned 1 or 0 according to whether the butterfly crossed the boundary with tree plantations or not, respectively. Butterflies were not tracked after crossing the boundary except for Pieridae species which, due to their larger size, were possible to track through the forest.

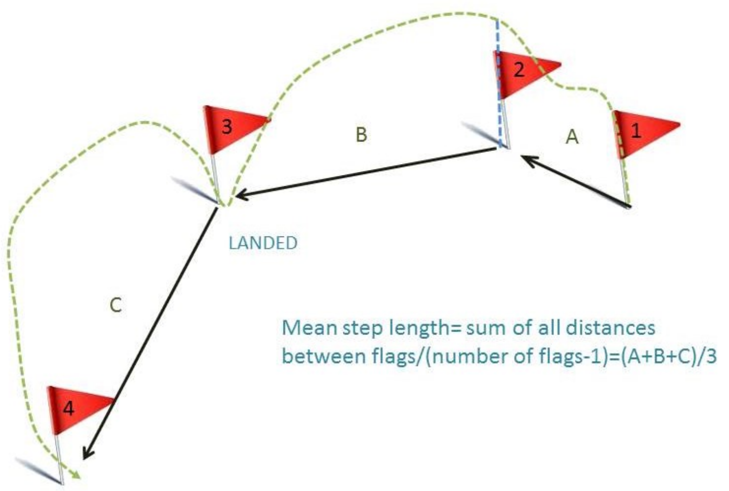


Figure 1. *Example of a butterfly flight path shown in green. The blue line shows location where butterfly was after 20 seconds of flight. Flags were dropped every time the butterfly landed or after every 20 seconds of flight. The equation from which mean step length for each individual was calculated is shown.*

Data analysis

Previous studies using the flag method showed no effect of handling on butterfly behaviour (Root & Kareiva, 1984; Schultz, 1998). Nevertheless, because the butterflies were cooled, we excluded the first move from all analyses. Only paths with three or more flags were included in the analysis. Each butterfly was followed once and individual paths were the sampling unit. Mean step length (sum of distances between consecutive flags divided by the number of moves in the path) is correlated with net distance moved (Pearson correlation=0.718). Mean step length was used as our movement parameter because it is easy to measure and it is not dependent on the number of moves or time an individual was tracked for. Mean step length was $\log_e$-transformed prior to analyses. Differences among species were examined by a linear model (LM) that had step length as the dependent variable and species as an explanatory variable. A second model including sex and species as explanatory variables was created for those five species for which sex was known. A further model was built for four of the species for which sex was known to test the effect of adult age, sex and species on step length (*A. cardamines* was excluded as only two individuals were classified as old). Full models included all interactions and model selection was performed by backward stepwise regression using $P < 0.05$.

The effect of mean step length and species on the likelihood of crossing a boundary was determined using generalized LMs with binomial distribution and logit link. A similar model was created incorporating sex as an explanatory variable for *A. cardamines, P. icarus* and *M. jurtina* for which some individuals of both sexes had crossed. A further model that included the effect of adult age was built for the four species with large enough sample size of young and old individuals. Full models including all possible interactions were fitted and model selection was performed using backward stepwise regression with $P < 0.05$. Pair-wise differences among species were tested with Tukey's HSD test. All analyses were conducted in R 2.15.0 (R Development Core Team, 2012).

Results

There were clear differences between species from different families in mean step lengths, with butterflies from the Pieridae family having on average three to four times longer step lengths than those of the two other families (Table 1 and 2). The only significant difference in step length between species from the same family was between *A. agestis* and *P. icarus* (Tukey's HSD P=0.024); *Aricia agestis* had shorter step lengths (Table 2). Males had longer step lengths than females with the exception of *M. jurtina* for which females and males had similar step lengths (Table 2, Figure 2). Young individuals moved, in general, with longer step lengths than old ones (Table 3).

Table 1. *Results from regression analyses of differences in step lengths among species for (a) all species, (b) species for which the sex of individual could be determined and (c) model with adult age included.*

Dependent variable	Independent variable	d.f.	*F*-value	*P*-value
a. Log_e (step length)	species	6	24.63	<0.0001
	residuals	307		
b. Log_e (step length)	species	4	27.11	<0.0001
	sex	1	17.84	<0.0001
	residuals	265		
c. Log_e (step length)	species	3	5.51	<0.01
	adult age	1	9.76	<0.01
	sex	1	19.38	<0.0001
	residuals	232		

Table 2. *Sample sizes (n total), numbers of individuals that crossed (n crossed), number of moves per track and mean step lengths (m±SE) of all individuals tracked for ≥3 flags for each species and sex.*

Species	**Sex**	**n total**	**n crossed**	**Mean number of moves per track**	**Mean step length ±SE**
Anthocharis cardamines	Female	17	7	13.2	16.8±2.2
	Male	14	2	12.2	22.2±2.7
	All	31	9	12.8	19.2±1.8
Pieris napi	All	14	9	11.3	21.5±3.3
Aricia agestis	Female	19	0	9.26	3.64±0.61
	Male	29	0	9.86	6.01±0.71
	All	48	0	9.63	5.07±0.52
Polyommatus icarus	Female	29	3	9.86	5.10±0.59
	Male	36	2	8.94	9.93±1.40
	All	65	5	9.35	7.77±0.87
Coenonympha pamphilus	All	28	4	11.0	6.74±0.58
Pyronia tithonus	Female	29	2	11.6	7.03±0.63
	Male	8	0	12.8	10.2±1.90
	All	37	2	11.8	7.71±0.66
Maniola jurtina	Female	39	5	9.64	6.54±0.70
	Male	51	7	13.5	6.34±0.47
	All	91	12	11.9	6.39±0.40

Table 3. *Table of sample sizes (n) and mean step lengths (m±SE) of young and old individuals of all species and sex.*

Species	Sex	n young/old	Young Mean step length ±SE	Old Mean step length ±SE
Anthocharis cardamines	Female	16/1	16.5±2.3	21.6
	Male	13/1	23.3±2.7	7.8
	All	29/2	19.5±1.8	14.7±0.7
Pieris napi	All	14/0	NA	NA
Aricia agestis	Female	9/10	4.3±1.1	2.9±0.6
	Male	17/11	7.3±1.1	4.4±0.5
	All	26/21	6.2±0.8	3.7±0.4
Polyommatus icarus	Female	12/16	6.4±1.2	4.0±0.5
	Male	21/15	10.9±1.9	8.5±2.1
	All	33/31	9.3±1.3	6.2±1.1
Coenonympha pamphilus	All	22/3	7.1±0.7	5.0±1.6
Pyronia tithonus	Female	15/14	7.3±1.1	6.7±0.7
	Male	4/4	11.1±3.7	9.2±1.2
	All	19/18	8.1±1.1	7.3±0.6
Maniola jurtina	Female	26/13	7.4±0.9	4.8±0.8
	Male	20/31	6.3±0.7	6.4±0.7
	All	47/44	6.9±0.6	5.9±0.5

Species differed in their rate of crossing the boundary from 0% for *A. agestis* to 64% for *P. napi* (Table 2, Figure 2). Within the same family, however, there were no between-species differences in the rate of crossing boundaries (Appendix A). Young and old individuals did not differ in likelihood of crossing boundaries (likelihood ratio test (LRT) =0.450, *P*=0.502). In the model obtained for all study species, individuals and species that crossed the boundaries had on average longer steps than those that did not cross the boundaries (coefficient: 0.629 ±0.32, Table 4, Figure 3). Step length was not correlated with the number of moves for which an individual was tracked, except for *P. icarus* for which individuals with longer steps were tracked for fewer flags. Thus our results are conservative for *P. icarus*, as there were fewer chances of observing individuals that moved with long steps crossing a boundary.

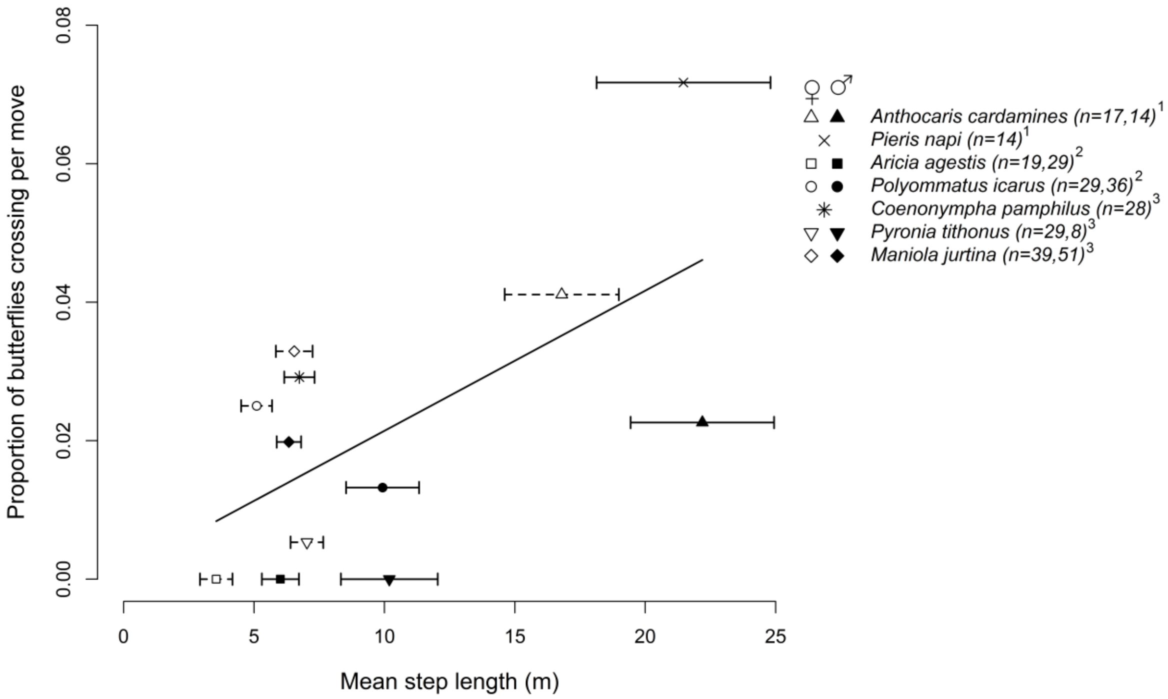


***Figure 2**. Relationship between step length ±SE and the proportion of butterflies crossing a boundary per species and sex when known. Sample sizes are given in the legend for females and males respectively. Line is fitted based on linear model of likelihood of crossing per flag of each species and sex. [1]Pieridae, [2]Lycaenidae and [3]Nymphalidae.*

The model for probability of crossing the boundary revealed interactions between species and step length and between sex and step length (Table 4). For the species for which the sexes were known, females had shorter step lengths than males but were more likely to cross the boundaries than males. *Anthocaris cardamines* males that crossed boundaries had shorter step lengths that those that did not cross boundaries. For all other species individuals with longer steps were more likely to cross boundaries or there was no difference between the step length of individuals that crossed or did not cross boundaries (Figure 3). *Polyommatus icarus, M. jurtina* and *P. tithonus* females that crossed boundaries had longer step lengths than those that did not cross although this was only significant for *M. jurtina* (Figure 3).

Table 4. *Tests of differences among species in likelihood of boundary crossing for (a) all species and (b) species for which the sex is known. LRT= likelihood ratio test.*

Dependent variable	Independent variable	d.f.	Deviance	LRT	P-value
a. Crossing	none		196.36		
	Log$_e$ (step length)	1	200.35	3.99	0.046
	species	6	219.61	23.2	<0.001
b. Crossing	none		128.52		
	Log$_e$(step length)×species	2	134.53	6.01	0.050
	Log$_e$(step length)×sex	1	133.79	5.27	0.022

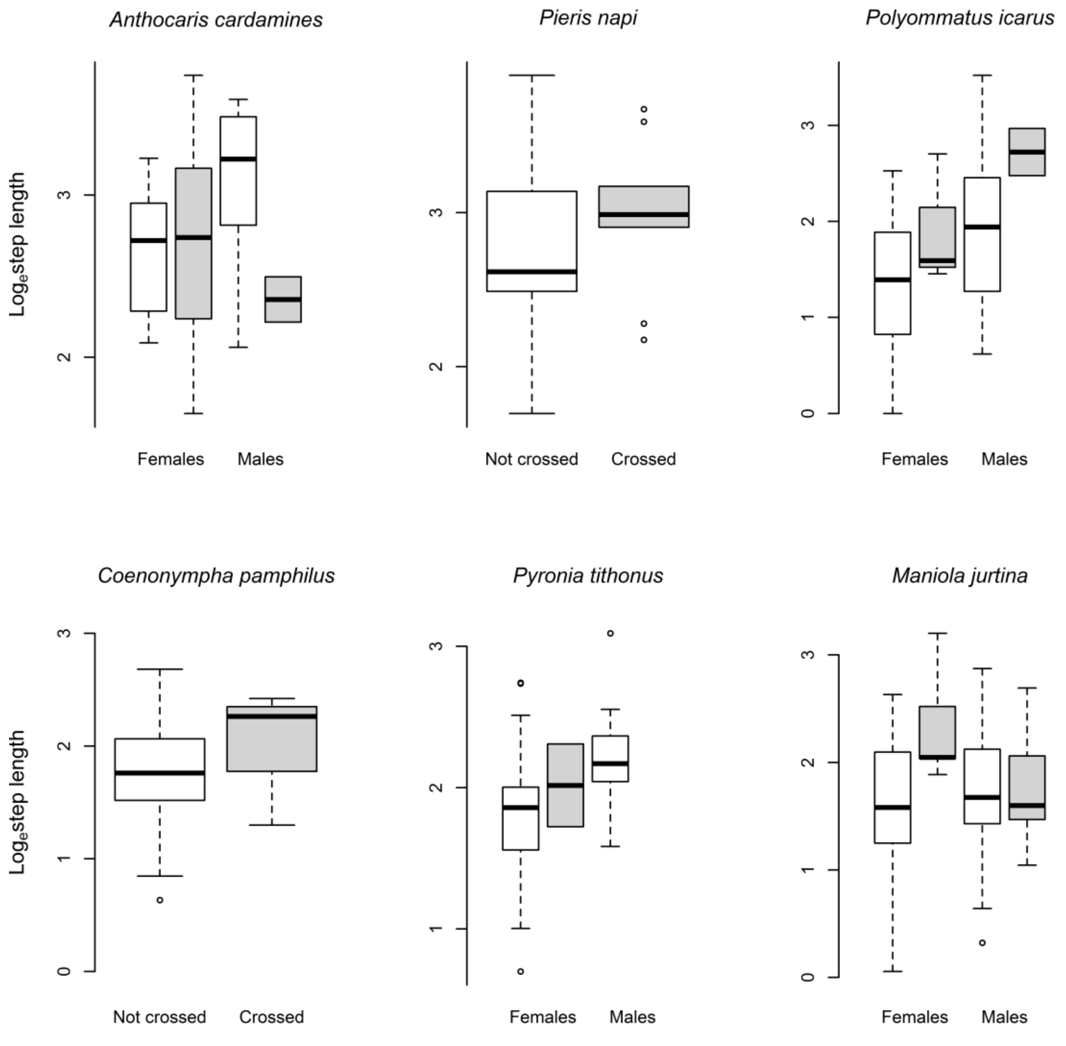


Figure 3. *Step length (m) for individuals that did not cross (white) and individuals that crossed the boundary (shaded).*

Discussion

Our findings show large differences in movement and propensity to cross habitat boundaries among seven butterfly species in Britain. Individuals and species that moved more were more likely to cross boundaries; an increase in step length from 5m to 10.5m doubled the per move probability of a butterfly crossing a boundary. Female butterflies had shorter steps than males but were more prone to cross boundaries.

Differences between species in step lengths and behaviour at boundaries

Both Pieridae species (*A. cardamines* and *P. napi*) were highly dispersive; a high proportion of individuals moved into the tall and dense tree plantation. Step lengths and the likelihood of crossing the boundary with tall tree plantation were similar for these two species which is congruent with previous mobility estimates (Cowley et al., 2001; Dennis, 2010, but see Cook et al., 2001). Our results show that *A. agestis* individuals had the shortest mean step length and none of the 48 individuals sampled crossed the tall tree plantation line. Butterfly displacement results either from “routine” movements (associated e.g. with foraging) or from rare long distance movements that enable species dispersal (Van Dyck & Baguette, 2005). It is possible that *A. agestis* disperse using rare long-distance movements and avoid crossing tall boundaries preferring to move in open habitat. Our results coincide with a mark release recapture (MRR) study that indicates that in general *A. agestis* individuals moved short distances, and only a few individuals (1.5%) moved more than 500 m (Wilson & Thomas, 2002). Because of the low frequency of the long-distance movements and taking into account that our study only captured the routine daily movements it is not possible to make meaningful conclusions on impact of tall forest plantation on *A. agestis*. Despite having been able to expand its range recently (Asher et al., 2001; Wilson & Thomas, 2002), this species may be vulnerable to changes in the landscape that hinder its likelihood of leaving a habitat patch.

The declining and conservation concern species *C. pamphilus* was able to cross tall and dense boundaries more often than *P. icarus* which is a widespread species that has been previously considered more dispersive than *C. pamphilus* (Cowley et al., 2001; Gutierrez et al., 2001). A MRR study conducted in Sweden showed that 6% of *C. pamphilus* individuals moved distances greater than 1000 m which also shows that this is a fairly mobile species (Öckinger &

Smith, 2007), but there were no previous studies on rates of boundary crossing for this species. Our results suggest that dispersal ability is unlikely to be important factor in the decline of *C. pamphilus* in the UK, assuming that the within-habitat movement reflects dispersal and colonisation rates at a landscape level. We did not find significant differences in the step lengths or rates of crossing boundaries between the three Nymphalidae species despite earlier study by Merckx & van Dyck (2002) showed that *M. jurtina* moves through landscape at higher rate than *P. tithonus*.

Our results show that, although some of the study species are able to move through tall tree plantations, for open habitat species these tree plantations can act as potential barriers to movement. Therefore, afforestations should be carefully planned to enhance both woodland and open habitat species movement. This is especially relevant in the current context of global environmental change as some species are already lagging behind climate change (Devictor et al., 2012).

It has been previously recommended that species-specific information should be used to understand spatial population dynamics (e.g. Gutierrez et al., 2001; Turlure et al., 2011). Due to the low impact of phylogeny on dispersal patterns, instead of closest relative, preference should be given to using species with similar demography as a surrogate for species-specific information (Stevens et al., 2012). Furthermore, due to large intra-species variation in movement behaviour, care should be taken even when extrapolating behaviour of one population to another (Stevens et al., 2010b; Bergerot et al., 2012). Contrarily to previous recommendations our results shows that there were no significant differences in step lengths within families for five out of seven species included in this study. Our results suggest that when species-specific data is not available, data on closely related species that share similar habitats can potentially be used.

Relationship between step length and likelihood of crossing boundaries

Step length in suitable habitat was positively associated with likelihood of crossing a boundary and therefore likelihood of leaving the habitat patch. Individuals and species that had longer step lengths were more likely to cross the boundaries probably because it is less risky for dispersive individuals/species to move outside habitat as they are more likely to cross long distances and encounter suitable habitat. These results are particularly relevant and useful because step length in suitable habitat is relatively easy to measure. Our results indicate that step length can potentially be used for estimating movement capacity of different species and thereby the impacts of different landscape management actions on species. The relationship between step length and probability of crossing boundaries, however, should be confirmed for more species. Furthermore, these results represent the responses of open habitat butterflies to tall and dense tree plantations which can act as a hard barrier to dispersal while open and shorter woodlands can be more permeable for all the species studied.

Sex- and age-dependent dispersal and responses to boundaries

Although species that were more mobile were more likely to cross boundaries, this result did not hold within individuals of the same species. In four of the five study species (*A. cardamines, A. agestis*, *P. icarus* and *P. tithonus*) for which sexes were compared, males moved with longer steps than females (for *M. jurtina* there were no differences in the step lengths between sexes). As expected, in general, males were more mobile in the habitat than females. For four of these species (except *A.agestis* for which no boundary crossings were observed), females were more likely to cross boundaries. This indicates that although males move more in habitat, females are more likely to move outside the habitat which has been previously found for *Phengaris teleius* butterfly (Skórka et al., 2013). This result may be explained by selection pressure on females to bet-hedge by laying their eggs in different habitat patches (Gall, 1984; Baguette et al., 1996; Delattre et al., 2010). Dispersing may be a risky strategy for males as it may be difficult to find unmated females if males disperse to a new area (Baguette & Neve, 1994; Baguette et al., 1998; Hirota, 2004).

Previous studies found that flight endurance decreases with adult age for both sexes (Åhman & Karlsson, 2009) and that older males move less (Warren, 1987; Kemp, 2001). These findings were confirmed by our results of young individuals having longer steps than older individuals. Furthermore, as most butterfly species mate once or only a few times (e.g. Ehrlich & Ehrlich, 1978), young females are likely to spend more time in flight to increase the likelihood of encountering a male (Bergman et al., 2011), whereas older females may reduce their activity after mating to avoid male harassment (Wiklund, 1982; Wickman, 1986; Ide, 2011). This could also explain why females in our study moved with shorter steps than males while the in habitat.

In general, females that crossed boundaries had longer step lengths than those that did not cross. For males, there was no relationship between likelihood of crossing boundaries and step length except for *A. cardamines* for which males that crossed had shorter step lengths. This suggests that there may be a behavioural shift in the movement that is associated with greater willingness or likelihood of crossing the boundary particularly in females. Adult age did not appear to be linked to likelihood of crossing the boundary and therefore our results reflect differences in movement within habitat.

The differences in step length and likelihood of crossing boundaries observed for different sexes and individuals of different adult ages emphasize the importance to measure dispersal ability separately for males and females and distinguishing different adult age groups when doing population level analyses.

Conclusions

There are large differences between butterfly families in their movements and responses to boundaries. Here we show the link between likelihood of crossing a boundary and step length in suitable habitat which is fairly easy to measure across several species. The relationship between step length and probability of crossing boundaries should be investigated for other species to examine the generality of the results of this study, which can have useful applications (e.g. modelling species movement at a landscape level). Our results show that tall tree plantations have the potential to act as a dispersal barrier for some species and therefore this should be taken into account for landscape management. In general, individuals and species that were more mobile within habitat were more likely to cross boundaries. Within the same species, in general males were, however, more mobile than females and yet less likely to cross boundaries. Our study indicates that, whenever possible, dispersal information should be obtained for males and females separately as there are large differences between males and females in movement behaviour and behaviour at boundaries.

Acknowledgements

We thank Paul Dolman for his comments on the manuscript, Butterfly Conservation for providing us with butterfly distribution data, Neal Armour-Chelu for his help with field sites and Forestry commission for facilitating access. We also thank three anonymous referees for their valuable comments to the manuscript. This study was funded by the School of Environmental Sciences, University of East Anglia, Kone Foundation and NERC (NE/H007237/1).

Literature

Åhman, M. & Karlsson, B. (2009) Flight endurance in relation to adult age in the green-veined white butterfly *Pieris napi*. *Ecological Entomology*, **34**, 783–787.

Asher, J., Warren M., Fox R., Harding P., G. Jeffcoate, G. & Jeffcoate, S. (2001) *Millennium Atlas of Butterflies in Britain and Ireland*. Oxford University Press, Oxford, United Kingdom.

Baguette,M., Convie I. & Neve G. (1996) Male density affects female spatial behaviour in the butterfly *Proclossiana eunomia*. *Acta Oecologica,* **17**, 225–232.

Baguette, M. & Neve G. (1994) Adult movements between populations in the specialist butterfly *Proclossiana eunomia* (Lepidoptera, Nymphalidae). *Ecological Entomology,* **19**, 1–5.

Baguette, M., Vansteenwegen, C., Convi I. & Neve G. (1998) Sex-biased density-dependent migration in a metapopulation of the butterfly *Proclossiana eunomia*. *Acta Oecologica-International Journal of Ecology,* **19**, 17–24.

Bergerot, B., Merckx, T., Dyck H.V. & Baguette M. (2012) Habitat fragmentation impacts mobility in a common and widespread woodland butterfly: do sexes respond differently? *BMC Ecology***, 12,** 5.

Bergman, M., Gotthard K. & Wiklund C. (2011) Mate acquisition by females in a butterfly: the effects of mating status and age on female mate-locating behaviour. *Animal Behaviour,* **81**, 225–229.

Brooker, R. W., Travis J. M. J., Clark E. J. & Dytham C. (2007) Modelling species' range shifts in a changing climate: The impacts of biotic interactions, dispersal distance and the rate of climate change. *Journal of Theoretical Biology***, 245**, 59–65.

Buse, J. & Griebeler E. M. (2011) Incorporating classified dispersal assumptions in predictive distribution models – A case study with grasshoppers and bush-crickets. *Ecological Modelling*, **222,** 2130–2141.

Conradt, L. & Roper T. J. (2006) Nonrandom movement behavior at habitat boundaries in two butterfly species: Implications for dispersal. *Ecology* **87,** 125–132.

Cook, L. M., Dennis, R. L. H. & Hardy P. B. (2001). Butterfly-hostplant fidelity, vagrancy and measuring mobility from distribution maps. *Ecography*, **24**, 497–504.

Cowley, M. J. R., Thomas, C. D. , Roy, D. B., Wilson, R. J., Leon-Cortes, J. L., Gutierrez, D. , Bulman, C. R., Quinn, R. M., Moss, D. & Gaston K. J. (2001) Density-distribution relationships in British butterflies. I. The Effect of Mobility and Spatial Scale. *Journal of Animal Ecology*, **70**, 410–425.

Delattre, T., Burel, F., Humeau A., Stevens, V. M., Vernon, P. & Baguette, M. (2010) Dispersal mood revealed by shifts from routine to direct flights in the meadow brown butterfly *Maniola jurtina*. *Oikos* **119**, 1900–1908.

Dennis, R. L. H. (Ed.) (1992) *The Ecology of Butterflies in Britain*. OUP Oxford, United Kingdom.

Dennis, R. L. H. (2010) *A resource-based habitat view for conservation: butterflies in the British landscape*. John Wiley and Sons, Oxford, United Kingdom.

Devictor, V., Van Swaay, C., Brereton, T., Brotons, L., Chamberlain, D., Heliölä, J. , Herrando, S., Julliard, R., Kuussaari, M., Lindström, Å., Reif, J., Roy, D.B., Schweiger, O., Settele, J., Stefanescu, C., Van Strien, A., Van Turnhout, C., Vermouzek, Z., WallisDeVries, M., Wynhoff, I. & Jiguet F. (2012) Differences in the climatic debts of birds and butterflies at a continental scale. *Nature Climate Change,* **2**, 121–124.

Ehrlich, A. & Ehrlich P. (1978) Reproductive strategies in the butterflies Part 1 Mating frequency plugging and egg number. *Journal of the Kansas Entomological Society,* **51**, 666–697.

Fahrig, L. (2003) Effects of habitat fragmentation on biodiversity. *Annual Review of Ecology Evolution and Systematics*, **34**, 487–515.

Fox, R., Asher, J., Brereton, T., Roy, D. & Warren, M. (2006) *State of Butterflies in Britain and Ireland.* Pisces Publications, Newbury, United Kingdom.

Fric, Z., Hula, V., Klimova, M., Zimmermann, K. & Konvicka, M. (2010) Dispersal of four fritillary butterflies within identical landscape. *Ecological Research* **25**, 543–552.

Gall, L. (1984) Population structure and recommendations for conservation of the narrowly endemic alpine butterfly, *Boloria-Acrocnema* (Lepidoptera, Nymphalidae). *Biological Conservation,* **28**, 111–138.

Gros, A., Hovestadt, T. & Poethke H. J. (2008) Evolution of sex-biased dispersal: The role of sex-specific dispersal costs, demographic stochasticity, and inbreeding. *Ecological Modelling,* **219**, 226–233.

Gros, A., Poethke, H.J. & Hovestadt, T. (2009) Sex-specific spatio-temporal variability in reproductive success promotes the evolution of sex-biased dispersal. *Theoretical Population Biology,* **76**, 13–18.

Gutierrez, D., Leon-Cortes, J. L., Menendez, R., Wilson, R. J., Cowley, M. J. R. & Thomas, C. D. (2001) Metapopulations of four lepidopteran herbivores on a single host plant, *Lotus corniculatus. Ecology,* **82**, 1371–1386.

Haddad, N. M. (1999) Corridor use predicted from behaviors at habitat boundaries. *American Naturalist,* **153**, 215–227.

Hickling, R., Roy, D. B., Hill, J. K., Fox, R. & Thomas, C. D. (2006) The distributions of a wide range of taxonomic groups are expanding polewards. *Global Change Biology* **12,** 450–455.

Hirota, T. (2004) The evolution of sex-biased dispersal by pre-dispersal copulation and fluctuating environment. *Journal of Animal Ecology* **73**, 1115–1120.

Huntley, B., Green, R., Collingham, Y. & Wills, S.G. (2007) *A Climatic Atlas of European Breeding Birds.* Durham University, RSPB, Lynx Edicions, Barcelona, Spain.

Ide, J.-Y. (2011) Avoiding male harassment: wing-closing reactions to flying individuals by female small copper butterflies. *Ethology* **117**, 630–637.

Ims, R.A., & Yoccoz N.G. (1997) Studying transfer processes in metapopulations: emigration, migration, and colonization. *Metapopulation Biology, Ecology, Genetics and Evolution*, ed. by I. Hanski & M. Gilbin, pp. 247 – 265, Academic Press, San Diego, USA.

Jaeschke, A., Bittner, T., Reineking, B. & Beierkuhnlein, C. (2013) Can they keep up with climate change? – Integrating specific dispersal abilities of protected Odonata in species distribution modelling. *Insect Conservation and Diversity,* **6,** 93–103.

Kemp, D. J. (2001) Age-related site fidelity in the territorial butterfly *Hypolimnas bolina* (L.) (Lepidoptera: Nymphalidae). *Australian Journal of Entomology,* **40**, 65–68.

Kuefler, D., Hudgens, B., Haddad, N. M., Morris, W. F. & Thurgate, N. (2010) The conflicting role of matrix habitats as conduits and barriers for dispersal. *Ecology* **91**, 944–950.

Kuussaari, M., Nieminen, M. & Hanski, I. (1996) An experimental study of migration in the Glanville fritillary butterfly *Melitaea cinxia*. *Journal of Animal Ecology* **65**, 791–801.

Lawton, J.H., Brotherton, P.N.M., Brown, V.K., Brown, V.K., Elphick, C., Fitter, A.H., Forshaw, J., Haddow, R.W., Hilborne, S., Leafe, R.N., Mace, G.M., Southgate, M.P., Sutherland, W.J., Tew, T.E., Varley, J. & Wynne, G.R. 2010. Making Space for Nature: a review of England's wildlife sites and ecological network. Report to Defra . <http://www.defra.gov.uk/environment/ biodiversity/documents/201009space-for-nature.pdf>23rd January 2012.

Mattila, N., Kaitala, V., Komonen, A., Päivinen, J. & Kotiaho, J. S. (2011) Ecological correlates of distribution change and range shift in butterflies. *Insect Conservation and Diversity* **4**, 239–246.

Merckx, T. & Van Dyck, H. (2002) Interrelations among habitat use, behavior, and flight-related morphology in two cooccurring satyrine butterflies, *Maniola jurtina* and *Pyronia tithonus*. *Journal of Insect Behavior,* **15**,541–561.

Merckx, T., Van Dyck, H.**,** Karlsson, B. & Leimar, O. (2003) The evolution of movements and behaviour at boundaries in different landscapes: a common arena experiment with butterflies. *Proceedings of the Royal Society of London Series B-Biological Sciences* **270,** 1815–1821.

Mousson, L., Neve, G. & Baguette, M. (1999) Metapopulation structure and conservation of the cranberry fritillary *Boloria aquilonaris* is (Lepidoptera, Nymphalidae) in Belgium. *Biological Conservation*, **87**,285–293.

Norberg, U., Enfjäll, K. & Leimar, O. (2002) Habitat exploration in butterflies – an outdoor cage experiment. *Evolutionary Ecology,* **16**, 1–14.

Öckinger, E. & Smith, H. G. (2007) Asymmetric dispersal and survival indicate population sources for grassland butterflies in agricultural landscapes. *Ecography,* **30,** 288–298.

Parmesan, C. (2006) *Ecological and evolutionary responses to recent climate change*. pp. 637–669 Annual Review of Ecology Evolution and Systematics. Annual Reviews, Palo Alto, California, USA.

Pöyry, J., Luoto, M., Heikkinen, R. K., Kuussaari, M., & Saarinen, K. (2009) Species traits explain recent range shifts of Finnish butterflies. *Global Change Biology,* **15**, 732–743.

Rabasa, S., G., Gutiérrez, D., & Escudero, A., 2007. Metapopulation structure and habitat quality in modelling dispersal in the butterfly Iolana iolas. *Oikos,* **116,** 793–806.

R Development Core Team (2012) R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, <http://www.R-project.org> 15th February, 2012.

Ries, L., & Debinski, D. M. (2001) Butterfly responses to habitat edges in the highly fragmented prairies of Central Iowa. *Journal of Animal Ecology,* **70,** 840–852.

Root, R. B. & Kareiva, P. M. (1984) The search for resources by cabbage butterflies (*Pieris rapae*) - ecological consequences and adaptive significance of Markovian movements in a patchy environment. *Ecology,* **65**, 147–165.

Schultz, C. B. (1998) Dispersal behavior and its implications for reserve design in a rare Oregon butterfly. *Conservation Biology,* **12,** 284–292.

Schultz, C. B. & Crone, E. E. (2001) Edge-mediated dispersal behavior in a prairie butterfly. *Ecology* **82,** 1879–1892.

Schultz, C. B., Franco, A. M. A. & Crone, E. E. (2012) Response of butterflies to structural and resource boundaries. *Journal of Animal Ecology* **81**, 724–734.

Settele, J. *et al.* (2008) Climatic Risk Atlas of European Butterflies. BIORISK – Biodiversity and Ecosystem Risk Assessment **1**. <http://www.pensoft.net/journals/biorisk/article/568/> 21st September 2012.

Shreeve, T. (1981) Flight patterns of butterfly species in woodlands. *Oecologia,* **51**, 289–293.

Skórka, P., Nowicki, P., Lenda, M., Witek, M., Sliwinska, E. B., Settele, J. & Woyciechowski, M. (2013) Different flight behaviour of the endangered scarce large blue butterfly *Phengaris teleius* (Lepidoptera: Lycaenidae) within and outside its habitat patches. *Landscape Ecology* **28**, 533–546.

Stevens, V. M., Turlure, C. & Baguette, M. (2010a). A meta-analysis of dispersal in butterflies. *Biological Reviews,* **85,** 625–642.

Stevens, V. M., Pavoine, S. & Baguette, M. (2010b) Variation within and between closely related species uncovers high intra-specific variability in dispersal. *Plos One,* **5**.

Stevens, V.M., Trochet, A., Van Dyck, H., Clobert, J. & Baguette, M. (2012) How is dispersal integrated in life histories: a quantitative analysis using butterflies. *Ecology Letters* **15**, 74-86.

Thomas, J. (2005) Monitoring change in the abundance and distribution of insects using butterflies and other indicator groups. *Philosophical Transactions of the Royal Society B-Biological Sciences,* **360**, 339–357.

Thomas, J. A., Simcox, D. J. & Hovestadt, T. (2011) Evidence based conservation of butterflies. *Journal of Insect Conservation* **15**, 241–258.

Turlure, C., Baguette, M., Stevens, V. M. & Maes, D. (2011) Species-and sex-specific adjustments of movement behavior to landscape heterogeneity in butterflies. *Behavioral Ecology,* **22**,967–975.

Van Dyck, H. & Baguette, M. (2005) Dispersal behaviour in fragmented landscapes: Routine or special movements? *Basic and Applied Ecology,* **6**, 535-545.

Wang, R., Wang, Y., Chen, J., Lei, G. & Xu, R. (2004) Contrasting movement patterns in two species of chequerspot butterflies, *Euphydryas aurinia* and *Melitaea phoebe*, in the same patch network. *Ecological Entomology,* **29,** 367–374.

Warren, M. (1987) The ecology and conservation of the Heath fritillary butterfly, *Mellicta athalia* 2. Adult-population structure and mobility. *Journal of Applied Ecology***, 24**, 483–498.

Warren, M. S., Hill, J.K., Thomas, J.A., Asher, J., Fox, R., Huntley, B., Roy, D.B., Telfer, M.G., Jeffcoate, S., Harding, P., Jeffcoate, G., Willis, S.G., Greatorex-Davies, J.N., Moss, D. & Thomas, C.D. (2001) Rapid responses of British butterflies to opposing forces of climate and habitat change. *Nature* **414,** 65–69.

Wickman, P.-O. (1986) Courtship solicitation by females of the small heath butterfly*,* *Coenonympha pamphilus* (L.) (Lepidoptera: Satyridae) and their behaviour in relation to male territories before and after copulation. *Animal Behaviour,* **34,** 153–157.

Wiklund, C. (1982). Behavioral shift from courtship solicitation to mate avoidance in female ringlet butterflies (*Aphantopus hyperanthus*) after copulation. *Animal Behaviour,* **30**, 790–793.

Wilson, R. & Thomas, C.D. (2002) Dispersal and the spatial dynamics of butterfly populations. *Dispersal Ecology*. Ed. by Bullock, J.M., Kenward, R.E. & Hails, R.S. pp. 257–278. Blackwell Science Ltd., Oxford, United Kingdom.

Wood, P. & Samways, M. (1991) Landscape element pattern and continuity of butterfly flight paths in an ecologically landscaped botanical garden, Natal, South-Africa. *Biological Conservation,* **58**, 149–166.

Chapter 3: Effects of wing length and ambient temperature on the movement behaviour of range-expanding butterfly species

Abstract

Understanding factors affecting species dispersal is becoming increasingly important because habitat degradation and fragmentation may constrain the ability of individuals to colonise habitat fragments. Hence, individuals with better dispersal capacity are likely to be selected during range expansion. There are documented differences in morphology in newly established populations that have been have been assumed to be related to increased flight capacity. It is not clear, however, how morphological traits relate to flight capacity in the field or if there is an interactive effect of morphology and temperature on movement ability which can potentially impact a species ability to colonise new areas.

Here I investigate the links between morphology and flight capacity by comparing the wing length of 135 individuals of two range-expanding butterfly species from long established and newly colonised areas (with 123 individuals of two widespread species studied as a control). Movement parameters of these individuals were also measured in natural field conditions. I investigated the effect of wing length and the combined effects of wing length and temperature on four different movement parameters.

I found no evidence of differences in wing length between long established and recently colonised sites, for the range-expanding species, that could be attributed to the recent range expansion. Speed increased with wing length for the control species *Maniola jurtina* and females of *P. napi,* while *P. napi* males with longer wings flew slower. Temperature increased the mobility for the range-expanding *Pyronia tithonus* and control species *M. jurtina* and no interaction between temperature and wing length was detected.

This study shows that wing length does not provide a good proxy for butterfly flight in the field for these species, while temperature had a strong but highly species dependent effect on the movement parameters.

Introduction

There is evidence that a large number of species across several taxa have shifted their ranges towards the poles or higher elevations in response to recent global warming (Parmesan & Yohe 2003; Franco et al. 2006; Hickling et al. 2006; Parmesan 2006). Despite the assumed benefit of generally warmer temperatures, three quarters of the British butterflies that are at their northern climatic distribution limit in the UK have declined in distribution area in recent decades due to habitat modification and climate change (Warren 2001). European butterfly and bird species are lagging behind their climatic range on average by 135km and 212km respectively (Devictor et al. 2012), most likely because for some species habitat patches are too isolated for successful colonisation (Warren et al. 2001). The specialist and more sedentary species have been in general less effective than generalists at shifting their ranges to climatically suitable areas (Warren et al. 2001; Pöyry et al. 2009). To design effective conservation measures it is, therefore, important to understand species dispersal capacities and the environmental factors affecting them.

Individuals that colonise new areas are likely to have traits, such as increased dispersal ability, that make them better colonisers (Dytham 2009). Hence individuals from recently colonised sites have been found to have increased dispersal capacity when compared with individuals occurring in sites with long established populations (Hill et al. 1999; Merckx & Van Dyck 2002; Hanski et al. 2004; Mitikka & Hanski 2010). The increased dispersal capacity of newly established populations has been shown to be a transient feature; Simmons and Thomas (2004) found that the frequency of individuals with high dispersal capacity decreases with time since colonisation and was not found to differ for long (5-10 years after colonisation) established sites. However, Hughes et al. (2007) found morphological differences in *Pararge aegeria* butterflies from areas that were colonised up to 30 years earlier. Because dispersal and flight capacity are often difficult to measure in the field, potential differences in dispersal ability between margin and core populations have mainly been inferred from morphological differences such as wing size and thorax mass (Hill et al. 1999; Simmons & Thomas 2004;

Hughes et al. 2007). Thus, it is assumed that greater investment in flight morphology (longer/larger wings and heavier thorax) will represent better dispersal capacity, but it is not well known how this translates into differences in movement in the field (Turlure et al. 2010). Few studies have linked movement parameters with morphological differences directly; Dudley (1990) found flight speed to be positively correlated with different morphological measurements, including wing length, for several butterfly species. Hence, it could be expected that populations of range-expanding species located at the expanding range edge would have longer wings, either involving relatively larger wings or overall body size, both of which have been linked with better flight ability in butterfly species (Dudley 1990; Dudley & Srygley 1994; Berwaerts et al. 2002).

In temperate environments, weather conditions as well as morphology are likely to affect butterfly flight capacity. Butterflies spend more time in flight at higher temperatures (Shreeve 1984) and with increased sunshine (Dennis & Sparks 2006). Cormont et al. (2011) found a positive correlation between warmer temperatures and frequency of colonisations of new areas for three butterfly species in the Netherlands. Despite the importance of both temperature and morphology on insect flight it is not well known how flight is affected by morphology or how this interacts with temperature (Gilchrist 1990; Berwaerts & Van Dyck 2004; Samejima & Tsubaki 2010). An interaction between temperature and butterfly size can be expected because larger individuals are better buffered against convective cooling during flight due to smaller surface volume ratio (Shreeve 1984; Heinrich 1986; Dennis & Shreeve 1989; Gilchrist 1990) and they can differ in the time they take to warm up (Gilchrist 1990), but knowledge of the relative importance and interaction of temperature and morphology on butterfly flight is still lagging. I predict that both temperature and morphology will impact butterfly movement so that individuals with longer wings (which is positively correlated with total body mass) are likely to be more mobile, particularly at lower temperatures.

In this study I tested three hypotheses: i) individuals from margin populations of range-expanding species will have longer wings than individuals from core (long-established) populations, due to selection towards larger investment in dispersal or larger sizes during range expansion; ii) individuals with longer wings will be more mobile and cover longer distances than short-winged individuals; iii) individuals with longer wings are particularly more mobile at low temperatures compared to those with short wings. I aimed to answer these questions by tracking individuals of two range-expanding butterfly species in recently colonised and long established areas, and individuals of two widespread control species.

Materials and methods

Study species

Four generalist species that occur in a range of habitats in the UK (Asher et al. 2001) were included in this study: two Pieridae species; *Anthocaris cardamines* (orange-tip L., range-expanding) and *Pieris napi* (green-veined white, L., widespread control species) and two Nymphalidae species; *Pyronia tithonus* (gatekeeper L., range-expanding) and *Maniola jurtina* (meadow brown, L., widespread control species). These species were selected because they are common, abundant and easy to track individually. The control species *P. napi* and *M. jurtina* are from the same family as the corresponding range-expanding species, with which they share similar habitats and the same host plants (Asher et al. 2001).

Anthocaris cardamines and *P. napi* were studied at seven sites in Northern England and Western Scotland in April and May 2011. *P. tithonus* and *M. jurtina* were studied at five sites in Yorkshire, England in July and August 2011. The study was carried out at four Pieridae and two Nymphalidae range margin sites (<15 years old) and at three core sites of both families. The core sites were in areas with long-established populations (core >28 year old) as shown by UK Butterfly Conservation volunteer records (Asher et al. 2001; Fox et al. 2006). The widespread control species (*P. napi* and *M. jurtina*) occurred at all sites and had long-established populations. Control species were collected from the same sites as the range-expanding species, to control for the effect of environmental variables on morphology.

Butterfly tracking

Butterflies were caught with sweep nets and cooled to 7-15°C in a cool box for a minimum of 20 minutes to facilitate marking, photographing and determining the sex. Previous studies on other butterfly species have shown that butterfly flight behaviour is not affected by handling (including cooling) (Schultz 1998) or following of the butterflies (Root & Kareiva 1984). However, as a precautionary measure, the first move of each track was excluded. After cooling, each butterfly was individually coded by a permanent marker on their hindwing and butterfly condition was recorded (1- fresh, no apparent wing wear, or 2-old, individuals with wing wear).

Photographs for subsequent wing measurement were taken when butterflies were lying flat at the bottom of a container with wings folded, using a digital camera (Olympus µ touch-801, 3MB, 4300 × 3200 pixels) placed at the top of the container, so that the distance to the butterfly was constant between photographs. Wing length of each butterfly was measured as distance between two landmarks in the wing: the base of the wing where the veins meet up and end of vein V6 at the end of the hindwing (Tolman 1997, Figure 2 p. 13). Wing lengths were measured using program ImageJ by Rasband (ImageJ, http://imagej.nih.gov/ij/, 1997-2012). Only individuals with intact wings with visible landmarks were included in the study, therefore excluding a total of 37 butterflies from the analysis. Landmarks were measured only from the hindwing because forewing measurements were not feasible in the field. However, fore- and hindwing length were found to be highly correlated (*r*= 0.85-0.94 for all four species, from a sample of n= 15-17 individuals/species, E. Kallioniemi unpublished data) and high similarity of hind and forewings have also been found for other butterfly species (Breuker et al. 2007). Accurate weight measures were difficult to obtain in the field due to the need to minimize handling and its impact on the butterflies, thus were not included in this study. However, hindwing length is also correlated with butterfly total dry weight (*r*= 0.736-0.814, n=50-134, for all four species, E. Kallioniemi unpublished data for individuals sampled from the same populations). Wing aspect ratio (wingspan^2/wing area) was also measured from the pictures. Aspect ratio, however, has shortcomings in describing wing shape (Betts & Wootton 1988) and due to wing wear sample size was lower for aspect ratio than for wing length. Therefore, the final analyses used wing length (which is positively correlated with butterfly size) to explore the interaction of morphology and temperature on butterfly flight. After marking, butterflies were cooled again for a minimum of 20 minutes prior to release. Individuals were released up to 24 hours after being caught and, to minimise any potential effects of capture time, I sequentially alternated species, sex and capture site of the butterflies released.

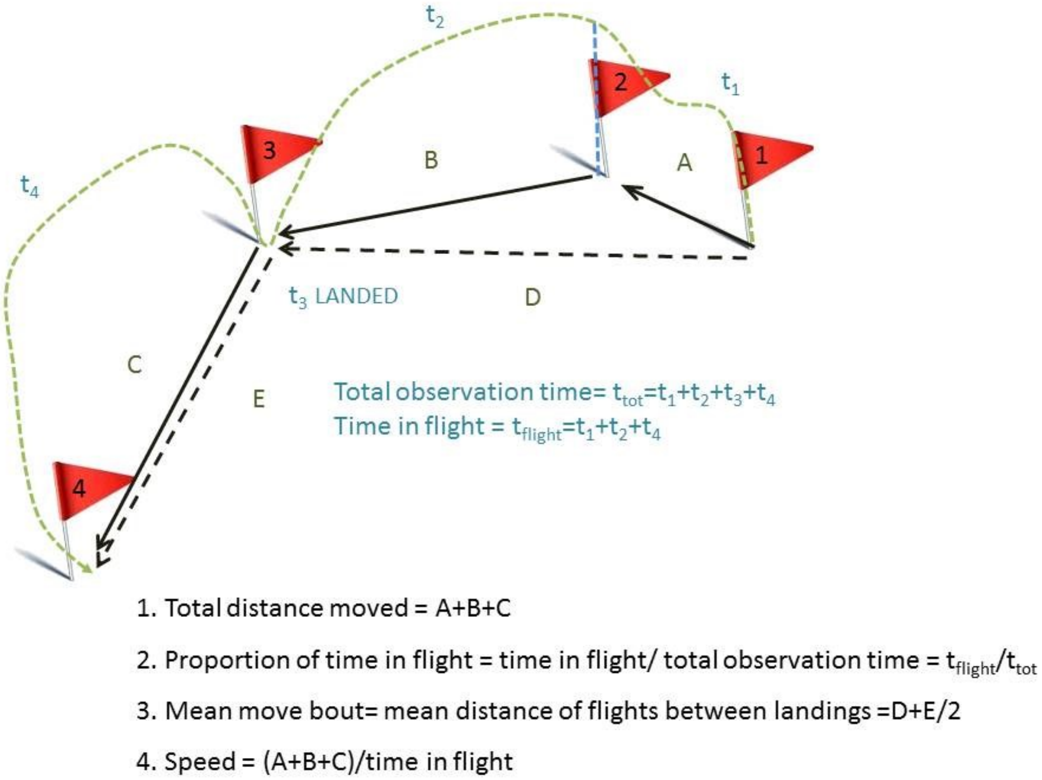
Each butterfly was released at a site that was >500m from its capture location and therefore in an area that was considered unknown for the butterfly. Butterflies were released one at a time, within a suitable habitat patch with both host plants and nectar sources. Depending on the weather, butterflies would bask for a few minutes to warm up before starting their voluntary flight. Butterflies were followed at a distance of 2-5 meters for a maximum of 5 minutes of flight, or until lost. Two comparable methods were used for recording the flight paths: *P. tithonus* and *M. jurtina* butterfly flight paths were recorded using a flag technique with numbered flags dropped on the butterfly flight path every 20 seconds, or when the individual lands (flight bout) (details of the methods in Schultz 1998 and Figure 1).

Butterflies were followed for up to 15 flags or until lost. Tracks with fewer than 3 flags were excluded from the analyses. The flag locations of each track were recorded with differential GPS with 10 cm accuracy (Magellan ProMark III GPS) at the end of each day. Individual *A. cardamines* and *P. napi* butterflies were followed while the observer carried a differential GPS (Magellan ProMark III GPS) that recorded the butterfly location every second. Locations of every 20 s of flight and positions of where butterflies landed were obtained from these detailed GPS tracks, producing a comparable dataset to the flag technique that could be analysed with the same methods. Similarly to *P. tithonus* and *M. jurtina*, *A. cardamines* and *P. napi* butterflies were followed up to maximum of 5 minutes of flight (=15 flags) or until the butterfly was lost. Timings of butterfly flights and landings were recorded using handheld computers for all species studied. Each butterfly was followed only once and temperature during the track was measured as the mean of ambient temperature in shade at the start and end of each track. Butterflies were released only when ambient temperature was above 12°C and wind speed did not exceed 5 on Beaufort scale.

Analyses

For each species, wing length of core and margin butterflies was compared using a linear mixed model (LMM) in the nlme package (Pinheiro et al. 2013) in R (R Core Team 2013), with origin (core / margin), sex and their interaction included as fixed variables and capture site as a random effect in the full model. The interaction between sex and origin was not statistically significant and was removed from the model if retaining it in the model did not improve the model more than 2 ΔAIC (Akaike information criterion) value. Differences between areas of origin were tested using Tukey pairwise comparison when area was significant in the model. When area × sex interaction was significant, Tukey pairwise comparisons between males and females from core and margin areas were carried out.

Butterfly dispersal is complex to assess, thus I used four different measurements of flight within suitable butterfly habitat as a proxy for dispersal and assumed that dispersal increases when butterflies (1) fly longer distances, (2) spend more time in flight (Cormont et al. 2011 and references therein) (3) have longer movement bouts and (4) fly faster. Butterfly mean step length (mobility) at suitable habitat is positively correlated with a likelihood of crossing barriers that can be linked with likelihood of dispersal (Kallioniemi et al. in press). The four movement parameters were determined based on the track data for each individual: 1) Total distance moved = sum of all distances between consecutive flags which measures the overall mobility (2) Proportion of time in flight = the total time spent in flight/total observation time which measures overall activity (3) Mean move bout = sum of distances between landings/ number of move bouts (4) Speed = total distance moved/ time spent in flight (Figure 1). All the movement parameters were square root or $\log_{10}$ -transformed as required for normality.



Total observation time= $t_{tot}=t_1+t_2+t_3+t_4$
Time in flight = $t_{flight}=t_1+t_2+t_4$

1. Total distance moved = A+B+C
2. Proportion of time in flight = time in flight/ total observation time = t_{flight}/t_{tot}
3. Mean move bout= mean distance of flights between landings =D+E/2
4. Speed = (A+B+C)/time in flight

Figure 1. Example flight path shown in green. Blue line shows the location where the buttefly was after 20 seconds of flight. This track as two flight bouts and 3 flags dropped on the path, and subsequent calculations of the four movement parameters.

For each species, the four measures of flight behaviour were related to wing length and temperature with LMMs. Data were visually checked prior to analyses to ensure that any relationships between the movement parameters and wing length as well as movement parameters and temperature were linear. Full models included sex, wing length and mean ambient temperature and all three and two way interactions between them. Butterfly condition (1 or 2) was added as a fixed effect, except for *A. cardamines* butterflies for which there were no individuals in condition 2. In models of total distance moved, track length (number of flags) was included as a covariate. Release site was included as a random factor to control for the possible differences in flight due to release habitat or micro-topography. For some *P. tithonus* and *M. jurtina* models, variance was different between sexes or larger at higher values in which case model fit was improved by including variance structure. Models were simplified by backwards elimination, with retention of fixed effects and their interactions based on the change in the value of the Akaike information criterion (ΔAIC ≥2) on variable removal. All analyses were performed with nlme package (Pinheiro et al. 2013) in R (R Core Team 2013).

Results

Wing length of core and margin butterflies

This study included 258 individual butterflies of the four species. 50-84 individual flight tracks were obtained per species, of which 35-57% was from core and the remaining from margin populations (Table 1 and 2). Wing lengths of the range-expanding and control Pieridae species (*A. cardamines and P. napi*) did not differ between core and margin (Figure 2, Table 3). In contrast *P. tithonus* (range-expanding species) individuals from range margin sites had longer wings than core individuals (Tukey P=0.005). However, *M. jurtina* (control species) females from margin sites also had longer wings than those from core sites (Tukey P=0.014) while *M. jurtina* males from different origin populations did not differ in wing length (Tukey P=0.786). These results did not confirm my initial hypothesis for Nymphalidae butterflies (Hypothesis 1: *individuals from margin populations of range-expanding species will have longer wings than individuals from core (long-established) populations, due to selection towards larger investment in dispersal or larger sizes during range expansion*).

Table 1. *Sample sizes for each species per area, with numbers of females and males per area in parenthesis. Mean temperature during flight ±SD for core and margin butterflies.*

Species	Area	n (females/males)	Mean temperature °C ± SD
A. cardamines (range-expanding)	Core	29 (13/16)	17.0±2.4
	Margin	22 (8/14)	16.1±2.3
P. napi (control)	Core	25 (12/13)	17.4±2.8
	Margin	25 (11/14)	15.9±2.3
P. tithonus (range-expanding)	Core	29 (25/4)	20.4±3.2
	Margin	55 (29/26)	20.6±3.5
M. jurtina (control)	Core	38 (21/17)	20.4±2.7
	Margin	35 (23/12)	20.0±3.3

Table 2. *Mean values of movement parameters quantified (±SE) for species and sexes separately.*

Species	sex	Total distance (m)	Proportion of time in flight	Mean step length (m)	Speed (m/s)
A. cardamines	Females	63.8±47.6	0.249±0.280	13.7±10.3	0.772± 0.098
	Males	72.0±54.0	0.343±0.302	26.7±34.5	0.859± 0.082
P. napi	Females	54.7±43.4	0.327±0.262	14.0±15.3	0.748± 0.079
	Males	52.6±46.5	0.355±0.342	15.6±28.0	0.738± 0.049
P. tithonus	Females	56.0±42.2	0.108±0.174	10.2±11.0	0.734± 0.056
	Males	62.1±47.1	0.237±0.223	15.0±16.8	0.767± 0.107
M. jurtina	Females	57.6±36.6	0.148±0.227	11.0±8.3	0.943± 0.058
	Males	73.0±45.1	0.324±0.312	13.3±8.3	0.844± 0.068

Table 3*. Models examining the effects of sex and origin and their interaction on wing length for each species. Origin × sex interaction removed from the model when change in AIC> 2. Origin marked with bold when ΔAIC>2.*

Species	*Independent variable*	*value*	*SE*	*df*	*ΔAIC*
A. cardamines	sex	0.095	0.3291	41	-1.91
	origin	-0.080	0.3270	7	-1.93
P. napi	sex	0.486	0.3368	40	29.50
	origin	-0.012	0.3701	7	-2.00
P. tithonus	sex	-2.966	0.2510	79	81.17
	origin	**0.705**	**0.2530**	**2**	**3.36**
M. jurtina	sex	-1.160	0.5255	66	-
	origin	1.463	0.4861	3	-
	sex × origin	**-2.030**	**0.7779**	**66**	**4.87**

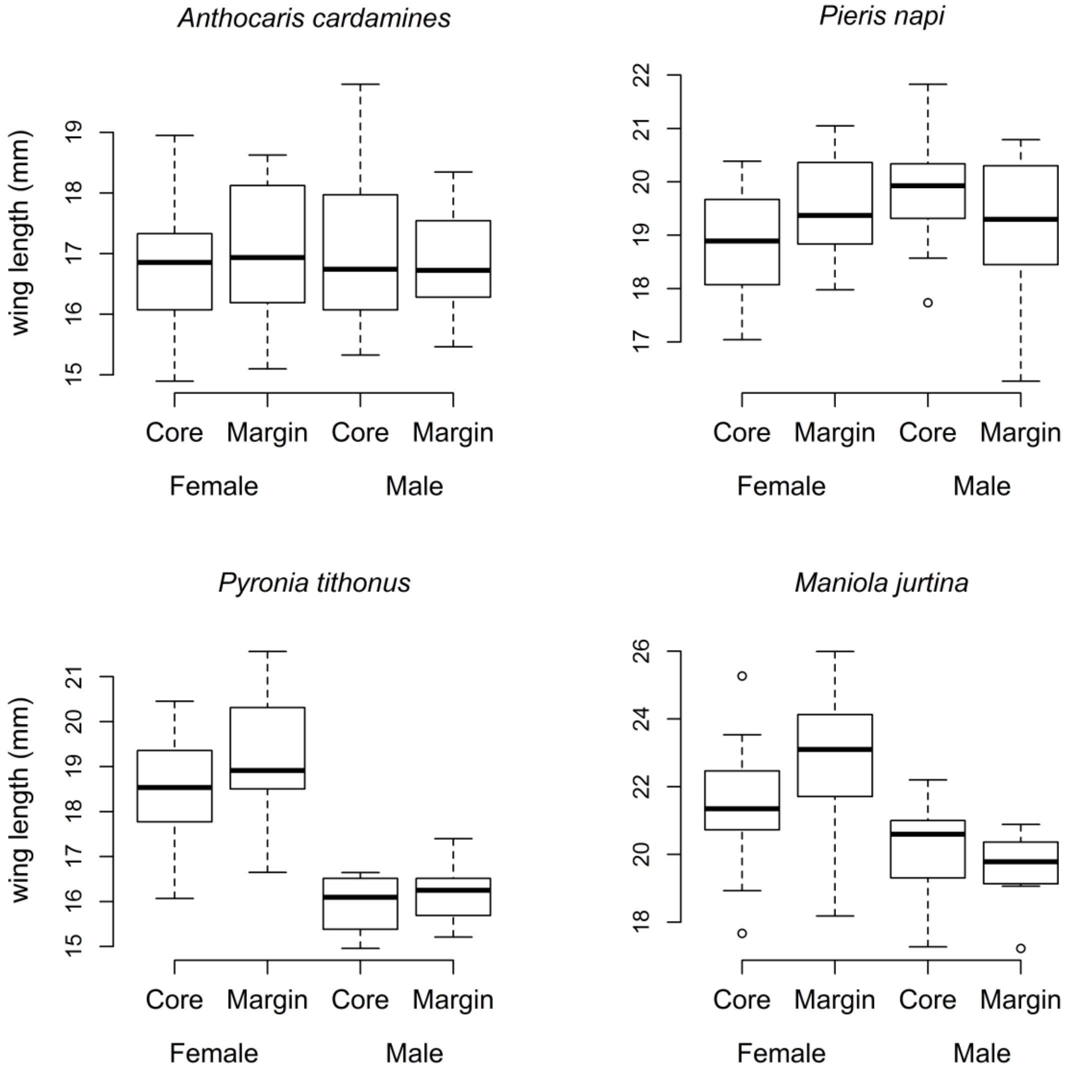


Figure 2. *Median, quartiles, minimum and maximum values of wing length for the four butterfly species, representing separately females and males from core and margin populations. For* A. cardamines *and* P. napi *there are no difference between populations or sexes (Table 3).* Pyronia tithonus *individuals from the margin populations have longer wings and for* M. jurtina *there was an interaction between population origin and sex.*

Effect of wing length on the movement parameters

Anthocaris cardamines individuals with longer wings did not differ in their movement parameters from individuals with shorter wings (Table 4). Contrary to the initial hypothesis, *P. napi* males with longer wings were slower than shorter-winged males, while female speed increased with increasing wing length (Table 4, Figure 3); a 5 mm increase in wing length was predicted to reduce *P. napi* male speed by 54% and increase female flight speed by 30%. Wing length had no influence on any other movement parameter measured for *P. napi*. As predicted*,* *M. jurtina* with longer wings flew faster than shorter-winged individuals (Table 4); a 5 mm increase in wing length was predicted to increase *M. jurtina* flight speed by 30%. However, wing length did not explain variation in any of the other movement parameters for *M. jurtina* and none for *P. tithonus.*

Interaction between wing length and temperature

There was no interaction between temperature and wing length in either of the Pieridae species nor did the ambient temperature as a main effect explain variation in any the movement parameters measured (Table 4). Similarly for *M. jurtina* or *P. tithonus* there was no combined effect of temperature and wing length on any of the movement parameters measured (Table 4). Temperature, however, influenced *P. tithonus* and *M. jurtina* flight in various ways: *P. tithonus* flew slower at higher temperatures but the proportion of time spent in flight increased with increasing temperature particularly for females (Table 4, Figure 4); a 10 °C increase in temperature was predicted to reduce *P. tithonus* speed by 51%, while the proportion of time spent in flight increased almost 6-fold. *Maniola jurtina* flew with longer mean steps, spent more time in flight and the females flew longer total distances at higher temperatures (Table 4, Figure 5). For example, a 10 °C increase in temperature was predicted to increase the total distance moved for *M. jurtina* by 130%.

Table 4. *Final models examining effects of wing length, temperature and sex and their 3-way and 2-way interactions and butterfly condition, on movement parameters of each of the four species analysed, showing parameter coefficients and change in AIC (ΔAIC) on variable removal. Wing length, when retained in the model, is presented in bold. Degrees of freedom= df. Variance structure when included in the model is: [1]= varPower, [2]= varIdent(form=~1|sex). Wing length marked with bold when retained in the final model.*

Dependent variable	*Species*	*Independent variable*	*value*	*df*	*SE*	*ΔAIC*
Sqrt(Total distance (m))	*A. cardamines*	number of flags	0.523	42	0.0868	26.23
	P. napi	number of flags	0.436	42	0.0702	27.47
	P. tithonus	number of flags	0.431	79	0.0482	54.70
	M. jurtina[1]	number of flags	0.366	65	0.0463	36.34
		sex × temperature	-	65	-	2.52
Sqrt(Proportion of time in flight)	*A. cardamines*	no variable remains in the model				
	P. napi	no variable remains in the model				
	P. tithonus[1]	sex × temperature	0.171	77	0.0444	2.438
	M. jurtina[1]	sex	0.241	67	0.0544	29.62
		temperature	0.032	67	0.0074	31.20
Log(Mean move bout (m))	*A. cardamines*	no variable remains in the model				
	P. napi	no variable remains in the model				
	P. tithonus	butterfly condition	0.234	79	0.0866	5.173
	M. jurtina[2]	temperature	0.042	68	0.0132	3.167
Sqrt(Speed (m/s))	*A. cardamines*	no variable remains in the model				
	P. napi	**sex × wing length**	-	40	-	**2.393**
	P. tithonus	temperature	-0.029	79	0.0085	6.522
	M. jurtina	**wing length**	0.024	73	0.0113	**2.467**

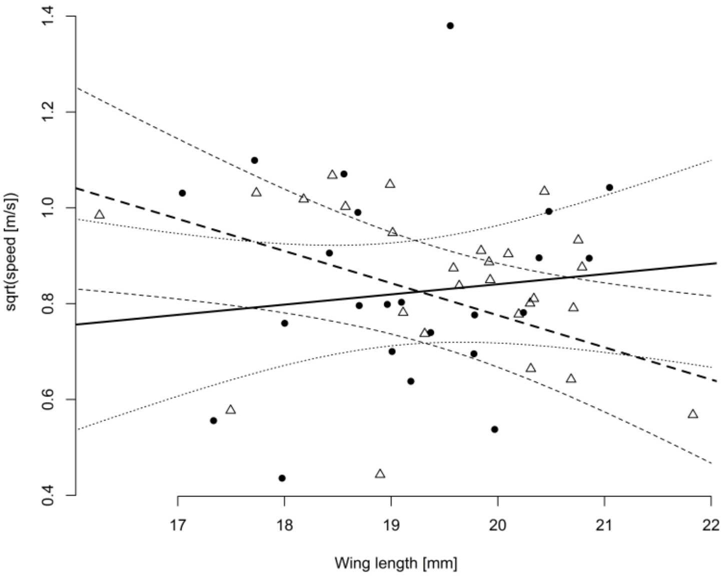


***Figure 3**. Speed of* P. napi *females (solid circles and line) and males (open triangle and dashed line) shown in relation to wing length. Lines and 95 % confidence intervals are based on predicted values from the most parsimonious model (detailed in Table 4). Female speed= 0.41+0.02*wing length; male speed= -.067+2.12*wing length.*

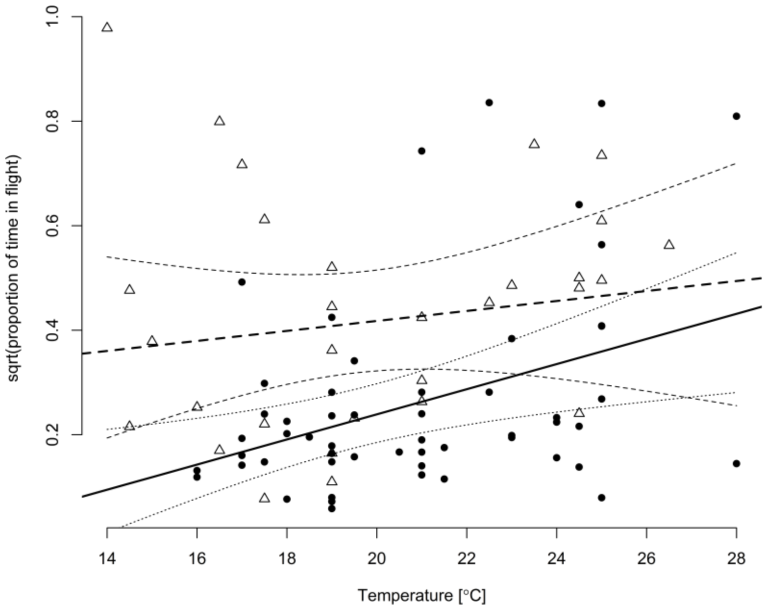


***Figure 4**. Proportion of time in flight of* P. tithonus *females (solid circles and line) and males (open triangle and dashed line) in relation to ambient temperature at the time of tracking. Lines and 95 % confidence intervals are based on predicted values from the most parsimonious model (detailed in Table 4). Female proportion of time in flight= -0.191+0.022*temperature; male proportion of time in flight= 0.246+0.009*temperature.*

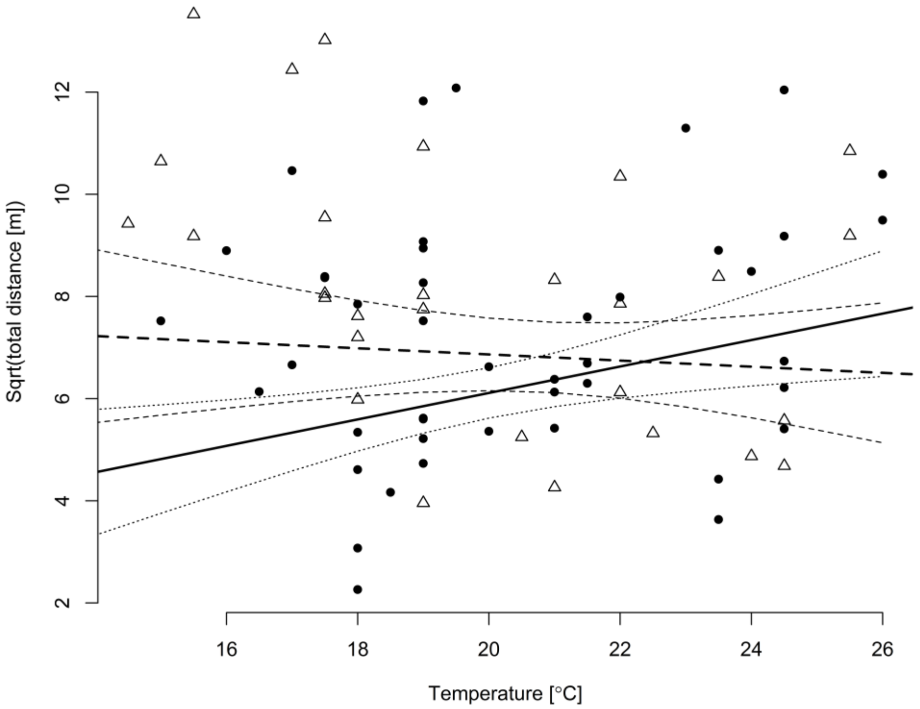


Figure 5. *Total distance moved of* M. jurtina *females (solid circles and line) and males (open triangle and dashed line) in relation to ambient temperature at the time of tracking. Lines and 95 % confidence intervals are based on predicted values from the most parsimonious model (detailed in Table 4) calculated at 7.5 flags. Female total distance moved= -0.81+0.259*temperature; male total distance moved= 5.32-0.060*temperature.*

Discussion

Morphological differences between the core and margin populations studied here appear to be influenced more by environmental factors than selection for long-winged individuals during range expansion. I found differences between the areas in wing lengths for *M. jurtina* which is not a range-expanding species and no differences in the wing lengths for the range-expanding *A. cardamines* from core and margin sites. Movement parameters measured in the field were not found tightly linked with wing length; flight speed was the only movement parameter affected by wing length for two out of the four species studied.

Wing length differences between margin and core populations

Earlier studies have found that individuals from more recently colonised areas have greater investment in flight morphology (relative wing area and thorax mass) (Hill et al. 1999) and longer wings and bigger body size have been connected with better flight ability such as forward air speed and acceleration ability in butterflies (Dudley 1990; Berwaerts et al. 2002). Therefore it was predicted that individuals of range-expanding species occupying newly colonised areas would have longer wings, while no differences in wing length were expected for the control species. However, no differences were found in wing lengths between areas for the range-expanding species *A. cardamines* while both *P. tithonus* (range-expanding) and *M. jurtina* females (control species) from margin sites had longer wings. Firstly, this may be due to phenotypic plasticity in response to environmental factors during development (Gibbs et al. 2011). For example, for *Polygonia c-album* rearing temperature and host plant caused larger differences in insect performance and morphology compared with differences between core and margin populations (Braschler & Hill 2007). It is known that food stress during larval development affects butterfly wing morphology (Pellegroms et al. 2009). Although study areas for *P. tithonus* and *M. jurtina* only differed 40 km in average latitude, they differed 170 meters in altitude causing on average 1.85 °C colder temperatures at the margin areas and possibly therefore causing butterflies to develop longer wings (and larger bodies) at the margin area in both species as is suggested by Bergmann's rule (Blanckenhorn & Demont 2004). Secondly, it is possible that there were no differences in range-expanding species because there has not been strong selection for longer wings during range expansion. Butterflies use flight not only for dispersal but also for other activities such as mate location, finding oviposition sites and nectaring, thereby making it harder to understand possible differences in wing morphology and possible selection pressures on wing length (Shreeve et al. 2009). Thirdly, it is possible that instead of margin populations consisting of individuals that are different in their morphology, range expansion has instead resulted in selection, through environmental or dispersal based filtering, for morphology that represents a sub-selection of morphological traits that are also present at the core areas. This makes it harder to detect the differences between the populations. If this was the case, I would have expected to see differences in the variances between the areas in wing lengths but this was not detected (F-test for each species and sex $P>0.05$). Fourthly, increased flight dispersal morphology can be of transient feature (Simmons et al 2004) if there is a trade-off with fecundity (Hill et al. 1999; Hughes et al. 2003), thus possible differences in investment in flight may have already been selected against at the sites surveyed. However, Hughes et al. (2003) found morphological differences, which are linked with increased flight capacity, in *Pararge aegeria* butterflies from areas that were colonised more than 30 years ago.

Previous studies have not always found morphological differences between newly colonised range margins and core areas. Range-expanding *Araschnia levana* butterfly did not show any morphological differences between old and recently colonised populations despite there being differences in *Pgi* allele frequencies that have been linked to more active flight metabolism (Mitikka & Hanski 2010). Hassall et al. (2009) found systematic morphological differences between core and margin individuals only in one out of three species of damselflies. They suspected that rate of expansion can also be crucial for this variation (Hassall et al. 2009). My results indicate that for the species included in this study there were no differences in wing length between core and margin populations that could be linked with range expansion. However, it is also important to establish whether differences in wing length actually affect movement parameters of individuals.

Impact of wing size on movement parameters

It was expected that butterflies with longer wings would fly faster and longer distances than individuals with shorter wings because for example Dudley (1990) had found a positive correlation between wing length and airspeed. However, in the present study, a positive correlation between speed and wing length was detected for only one (*P. napi*) out of four species, while for male *M. jurtina* wing length was negatively related to speed. Also, there was no correlation between wing length and any of the other movement parameters measured. Similarly, Kingsolver (1999) found for the *Pontia occidentalis* butterfly that a reduction in wing area did not translate into differences in dispersal in the field, suggesting that wing size, or wing length, may not always be the main determinant of flight ability. Greater dispersal capacity may not always be associated with differences in morphological traits; *Araschnia levana* butterflies from newly colonised areas at a range margin had higher frequency of the *Pgi*-allele, which is associated with superior flight metabolic rate, but did not display morphological differences that would reflect the increased dispersal capacity (Mitikka & Hanski 2010). Furthermore, earlier studies that had linked morphology with flight have used different movement parameters for measuring flight performance; for example long winged individuals of *Pararge aegeria* had better acceleration capacity (Berwaerts et al. 2002), but it is not clear if and how acceleration capacity is correlated with the parameters measured here. There could be trade-offs between acceleration capacity and different aspects of flight such as speed and endurance (Berwaerts et al. 2002). I would expect movement parameters used in this study, particularly mean move bout, to better reflect the long distance movements required for effective dispersal, as similar movement parameter mean step length has been shown to be correlated with the rate of crossing boundaries across several species (Kallioniemi et al. in press) and therefore indicate the likelihood of dispersal out of habitat. Tiple *et al*. (2010) showed that heavier and larger butterflies are more defensive and display greater site fidelity which could explain the slower flight of long winged *P. napi* males in this study via behavioural differences between sexes. My results indicate that wing length is not strongly correlated with butterfly flight in the field, reinforcing the importance of caution in using morphological measures as proxies for butterfly flight or dispersal (but see Turlure et al. 2010; Sekar 2012).

Impact of wing size and temperature on dispersal

Several earlier studies have found butterflies to be more active at high temperatures (Kingsolver 1983a; Shreeve 1984; Wickman 1985; Dennis & Sparks 2006; Cormont et al. 2011) which is likely due to need for ectothermic butterflies to increase their body temperature in temperate environments above ambient temperature. Effect of temperature on flight is important because time available for flight can be a limiting resource for butterflies (Kingsolver 1983b) and climate change might enhance butterfly flight by increasing temperatures and decreasing cloudiness in certain areas, thereby promoting colonisation of new habitats (Mitikka et al. 2008; Cormont et al. 2011). I found that *P. tithonus* females and males, and *M. jurtina* females spend more time in flight at higher temperatures. *Maniola jurtina* also had longer mean step lengths and *M. jurtina* females moved longer total distances at higher temperatures. More time in flight did not, however, translate into moving faster but instead, *P. tithonus* moved slower at high temperatures. It is possible that temperature influences these species in interaction with radiation which was not assessed here, but has been shown to influence movement of other species (Cormont et al. 2011). In conclusion, these results suggest that temperature can potentially have a large impact on flight, but this is species dependent and higher temperatures do not necessarily mean that individuals move further and are therefore more likely to colonise new areas at higher temperatures.

Body size, which is positively correlated with wing size (Nylin & Svard 1991), is likely to affect thermoregulation of butterflies and butterflies with shorter wings are expected to be able to reach optimum flight temperatures faster by basking but also cool down more rapidly during flight due to convective cooling (Shreeve 1984; Gilchrist 1990). Therefore long winged individuals were expected to move with longer move bouts particularly at low temperatures. Berwaerts et al. (2004) showed for *Pararge aegeria* butterfly that how morphology relates to flight performance depends, at least to some extent, on body temperature but interactive impact of morphology and temperature on flight has not been exhaustively addressed. These results, however, did not show an interaction between wing length and temperature for any of the flight parameters measured for the four species. It is possible that because the current study was conducted at temperatures at which butterflies start their flight voluntary it might not represent enough variation to show differences between butterflies with different morphology. Furthermore, it is possible that the impact of differential morphology on flight is only apparent during maximal performance, e.g. when a butterfly is forced to fly for extended period or fast, and therefore differences in flight performance were not captured in this study. More detailed information including butterfly mass, thorax size, and amount of fur in the body and pigmentation of butterfly wings would bring further understanding to the question. Based on the findings here it appears that while temperature has a big influence on butterfly flight wing length does not link strongly with flight nor is there interactive effect of wing length and temperature on flight.

Conclusions

I did not detect differences in butterfly wing length at the range margin that could be attributed to range expansion, and environmental effects may be more important determinants of morphology in these species. My results challenge the view that wing size can be used as a measure of dispersal capacity in butterflies in the field since it was positively related to flight speed in only one of four species studied and failed to explain differences in total distances moved, mean move bout or time spent in flight. There were also no interaction between wing length and temperature in how butterflies flew, but temperature was more important in determining butterfly flight aspects than wing length.

Literature

Asher, J., M. Warren, R. Fox, P. Harding, G. Jeffcoate, and M. A. Jeffcoate, Stephens. 2001. Millennium Atlas of Butterflies in Britain and Ireland. Oxford University Press, Oxford United Kingdom.

Berwaerts, K., and H. Van Dyck. 2004. Take-off performance under optimal and suboptimal thermal conditions in the butterfly *Pararge aegeria*. Oecologia **141**:536–545.

Berwaerts, K., H. Van Dyck, and P. Aerts. 2002. Does flight morphology relate to flight performance? An experimental test with the butterfly *Pararge aegeria*. Functional Ecology **16**:484–491.

Betts, C. R., and R. J. Wootton. 1988. Wing shape and flight behavior in butterflies (Lepidoptera, Papilionoidea and Hesperioidea) - a preliminary-analysis. Journal of Experimental Biology **138**:271–288.

Blanckenhorn, W. U., and M. Demont. 2004. Bergmann and converse Bergmann latitudinal clines in arthropods: Two ends of a continuum? Integrative and Comparative Biology **44**:413–424.

Braschler, B., and J. K. Hill. 2007. Role of larval host plants in the climate-driven range expansion of the butterfly *Polygonia c-album*. Journal of Animal Ecology **76**:415–423.

Breuker, C. J., P. M. Brakefield, and M. Gibbs. 2007. The association between wing morphology and dispersal is sex-specific in the glanville fritillary butterfly *Melitaea cinxia* (Lepidoptera : Nymphalidae). European Journal of Entomology **104**:445–452.

Cormont, A., A. H. Malinowska, O. Kostenko, V. Radchuk, L. Hemerik, M. F. WallisDeVries, and J. Verboom. 2011. Effect of local weather on butterfly flight behaviour, movement, and colonization: significance for dispersal under climate change. Biodiversity and Conservation **20**:483–503.

Dennis, R. L. H., and T. H. Sparks. 2006. When is a habitat not a habitat? Dramatic resource use changes under differing weather conditions for the butterfly *Plebejus argus*. Biological Conservation **129**:291–301.

Dennis, R., and T. Shreeve. 1989. Butterfly wing morphology variation in the British Isles: the influence of climate, behavioural posture and the hostplant-habitat. Biological Journal of the Linnean Society **38**:323–348.

Devictor, V. et al. 2012. Differences in the climatic debts of birds and butterflies at a continental scale. Nature Climate Change **2**:121–124.

Dudley, R. 1990. Biomechanics of flight in neotropical butterflies - morphometrics and kinematics. Journal of Experimental Biology **150**:37–53.

Dudley, R., and R. Srygley. 1994. Flight physiology of neotropical butterflies - Allometry of airspeeds during natural free-flight. Journal of Experimental Biology **191**:125–139.

Dytham, C. 2009. Evolved dispersal strategies at range margins. Proceedings of the Royal Society B-Biological Sciences **276**:1407–1413.

Fox, R., J. Asher, T. Brereton, D. Roy, and M. Warren. 2006. State of butterflies in Britain and Ireland. Pisces Publications, Newbury, United Kingdom.

Franco, A. M. A., J. K. Hill, C. Kitschke, Y. C. Collingham, D. B. Roy, R. Fox, B. Huntley, and C. D. Thomas. 2006. Impacts of climate warming and habitat loss on extinctions at species' low-latitude range boundaries. Global Change Biology **12**:1545–1553.

Gibbs, M., C. Wiklund, and H. Van Dyck. 2011. Phenotypic plasticity in butterfly morphology in response to weather conditions during development. Journal of Zoology **283**:162–168.

Gilchrist, G. 1990. The consequences of sexual dimorphism in body size for butterfly flight and thermoregulation. Functional Ecology **4**:475–487.

Hanski, I., C. Erälahti, M. Kankare, O. Ovaskainen, and H. Sirén. 2004. Variation in migration propensity among individuals maintained by landscape structure. Ecology Letters **7**:958–966.

Hassall, C., D. J. Thompson, and I. F. Harvey. 2009. Variation in morphology between core and marginal populations of three British damselflies. Aquatic Insects **31**:187–197.

Heinrich, B. 1986. Comparative thermoregulation of 4 montane butterflies of different mass. Physiological Zoology **59**:616–626.

Hickling, R., D. B. Roy, J. K. Hill, R. Fox, and C. D. Thomas. 2006. The distributions of a wide range of taxonomic groups are expanding polewards. Global Change Biology **12**:450–455.

Hill, J. K., C. D. Thomas, and D. S. Blakeley. 1999. Evolution of flight morphology in a butterfly that has recently expanded its geographic range. Oecologia **121**:165–170.

Hughes, C. L., C. Dytham, and J. K. Hill. 2007. Modelling and analysing evolution of dispersal in populations at expanding range boundaries. Ecological Entomology **32**:437–445.

Hughes, C. L., J. K. Hill, and C. Dytham. 2003. Evolutionary trade-offs between reproduction and dispersal in populations at expanding range boundaries. Proceedings of the Royal Society of London Series B-Biological Sciences **270**:S147–S150.

Kingsolver, J. 1983a. Thermoregulation and flight in Colias butterflies - Elevational patterns and mechanistic limitations. Ecology **64**:534–545.

Kingsolver, J. 1983b. Ecological significance of flight activity in Colias butterflies - implications for reproductive strategy and population-structure. Ecology **64**:546–551.

Kingsolver, J. G. 1999. Experimental analyses of wing size, flight, and survival in the western white butterfly. Evolution **53**:1479–1490.

Merckx, T., and H. Van Dyck. 2002. Interrelations among habitat use, behavior, and flight-related morphology in two cooccurring satyrine butterflies, *Maniola jurtina* and *Pyronia tithonus*. Journal of Insect Behavior **15**:541–561.

Mitikka, V., and I. Hanski. 2010. Pgi genotype influences flight metabolism at the expanding range margin of the European map butterfly. Annales Zoologici Fennici **47**:1–14.

Mitikka, V., R. K. Heikkinen, M. Luoto, M. B. Araujo, K. Saarinen, J. Pöyry, and S. Fronzek. 2008. Predicting range expansion of the map butterfly in Northern Europe using bioclimatic models. Biodiversity and Conservation **17**:623–641.

Nylin, S., and L. Svard. 1991. Latitudinal patterns in the size of European butterflies. Holarctic ecology **14**:192–202.

Parmesan, C. 2006. Ecological and evolutionary responses to recent climate change. Annual Review of Ecology Evolution and Systematics **37**:637–669.

Parmesan, C., and G. Yohe. 2003. A globally coherent fingerprint of climate change impacts across natural systems. Nature **421**:37–42.

Pellegroms, B., S. Van Dongen, H. Van Dyck, and L. Lens. 2009. Larval food stress differentially affects flight morphology in male and female speckled woods (*Pararge aegeria*). Ecological Entomology **34**:387–393.

Pinheiro J., D. Bates, S. DebRoy, D. Sarkar and the R Development Core Team (2013). nlme: Linear and Nonlinear Mixed Effects Models. R package version 3.1-110.

Pöyry, J., M. Luoto, R. K. Heikkinen, M. Kuussaari, and K. Saarinen. 2009. Species traits explain recent range shifts of Finnish butterflies. Global Change Biology **15**:732–743.

Rasband, W.S., ImageJ, U. S. National Institutes of Health, Bethesda, Maryland, USA, http://imagej.nih.gov/ij/, 1997-2012.

R Core Team. 2013. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. Retrieved from http://www.R-project.org.

Root, R., and P. Kareiva. 1984. The search for resources by cabbage butterflies (*Pieris Rapae*) - Ecological consequences and adaptive significance of Markovian movements. Ecology **65**:147–165.

Samejima, Y., and Y. Tsubaki. 2010. Body temperature and body size affect flight performance in a damselfly. Behavioral Ecology and Sociobiology **64**:685–692.

Schultz, C. B. 1998. Dispersal behavior and its implications for reserve design in a rare Oregon butterfly. Conservation Biology **12**:284–292.

Sekar, S. 2012. A meta-analysis of the traits affecting dispersal ability in butterflies: can wingspan be used as a proxy? The Journal of animal ecology **81**.

Shreeve, T. 1984. Habitat selection, mate location, and microclimatic constraints on the activity of the Speckled wood butterfly *Pararge aegeria*. Oikos **42**:371–377.

Shreeve, T., Konvicka, M. & Van Dyck, H. 2009 Functional significance of butterfly wing morphology variation. In: Settele, J., Shreeve, T., Konvicka M. & Van Dyck, H. (Eds.): Ecology of Butterflies in Europe. pp 171-188. Cambridge University Press. Cambridge, United Kingdom.

Simmons, A. D., and C. D. Thomas. 2004. Changes in dispersal during species' range expansions. American Naturalist **164**:378–395.

Tiple, A. D., S. V. Padwad, L. Dapporto, and R. L. H. Dennis. 2010. Male mate location behaviour and encounter sites in a community of tropical butterflies: taxonomic and site associations and distinctions. Journal of Biosciences **35**:629–646.

Tolman, T. 1997. Butterflies of Britain and Europe. 4th edition. HarperCollins Publishers Ltd., London, United Kingdom.

Turlure, C., N. Schtickzelle, and M. Baguette. 2010. Resource grain scales mobility and adult morphology in butterflies. Landscape Ecology **25**:95–108.

Warren, M. S. et al. 2001. Rapid responses of British butterflies to opposing forces of climate and habitat change. Nature **414**:65–69.

Wickman, P. 1985. The influence of temperature on the territorial and mate locating behavior of the small heath butterfly, *Coenonympha pamphilus* (l) (Lepidoptera, Satyridae). Behavioral Ecology and Sociobiology **16**:233–238.

Chapter 4: Morphology characteristics of range-expanding species under climate change

Abstract

In recent decades many species have changed distributions and colonised new areas that are now climatically suitable due to climate warming. Individuals at the expanding range margin are predicted to differ in their dispersal capacity due to selection towards increased colonisation ability. This study tests the hypothesis that during range expansion there is selection for morphological traits that enhance dispersal and uses butterflies as the study system to test the predictions. Butterfly dispersal capacity is often measured as morphological traits such as thorax mass and wing length. Despite reported differences in butterflies at the expanding range margin, it is not clear if the differences at newly colonised sites are found across species for individuals collected from the field.

Here I measured morphological traits of three range-expanding species collected from newly colonised and core areas of their distribution. The same measurements were also taken from three widespread control species from the same families that use the same habitats and historically occur in both areas.

I found the expected differences in morphology, measured as a heavier thorax mass and longer wings, for one of the range-expanding species (*Aricia agestis*). This species is relatively limited in its dispersal ability but has expanded its range considerably in recent decades. No differences attributable to origin of the butterflies were identified for the other two range-expanding species (*Anthocaris cardamines* and *Pyronia tithonus*).

I hypothesise that the increase in dispersal capacity is likely to be found for less dispersive species and species with more fragmented habitat while highly mobile species are less likely to benefit from increased dispersal capacity during range expansion. Therefore selection at the range margin can be highly species dependent and no general pattern of selection for morphological traits associated with higher dispersal capacity is likely to occur across all species that are expanding their ranges.

Introduction

Several species have expanded their range recently and these distribution changes have been linked to climate change (Warren et al. 2001; Root et al. 2003; Hickling et al. 2005; Parmesan 2006). Which species are able to expand their range and the rate of the range shift are likely to depend on habitat availability (Hill et al. 2001), configuration (e.g. Hughes et al. 2007), and species dispersal ability (Pöyry et al. 2009). Species ability to shift distributions and track climatically suitable areas (Thomas et al. 2004) together with variability of natural populations (Hill et al. 2011) can potentially be crucial to the responses of biodiversity and ecosystems to climate change. Understanding the differences between populations at the expanding range margin compared with populations at the core of the distribution will help scientists to predict the speed of the responses to climate change that has implications for conservation and biodiversity, as well as future species community structure (Gilman et al. 2010).

Dispersal ability is a trait that is expected to evolve during range expansion and thereby alter the rate of range shifts (Dytham 2009). Colonising individuals are expected not to be a random selection from the source population but be the more dispersive individuals as these are more likely to reach and colonise new habitat patches (Simberloff 1981; Haag et al. 2005; Dytham 2009). Theoretical studies support the idea of evolution of dispersiveness at expanding range margins (Travis et al. 2009; Burton et al. 2010) and the models are backed up by empirical studies documenting increased dispersal at the range margins and at newly established populations (Hill et al. 2011 and references therein).

The differences in relative investment in dispersal or reproduction between core and margin populations are often inferred from morphological characteristics measured in butterflies as a total body mass, thorax mass, wing length, wing aspect ratio and thorax width (e.g. Hill et al. 1999a; Hughes et al. 2003) but such relationships are not always clear. Several studies on insect have found heavier total body mass, longer wing-span, larger and wider thoraxes to increase flight ability (McLachlan 1983; Davis 1984; Chai & Srygley 1990; Dudley & Srygley 1994; Berwaerts et al. 2002; Sekar 2012) while increased abdomen mass in relation to body size shows larger investment in reproduction (Wickman & Karlsson 1989; Hughes et al. 2003). Larger body size can be argued to be beneficial to a disperser, as it increases the distance that can be travelled without refuelling (Roff 1991), but can also lead to increased fecundity (Honek 1993) making it therefore difficult to interpret the meaning of body size in terms of investment in dispersal. Earlier studies of morphological differences between margin/newly colonised areas vs. core/older sites have repeatedly found mobility differences only in females or the differences to be more pronounced in females (Hanski et al. 2004; Hughes et al. 2007). This is because male morphology is expected to respond more to the usage of local resources (such as acquiring mates and nectaring) rather than long distance dispersal.

Increased investment in flight has been found sometimes to have a trade-off with fecundity (Zera & Denno 1997; Hughes et al. 2003; Simmons & Thomas 2004; Karlsson & Johansson 2008). For example, Hughes et al (2003) documented a trade-off between dispersal and fecundity for *Pararge aegeria* butterfly at expanding range boundaries, where individuals from the expanding range margin had heavier thoraxes indicating higher investment to dispersal but laid fewer eggs than individuals from the core of the distribution. For two wing-dimorphic bush crickets *Conocephalus discolour* and *Metrioptera roeselii* higher frequency of long-winged individuals at newly colonised areas was found only for 5-10 years after colonisation, most likely due to lower reproductive capacity of long-winged individuals (Simmons & Thomas 2004). However, in wing-monomorphic species like butterflies this trade-off between investment in flight and reproduction is not as straightforward and appears to be species dependent (Hughes et al. 2003; Saastamoinen 2007). For some species, increased investment in reproduction has been found coupled with increased mobility (Hanski et al. 2006; Saastamoinen 2007), and theoretical models also predict that trade-off occurs with competitive ability instead (Burton et al. 2010). Also, differences in the dispersal ability at the expanding range margin have not always been found (Mitikka and Hanski, 2010). Whether a species shows increased dispersal traits at the margin or not, can be dependent on interactions with landscape structure that have been shown to affect species dispersal patterns (Hill et al., 1999b). Based on these earlier studies there is still no consensus of how butterflies from expanding margin populations across different butterfly families might differ in their morphology from those at the core of the distribution. It is not clear if the trade-off between investment in dispersal and reproduction (measured as morphological traits) exists for a range of different species and across families.

Here I studied three range-expanding species and compared morphology of individuals from recently colonised range margin areas to those from core areas that have been occupied for many decades. I also compared the morphology of widespread control species that have been found in these areas for long. It was hypothesised that: i) for range-expanding species, individuals from margin sites have invested more in dispersal than individuals from the core areas. No difference in investment in dispersal was anticipated for control species. ii) A trade-off between investment in dispersal and reproduction would be observed for range-expanding species only. iii) Females have bigger differences in their investment in flight and reproduction than males.

Materials and methods

Study species

Three range-expanding species (*Anthocaris cardamines* (L., orange-tip, Pieridaea)*, Aricia agestis* (Denis & Schiffermüller, brown argus, Lycaenidae) and *Pyronia tithonus* (L., gatekeeper, Nymphalidae) were selected to investigate if differences in investment in flight are found across a range of butterfly families. These species are found in good abundances and the new areas of range expansion are well known. Furthermore, each of the range-expanding species has a closely related control species that is similar in size, use same/similar host plants, is found at the same habitats and is widespread and present at both core and range margin of the range-expanding species distribution (Asher et al. 2001; Fox et al. 2006). The control species are *Pieris napi* (L., green-veined white), *Polyommatus icarus* (Rottemburg, common blue) and *Maniola jurtina* (L., meadow brown), respectively, and were included in this study to control for potential confounding effects of study sites and environmental factors that may differ between core and margin. Sample sizes of each species are presented in table 1. *Aricia agestis* has gone through a recent host plant shift, historically this species has been feeding in the northern parts of its range only on *Helianthemum nummularium* while more recently it has also been observed using plants of the Geranium family, *Geranium molle* and *Erodium cicutarium* in particular, throughout its distribution (Heath et al. 1984; Asher et al. 2001, Pateman et al. 2012). These species were studied for differences between morphological traits of individuals collected from areas where they have recently expanded their range (margin <18 years) and areas where they have been present for longer (core >28 years) which is known based on Butterfly Conservation volunteer records (Asher et al. 2001; Fox et al. 2006) (Figure 1). Investment in movement was measured as thorax mass and wing length while investment in reproduction was measured as abdomen mass.

Table 1. *Sample sizes of males and females of each species from core and margin areas.*

Species		*Margin females*	*Core females*	*Margin males*	*Core males*
A. cardamines	range-expanding	21	19	19	25
P. napi	widespread	24	17	24	23
A. agestis	range-expanding	26	15	18	11
P. icarus	widespread	23	21	23	21
P. tithonus	range-expanding	32	28	25	32
M. jurtina	widespread	27	28	18	22

Study sites and collection of butterflies

Study sites were chosen based on Butterfly Conservation's casual observation records and Atlas data (Asher et al. 2001; Fox et al. 2006). Core sites were in 10×10 km grid cells that had been occupied at least since the first atlas of butterflies (1970-1982) and were surrounded by at least 3 10×10 km grid cells occupied at least since that period. Grid cells were considered margin if they were first found to be occupied after 1995. The margin sites were on average 53km ± 1.8 km for *A. cardamines* 24 km ± 9.7 km for *A. agestis* and 23 km ± 5.4 km for *P. tithonus* from the nearest old (before 1982) observation. Within the defined grid cells, study sites were selected based on access and where populations were found. Each control species had been present at the respective study areas since 1970-1982 and therefore both areas were considered core for the control species.

88 *A. cardamines* and 84 *P. napi* butterflies were collected with sweep nets from 19 sites in Northern England, Western Scotland and Eastern Scotland during April-May 2011 and 2013 (Figure 1 a and b). A total of 70 *A. agestis* and 88 *P. icarus* butterflies were collected during August 2010 and 2011, from 13 different sites in Eastern England, South East England and East Midlands (Figure 1 c and d). *Aricia agestis* host plants *Geranium molle* and/or *Erodium cicutarium* was found at all collection sites. At two of the core sites *Helianthemum nummularium* was also found, but not at the locations where our samples were collected so I do not expect this to affect these results. 117 *P. tithonus* and 95 *M. jurtina* butterflies were collected from 14 sites in Northern England June to August in 2011 and 2012 (Figure 1 e and f).

Only fresh individuals (negligible wing wear at the time of collection) were used to reduce the possible effects of age and allow reliable comparison of investment in different body parts, particularly in females for which abdomen mass is likely to decline greatly with age and thereby the quantity of eggs in the abdomen.

Butterflies were kept frozen at -20°C until measured in the laboratory. After being thawed the butterflies were dissected and wings were photographed under a microscope with 8-16 times magnification. ImageJ 1.45r software (Rasband 1997-2012, <http://rsbweb.nih.gov/ij/>) was used for measuring the forewing lengths from the base of the wing where veins meet to the end of vein V6 (Tolman 1997 p. 13 Figure 2.). Butterfly parts were dried in an oven at 60°C for 24h hours and measured for total body, abdomen and thorax dry mass with Mettler Toledo XS205 scale (accuracy of 0.1 mg).

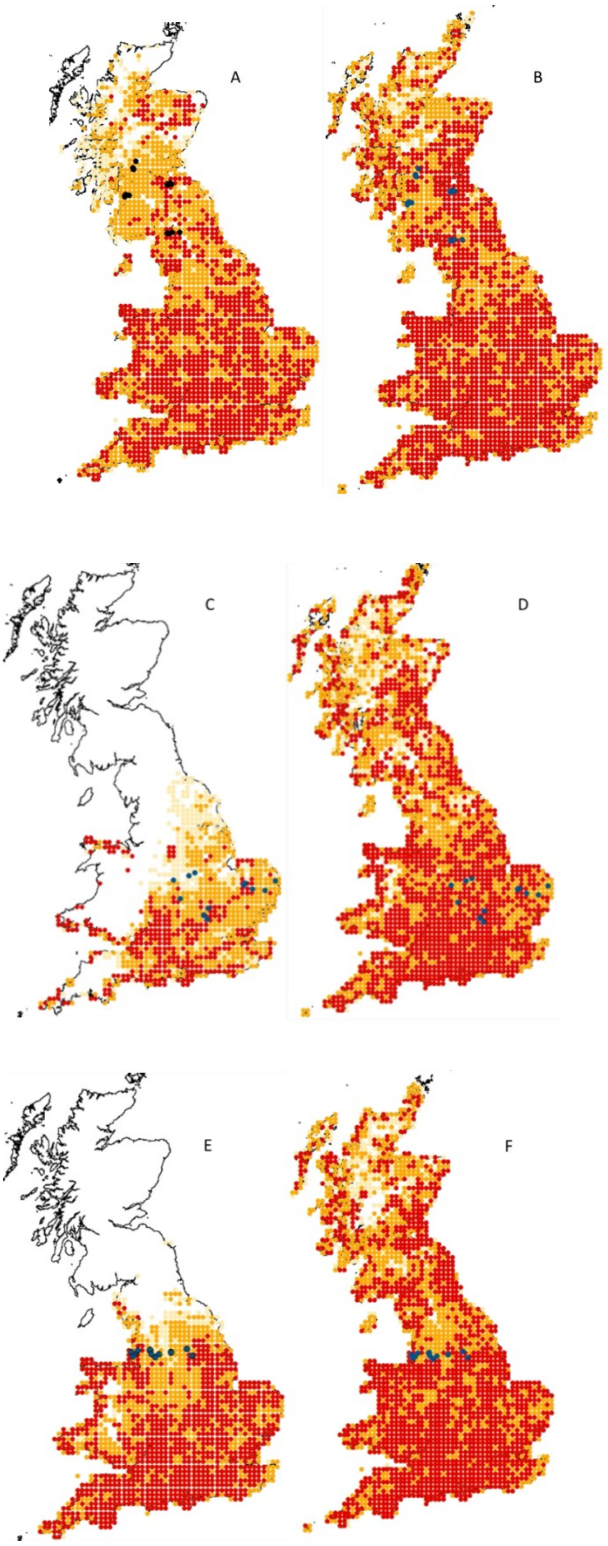


Figure 1. *Occurrence of A)* A. cardamines *B)* P. napi *C)* A. agestis *D)* P. icarus *E)* P. tithonus *and F)* M. jurtina *in 10x10 km squares based on Butterfly Conservation volunteer records. Records from 1970-1982 are in red, 1995-1999 records are dark yellow and 2000-2011 records are in light yellow. Collection sites are marked with blue.*

Analysis

Each species pair (*A. agestis* and *P. icarus*, *A. cardamines* and *P. napi*, *P. tithonus* and *M. jurtina*) and both sexes were analysed separately. To study relative investment in flight morphology, body, thorax and abdomen mass and wing length were compared between areas (core vs. margin) and species (range-expanding vs. control) for each sex, of each species pair with linear mixed models (LMM) using the nlme package (Pinheiro et al. 2012) in R (R Core Team 2013). Body mass was added in the models for thorax, abdomen and wing length as covariate to account for the allometry so that these would represent relative investment into different body parts. When required, variables were $\log_{10}$-transformed to achieve normality. Capture site was nested within the study year as a random factor. Variance structure (vs) was included in the models where needed if variance differed between species (varIdent(form=~1|Species) or increased for larger values (varPower). Although differences in body mass between areas was also analysed I focus on the results on thorax mass and wing length as indicators of investment in dispersal and abdomen mass as indicator of investment in reproduction because body mass can be related to both and it is therefore difficult to assess how it relates to relative investment of dispersal or reproduction. Full models, including interactions between capture area and species were examined and the area × species interaction was excluded when $P>0.05$. Pair-wise differences between areas for both species were tested with Tukey tests when area had a significant effect in the model.

Results

The ratio of thorax to body mass, abdomen to body mass and wing length to body mass was similar for all six species and both sexes (Table 2); Average thorax mass to body mass ratio varied between 0.241 and 0.323 for females of all species and 0.303 and 0.422 for males. Average abdomen to body ratio was between 0.388 and 0.472 for females of all species and 0.241 and 0.324 for males. Average wing length to body ratio was from 0.699 to 1.374 for females of all species and 0.947 to 1.481 for males.

Table 2. *Ratio of thorax mass to body mass, abdomen mass to body mass and wing length to body mass for each species and sex. All mass measurements are in mg and wing lengths in mm.*

Species	*sex*	*Thorax/ body ±SE*	*Abdomen/ body ±SE*	*Wing length /body ±SE*
A. cardamines	female	0.241±0.005	0.438±0.012	1.042±0.060
	male	0.303±0.004	0.277±0.007	1.268±0.037
P. napi	female	0.243±0.011	0.472±0.018	0.792±0.040
	male	0.318±0.005	0.324±0.010	0.947±0.036
A. agestis	female	0.323±0.006	0.388±0.010	1.337±0.065
	male	0.387±0.010	0.258±0.012	1.638±0.046
P. icarus	female	0.314±0.006	0.408±0.010	1.374±0.061
	male	0.422±0.008	0.241±0.006	1.481±0.027
P. tithonus	female	0.286±0.006	0.443±0.013	0.953±0.039
	male	0.350±0.007	0.263±0.010	1.321±0.034
M. jurtina	female	0.295±0.006	0.438±0.012	0.699±0.030
	male	0.392±0.007	0.261±0.009	1.013±0.023

Total body mass

There were no differences in total body mass for *A. cardamines* (range-expanding) and *P. napi* (control) females (Table 3, Figure 2a) or males (Table 4, Figure 2b) from core and margin areas. Similarly *A. agestis* (range-expanding) and *P. icarus* (control) females from core and margin areas did not differ in their body mass (Table 3, Figure 2c). The difference between core and margin *A. agestis* and *P. icarus* males in body mass was not significant in a pairwise comparison (*A. agestis*: estimate=1.096, SE= 0.531, z=2.06, *P*=0.16; *P. icarus*: SE=0.419, z=-0.535, *P*=0.95) (Table 4, Figure 2d). Both *P. tithonus* (range-expanding) and *M. jurtina* (control) females from margin sites were heavier than individuals collected from core areas (Table 3, Figure 2e) but this difference was significant only for *M. jurtina* females (*P. tithonus*: estimate=4.999, SE=2.425, z=2.061, *P*=0.16; *M. jurtina*: estimate=6.669, SE=2.441, z=2.732, *P*<0.03). No differences between areas were found for males (Table 4, Figure 2f).

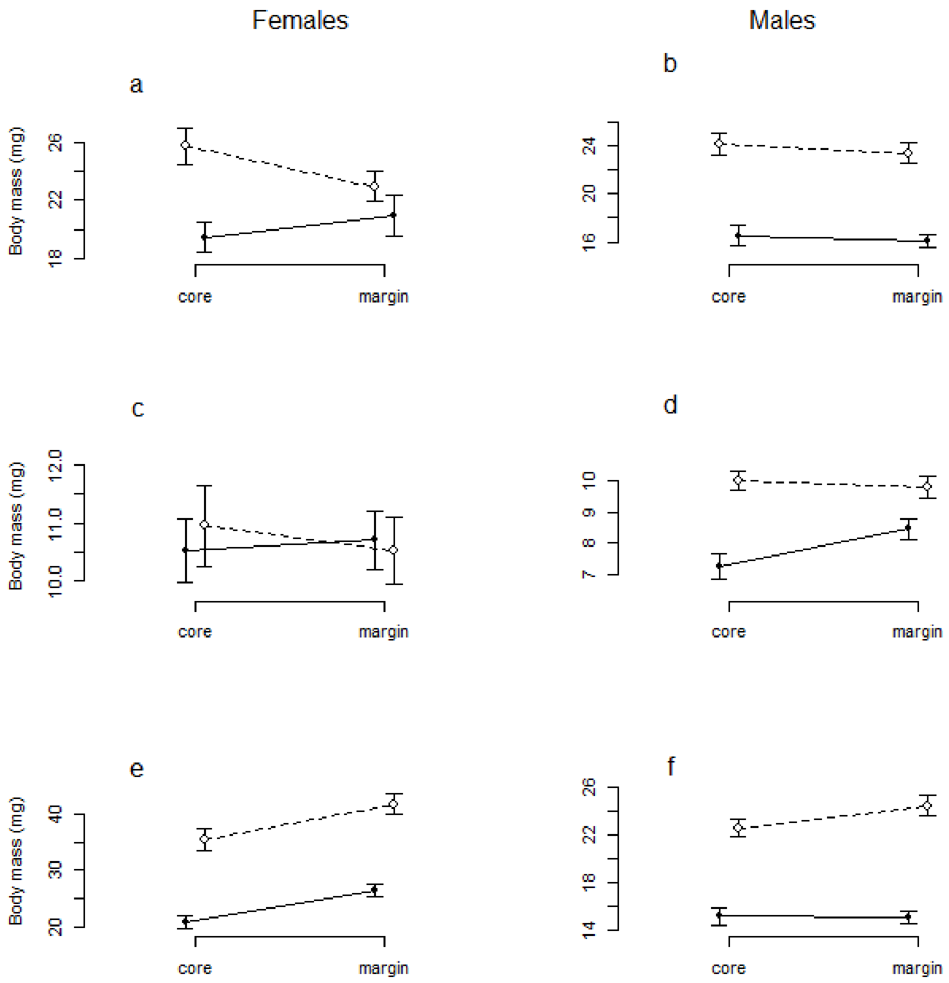


Figure 2. *Body mass ±SE for all species pairs at core and margin. Range-expanding species are marked with solid circles and solid line, control species with open circle and dashed line.* A. cardamines *and* P. napi *females (a) and males (b);* A. agestis *and* P. icarus *females (c) and males (d);* P. tithonus *and* M. jurtina *females (e) and males (f).*

Investment in dispersal morphology

Anthocaris cardamines and *P. napi* females (Table 3, Figure 3a and b) and males (Table 4, Figure 4a and b) from core and margin areas did not differ in their wing lengths or thorax mass relative to body mass.

Aricia agestis and *P. icarus* females had interactions between area and species for the wing length and thorax mass in relation to body mass; *A. agestis* females from margin areas had longer wings and heavier thoraxes than those from core areas (wing: estimate=0.527, SE=0.184, z=2.86, *P*=0.022; thorax: estimate=0.310, SE=0.112, z=2.76, *P*=0.029) while there was no difference for *P. icarus* females (wing: estimate=-0.216, SE=0.241, z=-0.896, *P*=0.805; thorax: estimate=-0.011, SE=0.105, z=-0.110, *P*=0.9995) (Table 3, Figure3c and d). *Aricia agestis* and *P. icarus* males from different areas did not differ in their wing length or thorax mass in relation to body mass (Table 4, Figure 4c and d).

There was an interaction between area and species for wing length and thorax mass in relation to body mass for *P. tithonus* and *M. jurtina* females. *Maniola jurtina* females from margin areas had longer wings and heavier thoraxes than those from the core areas (pair-wise comparison; wings: estimate=0.090, SE=0.026, z=3.5, *P*=0.0024; thorax: estimate=1.021, SE =0.31, z=3.3, *P*=0.0059) while the differences between *P. tithonus* females from core and margin were not significant (wings: estimate=0.028, SE= 0.025,z=1.1, *P*=0.69; thorax: estimate=-0.118, SE=0.26, z=-0.45, *P*=0.97) (Table 3, Figure 3e and f). Both *P. tithonus* and *M. jurtina* males from margin had longer wings and heavier thorax than males from core (Table 4, Figure 4e and f) but the there was no significant within-species difference between individuals from core and margin areas (*P. tithonus* wings: estimate=0.239, SE=0.210, z=1.1, *P*=0.66; thorax: estimate=-0.260, SE=0.12, z=2.25, *P*=0.10; *M. jurtina*: wings: estimate=0.441, SE=0.26,z=1.7, *P*=0.30; thorax: estimate=0.307, SE=0.26, z=1.2, *P*=0.61).

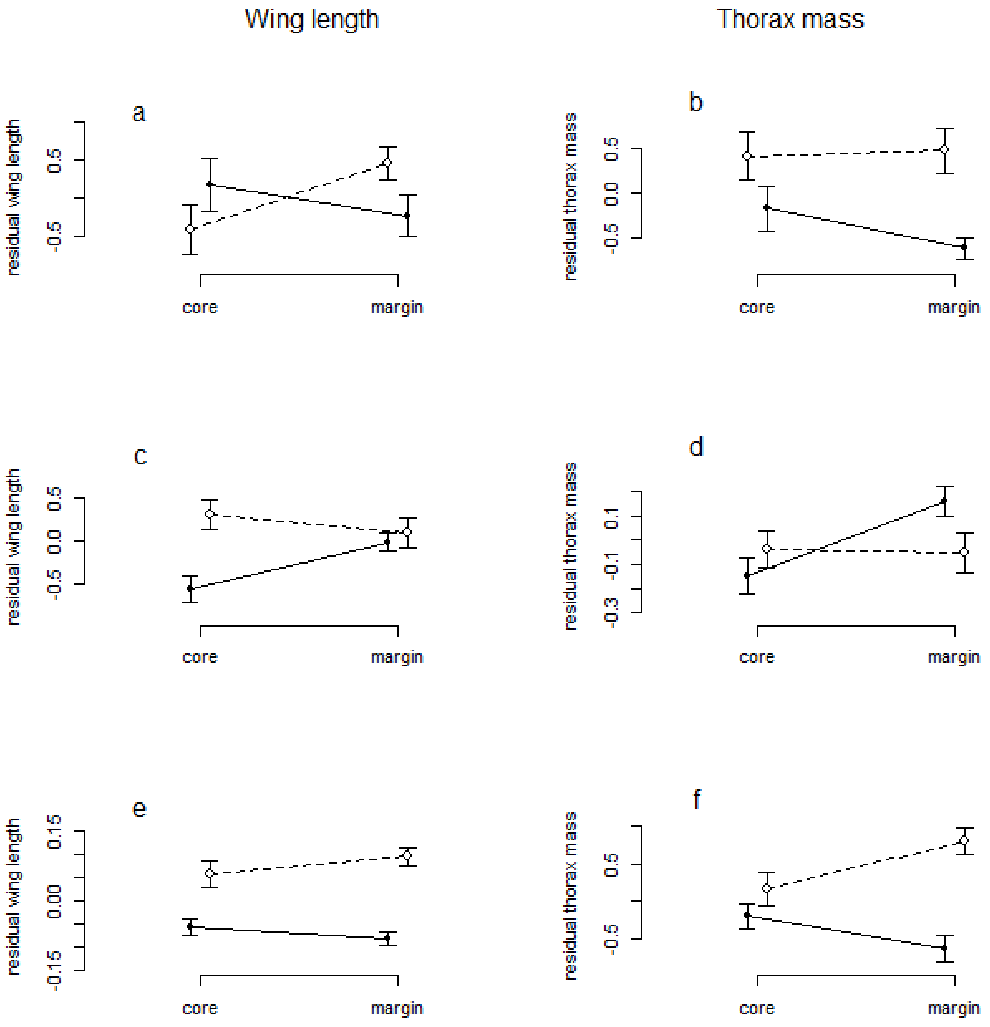


Figure 3. *Residual wing length and thorax mass ± SE for females* A. cardamines *and* P. napi *(a,b),* A. agestis *and* P. icarus *(c,d) and* P. tithonus *and* M. jurtina *(e,f) from core and margin sites. Range-expanding species are marked with solid circles and solid line, control species with open circle and dashed line. Residual values are calculated from regression of wing length or thorax mass against body mass combining both species and areas.*

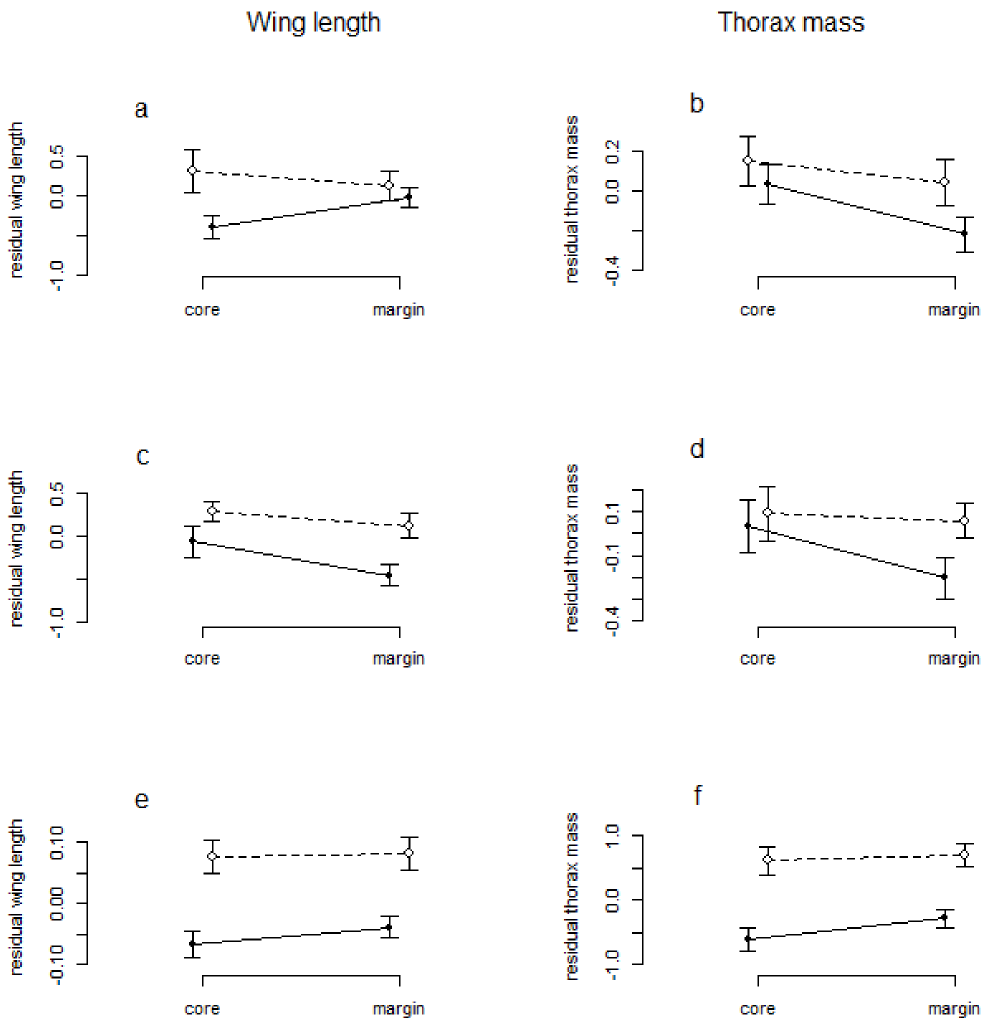


Figure 4. *Residual wing length and thorax mass ± SE for males* A. cardamines *and* P. napi *(a,b);* A. agestis *and* P. icarus *(c,d);* P. tithonus *and* M. jurtina *(e,f) from core and margin sites. Range-expanding species are marked with solid circles and solid line, control species with open circle and dashed line. Residual values are calculated from regression of wing length or thorax mass against body mass combining both species and areas.*

Investment in reproduction

There were no differences between *A. cardamines*, *P. napi*, *A. agestis* or *P. icarus* butterflies from core and margin areas in their abdomen mass in relation to total body mass in either of the sexes (Table 3 and 4, Figure 5a and b). For *P. tithonus* and *M. jurtina* females there was an interaction between area and species for abdomen mass in relation to body mass; *M. jurtina* females from margin areas had lighter abdomens in relation to body mass than those from the core areas (estimate=-1.7728, SE=0.42, z= -4.3, P=0.0001) while the differences between core and margin *P. tithonus* were not significant (estimate=0.2296, SE=0.35, z=0.65, P=0.91) (Table 3, Figure 5e). As predicted, no difference in abdomen mass in relation to body mass was found for *P. tithonus* and *M. jurtina* males from core and margin areas (Table 4, Figure 5f).

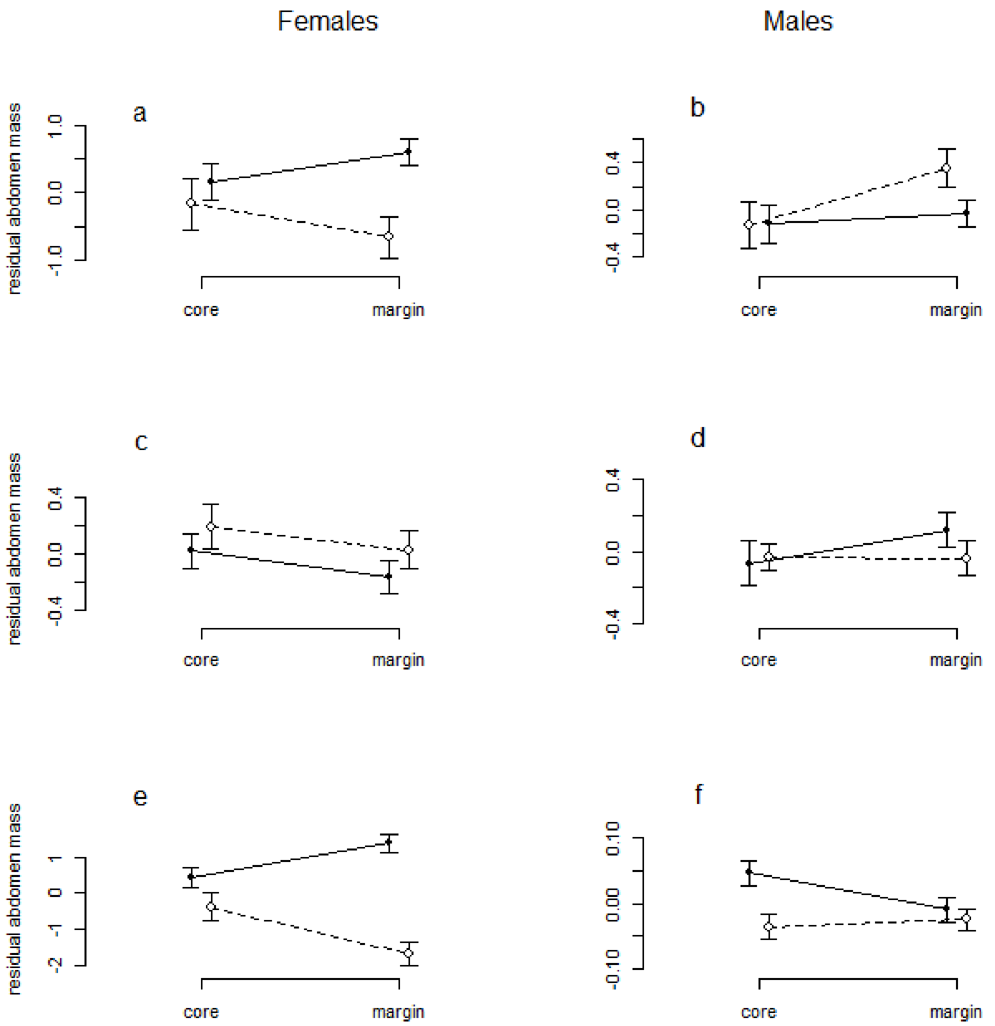


Figure 5. *Residual abdomen mass ±SE for* A. cardamines *and* P. napi *females (a) and males (b);* A. agestis *and* P. Icarus *females (c) and males (d) and* P. tithonus *and* M. jurtina *females (e) and males (f). Range-expanding species are marked with solid circles and solid line, control species with open circle and dashed line. Residual values are calculated from regression of abdomen mass against body mass combining both species and areas. Abdomen mass was log-tranformed for* P. tithonus *and* M. jurtina*.*

Table 3. *Results for females from linear mixed models on morphology variables with F-values and degrees of freedom and P-values. The full model included area, species, area×species interaction and body mass as a dependent variables. vs. =variance structure of the model: 1=none, 2=varPower, 3=varIdent(form=~1|Species).*

		A. cardamines/ P. napi		*A. agestis/ P. icarus*		*P. tithonus/ M. jurtina*
Body mass	*vs*		*vs*		*vs*	
Area	1	$F_{1,62}$=0.19 P=0.67	1	$F_{1,73}$=0.44 P=0.44	3	$F_{1,99}$=13.12 P= 5e-04
Species		$F_{1,62}$=18.48 P=0.0001		$F_{1,73}$=0.03 P=0.85		$F_{1,62}$=95.26 P<0.0001
Wing length						
Area	1	$F_{1,61}$=0.63 P=0.4403	3	$F_{1,70}$=1.94 P=0.20	1	$F_{1,94}$=21.42 P<0.0001
Species		$F_{1,61}$=1.47 P=0.23		$F_{1,70}$=10.02 P=0.002		$F_{1,94}$=667.9 P<0.0001
Body mass		$F_{1,61}$= 59.59 P <0.0001		$F_{1,70}$=56.94 P<0.0001		$F_{1,94}$=71.41 P= <.0001
Area × species		-		$F_{1,70}$=6.00 P=0.017		$F_{1,94}$= 4.72 P=0.032
Thorax mass						
Area	2	$F_{1,61}$=20.04 P<0.0001	1	$F_{1,71}$=1.62 P=0.24	3	$F_{1,97}$=42.40 P <0.0001
Species		$F_{1,61}$=97.40 P<0.0001		$F_{1,71}$=0.49 P=0.49		$F_{1,97}$=717.3 P<0.0001
Body mass		$F_{1,61}$=273.20 P<0.0001		$F_{1,71}$=467.4 P=<.0001		$F_{1,97}$=314.9 P<0.0001
Area × species		-		$F_{1,71}$=4.39 P=0.04		$F_{1,97}$=11.52 P=0.001
Abdomen mass						
Area	3	$F_{1,59}$=0.59 P=0.45	3	$F_{1,71}$=6.35 P=0.0358	2	$F_{1,97}$=241.6 P<0.0001
Species		$F_{1,59}$=114.36 P<0.0001		$F_{1,71}$=1.61 P=0.2081		$F_{1,97}$=556.2 P<0.0001
Body mass		$F_{1,59}$=655.57 P<0.0001		$F_{1,71}$=369.0 P<0.0001		$F_{1,97}$=2149 P<0.0001
Area × species		-		-		$F_{1,97}$=16.70 P<0.001

Table 4. *Results for males from linear mixed models on morphology variables with F-values and degrees of freedom and P-values. The full model included area, species, area×species interaction and body mass as a dependent variables. vs. =variance structure of the model: 1=none, 2=varPower, 3=varIdent(form=~1|Species). *Abdomen mass was log-transformed for* P. tithonus *and* M. jurtina *males.*

		A. cardamines/ P. napi		*A. agestis/ P. icarus*		*P. tithonus/ M. jurtina*
Body mass	*vs*		*vs*		*vs*	
Area	1	$F_{1,73}$=1.96 P=0.17	1	$F_{1,58}$= 0.10 P=0.76	2	$F_{1,83}$= 0.00 P= 0.99
Species		$F_{1,73}$=79.40 P<0.0001		$F_{1,58}$=33.10 P<0.0001		$F_{1,83}$=138.2 P<0.0001
Area × species		-		$F_{1,58}$=3.79 P=0.056		-
Wing length						
Area	3	$F_{1,71}$=0.00 P=0.99	1	$F_{1,58}$=0.39 P=0.55	3	$F_{1,82}$=7.11 P= 0.024
Species		$F_{1,71}$=158.1 P<0.0001		$F_{1,58}$=173.2 P<0.0001		$F_{1,82}$=572.4 P= <.0001
Body mass		$F_{1,71}$=115.6 P<0.0001		$F_{1,58}$=100.5 P<0.0001		$F_{1,82}$=26.04 P<0.0001
Thorax mass						
Area	1	$F_{1,72}$=17.24 P=0.0001	1	$F_{1,58}$=0.53 P=0.48	2	$F_{1,82}$=6.59 P=0.028
Species		$F_{1,72}$=538.2 P<0.0001		$F_{1,58}$=102.2 P<0.0001		$F_{1,82}$=824.8 P<0.0001
Body mass		$F_{1,72}$=445.5 P<0.0001		$F_{1,58}$= 123.0 P<0.0001		$F_{1,82}$=47.43 P<0.0001
Abdomen mass						
Area	2	$F_{1,72}$=11.88 P=0.001	2	$F_{1,58}$=0.52 P=0.49	1	$F_{1,82}$=2.02 P= 0.19
Species		$F_{1,72}$=480.0 P<0.0001		$F_{1,58}$=11.90 P=0.0011		$F_{1,82}$=103.7 P<0.0001
Body mass		$F_{1,72}$=423.9 P<0.0001		$F_{1,58}$=50.99 P<0.0001		$F_{1,82}$=172.4 P<0.0001

Discussion

There were no consistent differences between core and margin populations for all range-expanding species. *Anthocaris cardamines* and its control species *P. napi* did not show any differences between areas in the morphological traits measured. *Aricia agestis* females had larger investment in thorax and wing length at the margin sites as predicted while no similar differences were found for control species *P. icarus*. This would suggest increased investment in dispersal at the range margin in *A. agestis* possibly due to recent range expansion. *Pyronia tithonus* females were heavier at the margin but similar differences in morphology were also found in the control species *M. jurtina* suggesting that environmental effects are likely to be responsible for the morphological differences found in these species.

Investment in dispersal and reproduction

There were no detectable differences between core and margin individuals in investment in dispersal for Pieridae (*A. cardamines* and *P. napi)* females or males. Other factors such as food plant availability and environmental variables may have influenced the development of the butterflies and their morphology as shown in other species (Braschler & Hill 2007; Pellegroms et al. 2009; Gibbs et al. 2011) possibly masking the differences in morphology caused by range expansion. For example food plants have been shown to have a larger influence on *Polygonia c-album* butterfly morphology than range expansion (Braschler & Hill 2007). However, if environmental factors had a strong impact, one would expect to see a similar differences in morphology on the control species *P. napi* that were collected from same sites and that use the same host plants. It is possible that for a dispersive species there is no strong selection towards increased dispersal ability particularly because *A. cardamines* is able to use several different crucifer species that are common and widely distributed as its host plants (Asher et al. 2001; Grime et al. 2007) and therefore its habitat is not likely to be highly fragmented. It is also possible that for this habitat generalist and highly mobile species the frequency of highly dispersive phenotypes may decrease very quickly in newly colonised sites due to the high influx of individuals from older populations, hence not having been detected by this study. However, a study of *Pararge aegeria* showed increased investment in dispersal at the areas that were colonised up to 30 years earlier (Hughes et al. 2007). Therefore in the absence of strong trade-offs e.g. with reproduction like in the case of wing-dimorphic bush crickets *C.*

discolour and *M. roeselii* (Simmons & Thomas 2004), I would expect that differences in morphology, if there were any, would still be detectable.

As predicted, range-expanding *A. agestis* females from margin areas had heavier thoraxes and longer wings in relation to body size and the same pattern was found for both years of the study and at two different range expansion areas (East of England and North Midlands) indicating phenotypic selection during range expansion (Chai & Srygley 1990; Dudley & Srygley 1994; Berwaerts et al. 2002). These differences were not detected for males or for the control species *P. icarus*. It could be argued that, similarly to *Polygonia c-album* (Braschler & Hill 2007), the morphology of *A. agestis* could have been affected by recent host plant shifts. However, in this study, host plants at the study sites did not differ between core and margin areas and would have therefore unlikely to have caused the differences. Lycaenidae species have more restricted dispersal capacity than the Pieridae species (e.g. Cowley et al. 2001; Kallioniemi et al. in press) and therefore habitat is likely to be perceived more fragmented by *A. agestis* making dispersal ability more crucial for successful colonisation of new areas. Despite *A. agestis* females having heavier thoraxes at the margin than at the core, no difference in the abdomen mass were detected. This would suggest that there is no direct trade-off between reproduction and flight in this species which indicates that higher dispersal capacity might not always come with a trade-off. Also, it is possible that range expansion favours increased fecundity as suggested by theoretical models (Burton et al. 2010).

Both Nymphalidae species *P. tithonus* and *M. jurtina* females were heavier at the margin area, although for the range-expanding *P. tithonus* the difference was not significant. For *P. tithonus* the newly colonised areas coincide with higher altitudes. This pattern may follow Bergmann’s rule (Blanckenhorn & Demont 2004) as higher altitude sites have on average lower temperatures than lower elevation sites, which can increases larval development time resulting in larger individuals at colder temperatures. This may explain the heavier body mass found for females of both the range-expanding and the control species. *Maniola jurtina* had longer wings and heavier thorax in relation to body mass in margin sites while the females had smaller abdomens at the margin in relation to body mass. No significant differences in wing size or thorax mass in relation to body mass were detected for the range-expanding *P. tithonus.* While thorax and abdomen ratio to body mass are very similar in both *P. tithonus* and *M. jurtina,* female wing length/body mass ratio is higher for *P. tithonus* in comparison to *M. jurtina* females (Table 2). Because both of these species use several grass species as host plants (Asher et al. 2001) they are not likely to be limited by suitable habitat.

However, if there are differences in the degree of fragmentation between the core and margin areas it is possible that *M. jurtina,* which in general has lower investment in wing in relation to body size, might be more likely to display morphological differences in response to landscape structure which has been shown to influence butterfly morphology (Thomas et al. 1998; Hill et al. 1999b; Merckx & Van Dyck 2006). It is also possible that continuous suitable habitat might not select for increased dispersal capacity at the expanding range margin while increases in fecundity may be more important. Increases in fecundity could result at the margin if there is a trade-off with other factors such as competitive ability (Burton et al. 2010) or selection differs from those at the core areas for other reasons, for example due to decrease in parasitism at the margin areas (Menendez et al. 2008).

Previous studies have shown differences in dispersal of the range-expanding butterfly *P. aegeria* to be more evident in females (Hughes et al. 2007). Similarly, mobility differences between individuals from new and old habitat patches were only detected in females for *Melitaea cinxia* butterfly (Hanski et al. 2004). In agreement with these studies and my predictions most differences between core and margin areas were found only in females. This does not come as a surprise since flight serves as a different function in males and females and male morphology is thought to be affected more by mating system in comparison to dispersal (Wickman 1992).

Species dependent changes in morphology

Why would there be changes morphology between areas in some range-expanding species but not in others? There are several possible explanations for this; Firstly butterflies use flight for dispersal and for other purposes such as nectaring, finding oviposition sites, predator avoidance and mate location. Therefore species might not always show (evolutionary) change in morphological traits corresponding to increased dispersal at expanding range edge (Mitikka & Hanski 2010). For example, for *Araschania levana* butterfly, despite more dispersive genotypes being more frequent at the range-expanding areas, no morphological differences between the areas were detected (Mitikka & Hanski 2010). Secondly, my previous results on morphology and movement parameters for four of the species studied here (*A. cardamines, P. napi, P. tithonus, M. jurtina*) did not find a strong link between the wing length and movement in the field but instead only flight speed was related to wing length in two of the species studied (Kallioniemi et al. unpublished). It is therefore possible that even if there is selection towards increased dispersal capacity or propensity at the expanding range margin it might not be displayed as morphological differences between areas or how it is displayed may be species dependent. Thirdly, the strength of selection towards larger investment in dispersal at the expanding range margin may depend on species dispersal ability, how fragmented the suitable habitat is for that species and how limited a species is in the time for flight. Differences in investment in flight measured as larger wing area and thorax mass of individuals from newly colonised range edge was found for *Pararge aegeria* butterfly (Hill et al. 1999a; Hughes et al. 2007) that particularly at the northern margins of its range are found in woodland habitats (Asher et al. 2001). Although it is considered a relatively mobile species (Cowley et al. 2001), due to the low percentage of cover of woodland remaining in England (Lawton et al. 2010), it is possible that higher dispersal capacity or propensity is essential for colonisation of new sites. *Polygonia c-album* butterfly on the other hand is a highly mobile species (Cowley et al. 2001) and it uses host plants (*Humulus lupulus*, *Ulmus glabra* and *Urtica dioica*) that are widely distributed (Braschler & Hill 2007). For this species no difference in morphology for individuals from margin populations reared in laboratory were detected (Braschler & Hill 2007). The species studied here differ in their mobility and dispersal capacity (e.g. Cowley et al. 2001; Dennis 2010; Kallioniemi et al. in press). Larger investment in dispersal at the margin were found only for *A. agestis* that is the least mobile of the species studies here while highly mobile *A. cardamines* and grassland species *P. tithonus,* that is not likely to be limited by available habitat, did not indicate change in morphological traits that could be contributed to selection during range expansion. The range margins of the species also differ in the amount of suitable weather for flight (high enough ambient temperature and sunshine) which, together with differences in species life spans, can alter the time available for flight particularly at temperate environments (Kingsolver 1983) and therefore affect the selective pressure on increased flight capacity. These results together with earlier studies suggest that evolutionary changes during range expansion could be dependent on species dispersal capacity as well as the amount of habitat available for the species (habitat fragmentation). Finally, although by collecting samples from the wild we are able to study the actual phenotypes present in the field, due to differences in environmental conditions and possibly in the ages of the butterflies at the time of collection it is possible that the sample sizes were not large enough to detect possible differences in morphology even if they were present.

Conclusions

These results show no consistent inter-family pattern of increased investment in dispersal at the range margins across three range-expanding species. I hypothesise that selection towards increased dispersal capacity is stronger for less mobile species and species with less continuous habitat available thereby making increased dispersal more crucial during range expansion. Environmental variables are also likely to influence the morphology of butterflies collected from the wild. Further investigation would clarify if the differences observed are genetic or caused by plastic responses to environmental conditions or the interaction between genes and the environment in which individuals developed, which can alter the reaction norm through genetic assimilation or accommodation (Fusco and Minelli, 2010). However, individuals studied here represent the phenotypes that are responsible for the colonisation of new areas in the wild in comparison to the individuals that are reared at optimal conditions in the laboratories. This study shows that morphological responses to selection during range expansion are likely to be species dependent and therefore accelerated increase in dispersal ability during range expansion cannot be assumed.

Literature

Asher, J., M. Warren, R. Fox, P. Harding, G. Jeffcoate, and M. A. Jeffcoate, Stephens. 2001. Millennium Atlas of Butterflies in Britain and Ireland. Oxford University Press, Oxford United Kingdom.

Berwaerts, K., H. Van Dyck, and P. Aerts. 2002. Does flight morphology relate to flight performance? An experimental test with the butterfly *Pararge aegeria*. Functional Ecology **16**:484–491.

Blanckenhorn, W. U., and M. Demont. 2004. Bergmann and converse Bergmann latitudinal clines in arthropods: Two ends of a continuum? Integrative and Comparative Biology **44**:413–424.

Braschler, B., and J. K. Hill. 2007. Role of larval host plants in the climate-driven range expansion of the butterfly *Polygonia c-album*. Journal of Animal Ecology **76**:415–423.

Burton, O. J., B. L. Phillips, and J. M. J. Travis. 2010. Trade-offs and the evolution of life-histories during range expansion. Ecology Letters **13**:1210–1220.

Chai, P., and R. Srygley. 1990. Predation and the flight, morphology, and temperature of neotropical rain-forest butterflies. American Naturalist **135**:748–765.

Cowley, M. J. R., C. D. Thomas, D. B. Roy, R. J. Wilson, J. L. Leon-Cortes, D. Gutierrez, C. R. Bulman, R. M. Quinn, D. Moss, and K. J. Gaston. 2001. Density-distribution relationships in British butterflies. I. The effect of mobility and spatial scale. Journal of Animal Ecology **70**:410–425.

Davis, M. 1984. The flight and migration ecology of the red milkweed beetle (*Tetraopes tetraophthalmus*). Ecology **65**:230–234.

Dennis, R. L. H. 2010. A resource-based habitat view for conservation: butterflies in the British landscape. John Wiley and Sons, Oxford, United Kingdom.

Dudley, R., and R. Srygley. 1994. Flight physiology of neotropical butterflies - Allometry of airspeeds during natural free-flight. Journal of Experimental Biology **191**:125–139.

Dytham, C. 2009. Evolved dispersal strategies at range margins. Proceedings of the Royal Society B-Biological Sciences **276**:1407–1413.

Fox, R., J. Asher, T. Brereton, D. Roy, and M. Warren. 2006. State of butterflies in Britain and Ireland. Pisces Publications, Newbury, United Kingdom.

Fusco, G. and A. Minelli. 2010. Phenotypic plasticity in development and evolution: facts and concepts. Philosophical Transactions of The Royal Society B Biological Sciences **365**: 547–556.

Gibbs, M., C. Wiklund, and H. Van Dyck. 2011. Phenotypic plasticity in butterfly morphology in response to weather conditions during development. Journal of Zoology **283**:162–168.

Gilman, S. E., M. C. Urban, J. Tewksbury, G. W. Gilchrist, and R. D. Holt. 2010. A framework for community interactions under climate change. Trends in Ecology & Evolution **25**:325–331.

Grime, J. P., J. G. Hodgson, and R. Hunt. 2007. Comparative plant ecology - a functional approach to common British speciessecond edition. Castlepoint press, Colvend, United Kingdom.

Haag, C. R., M. Saastamoinen, J. H. Marden, and I. Hanski. 2005. A candidate locus for variation in dispersal rate in a butterfly metapopulation. Proceedings of the Royal Society B-Biological Sciences **272**:2449–2456.

Hanski, I., C. Erälahti, M. Kankare, O. Ovaskainen, and H. Sirén. 2004. Variation in migration propensity among individuals maintained by landscape structure. Ecology Letters **7**:958–966.

Hanski, I., M. Saastamoinen, and O. Ovaskainen. 2006. Dispersal-related life-history trade-offs in a butterfly metapopulation. Journal of Animal Ecology **75**:91–100.

Heath, J., E. Pollard, and J. A. Thomas. 1984. Atlas of butterflies in Britain and Ireland. Viking, Harmondsworth, United Kingdom.

Hickling, R., D. B. Roy, J. K. Hill, and C. D. Thomas. 2005. A northward shift of range margins in British Odonata. Global Change Biology **11**:502–506.

Hill, J. K., Y. C. Collingham, C. D. Thomas, D. S. Blakeley, R. Fox, D. Moss, and B. Huntley. 2001. Impacts of landscape structure on butterfly range expansion. Ecology Letters **4**:313–321.

Hill, J. K., H. M. Griffiths, and C. D. Thomas. 2011. Climate change and evolutionary adaptations at species' range margins. Pages 143–159 in M. Berenbaum, R. Carde, and G. Robinson, (eds). Annual Review of Entomology, Vol 56. Annual Reviews, Palo Alto.

Hill, J. K., C. D. Thomas, and D. S. Blakeley. 1999a. Evolution of flight morphology in a butterfly that has recently expanded its geographic range. Oecologia **121**:165–170.

Hill, J. K., C. D. Thomas, and O. T. Lewis. 1999b. Flight morphology in fragmented populations of a rare British butterfly, *Hesperia comma*. Biological Conservation **87**:277–283.

Honek, A. 1993. Intraspecific variation in body size and fecundity in insects - A general relationship. Oikos **66**:483–492.

Hughes, C. L., C. Dytham, and J. K. Hill. 2007. Modelling and analysing evolution of dispersal in populations at expanding range boundaries. Ecological Entomology **32**:437–445.

Hughes, C. L., J. K. Hill, and C. Dytham. 2003. Evolutionary trade-offs between reproduction and dispersal in populations at expanding range boundaries. Proceedings of the Royal Society of London Series B-Biological Sciences **270**:S147–S150.

Karlsson, B., and A. Johansson. 2008. Seasonal polyphenism and developmental trade-offs between flight ability and egg laying in a pierid butterfly. Proceedings of the Royal Society B-Biological Sciences **275**:2131–2136.

Kingsolver, J. 1983. Ecological significance of flight activity in Colias butterflies - implications for reproductive strategy and population-structure. Ecology **64**:546–551.

Lawton, J.H. et al. 2010. Making Space for Nature: a review of England's wildlife sites and ecological network. Defra, United Kingdom.

McLachlan, A. 1983. Life-history tactics of rain-pool dwellers. Journal of Animal Ecology **52**:545–561.

Menendez, R., A. Gonzalez-Megias, O. T. Lewis, M. R. Shaw, and C. D. Thomas. 2008. Escape from natural enemies during climate-driven range expansion: a case study. Ecological Entomology **33**:413–421.

Merckx, T., and H. Van Dyck. 2006. Landscape structure and phenotypic plasticity in flight morphology in the butterfly *Pararge aegeria*. Oikos **113**:226–232.

Mitikka, V., and I. Hanski. 2010. Pgi genotype influences flight metabolism at the expanding range margin of the European map butterfly. Annales Zoologici Fennici **47**:1–14.

Parmesan, C. 2006. Ecological and evolutionary responses to recent climate change. Pages 637–669 Annual Review of Ecology Evolution and Systematics. Annual Reviews, Palo Alto.

Pateman, R. M., J. K. Hill, D. B. Roy, R. Fox, and C. D. Thomas. 2012. Temperature-dependent alterations in host use drive rapid range expansion in a butterfly. Science **336**:1028–1030.

Pellegroms, B., S. Van Dongen, H. Van Dyck, and L. Lens. 2009. Larval food stress differentially affects flight morphology in male and female speckled woods (*Pararge aegeria*). Ecological Entomology **34**:387–393.

Pinheiro J., D. Bates, S. DebRoy, D. Sarkar and the R Development Core Team (2012). nlme: Linear and Nonlinear Mixed Effects Models. R package version 3.1-106.

Pöyry, J., M. Luoto, R. K. Heikkinen, M. Kuussaari, and K. Saarinen. 2009. Species traits explain recent range shifts of Finnish butterflies. Global Change Biology **15**:732–743.

Rasband, W.S., ImageJ, U. S. National Institutes of Health, Bethesda, Maryland, USA, http://imagej.nih.gov/ij/, 1997-2012.

R Core Team. 2013. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. Retrieved from http://www.R-project.org.

Roff, D. A. 1991. Life history consequences of bioenergetic and biomechanical constraints on migration. American Zoologist **31**:205–216.

Root, T. L., J. T. Price, K. R. Hall, S. H. Schneider, C. Rosenzweig, and J. A. Pounds. 2003. Fingerprints of global warming on wild animals and plants. Nature **421**:57–60.

Saastamoinen, M. 2007. Mobility and lifetime fecundity in new versus old populations of the Glanville fritillary butterfly. Oecologia **153**:569–578.

Sekar, S. 2012. A meta-analysis of the traits affecting dispersal ability in butterflies: can wingspan be used as a proxy? The Journal of animal ecology **81**.

Simberloff, D. 1981. What makes a good island colonist? Pages 195–205 Insect life history patterns: habitat and geographic variation. Springer, Berlin, Germany.

Simmons, A. D., and C. D. Thomas. 2004. Changes in dispersal during species' range expansions. American Naturalist **164**:378–395.

Thomas, C. D. et al. 2004. Extinction risk from climate change. Nature **427**:145–148.

Thomas, C. D., J. K. Hill, and O. T. Lewis. 1998. Evolutionary consequences of habitat fragmentation in a localized butterfly. Journal of Animal Ecology **67**:485–497.

Tolman, T. 1997. Butterflies of Britain and Europe, 1997. 4th edition. HarperCollins Publishers Ltd., London, United Kingdom.

Travis, J. M. J., K. Mustin, T. G. Benton, and C. Dytham. 2009. Accelerating invasion rates result from the evolution of density-dependent dispersal. Journal of Theoretical Biology **259**:151–158.

Warren, M. S. et al. 2001. Rapid responses of British butterflies to opposing forces of climate and habitat change. Nature **414**:65–69.

Wickman, P. 1992. Sexual Selection and Butterfly Design - a Comparative-Study. Evolution **46**:1525–1536.

Wickman, P.-O., and B. Karlsson. 1989. Abdomen size, body size and the reproductive effort of insects. Oikos **56**:209.

Zera, A. J., and R. F. Denno. 1997. Physiology and ecology of dispersal polymorphism in insects. Annual Review of Entomology **42**:207–230.

Chapter 5: Different movement responses of two Lycaenidae species in relation to habitat quality and structure

Abstract

Several dispersal studies have focused on the impact of patch area and isolation on butterfly movement. More recently there has been more interest on effects of habitat structure and quality and their relative importance for movement within a habitat. This is important because it builds understanding of how environmental factors influence movement behaviour and this will affect dispersal at a larger spatial scale. This information is essential for designing efficient conservation measures in the face of global environmental change.

This study focuses on determining the influence of habitat quality and structure and temperature on the movement behaviour of species with limited dispersal. The movement parameters of two Lycaenidae species; (1) range-expanding *Aricia agestis* and (2) widely distributed and common *Polyommatus icarus* were measured within habitats that varied in their quality and structure. Mean bout (step length) and speed was calculated for each flight track. Hierarchical partitioning method was used for assessing the relative importance of the habitat quality, structure and temperature on the movement parameters.

Speed and step length of the range-expanding *Aricia agestis* was not significantly influenced by the variation in the habitat or temperatures to which they were exposed suggesting that this species might be responding to coarser environmental cues. This tolerance to wide environmental conditions may be one of the reasons why this fairly sedentary species has been able to expand its range in response to recent global warming. Responses of *Polyommatus icarus* to the variables were sex-dependent: female flight varied with area, edge ratio and temperature while males flew less in tall vegetation. These results indicate that responses of butterflies to habitat quality and structure are likely to be highly species dependent and therefore habitat management should take into account individual species requirements. The results also imply that there is plasticity in how much environmental cues are used by species during their dispersal.

Introduction

The combined effects of habitat fragmentation and climate change present a big challenge for conservation, especially of small range habitat-specialist species (Saunders et al. 1991; Thomas et al. 2004; Pöyry et al. 2009). Habitat fragmentation and degradation can result in the remaining suitable habitats being more isolated, meaning that species are required to move increasingly long distances to colonize new climatically suitable areas (Warren et al. 2001). Hence understanding animal movement is of critical importance, and dispersal and animal movement are currently hot topics in ecology (Stevens et al. 2010).

Butterflies are known to be able to adapt their flight behaviour in relation to their environment (Turlure et al. 2011) and structural habitat as mapped and perceived by humans may have features that promote different behaviours and movement patterns for different species (Van Dyck 2012). The speed at which butterflies traverse across different habitats and landscapes is often faster outside suitable habitat (Schultz 1998; Turlure et al. 2011; Skorka et al. 2013) and in areas with low host plant density (Odendaal et al. 1989). Schultz et al. (2012) highlighted the impact of both physical structures and food resources on butterfly flight as butterflies moved slower in open woods and in areas where food resources were present. The abundance of nectar sources has been shown to decrease butterfly emigration rate from a patch (Kuussaari et al. 1996). Butterfly movement will be dependent on nectar and host plants that influence the quality of the habitat; *Parnassius apollo* were found more often at host plant patches located close to nectar sources (Brommer & Fred 1999), *Danaus plexippus* were less likely to cross edges of habitats with high nectar abundance (Ries & Debinski 2001) and *Parnassius smitheus* females moved more randomly in unsuitable meadows than in suitable meadows with larval host plants (Fownes & Roland 2002). Furthermore, vegetation height, amount of bare ground, shelter and insolation has an effect on butterfly habitat usage and patch occupancy (Dover 1996; Dover et al. 1997; Merckx & Van Dyck 2002; Pradel & Fischer 2011) and are therefore also likely to influence butterfly movement within habitat. Additionally, structural elements such as shrubs or tree lines are important resources for butterflies providing shelter and flyways (Dover & Fry 2001; Dennis & Sparks 2006). Detailed studies of within patch movements are still sparse (Fownes & Roland 2002; Auckland et al. 2004; James 2006) and few studies have looked at the relative importance of structural and habitat quality measures on butterfly movement parameters within habitat.

Butterfly flight is known to vary depending on the weather and temperature in particular. For example *Pararge aegeria* butterflies spend more time in flight at higher temperatures (Shreeve 1984) and flight bout duration increased in general with temperature and decreased with cloudiness for four different butterfly species (Cormont et al. 2011). When studying butterfly movement it is therefore important to also take into account the prevailing weather during flight to be able to distinguish how flight is affected by other parameters in the butterfly's environment.

This study uses hierarchical partitioning to assess individual and relative contributions of several co-varying explanatory variables on butterfly movement parameters. This method has been used successfully for assessing the relative importance of different factors on butterfly patch use and abundance, for example for *Parnassius mnemosyne* (Heikkinen et al. 2005) and *Lycaeides melissa samuelis* butterflies (Grundel & Pavlovic 2007). However, the relative impact of different habitat quality and structure on butterfly flight within habitat patches is not well known despite its importance for conservation measures and habitat management. As dispersal can also occur as a by-product of so called "routine movements" that are daily movements related to resource use such as finding mates or nectar sources (Van Dyck & Baguette 2005), knowledge of movement at a smaller scale and within a habitat patch can prove valuable, for example in explaining events such as immigration (Auckland et al. 2004; Kallioniemi et al. in press) and thereby help understand dispersal over longer distances and time scales (Wiens et al. 1993; Ims & Yoccoz 1997).

Aricia agestis (brown argus, L.) is a good model species for studying the effects of habitat structures and quality on its flight because it is highly restricted in its movements within habitat and tall forest can act as a barrier to its movement (Wilson & Thomas 2002; Kallioniemi et al. in press). Yet, this species has managed to shift its range in the UK in the recent three decades (Asher et al. 2001; Fox et al. 2006). Another Lycaenidae species *Polyommatus icarus* (common blue, Rottemburg) was included as a comparison as it occurs in the same habitats and by including two species in the study it will be possible to assess variation in species responses to both habitat quality and structure.

This study has two main questions: 1) is butterfly movement behaviour (speed and mean move bout length) affected by habitat quality (measured as a nectar and host plant abundance and vegetation height), habitat structure measures (habitat area, shelter and ratio of area to edge) and environmental factors (temperature)? 2) What is the relative importance of each of these factors on butterfly flight?

Material and methods

Study sites and butterfly tracking

Butterflies were tracked at four different areas in Eastern England in 2010 and at eight areas located in the midlands of England in 2011. These 12 areas had 18 release sites that were considered suitable habitat for the butterflies but varied in their quality and structural characteristics (Table 1). Although some variables measured were highly correlated (e.g. area and edge ratio Pearson correlation=0.85), sites differed in how habitat structure was coupled with habitat quality measures (see Table 1 for variable values at each site and Appendix B for between site variation).

A total of 187 *Aricia agestis* and 206 *Polyommatus icarus* butterflies were caught and tracked during their second flight season July-September, in 2010 and 2011 (Table 1). Butterflies were cooled down to 7-15°C and marked individually on the hindwing prior to release. Butterfly flight paths were tracked individually using a flag technique in which numbered flags are dropped on the butterfly flight path, either every 20 seconds or when the butterfly lands, continuing for up to maximum of 15 flags or until the butterfly was lost (see details in Schultz 1998). At the end of each day, flag locations were recorded using a differential GPS with 10 cm accuracy (Magellan ProMark III and 100 GPS). For each track I calculated i) mean step length = sum of distances between consecutive flags where each butterfly had landed / number of moves (no. flags-1) and ii) speed = sum of distances between each consecutive flag / total time spent in flight. These were used as general measures of butterfly mobility because mean step length has been previously connected with butterfly likelihood of crossing habitat boundaries (Kallioniemi et al. in press) and butterflies have been shown to adjust their flight speed in response to habitat and matrix types (Turlure et al. 2011). These movement parameters are also independent of the length of the track. Each butterfly was tracked only once. Tracking of the butterflies was done only on sunny days when temperature was >15°C and wind speed did not exceed 5 on the Beaufort scale. Ambient temperature in the shade was measured at the beginning and at the end of each track and the average was used as a value for the track. To standardise the procedure butterflies were always released in a site that was >500 m from

Table 1. *Site information and sample sizes for* A. agestis *(AAf) females and (AAm) males and* P. icarus *(PIf) females and males (PIm). AA host=* Aricia agestis *host plant cover index, PI host=* P. icarus *host plant cover index. Edge ratio= habitat area (ha)/edges around and in the area (m).*

Site	*Year*	*AA f*	*AA m*	*PI f*	*PI m*	*Temperature °C*	*Nectar cover*	*Vegetation height (cm)*	*AA host*	*PI host*	*Shelter*	*Edge ratio ha /m*	*Sqrt(area(ha))*
1	2011	-	5	4	8	20.5	4	8	0	3	4	5	0.072
2	2011	7	7	8	10	23.0	2	12	0	2	1	40	0.111
3	2010	14	13	21	14	21.2	2	35	0	1	2	67	0.224
4	2010	8	7	6	10	18.8	2	14	1	1	3	19	0.089
5	2011	-	8	5	4	26.2	2	31	1	1	4	26	0.139
6	2011	-	4	7	3	19.9	3	15	1	1	8	3	0.044
7	2010	12	7	6	13	19.1	2	8	1	1	3	90	0.254
8	2010	4	3	3	-	21.8	2	13	1	1	2	90	0.254
9	2011	3	3	4	6	21.0	2	24	0	0	4	88	0.130
10	2011	-	-	4	-	20.8	1	3	1	2	4	18	0.100
11	2011	3	-	-	-	19.3	3	16	0	1	3	32	0.119
12	2011	6	6	-	6	20.7	2	11	0	1	2	48	0.158
13	2011	-	4	3	-	27.6	2	16	0	1	4	17	0.068
14	2010	12	15	13	10	21.0	2	2	2	0	4	24	0.096
15	2011	6	5	10	-	21.0	3	16	0	3	5	22	0.110
16	2011	3	-	-	3	22.9	2	14	0	1	4	37	0.132
17	2010	8	-	5	7	18.1	1	13	1	1	8	6	0.063
18	2010	6	8	7	6	21.8	2	16	1	1	5	7	0.080

where they were caught, thus in sites that were considered unknown for the butterflies. Release sites all had nectar sources and host plants; the butterflies were placed on a host plant or nectar source when released. Release sites were alternated between days and butterflies were released at each site throughout the field season. Studies of other butterfly species have indicated that handling and following of butterflies did not impact the flight behaviour of the butterflies (Root & Kareiva 1984; Schultz 1998; Skorka et al. 2013). However, as a precautionary measure I excluded the first flag of each track and only included tracks that had three or more flags.

All butterflies were released within 5-20m from a habitat boundary or shelter which is thought to be within the perceptual range of these butterflies based on previous study of boundary crossing (Kallioniemi et al. in press) and data on other Lycaenidae species *Icaricia icarioides fenderi* (Schultz & Crone 2001). Butterflies were released close to the these structures because most individuals of the study species were naturally observed at sheltered locations and therefore flight behaviour there is thought to represent their normal movement behaviour. The variability in shelter conditions within a path reflects the habitat characteristics of each site such as area size and quantity of shelter/boundary available.

Habitat quality and site structure

Habitat quality was measured at each release location in the area where butterflies had been flying (area in which ~95% of the flags at that site were placed) at the end of the flight season (15-17th of September in 2010 and 6-8th of September 2011). Vegetation was surveyed using 1m^2 quadrats placed randomly in the habitat, with 7-30 quadrats were recorded at each site depending on the size of the area (~ 1 quadrat/400m^2) and the following measurements were taken at each quadrat location: 1) the percentage cover of each host plant for both species separately using Braun-Blanquet scale with increasing cover percentage from 0 to 5 (Table 2): 1 equals <5 % cover and 5 means 75-100% of the surface was covered with the host plant in question; 2) mean vegetation height in the quadrat estimated using a tape measure; 3) the total % cover of all nectar species (defined as species that butterflies had been observed nectaring on during the flight season) again using Braun-Blanquet scale (Table 2). Mean of the host plant and nectar plant cover per site was converted to scale 0-5 again to reflect the Braun Blanquet scale (see appendix C).

For quantifying habitat structure I used three parameters: 1) Shelter of a site, considered as the amount of protection from wind as well as shade, was estimated on a scale from 0-9 with the following formula:

$$Shelter = \sum_{i=1}^{4} a_i \times \sum_{i=1}^{4} b_i + \begin{cases} 1 & if\ additional\ trees\ or\ bushes\ at\ site \\ 0 & else \end{cases}$$

Where a=1 if there is a shelter within 30 m and 0 if not. This was estimated separately for the four cardinal directions. b is a coefficient of 1 or 2 if the shelter is dense (cannot see through) and or tall (>2m) and or close <20m in that direction forming an continuous edge. +1 was added to the shelter index if there were several trees or other structures other than edges within the site. Thus, shelter values represent increasing amount of shelter: 0 indicates no shelter at all within 30 meters in any direction and 9 means site was sheltered from all directions and had extra trees/bushes at the open area. Shelter value of 4 and 5 preresent intermediate shelter where area can be surrounded by shelter from all sides but these are low or far away. Alternatively site can have same number if it is surrounded by tall or dense shelter from two sides. 2) Area = size of the release area. This was determined based on open area that was surrounded by either boundary with forest or unsuitable habitat such as crop field; 3) Edge ratio = area/amount of tall (>1m) edges around and within the habitat patch, ha/m. Large trees and bushes within the area were included as an edge.

Table 2. Braun Blanquet scale that was used for measuring nectar and host plant vegetation cover % in 1m × 1m quadrats.

Coeffient	Vegetation cover class %
5	>75%
4	50-75%
3	25-50%
2	5-25%
1	<5%
0	No host plants

Analysis

Because in ecological studies explanatory variables often co-vary and experimental manipulations are not always feasible in the field it can be difficult to distinguish the effects of different factors on the dependent variable of interest. Using regressions with methods such as backward elimination of non-significant variables can lead to removal of independent variables that influence the measured variable (such as species richness) but are collinear with other independent variables in the model. Hierarchical partitioning removes this problem by not aiming at the most reduced and predictive model but by calculating the mean effect of each independent variable on the dependent variable from all possible models (Mac Nally 2000). It can therefore be used for evaluating the independent and relative contribution of each independent variable on the dependent variable in question (Chevan & Sutherland 1991; Mac Nally 2000, 2002; Heikkinen et al. 2005).

Hierarchical partitioning was used, separately for both species and sexes, for separating the relative effects of habitat quality and structure and mean ambient temperature on the butterfly flight parameters i) mean step length and ii) speed. Hierarchical partitioning was done using hier.part package version 1.0-4 (Mac Nally & Walsh 2004) in statistical software R (R Core Team 2013) using R-squared as measure of goodness of fit. Patch area was log-transformed to increase linearity of relationships with the response variables. For each analysis I included only tracks from sites from where I had ≥3 tracks for the species and sex in question. Significance of each independent contribution of a variable on the movement parameter was tested with randomization procedure in hier.part package using 0.95 confidence limits (Z-score ≥ 1.65) (Mac Nally 2002).

Results

Mean step length varied between 1.4-4.3 m for *A. agestis* females and 2.7-6.2 m for males between sites (Table 3). Mean step lengths were 2.4-10.4 m for *P. icarus* females and 2.6-6.5 m for *P. icarus* males at different sites. Speed varied between 0.2-0.8 m/s for *A. agestis* females and 0.3-1.5 for males between the sites and 0.3-1.4 and 0.4-1 for *P. icarus* females and males, respectively.

Habitat quality

Nectar cover had no significant impact on the movement parameters studied (Table 4, Figures 1 and 2). *Polyommatus icarus* males had smaller mean step length and slower speed in sites with tall vegetation (Table 4, Figure 2). Host plant cover did not influence the movement parameters of either species (Table 4, Figures 1 and 2).

Habitat structure

Shelter had no significant influence on the movement parameters of either species (Table 4, Figure 1 and 2). Edge ratio and area were found to be strongly positively correlated across sites ($r = 0.85$) and for *P. icarus* females both mean step length and speed decreased with increasing edge ratio and area (Table 4, Figure 2). These habitat structure variables had no significant influence on the movement parameters of *P. icarus* males or *A. agestis* males or females.

Temperature

Mean step length of *P. icarus* females was longer at higher temperatures (Table 4, Figure 2) but speed was not affected. Within the range of temperatures examined (16.5-28.5°C), ambient temperature did not seem to influence the movement parameters of *P. icarus* males or *A. agestis* males or females.

Table 3. *Mean of mean step length (m) and speed ± se for both species and sexes per release site.*

	females		*males*		*females*		*males*	
Site	*Mean step*	*Speed*	*Mean step*	*Speed*	*Mean step*	*Speed*	*Mean step*	*Speed*
1	-	-	2.7±0.9	0.6±0.2	4.1±1.2	1.0±0.1	6.1±1.3	1.0±0.2
2	2.6±0.4	0.5±0.1	4.1±0.6	0.7±0.1	4.3±1.0	0.8±0.1	6.5±1.5	0.8±0.1
3	2.2±0.4	0.6±0.1	3.1±0.6	0.6±0.1	2.6±0.2	0.5±0.0	3.0±0.4	0.5±0.1
4	2.0±0.2	0.7±0.1	3.1±0.4	0.6±0.1	3.3±0.5	0.6±0.1	3.6±0.5	0.7±0.1
5	-	-	5.2±0.9	0.9±0.1	4.4±0.5	0.7±0.1	4.8±0.7	0.5±0.1
6	-	-	6.2±1.7	1.5±0.3	3.7±0.7	0.8±0.2	3.8±0.5	0.6±0.1
7	2.6±0.5	0.5±0.1	3.5±0.7	0.6±0.2	2.7±0.3	0.5±0.1	7.1±1.4	0.8±0.1
8	1.4±0.2	0.2±0.0	5.3±1.1	0.7±0.0	2.4±0.8	0.3±0.1	-	-
9	3.1±0.3	0.6±0.1	3.7±1.1	0.7±0.2	3.9±1.2	0.6±0.3	4.4±0.9	0.6±0.1
10	-	-	-	-	5.0±0.7	0.7±0.1	-	-
11	2.3±0.5	0.5±0.1	-	-	-	-	-	-
12	4.0±0.5	0.8±0.1	4.0±0.4	0.8±0.1	-	-	4.7±1.5	0.6±0.1
13	-	-	2.7±0.6	0.3±0.1	10.4±2.4	1.4±0.4	-	-
14	2.5±0.5	0.6±0.1	3.7±0.7	0.8±0.2	4.8±0.9	0.9±0.2	7.3±1.3	0.8±0.1
15	2.2±0.4	0.5±0.1	3.1±0.9	0.4±0.1	2.8±0.8	0.5±0.2	-	-
16	3.7±1.5	0.6±0.2	-	-	-	-	7.5±0.6	0.8±0.1
17	3.2±0.8	0.8±0.2	-	-	5.1±0.9	0.9±0.1	4.3±1.1	0.8±0.2
18	4.3±0.8	0.7±0.1	4.3±2.0	0.7±0.2	3.9±0.7	0.9±0.3	2.6±0.5	0.4±0.1

Table 4. *Independent contributions (I) of each variable, Z-scores (Z) and correlations (cor) for* A. agestis *and* P. icarus *males and females for mean step length and speed. Negative correlation indicates negative relation between the independent variable and the response variable. Significant (p<0.05) variables are presented in bold.*

	A. agestis						*P. icarus*					
	females			*males*			*females*			*males*		
	I	Z	cor	I	Z	cor	I	Z	cor	I	Z	cor
Step length												
Temperature	0.005	-0.38	0.03	0.017	0.17	0.14	**0.072**	**4.98**	**0.28**	0.013	0.2	-0.15
Nectar	0.019	0.5	-0.14	0.002	0.29	-0.04	0.019	0.79	-0.13	0.004	-0.42	0.08
Vegetation height	0.012	0.06	-0.08	0.001	-0.03	0.01	0.023	1.23	-0.23	**0.120**	**8.24**	**-0.34**
Host plant	0.015	0.26	-0.06	0.007	-0.62	-0.07	0.014	0.37	-0.10	0.002	-0.62	0.05
Shelter	0.014	0.18	0.16	0.015	-0.3	0.13	0.008	-0.11	0.14	0.005	-0.37	-0.07
Edge ratio	0.008	-0.19	-0.15	0.001	0.51	-0.05	0.029	1.34	-0.26	0.011	0.11	0.08
Area	0.011	-0.04	-0.16	0.001	0.45	-0.04	**0.048**	**3.06**	**-0.32**	0.004	-0.42	0.05
Speed												
Temperature	0.014	0.17	-0.13	0.005	-0.42	-0.05	0.006	-0.33	0.09	0.032	1.58	-0.21
Nectar	0.015	0.29	-0.15	0.003	-0.46	0.01	0.001	-0.65	0.02	0.005	-0.34	0.11
Vegetation height	0.010	-0.03	0.05	0.003	-0.5	-0.07	0.013	0.19	-0.19	**0.094**	**6.84**	**-0.34**
Host plant	0.002	-0.62	-0.11	0.028	1.3	-0.17	0.013	0.3	-0.07	0.023	0.96	0.16
Shelter	0.006	-0.3	0.14	0.031	1.23	0.22	0.010	0.03	0.17	0.001	-0.64	0.04
Edge ratio	0.020	0.51	-0.20	0.004	-0.47	-0.11	**0.038**	**2.43**	**-0.29**	0.006	-0.32	-0.05
Area	0.018	0.45	-0.19	0.003	-0.56	-0.09	**0.043**	**2.44**	**-0.31**	0.005	-0.36	-0.07

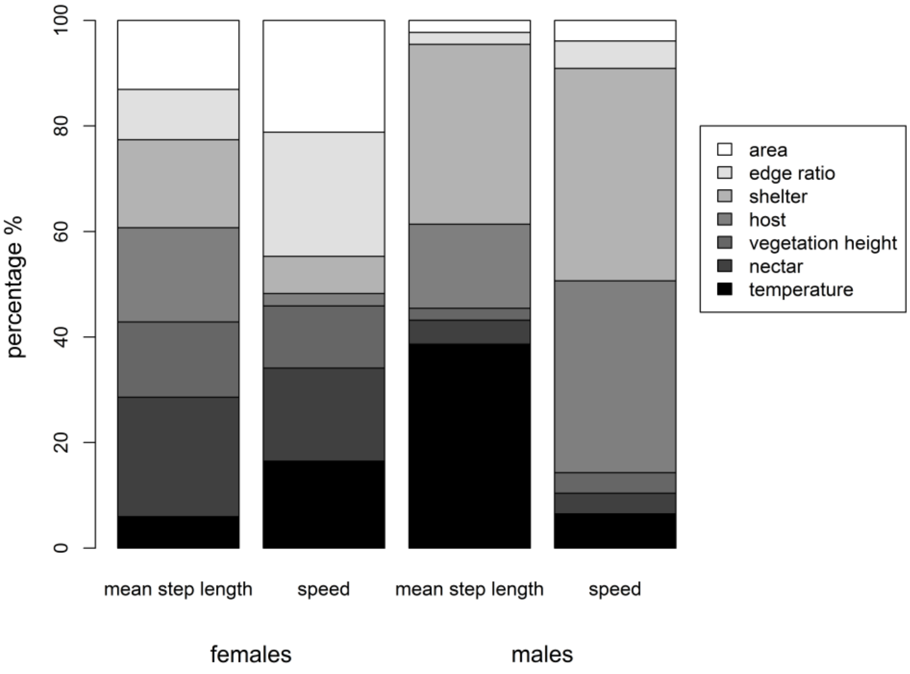


Figure 1. *Relative contribution of each independent variable on the mean step length and speed for* A. agestis *females and males.*

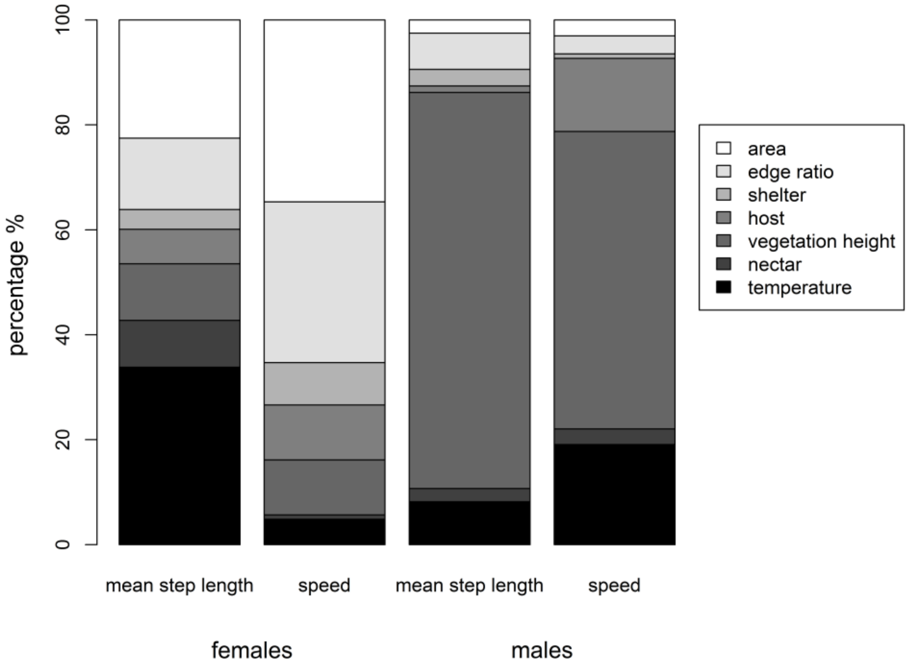


Figure 2. *Relative contribution of each independent variable on the mean step length and speed for* P. icarus *females and males.*

Discussion

Differences in habitat structure and resources, across the 18 sites, did not influence the movement parameters (speed and step length) of the range-expanding *A. agestis.* This species' movement parameters were not influenced by the variation in temperature to which individuals were exposed to during this study (16.5-28.5 °C), indicating that, for this species, movement behaviour in habitat is unaffected by the variability of conditions at which they were studied. The movement parameters of the widely distributed *P. icarus* were influenced by temperature, vegetation height, edge ratio and area but responses differed between the sexes indicating that this species may display more plastic responses depending on its environment. These results suggest that *A. agestis* response to habitat quality and structure at a coarse scale and highlights that responses to environment are species-and sex-specific.

Habitat quality

Nectar and host-plant cover did not have an effect on the movement parameters of the two Lycaenidae species included in this study. Other studies on resource availability found butterflies to be more likely to emigrate when the quantity of nectar in a habitat patch decreases (Kuussaari et al. 1996; Ries & Debinski 2001) although this has not been the case for all species (Ries & Debinski 2001; Matter & Roland 2002). In this study I quantified the percentage of cover of nectar plants used by the butterflies, but the indexed measurements of flower abundance might not be a sufficient estimate for nectar availability (Schultz & Dlugosch 1999). These authors noted that detailed nectar availability, taking into account the amount of nectar produced by each flower species might be needed for understanding the effect of resource availability on movement parameters; however this is likely to be unfeasible particularly for species that use several nectar species. Increase in vegetation height was found to decrease the mean step lengths and speed for *P. icarus* males but not for females. Vegetation height can easily be modified by management practices. Therefore, land managers could modify vegetation height in order to influence the movement of butterflies for example to encourage species to spread across a site or at a landscape level. However, for a common wide spread species such *P. icarus*, it is not likely that this would be management priority. Only male flight was affected by the vegetation height which could be due to its impact on mate searching strategies. This species is found both perching and patrolling and sites with high vegetation might offer more optimal places for perching behaviour. There was no significant influence of host plant cover on mean step or speed on either species. However, due to patchiness of the *A. agestis* host plant, and the methods used, I was able to detect only low variability in the host plant cover (Table 1), which could possibly hide the effect of host plant on the flight of *A. agestis*. Low effect of host plant cover on flight for *P. icarus* is unexpected particularly on female flight because female movement is thought to be linked with host plant abundance due to need for ovipositing and search behaviour (Thomas & Singer 1987; Fownes & Roland 2002). It is possible that females respond to host plant presence at the coarse scale rather than abundance as Fownes and Roland (2002) argued for *Parnassius smitheus* butterfly.

The relatively low linkage between habitat quality measures and movement parameters in the two species studied here is in line with findings of Fownes and Roland (2002) who found that movement was similar in suitable and unsuitable meadows for *Parnassius smitheus.* Sei (2009) also found that move lengths were not related to microhabitats for *Coenonympha nipisiquit* butterfly. Although habitat quality does not appear to have a strong influence on the butterfly flight characteristics within a habitat patch as such, an increase in habitat quality is likely to increase population size and thereby influence dispersal by producing more potential emigrants.

Habitat structures

Of the habitat structure measures (shelter, patch area and edge ratio) female *P. icarus* flight was affected by the area and edge ratio: increase in habitat area negatively affected both movement parameters and increase in edge ratio had a negative effect on speed. A similar effect of edge ratio and area on movement parameters was expected because these variables are highly correlated. Some butterflies follow habitat edges when flying (Ovaskainen et al. 2008) and smaller areas provide relatively more edges that species can follow which can alter the flight speed and step length of individuals. Large patches might have smaller emigration rate because individuals are less likely to encounter habitat edges and therefore enter unsuitable matrix. Because individuals that move with longer step lengths are more likely to cross boundaries (Kallioniemi et al. in press), for *P. icarus* females increase in step length with decreasing habitat area might add another mechanisms leading to higher emigration rates from smaller patches.

Temperature

Temperature varied from site to site (Table 1) and within the flight season, thus temperature was included in the models to understand its potential effect on flight behaviour. *Polymmatus icarus* females had longer mean step length at high temperatures, which is in line with findings for several other butterfly species (Kingsolver 1983; Wickman 1985; Dennis & Sparks 2006; Cormont et al. 2011). *Polyommatus icarus* males and *A. agestis* males and females showed no influence of temperature on the movement parameters analysed. It is possible that these butterflies were able to compensate for the colder temperatures by microsite selection and basking and therefore their movement parameters were not significantly influenced by it.

Hierarchical partitioning method in analysing movement

Hierarchical partitioning has been successfully used for analysing between patch movement and occupancy, and for understanding how these are influenced by habitat quality and structure (e.g. Heikkinen et al. 2005; Grundel & Pavlovic 2007). This method has not previously been used for analysing within habitat movement. This is a surprise since hierarchical partitioning is a convenient way of analysing relative importance of often co-varying explanatory variables in movement. One of the limitations of this technique is that the number of variables that can be analysed at once is limited to nine before the results become unreliable (Olea et al. 2010). This may be an issue if extensive habitat assessments are being carried out.

Management implications

No habitat quality or structural variables measured influenced the movement parameters for the range-expanding *A. agestis* inside suitable habitat. This may indicate that this species' flight behaviour is robust to the environmental characteristics measured here and this may be one of the possible explanations for its ability to expand its range recently (Asher et al. 2001; Fox et al. 2006). This species is reluctant to cross barriers and only occasionally (<1.5%) was found to disperse over long (>500) distances (Wilson & Thomas 2002) indicating that this species is resilient to habitat quality and structure. *Aricia agestis* may react to its environment at a more coarse scale. It is possible that *A. agestis* has therefore been able to take advantage of small and low quality patches that occur between, what humans recognise as, suitable habitat areas. This may have enabled the species to shift its range across seemingly large unsuitable areas, although this hypothesis would require further testing. For *A. agestis*, small patches of suitable habitat with some host plants within a landscape may serve as important stepping stones, enabling this species to colonise new sites and keep tracking climatic changes.

Temperature, area and structure were found to influence *P. icarus* female movement. This is interesting because *A. agestis* from the same butterfly family which occurs in the same sites did not respond to these variables. This highlights the fact that even species that are close relatives are likely to respond differently to their environment and, therefore, habitat management for one species may impact species that are not targeted by particular management actions. This indicates that management actions should take into account specific needs of several target species. Since *P. icarus* is a common and widespread species (Asher et al. 2001) and relatively mobile (Gutierrez et al. 2001), it is unlikely that any specific management actions will be needed. However, in the future, common species that are not extreme generalists might be the ones under greatest threat (Van Dyck et al. 2009; Dapporto & Dennis 2013). This is because at present, specialist species might be able to monopolise particular resources and are more likely to benefit from conservation measures (Dapporto & Dennis 2013). On the other hand, generalists are able to use variety of resources and move easily between suitable areas while common species that fall in between these two categories might not be able to compete with these strategies (Dapporto & Dennis 2013).

Conclusions

These results show that the range-expanding species *A. agestis* did not alter its movement in the habitat depending on the patch quality or structural variables measured. This tolerance to a wide range of conditions may have contributed to the successful range expansion of this species allowing it to take advantage of low quality habitats. Responses of the close relative *P. icarus* indicate that responses to habitat quality and structure are sex dependent. The differences between species found in this study indicate that there could be plasticity in the rate at which different species use environmental cues and management recommendations will need to take into account individual species requirements. Further studies with more species would be required to establish the relative importance of different environmental factors on butterfly flight behaviour. Knowing how habitat quality and structure affect species movement will enable more focused conservation and habitat management. Usage of hierarchical partitioning works well for this type of data that is likely to be highly correlated and will provide information on the importance of different factors for management purposes.

Literature

Asher, J., M. Warren, R. Fox, P. Harding, G. Jeffcoate, and M. A. Jeffcoate, Stephens. 2001. Millennium Atlas of Butterflies in Britain and Ireland. Oxford University Press, Oxford, United Kingdom.

Auckland, J. N., D. M. Debinski, and W. R. Clark. 2004. Survival, movement, and resource use of the butterfly *Parnassius clodius*. Ecological Entomology **29**:139–149.

Brommer, J. E., and M. S. Fred. 1999. Movement of the Apollo butterfly *Parnassius apollo* related to host plant and nectar plant patches. Ecological Entomology **24**:125–131.

Chevan, A., and M. Sutherland. 1991. Hierarchical partitioning. The American Statistician **45**:90–96.

Cormont, A., A. H. Malinowska, O. Kostenko, V. Radchuk, L. Hemerik, M. F. WallisDeVries, and J. Verboom. 2011. Effect of local weather on butterfly flight behaviour, movement, and colonization: significance for dispersal under climate change. Biodiversity and Conservation **20**:483–503.

Dapporto, L., and R. L. H. Dennis. 2013. The generalist-specialist continuum: Testing predictions for distribution and trends in British butterflies. Biological Conservation **157**:229–236.

Dennis, R. L. H., and T. H. Sparks. 2006. When is a habitat not a habitat? Dramatic resource use changes under differing weather conditions for the butterfly *Plebejus argus*. Biological Conservation **129**:291–301.

Dover, J. 1996. Factors affecting the distribution of satyrid butterflies on arable farmland. Journal of Applied Ecology **33**:723–734.

Dover, J. W., and G. L. A. Fry. 2001. Experimental simulation of some visual and physical components of a hedge and the effects on butterfly behaviour in an agricultural landscape. Entomologia Experimentalist et Applicata **100**:221 – 233.

Dover, J. W., T. H. Sparks, and J. N. Greatorex-Davies. 1997. The importance of shelter for butterflies in open landscapes. Journal of Insect Conservation **1**:89–97.

Fownes, S., and J. Roland. 2002. Effects of meadow suitability on female behaviour in the alpine butterfly *Parnassius smintheus*. Ecological Entomology **27**:457–466.

Fox, R., J. Asher, T. Brereton, D. Roy, and M. Warren. 2006. State of Butterflies in Britain and Ireland. Pisces Publications, Newbury, United Kingdom.

Grundel, R., and N. B. Pavlovic. 2007. Resource availability, matrix quality, microclimate, and spatial pattern as predictors of patch use by the Karner blue butterfly. Biological Conservation **135**:135–144.

Gutierrez, D., J. L. Leon-Cortes, R. Menendez, R. J. Wilson, M. J. R. Cowley, and C. D. Thomas. 2001. Metapopulations of four lepidopteran herbivores on a single host plant, *Lotus corniculatus.* Ecology **82**:1371–1386.

Heikkinen, R., M. Luoto, M. Kuussaari, and J. Pöyry. 2005. New insights into butterfly-environment relationships using partitioning methods. Proceedings of the Royal Society B-Biological Sciences **272**:2203–2210.

Ims, R. A., and N. G. Yoccoz. 1997. Studying transfer processes in metapopulations: emigration, migration, and colonization. In: Hansi I. and Gaggiotti O. (Eds.). *Metapopulation Biology, Ecology, Genetics and Evolution.* Pages 247 – 265. Elsevier Academic Press, Oxford, United Kingdom.

James, M. 2006. Intra-patch movement in the Sinai Baton Blue butterfly: influence of micro-habitat and individual characteristics. Egyptian Journal of Biology **8**: 51-66.

Kingsolver, J. 1983. Thermoregulation and flight in Colias butterflies - Elevational patterns and mechanistic limitations. Ecology **64**:534–545.

Kuussaari, M., M. Nieminen, and I. Hanski. 1996. An experimental study of migration in the Glanville fritillary butterfly *Melitaea cinxia*. Journal of Animal Ecology **65**:791–801.

Mac Nally, R. 2000. Regression and model-building in conservation biology, biogeography and ecology: The distinction between and reconciliation of “predictive” and “explanatory” models. Biodiversity and Conservation **9**:655–671.

Mac Nally, R. 2002. Multiple regression and inference in ecology and conservation biology: further comments on identifying important predictor variables. Biodiversity and Conservation **11**:1397–1401.

Mac Nally, R., and C. J. Walsh. 2004. Hierarchical partitioning public-domain software. Biodiversity and Conservation **13**:659–660.

Matter, S. F., and J. Roland. 2002. An experimental examination of the effects of habitat quality on the dispersal and local abundance of the butterfly *Parnassius smintheus*. Ecological Entomology **27**:308–316.

Merckx, T., and H. Van Dyck. 2002. Interrelations among habitat use, behavior, and flight-related morphology in two cooccurring satyrine butterflies, *Maniola jurtina* and *Pyronia tithonus*. Journal of Insect Behavior **15**:541–561.

Odendaal, F. J., P. Turchin, and F. R. Stermitz. 1989. Influence of host-plant density and male harassment on the distribution of female *Euphydryas anicia* (Nymphalidae). Oecologia **78**:283–288.

Olea, P. P., P. Mateo-Tomás, and Á. de Frutos. 2010. Estimating and modelling bias of the hierarchical partitioning public-domain software: implications in environmental management and conservation. PLoS One **5**:e11698.

Ovaskainen, O., A. D. Smith, J. L. Osborne, D. R. Reynolds, N. L. Carreck, A. P. Martin, K. Niitepõld, and I. Hanski. 2008. Tracking butterfly movements with harmonic radar reveals an effect of population age on movement distance. Proceedings of the National Academy of Sciences of the United States of America **105**:19090–19095.

Pöyry, J., M. Luoto, R. K. Heikkinen, M. Kuussaari, and K. Saarinen. 2009. Species traits explain recent range shifts of Finnish butterflies. Global Change Biology **15**:732–743.

Pradel, K., and K. Fischer. 2011. Living on the edge: Habitat and host-plant selection in the butterfly *Lycaena tityrus* (Lepidoptera: Lycaenidae) close to its northern range limit. Journal of Research on the Lepidoptera **44**:35–41.

R Core Team. 2013. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria.

Ries, L., and D. Debinski. 2001. Butterfly responses to habitat edges in the highly fragmented prairies of Central Iowa. Journal of Animal Ecology **70**:840–852.

Root, R. B., and P. M. Kareiva. 1984. The search for resources by cabbage butterflies (*Pieris rapae*) - ecological consequences and adaptive significance of Markovian movements in a patchy environment. Ecology **65**:147–165.

Saunders, D. A., R. J. Hobbs, and C. R. Margules. 1991. Biological consequences of ecosystem fragmentation: a review. Conservation Biology **5**:18–32.

Schultz, C. B. 1998. Dispersal behavior and its implications for reserve design in a rare Oregon butterfly. Conservation Biology **12**:284–292.

Schultz, C. B., and E. E. Crone. 2001. Edge-mediated dispersal behavior in a prairie butterfly. Ecology **82**:1879–1892.

Schultz, C. B., and K. M. Dlugosch. 1999. Nectar and hostplant scarcity limit populations of an endangered Oregon butterfly. Oecologia **119**:231–238.

Schultz, C. B., A. M. A. Franco, and E. E. Crone. 2012. Response of butterflies to structural and resource boundaries. Journal of Animal Ecology **81**:724–734.

Sei, M. 2009. Flight and Oviposition behavior of the adult maritime Ringlet (*Coenonympha nipisiquit* McDunnough) females in response to microhabitat. Journal of Insect Behavior **22**:87–100.

Shreeve, T. 1984. Habitat selection, mate location, and microclimatic constraints on the activity of the speckled wood butterfly *Pararge aegeria*. Oikos **42**:371–377.

Skorka, P., P. Nowicki, M. Lenda, M. Witek, E. B. Sliwinska, J. Settele, and M. Woyciechowski. 2013. Different flight behaviour of the endangered scarce large blue butterfly *Phengaris teleius* (Lepidoptera: Lycaenidae) within and outside its habitat patches. Landscape Ecology **28**:533–546.

Stevens, V. M., C. Turlure, and M. Baguette. 2010. A meta-analysis of dispersal in butterflies. Biological Reviews **85**:625–642.

Thomas, C. D. et al. 2004. Extinction risk from climate change. Nature **427**:145–148.

Thomas, C. D., and M. C. Singer. 1987. Variation in host preference affects movement patterns within a butterfly population. Ecology **68**:1262–1267.

Turlure, C., M. Baguette, V. M. Stevens, and D. Maes. 2011. Species-and sex-specific adjustments of movement behavior to landscape heterogeneity in butterflies. Behavioral Ecology **22**:967–975.

Van Dyck, H. 2012. Changing organisms in rapidly changing anthropogenic landscapes: the significance of the Umwelt'-concept and functional habitat for animal conservation. Evolutionary Applications **5**:144–153.

Van Dyck, H., and M. Baguette. 2005. Dispersal behaviour in fragmented landscapes: Routine or special movements? Basic and Applied Ecology **6**:535–545.

Van Dyck, H., A. J. Van Strien, D. Maes, and C. A. M. Van Swaay. 2009. Declines in common, widespread butterflies in a landscape under intense human use. Conservation Biology **23**:957–965.

Warren, M. S. et al. 2001. Rapid responses of British butterflies to opposing forces of climate and habitat change. Nature **414**:65–69.

Wickman, P. 1985. The influence of temperature on the territorial and mate locating behavior of the small heath butterfly, *Coenonympha pamphilus* (l) (Lepidoptera, Satyridae). Behavioral Ecology and Sociobiology **16**:233–238.

Wiens, J. A., N. C. Stenseth, B. V. Horne, and R. A. Ims. 1993. Ecological Mechanisms and Landscape Ecology. Oikos **66**:369.

Wilson, R., and C. D. Thomas. 2002. Dispersal and spatial dynamics of butterflies (Chapter 13). In: Bullock, J.M., Kenward R.E. and Hails R.S. (Eds.): *Dispersal Ecology*. pp 257-278. Blackwell Science Ltd., Oxford, United Kingdom.

Chapter 6: Concluding remarks

Key findings

Climate change is likely to have a negative impact on many species and populations as the distribution of climatically suitable areas may be shifting for some species (Thomas et al., 2004). Climate change is happening simultaneously with habitat fragmentation and degradation therefore presents a big challenge for many species. Some species will be able to respond, shift distributions and move large distances in order to colonise new climatic suitable areas, others will not (Fahrig, 2003; Hickling et al., 2006; Warren et al., 2001). To be able to predict species vulnerability and how they move in complex landscapes it is important to know how species respond to different factors in their environment and how dispersal traits may affect species capacity to respond to global environmental change. This information will be crucial to better model future ecological responses to climate change and habitat fragmentation and to improve our capacity to protect endangered species. This thesis aimed to enhance the current understanding of the role of dispersal in enabling species to respond to global environmental change, by answering four main questions; 1) Is butterfly movement within habitat linked to likelihood of crossing habitat boundaries? 2) Do core and margin populations of range-expanding species differ in their dispersal related morphology? 3) Is butterfly movement in the field correlated with morphological traits? 4) What is the relative importance of habitat quality and structure on butterfly movement within habitat? These results show varying responses of species and sexes to habitat boundaries (*Chapter 2*), habitat quality and structure (*Chapter 5*), temperature (*Chapter 3 and 5*) and selection during range expansion (*Chapter 3 and 4*) highlighting the complexity of factors affecting dispersal and movement traits of butterflies.

Behaviour at boundaries

By analysing the movement behaviour of seven butterfly species, this study showed that species that move more cross habitat boundaries more frequently (*Chapter 2*). The same applied also at an individual level; those individuals that moved with longer move bouts crossed boundaries more often. These results are not surprising as more mobile species and individuals are more likely to encounter habitat boundaries. Furthermore, crossing boundaries comes with less cost to highly mobile species and individuals due to higher likelihood of encountering suitable habitat again. However, the link between butterfly movement within habitat and likelihood of leaving habitat is not well known. More information of species dispersal abilities is likely to be required to confirm these results across more species but these results indicate that mean step length, which is relatively straightforward to measure, could be used as a proxy of species dispersal propensity as it was shown to be correlated with likelihood of crossing a habitat boundary and may therefore indicate butterfly likelihood of dispersal.

Changes at the expanding range margin

Because more dispersive individuals are more likely to reach new areas and colonise them it is expected that dispersal ability will evolve during range expansion (Dytham, 2009). Individuals at the expanding range margin have been found to display morphological traits associated with increased dispersal capacity (Hill et al., 1999; Hughes et al., 2003). This has implications for species ability to respond to climate change. Data, that was collected for three range-expanding butterfly species (*Anthocaris cardamines*, *Aricia agestis* and *Pyronia tithonus*) from the range margin and core areas of these species distributions, shows no consistent increase in dispersal capacity at the margin across the species (*Chapters 3 and 4*). Instead, only *A. agestis* females were found to have larger investment in thorax mass and wing length at the margin that could be attributed to range expansion. *Anthocaris cardamines* did not show differences between areas in either of the data sets (*Chapter 3 and 4*). *Pyronia tithonus* has longer wings at the margin sites (*Chapter 3*) but no difference was found in analysis that took into account allometry by including body mass in the model (*Chapter 4*). Also, *Maniola jurtina,* which is a close relative to *P. tithonus*, occurs at same habitats and has long established populations at both areas, females had heavier body and thorax mass and longer wings at the margin areas. This indicates that the differences for *P. tithonus* and *M. jurtina* were likely due to environmental factors rather than selection during range expansion. There are several reasons why increased investment in dispersal may have not been detected across all species: i) Dispersal in the field is not tightly linked with morphological traits in all species. In concordance with this are the results from *Chapter 3* that show only weak links between wing length and four different movement parameters across four butterfly species (*A. cardamines, P.*

napi, P. tithonus and *M. jurtina*); wing length correlated only with speed for *P. napi* and *M. jurtina*. ii) It is possible that increased dispersal is only necessary for successful range expansion for sedentary species with fairly fragmented habitat. *Aricia agestis* has been able to shift its range recently (Asher et al., 2001; Fox et al., 2006) and has shifted to using more host plants also in the northern parts of its distribution in the UK (Pateman et al., 2012; Thomas et al., 2001). However, it is considered relatively sedentary with only 1.5% of individuals moving distances over 500m (Wilson and Thomas, 2002) and the current study showed it to be highly restricted in its movement within habitat, and crossings of tall and dense boundaries were not documented probably due to their low frequency (*Chapter 2*). In comparison, *A. cardamines* and *P. tithonus* are less likely to require additional investment in dispersal ability to be able to expand their ranges. This is because *A. cardamines* is considered to be a species with high wanderlust (Cowley et al., 2001) and was found to move with long step lengths and cross boundaries readily (*Chapter 2*), and *P. tithonus,* that displays intermediate mobility (Cowley et al., 2001 and *Chapter 2*), uses a variety of grasses as its host plants (Asher et al., 2001) and is therefore not likely to be limited by the quantity of habitat available.

Butterfly movement in relation to habitat structure and quality

Butterfly movement between habitat patches has been investigated in relation to several variables including habitat isolation, host and nectar plant abundance (e.g. Grundel and Pavlovic, 2007; Heikkinen et al., 2005; Kuussaari et al., 1996). However, there are not many studies looking at species movement within habitat in relation to habitat quality and structure (Auckland et al., 2004; Fownes and Roland, 2002; James, 2006) despite it adding to understanding dispersal at a larger scale e.g. through influencing butterfly likelihood to leave a habitat patch (*Chapter 2*) and thereby the first stage of dispersal process (Ronce, 2007). In *Chapter 5*, I assessed the relative influence of habitat quality and structure and temperature on flight of range-expanding *A. agestis* and common and widespread *P. icarus* using a hierarchical partitioning method. The two species differed in their responses; *Aricia agestis* was not affected by any of the habitat quality or structure measures or temperature while *P. icarus* females responded to temperature, area and edge ratio and males to vegetation height. These results indicate that *A. agestis* is resilient to its environment and possibly responds to habitat quality at a coarser scale. This might have enabled this species to use low quality habitats that can function as stepping stones between high quality patches thereby facilitating its range expansion. The differences in responses to habitat quality and structure between these two species, that are close relatives, highlight how species specific responses to habitat quality and structure are and that there is likely to be plasticity in the way at which different species use environmental cues for dispersal. It also indicates that hierarchical partitioning is sensitive in picking these differences and is well suited for analysing movement data in which variables often co-vary and enables identifying variables that are important for management purposes.

Although butterfly flight was not found to be strongly affected by the habitat structure and quality variables measured here (*Chapter 5*), these are still likely to have an impact on population dynamics and range expansion through effects on other life stages. Habitat structure influences the microclimate within a site thereby affecting the temperatures at which eggs, larvae and pupae develop. These can influence the survival and morphology of the developing butterflies which can further influence the mobility of the species, although morphology and movement in the field might not be strongly linked (*Chapter 3*). Habitat structure, through its impact on temperature, shelter and shade, also influences vegetation composition and structure, which can also influence butterfly populations. Habitat quality may set limits to the population size a patch can support. Population size can influence the number of conspecifics encountered and therefore influence the rate of immigration and emigration to and from an area (Baguette et al., 1996; Baguette et al. 1998; Odendaal et al. 1989; Kuussaari et al. 1996). Based on these results, I would argue that habitat quality and structure has larger influence on butterfly flight and species capacity to colonise new areas, through the indirect influence on population sizes and though boundary effects (*Chapter 2*), than its direct influence on butterfly flight.

Impact of weather and temperature on butterfly flight

The impact of temperature on butterfly flight was studied in relation to morphology (*Chapter 3*) and habitat structure and quality (*Chapter 5*). Although temperature had a large influence on flight this was not found for all species and it was more pronounced in females: *Polyommatus icarus* females flew faster and with longer mean steps at high temperatures while *P. icarus* male and *A. agestis* female and male flight was not significantly influenced by temperature (*Chapter 5*). Of the four species studied in *Chapter 3* (*A. cardamines, P. napi, P. tithonus* and *M.*

jurtina) only the movement parameters of the Nymphalidae species (*P. tithonus* and *M. jurtina*) were affected by the temperature with mobility increasing more for females than males. Because mobility in the habitat has been correlated with likelihood of crossing boundaries (*Chapter 2*), changes in the climate, and increased temperature in particular, may increase rates of dispersal and therefore affect the rate of range expansion. This seems particularly likely because females, which are often responsible for colonising new areas, were the ones responding to temperature more (*Chapters 3 and 5*). This has already been suggested because i) time in flight can be a limiting factor for butterflies, particularly at temperate latitudes (Kingsolver 1983), ii) butterflies have been found to spend more time in flight with increasing temperatures (Cormont et al., 2011; Shreeve, 1984) and iii) more colonisations are reported during periods of warm weather (Cormont et al., 2011; Mitikka et al., 2008). As mentioned above, temperature is also likely to influence butterfly dispersal via earlier life stages and through host and nectar plants. Therefore, I would expect weather and environmental conditions to have more pronounced impact on butterfly dispersal than possible changes in dispersal capacity during range expansion.

Importance of measuring populations also in the field

Measuring butterfly movement and morphology in the field presents challenges related to factors that cause a lot of variation that could potentially be controlled for in laboratory settings. Field studies also have limitations in that they do not allow measurements of maximum performance or at more extreme conditions that might be relevant for dispersal. However, measurements and samples taken in the field provide more realistic information on butterfly behaviour and differences between populations in their natural environment. For example, although measurements of butterfly movement within population cages can provide valuable insights (Leimar et al., 2003; Saastamoinen, 2007), they also represent movement at a very small scale and the influence of the cage on butterfly behaviour is hard to estimate. Similarly, using tape or horticultural windbreaks as surrogate hedges has its justifications (Dover and Fry, 2001) but is inevitably lacking in realism. Here we found no clear effect of wing length on butterfly flight behaviour measured in the field conditions (*Chapter 3*). This is contradicting earlier studies conducted in laboratory settings that link wing length to flight capacity (e.g. Chai & Srygley 1990; Dudley & Srygley 1994; Berwaerts et al. 2002).

Studying butterflies at common garden conditions provides information on how individuals descending from different populations differ in their morphology when raised at the same conditions. However, these normally represent only outcome from one set of conditions which might not be close to what the larvae experience in the wild. There can also be selection towards certain morphological traits during development in the wild that is not present in laboratory. For example, range-expanding *Polygonia c-album* butterfly showed differences in morphology for margin individuals but only in field collected samples and not for individuals raised in common garden conditions (Braschler & Hill 2007). The results from this study indicate that not all species show increased investment in dispersal morphology at the expanding range margin (*Chapter 4*) and that there can be considerable differences between populations of species that have existed in the area for long periods (*Chapter 3*). Therefore, by measuring morphological and behavioural differences both in natural field conditions and experimental settings, it is possible to gain complementary information of factors affecting movement.

Future work and challenges

Studying morphology of individuals that were collected from the field provides information of the actual phenotypes that are responsible for the colonisation of new areas. However, environmental variables during larval development are likely to influence the variation in morphology (Braschler and Hill, 2007; Gibbs et al., 2011; Pellegroms et al., 2009). To control for the effects of environment on morphology, I also studied common wide spread species that are close relatives to the range-expanding species, occur in the same habitats and use same/similar host plants with long established populations in both areas. Because differences in morphology between areas were found in control species it is likely that there were environmental differences between the areas of core and margin areas of some range-expanding species. To be able to determine what are genotypic differences between individuals, further work in common garden conditions would be required. This was attempted but was not feasible due to difficulty in finding enough individuals at the newly colonised areas and the required resources.

Although I expected to find differences in morphology in margin areas that were sampled up to 18 years after colonisation it is possible that there was strong selection against increased dispersal ability or selection related to other uses of flight and possible differences in the morphology would have been already lost, particularly in highly mobile *A. cardamines.* Including only sites that have been colonised recently (<5 years) was not possible due to distance between core and margin sites and because newly colonised areas were less likely to have sufficiently large populations for this study and harder to find.

The study of influence of morphology on butterfly movement did not reveal any interaction between temperature and morphology. One of the reasons for this could be that temperatures at which the study was conducted only represented conditions at which butterflies start their flight voluntarily. Here the temperature also represented ambient air temperature while butterfly body temperature might differ from ambient temperature due to behavioural temperature regulation e.g. via basking. Therefore, in future work it would be good to study how body temperature influences butterfly flight instead of only measuring ambient temperature. Body temperature would be a more accurate measure of how butterflies experience weather and therefore also wind and sun intensity. This is also further affected by the amount of pigmentation and fur which affects the rate of heat absorption. It would be interesting to know if butterflies at the edge of the range differ in their pigmentation and amount of fur as this can potentially mediate how weather influences butterfly activity and flight ability at different temperatures and thereby also colonisations and rate of range expansion.

To fully understand species movement at landscape levels, information on within habitat movement should be complemented with data on species movement at areas considered as unsuitable habitat. Knowing how fast butterflies move within unsuitable habitats would make it possible to estimate the “resistance” of different land use types on movement. This together with information on species responses to different types of boundaries would allow construction of spatially explicit individual based models. This would enable estimation of species movement at a landscape level, for example to explore colonisation likelihood in different landscapes and effects of stepping stones or lower quality habitat on population dynamics. These could then be used further for informing conservation management.

Another possibly important factor influencing the movement behaviour of butterflies is the density of conspecifics, which has been shown to influence dispersal and movement (Baguette et al., 1996, 1998; Travis et al., 2009), but was out of the scope of this study. Investigating the link between habitat quality variables, population sizes and movement would increase the understanding of impact of habitat quality on colonisation capacity through its impact on both adults and larvae.

Movement within habitat was shown to be correlated with the likelihood of species to cross habitat boundaries (*Chapter 2*). Studying this relationship across more species would allow to test if mean step length, which is fairly easy to measure, could be used as a proxy of species' or populations' dispersal propensity. There is also a plan to compare this individual movement data with data from MRR studies that was conducted in the same areas by looking at the diffusion coefficient of each species and sex estimated using the two different methods. This allows us to further estimate how well movement measured within habitat reflects movement at a larger landscape scale. It is important to develop ways to easily assess dispersal capacity across several species and populations because due to climate change more species are likely to require management actions to be able to track the environmental changes. Furthermore, in the future increasing habitat fragmentation is likely to increase the number of species that exist as metapopulations, in which species ability to survive is dependent on the dynamics of colonisations and extinctions (Hanski, 1999). Thus, understanding general movement patterns across species and developing habitat management methods that are beneficial to a range of species e.g. via enhanced connectivity of the landscape, is crucial.

Literature

Asher, J., M. Warren, R. Fox, P. Harding, G. Jeffcoate, and M. A. Jeffcoate, Stephens. 2001. Millennium Atlas of Butterflies in Britain and Ireland. Oxford University Press, Oxford United Kingdom.

Auckland, J. N., D. M. Debinski, and W. R. Clark. 2004. Survival, movement, and resource use of the butterfly *Parnassius clodius*. Ecological Entomology **29**:139–149.

Baguette,M., I. Convie, and G. Neve. 1996. Male density affects female spatial behaviour in the butterfly *Proclossiana eunomia*. Acta Oecologica **17**:225–232.

Baguette, M., C. Vansteenwegen, I. Convi, and G. Neve. 1998. Sex-biased density-dependent migration in a metapopulation of the butterfly *Proclossiana eunomia*. Acta Oecologica-International Journal of Ecology **19:** 17–24.

Berwaerts, K., H. Van Dyck, and P. Aerts. 2002. Does flight morphology relate to flight performance? An experimental test with the butterfly *Pararge aegeria*. Functional Ecology **16**:484–491.

Braschler, B., and J. K. Hill. 2007. Role of larval host plants in the climate-driven range expansion of the butterfly *Polygonia c-album*. Journal of Animal Ecology **76**:415–423.

Chai, P., and R. Srygley. 1990. Predation and the flight, morphology, and temperature of neotropical rain-forest butterflies. American Naturalist **135**:748–765.

Cormont, A., A. H. Malinowska, O. Kostenko, V. Radchuk, L. Hemerik, M. F. WallisDeVries, and J. Verboom. 2011. Effect of local weather on butterfly flight behaviour, movement, and colonization: significance for dispersal under climate change. Biodiversity and Conservation **20**:483–503.

Cowley, M. J. R., C. D. Thomas, D. B. Roy, R. J. Wilson, J. L. Leon-Cortes, D. Gutierrez, C. R. Bulman, R. M. Quinn, D. Moss, and K. J. Gaston. 2001. Density-distribution relationships in British butterflies. I. The effect of mobility and spatial scale. Journal of Animal Ecology **70**:410–425.

Dover, J.W., and G.L.A. Fry. 2001. Experimental simulation of some visual and physical components of a hedge and the effects on butterfly behaviour in an agricultural landscape. Entomologia Experimentalis et Applicata **100**: 221 – 233.

Dudley, R., and R. Srygley. 1994. Flight physiology of neotropical butterflies - Allometry of airspeeds during natural free-flight. Journal of Experimental Biology **191**:125–139.

Dytham, C. 2009. Evolved dispersal strategies at range margins. Proceedings of the Royal Society B-Biological Sciences **276**:1407–1413.

Fahrig, L. 2003. Effects of habitat fragmentation on biodiversity. Annual Review of Ecology Evolution and Systematics **34**:487–515.

Fownes, S., and J. Roland. 2002. Effects of meadow suitability on female behaviour in the alpine butterfly *Parnassius smintheus*. Ecological Entomology **27**:457–466.

Fox, R., J. Asher, T. Brereton, D. Roy, and M. Warren. 2006. State of Butterflies in Britain and Ireland. Pisces Publications, Newbury, United Kingdom.

Gibbs, M., C. Wiklund, and H. Van Dyck. 2011. Phenotypic plasticity in butterfly morphology in response to weather conditions during development. Journal of Zoology **283**:162–168.

Grundel, R., and N. B. Pavlovic. 2007. Resource availability, matrix quality, microclimate, and spatial pattern as predictors of patch use by the Karner blue butterfly. Biological Conservation **135**:135–144.

Hanski, I. 1999. Metapopulation ecology. Oxford University Press, Oxford, United Kingdom.

Heikkinen, R., M. Luoto, M. Kuussaari, and J. Poyry. 2005. New insights into butterfly-environment relationships using partitioning methods. Proceedings of the Royal Society B-Biological Sciences **272**:2203–2210.

Hickling, R., D. B. Roy, J. K. Hill, R. Fox, and C. D. Thomas. 2006. The distributions of a wide range of taxonomic groups are expanding polewards. Global Change Biology **12**:450–455.

Hill, J. K., C. D. Thomas, and D. S. Blakeley. 1999. Evolution of flight morphology in a butterfly that has recently expanded its geographic range. Oecologia **121**:165–170.

Hughes, C. L., J. K. Hill, and C. Dytham. 2003. Evolutionary trade-offs between reproduction and dispersal in populations at expanding range boundaries. Proceedings of the Royal Society of London Series B-Biological Sciences **270**:S147–S150.

James, M. 2006. Intra-patch movement in the Sinai Baton blue butterfly: influence of micro-habitat and individual characteristics. Egyptian Journal of Biology **8**:51–66.

Kingsolver, J. 1983. Ecological significance of flight activity in Colias butterflies - Implications for reproductive strategy and population-structure Ecology **64**:546–551.

Kuussaari, M., M. Nieminen, and I. Hanski. 1996. An experimental study of migration in the Glanville fritillary butterfly *Melitaea cinxia*. Journal of Animal Ecology **65**:791–801.

Mitikka, V., R. K. Heikkinen, M. Luoto, M. B. Araujo, K. Saarinen, J. Pöyry, and S. Fronzek. 2008. Predicting range expansion of the map butterfly in Northern Europe using bioclimatic models. Biodiversity and Conservation **17**:623–641.

Leimar, O., U. Norberg, and C.Wiklund. 2003. Habitat preference and habitat exploration in two species of satyrine butterflies. Ecography **26**: 474–480.

Odendaal, F. J., P. Turchin, and F. R. Stermitz. 1989. Influence of host-plant density and male harassment on the distribution of female *Euphydryas anicia* (Nymphalidae). Oecologia **78**: 283–288.

Pateman, R. M., J. K. Hill, D. B. Roy, R. Fox, and C. D. Thomas. 2012. Temperature-dependent alterations in host use drive rapid range expansion in a butterfly. Science **336**:1028–1030.

Pellegroms, B., S. Van Dongen, H. Van Dyck, and L. Lens. 2009. Larval food stress differentially affects flight morphology in male and female speckled woods (*Pararge aegeria*). Ecological Entomology **34**:387–393.

Ronce, O. 2007. How does it feel to be like a rolling stone? Ten questions about dispersal evolution. Annual Review of Ecology, Evolution, and Systematics **38**:231–253.

Saastamoinen, M., 2007. Mobility and lifetime fecundity in new versus old populations of the Glanville fritillary butterfly. Oecologia **153**: 569–578.

Shreeve, T. 1984. Habitat selection, mate location, and microclimatic constraints on the activity of the speckled wood butterfly *Pararge aegeria*. Oikos **42**:371–377.

Thomas, C. D. et al. 2004. Extinction risk from climate change. Nature **427**:145–148.

Thomas, C. D., E. J. Bodsworth, R. J. Wilson, A. D. Simmons, Z. G. Davies, M. Musche, and L. Conradt. 2001. Ecological and evolutionary processes at expanding range margins. Nature **411**:577–581.

Travis, J. M. J., K. Mustin, T. G. Benton, and C. Dytham. 2009. Accelerating invasion rates result from the evolution of density-dependent dispersal. Journal of Theoretical Biology **259**:151–158.

Warren, M. S. et al. 2001. Rapid responses of British butterflies to opposing forces of climate and habitat change. Nature **414**:65–69.

Wilson, R., and C. D. Thomas. 2002. Dispersal and spatial dynamics of butterflies (Chapter 13). In: Bullock, J.M., Kenward R.E. and Hails R.S. (Eds.): *Dispersal Ecology*. pp 257-278. Blackwell, Oxford, United Kingdom.

Appendices

Appendix A. *Tukey HSD test results of comparison of within family differences in rates of crossings.*

Species	*Species*	*Estimate*	*SE*	*z-value*	*P-value*
Anthocaris cardamines	*Pieris napi*	-1.470	0.694	-2.118	0.29
Coenonympha pamphilus	*Pyronia tithonus*	1.158	0.910	1.273	0.83
Coenonympha pamphilus	*Maniola jurtina*	0.041	0.626	0.066	1.00
Maniola jurtina	*Pyronia tithonus*	1.117	0.795	1.404	0.76
Aricia agestis	*Polyommatus icarus*	15.83	928.2	0.017	1.00

Appendix B. *Table of minimum, maximum, mean values and standard deviations for all the independent variables measured. PI=* Polyommatus icarus*, AA=*Aricia agestis*.*

	Temperature	*Nectar mean*	*Vegetation height*	*shelter*	*Edge ratio*	*Mean step*	*PI host*	*AA host*
min	16.50	3	2.03	1.00	2.75	0.27	0.00	0.00
max	28.50	6065	34.94	8.00	90.41	19.32	2.75	2.10
mean	21.07	978	16.63	3.64	39.75	3.88	0.75	0.48
sd	2.85	779	10.84	1.84	29.46	2.77	0.88	0.72

Appendix C. *Host and nectar plant mean cover scaling*

Plant mean cover	Index	Approximate cover %
0	0	0
0.1-1.4	1	1-5%
1.5-2.4	2	6-25%
2.5-3.4	3	26-50%
3.5-4.4	4	51-75%
4.5-5	5	76-100%