Habitat fragmentation and evolutionary ecology of movement behaviour in the speckled wood butterfly (Pararge aegeria L.)

Antwerpen, 2005

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1.1 Habitat fragmentation

1.1.1 A Mini Review

Humans have become an evolutionary force of extraordinary influence (Palumbi 2001). The high selection pressures that result from their activities cause evolutionary changes that often occur on contemporary timescales, often even within decades (Kinnison & Hendry 2001, Reznick & Ghalambor 2001). For instance, human activities select for rapid changes in pathogen biology, and hence contribute to the emergence and spread of new diseases (Altizer et al. 2003). Another example of the evolutionary impact of human activity is the influence of fisheries on life history characteristics (e.g. size, maturity) of intensively exploited fish populations (Jennings et al. 1998). But, one of the most obvious effects of human activity is habitat fragmentation.

Habitat fragmentation can be viewed of as a landscape-scale process involving two co-occurring components: habitat loss and the breaking apart of habitat – or fragmentation sensu stricto (Lord & Norton 1990, Fahrig 2003). The process results in a transformation of a large habitat into a number of smaller patches isolated from each other by a landscape matrix unlike the original habitat (Wilcove et al. 1986). Since ancient times, man has modified landscapes and consequently caused the fragmentation of habitats. During the last century the rate of change ever has accelerated due to dramatic changes in land use (e.g. the intensification of agriculture). Nowadays, habitat fragmentation is almost ubiquitous (Wiens 1997, Hanski 1999). Habitat loss is generally considered to be one of the most important bottlenecks for biodiversity (Meffe & Carroll 1997): it is regarded as the main cause of the current mega-extinction crisis world-wide (Pimm & Raven 2000). However, other major players are global climate change and biological invasions (Thomas et al. 2001, 2004, Clark et al. 2003, Clobert et al. 2004). The dramatic, negative effects of habitat loss on biodiversity (a/o Fahrig 2002) mask in most cases the
weaker effects of habitat fragmentation sensu stricto. The latter can be either positive or negative (Fahrig 2003). Moreover, theoretical studies suggest that the effects of fragmentation sensu stricto should only become apparent below a critical threshold of habitat area in a landscape (e.g. 20-30% threshold: Andrén 1994, 1999, Wirth & Christ 1995, Fahrig 1997, 1998, Flather & Bevers 2002).

According to the classic theory of island biogeography (MacArthur & Wilson 1967), species richness increases with patch size and decreases with patch isolation (a/o Thomas & Hassan 1993, Hanski et al. 1994, Steffan-Dewenter & Tscharntke 1999, 2000, Rick- etts et al. 2001). Possible reasons for these species-area-distance relationships are colonisation-extinction dynamics and increased habitat diversity in large compared to small areas (MacArthur & Wilson 1967, Connor & McCoy 1979). Depending on the geometry of the fragment, edge effects may further affect habitat quality, and hence, species diversity and community structure (Didham et al. 1998, Thomas et al. 2001). However, due to the recent focus on spatial population dynamics (Bjornstad et al. 1999), metapopulation biology (Hanski & Gilpin 1997) and landscape ecology (Wiens et al. 1993), it has become apparent that the size and isolation of individual habitat patches alone is often insufficient for predicting population persistence. A landscape integrated view may be more appropriate (Hanski & Ovaskainen 2000, Weibull et al. 2000, Fahrig 2001, Söderström et al. 2001).


1.1.2 EVOLUTIONARY CONSEQUENCES

Given the nature of habitat fragmentation (see 1.1.1.), it is straightforward to see that habitat fragmentation can cause new selection regimes. Moreover, it can both provoke and impede adaptive responses to other selection pressures. Within the context of cli-
and the area where reproduction first takes place (i.e. natal dispersal), and on movements between two successive breeding areas (i.e. breeding dispersal). However, this is a view typical for territorial and/or nestbound species. The distinction between these and other types of movement is not clear-cut (Clobert et al. 2001, Wiens 2001), and as a consequence some scientists contrastingly consider any movement to be dispersal, or at least relevant for dispersal (a/o Ide 2002). Anyway, dispersal can be realised in two different ways: as a by-product of routine, explorative movements (e.g. foraging, mate location), or, as special, directed movements designed for displacement (see Johnson 1993, Shrestha 1992a, Van Dyck & Baguette 2001).

Changed mobility patterns may be associated with morphological adaptations since a number of morphological traits is found to be functionally related to flight performance and dispersal abilities (e.g. bees: Steffan-Dewenter & Tscharntke 1999; beetles: Niemela & Spence 1991; bugs: Palmer & Dingle 1989; butterflies: Kuussaari et al. 1996, Steffan-Dewenter & Tscharntke 1997, Berwaerts et al. 2001, Van Dyck 2003; crickets: Fairbairn & Roff 1990). Evidence for morphological change in association with fragmentation has accumulated. The striking wing reduction in birds on oceanic islands (McNab 1994, Williamson 1981), for instance, is a clear example that habitat isolation may have evolutionary consequences related to mobility. However, the fragmentation of terrestrial habitats may also affect the morphological ‘design’ in more subtle ways. Empirical research on insects has provided interesting suggestions for different directional evolutionary change in flight morphology with landscape structure. Time-series of Papilio machaon and Maculinea arion butterflies suggested that flight muscle mass decreased with isolation (Dempster et al. 1976, Dempster 1991), whereas Calopteryx maculata damselflies and Plebejus argus, Hesperia comma and Melitaea cinxia butterflies were found to have a more ‘mobile morphology’ in fragmented landscapes (Taylor & Merriam 1995, Thomas et al. 1998, Hill et al. 1999a, Norberg & Leimar 2002). The reasoning behind these evolutionary changes in flight morphology is that costs and benefits of dispersal alter with landscape structure (a/o Olivieri & Guyon 1997). If dispersers are not a random genetic sub-sample of populations, but if they are characterised by a more ‘costly’ mobile and heritable morphology, isolated fragments might evolve toward lower mobility (Thomas et al. 1998, Hill et al. 1999b, Van Dyck & Baguette 2003). Besides the cost of resource allocation toward flight muscles instead of toward fecundity, other costs associated with dispersal are the costs of time and energy which cannot be used for other activities, and the higher risk among mobile emigrants to get lost into the matrix, or to get predated. These individuals are not likely to be replaced by mobile immigrants (i.e. recolonisation) since individuals traversing the hostile matrix experience higher mortality (Schtickzelle & Baguette 2003). Hence, the resulting mobility pattern is achieved as a balance between selection at the local patch and selection at the metapopulation level (i.e. ‘metapopulation’ effect). Selection for a reduced emigration rate is likely to be strongest in small remnants due to relatively high proportions of emigrating individuals (Kareiva 1989, Hill et al. 1996, Sutcliffe et al. 1997, Thomas & Hanski 1997). On the other hand, when resources have a more scattered distribution in the landscape, and are no longer concentrated in single fragments, but neither too isolated from each other, selection for higher mobility is expected to occur (a/o Olivieri & Guyon 1997; Hill et al. 1999a, Van Dyck & Matthysen 1999).

Summarising, individuals may respond adaptively to habitat fragmentation by means of changing movement rates. Depending on the scale of isolation, the success of dispersers can be reduced, and hence selection against mobility is expected. However, one can also expect fragmented landscapes to favour higher mobility when necessary resources are no longer concentrated but rather spread over different fragments.

1.2.2 Behavioural Phenotypes

Persistence of wildlife populations in the face of landscape change depends not only on adaptive morphological variation but also on behavioural responses. Several behavioural consequences of habitat fragmentation have been described. For example, activity and hunting behaviour of tawny owls (Strix aluco) is greatly affected by fragmentation: they have longer inter-perch distances and perch times in fragmented woodlands, which is explained by an altered availability of small mammals (Redpath 1995). Another example is the trade-off between foraging and anti-predatory behaviour of black-capped chickadees (Poecile atricapilla). In more deforested landscapes, chickadees ventured farther into the open. This altered behaviour can be explained by a constraint (increased energy stress), which forces the birds to take more risks. Therefore, it may increase mortality through greater exposure to predators (Turcotte & Desrochers 2003).

Although these examples only make up a very small selection of numerous behavioural responses toward fragmentation, we now want to know whether movement behaviour, in particular, is affected by fragmentation. There exist several observations on intra-specific variation in movements among populations in different landscapes (a/o Roland et al. 2000, Tischendorf & Fahrig 2000, Ricketts 2001, Brotons et al. 2003, Mennechez et al. 2003). Such altered movement patterns may compensate for a reduced structural connectivity of the landscape: more mobile individuals may still be able to locate resources, in spite of the larger distances between them. However, compensation at the population-level may be hampered when fragmentation is too severe or happens too fast, leading to spatial isolation of patches. Then, fragmentation may result in reduced movement among patches (e.g. beetles: Goodwin & Fahrig 2002; butterflies: Baguette et al. 2003, Schtickzelle & Baguette 2005; voles: Wolff et al. 1997) (see also 1.2.1.).

In order to know the mechanism behind the observed patterns, several authors have recently pleaded for mechanistic studies, since different patterns can be explained by different mechanistic scenarios. Dissections of the observed behaviour, using experimental set-ups, allow to better understand and predict how animals react to landscape elements and how they move through the landscape. Dispersal is basically a three-stage
process, consisting of emigration, transience and immigration. A more thorough and mechanistic knowledge of the complete process will increase the ability to manipulate it in favour of conservation purposes (Lima & Zollner 1996, Morales & Ellner 2002, Wilson & Thomas 2002, Schooley & Wiens 2003, Schtickzelle & Baguette 2003). Recently, behavioural aspects of dispersal have received increasing attention (a/o Jonsen & Taylor 2000a, b, Conradt et al. 2001, Bowman et al. 2002, Hanski et al. 2002). Several studies focus on behaviour at patch boundaries and effects of corridors on dispersal (a/o Haddad 1999, Ries & Debinski 2001, Schultz & Crome 2001, Berggren et al. 2002, Harris & Reed 2002), or on the effect of patch area on emigration decisions (Sutcliffe et al. 1997, Baguette et al. 2000, Schtickzelle & Baguette 2003). Studies on perceptual ranges are also of high relevance to understand how animals moving through the landscape respond to their environment (a/o Yeomans 1995, Zollner & Lima 1997, Zollner & Lima 1999, Zollner 2000, Schooley & Wiens 2003, Bonte et al. 2004). Yet, for most groups, information on dispersal behaviour is scarce. This makes it difficult to assess how isolated or hostile a specific environment is for a specific population (Tscharntke et al. 2002). Nevertheless, such information is critical for predicting effects of fragmentation on populations as shown by simulation models (Fahrig 2003).

1.2.3 PLASTICITY VERSUS DIVERGENCE
Coping with changing environmental conditions, and hence with variable local selection pressures, is a fundamental aspect of organic evolution (Pigliucci 1996). The neo-Darwinian view of adaptation maintains that populations tend to evolve characteristics that make them well suited to the particular conditions experienced over a long period of time and a small spatial scale. However, organisms experience a variety of environments during their life cycle, and it is common that a given genotype can produce different phenotypes when exposed to different environments. So, in order to cope with varying or changing environments, populations can follow two general pathways. The first one is genetic adaptation and divergence: natural selection of present genetic variation results in local adaptation, at least when the amount of gene flow is not too high (e.g. Postma & van Noordwijk 2005). The second pathway is phenotypic plasticity. This option is characterised by single genotypes producing different phenotypes based on environmental cues (Agrawal 2001). Phenotypic plasticity is a measure of how different the phenotypes produced in distinct environments are from each other. In other words, it is a measure of sensitivity to external conditions. Reaction norms are functions describing these phenotypes over a certain range of environmental conditions. The study of phenotypic plasticity and reaction norms has attracted much attention in modern evolutionary ecology (Schlichting 1986, Sultan 1987, West-Eberhard 1989, Scheiner 1993, West-Eberhard 2003). Most of the time, plastic responses are highly specific and are co-ordinated by an array of regulatory genes acting at different hierarchical levels (Pigliucci 1996). The degree to which a genotype responds in a plastic way to environmental variation (i.e. the reaction norm) can be under genetic control (West-Eberhard 2003). Natural selection on traits can only occur when traits are variable and heritable (i.e. genetic basis). When considering phenotypic plasticity as a trait, the variability lies within the slopes of the reaction norms. If different genotypes possess different reaction norms one defines this variability as genotype-by-environment interactions. In that way, the pattern of sensitivity to external conditions may be altered adaptively (Blanckenhorn 1991, Price et al. 2003, West-Eberhard 2003).

Both pathways can be followed at the same time. For instance, we know that many speciation events result from colonisation of new environments. Such events go together with strong selection pressures but in the mean time they also directly induce changes in behaviour, morphology and physiology (i.e. phenotypic plasticity) (Price et al. 2003). Thus, phenotypic plasticity may permit recently colonised populations to survive in new environments during the first generations. In other words, phenotypic plasticity may bridge the time-gap during which individuals are not yet sufficiently adapted to the novel environment (Price et al. 2003, Yeh & Price 2004). Hence, phenotypic plasticity can be viewed as a trait that facilitates adaptation. In the absence of phenotypic plasticity, the trophic radiation and subsequent speciation as observed in Old World haplochromine cichlids, for example, most probably would have been less extensive and explosive (Stauffer & Gray 2004).

1.3. Butterfly movement biology
Butterflies have been popular study organisms for a long time: they were and are used to study questions relating to physiology, development, behaviour, ecology, evolution, and systematics (Boggs et al. 2003). Butterfly movements have attracted an increasing interest: Ehrlích (1961) was among the firsts to perform mark-release-recapture studies. Later, butterflies played a major role in the development of metapopulation theory (Hanski & Gilpin 1997). Nowadays, research on butterfly movements gradually focuses on the behavioural underpinning of movements in a landscape ecological context (Burel & Baudry 2003, Conradt et al. 2003, Schtickzelle & Baguette 2003).

1.3.1 MULTI-FUNCTIONALITY OF FLIGHT
In many insects, including butterflies, flight is multifunctional (Shreeve 1992a, Dudley 2000, Watt 2003). For instance, it is used for different activities like mate location, predator-avoidance, foraging, and finding oviposition sites. Thus, flight performance may have an important impact on realised fitness (Bervoets & Van Dyck 2004). In order to locate receptive females, male butterflies typically ‘wait or seek’. Patrollers actively search, while perchers sit-and-wait at particular spots (Scott 1974, Wickman & Wiklund 1983, Shreeve 1987). A study on several temperate butterfly species demonstrated that flight design was related to mating system. Males of perching species had
larger thorax/body mass ratio, higher wing loading and more pointed wings than patrolling species (Wickman 1992). A similar relationship was found at the intra-specific level in the speckled wood butterfly Pararge aegeria (Van Dyck et al. 1997a). Just as is the case for mate location strategy, the need to evade predatory birds by flight affects flight patterns and flight morphology. For example, neotropical butterflies that are palatable – and hence fly more erratically – have more of their mass allocated to flight muscles and tend to have shorter wings than unpalatable ones, that fly more slowly and straightly (Chai & Srygley 1990, Srygley & Chai 1990a, b, Marden & Chai 1991). Yet, whether and to what extent movements used for these different functions take part in dispersal between habitat patches is a matter of discussion (see also 1.2.1). In case they are not relevant for inter-patch movements, it would be best to refer to them as ‘trivial movements’, or as ‘station keeping’ (sensu Southwood 1962 and Dingle 1996, respectively). But in some cases these movements could have a significant impact on inter-patch movements and, hence, they could be of significance for gene flow and dispersal. Moreover, even truly trivial movements could confound interpretations with regard to dispersal. For instance, when predation pressure or mating system change in parallel with a change in habitat structure, it is difficult to disentangle the effects of habitat structure on dispersal capacity versus the effects on ability to escape predators or to locate mates. Merely looking at flight morphology is not sufficient, due to the multi-functionality of flight (Van Dyck & Matthysen 1999). Anyway, flight performance in ectothermic insects directly depends on thoracic (i.e. flight muscle) temperature, which is the outcome of both heat loss due to convective cooling and heat gained by means of thermoregulation (Watanabe & Imoto 2003).

### 2.2.2 THERMOREGULATION

Flight requires considerable quantities of energy. In ectotherms, flight performance and thermoregulation are inextricably linked (Kingsolver & Huey 1998, Chown & Nicolson 2004). In order to deliver the required amount of energy, body temperature typically needs to be situated within critical thermal limits (Dudley 2000, Chown & Nicolson 2004). Any factor that affects body temperature may have a strong impact on metabolic activity and flight performance and, hence, on individual fitness (Kingsolver 1987, Gilchrist 1995, Forsman 1999). Convective cooling is such a factor. It results from the impact of wind, but is also caused by flying itself. The endogenous heat produced when flying cannot compensate for the heat dissipated through convective cooling (Shelly & Ludwig 1985, Heinrich 1993), except for some larger insects (Heinrich 1975, Heinrich & Mommers 1985, Dudley 2000). Therefore, individuals need to stop frequently in order to behaviourally warm-up toward an optimal temperature by basking. Thermoregulatory adaptations are thought to account for a significant proportion of inter- and intra-specific phenotypic diversity in animals, including diversity due to phenotypic plasticity (Kingsolver 1987, Kingsolver & Wiernasz 1987, Kingsolver 1991).

Butterflies have been used extensively as ‘model’ animals for the study of thermoregulation and the co-evolution of morphology and thermoregulatory behaviour (i.e. Digby 1955, Clench 1966, Wasserthal 1975, Kingsolver 1985a, b, Heinrich 1986, Dennis 1993, Rutowski et al. 1994, Srygley 1994, Kemp & Krockenberger 2002, Watt 2003). In order to fly efficiently, butterflies need to elevate the temperature of their thoracic musculature considerably above air temperature (Watt 1968, Heinrich 1993): 34-37°C in several temperate butterflies (Vielmetter 1958, Douwes 1976, Rutowski et al. 1994, Dreissig 1995). Since internal heat production by shivering is rare in butterflies, which are typically heliothermic, most species select strategic microhabitats and apply specific basking body orientations and postures (Casey 1988, Dennis 1993, Rutowski et al. 1994). Heat transfer is further facilitated by morphological adaptations (Dennis & Shreeve 1989, Schmitz 1994, Berwaerts et al. 2001, Berwaerts & van Dyck 2004), such as colour pattern and amount of body fur. Fur functions as an insulator. It counteracts the convective cooling effect of cool air that moves over the body (Kingsolver & Moffat 1983, Kingsolver 1985, Watanabe & Imoto 2003). In this respect, size is known to be a crucial factor: smaller butterflies are more constrained to be on the wings during marginal weather conditions (Heinrich 1986). Melanisation of the basal wing parts increases solar absorption during basking (Van Dyck & Matthiesen 1998, Berwaerts et al. 2001). Trapped heat is then conducted to the nearby thorax and flight muscles. This mechanism allows dark-coloured individuals to raise their body temperature to the thermal optimum for activity faster than pale individuals (Kingsolver 1987, Van Dyck & Matthiesen 1998). Conversely, pale morphs can be at advantage in environments in which overheating is likely (Ottenheim et al. 1999). As a result, for instance, basal melanism increases with altitude and latitude (Guppy 1986, Kingsolver 1985, Kingsolver & Wiernasz 1987, Ellers & Bogg 2004), since even small increases in pre-flight warm-up rates owing to melanisation appear to have a strong selective pressure (Watt 1968). Wing surfaces play an important role in the process of heat transfer, either by conductance of absorbed heat from the basal wing areas to the thorax (Wasserthal 1975, Kingsolver 1987), reflectance of solar radiation onto body tissues (Kingsolver 1985a) or via shielding body tissues from solar radiation (Rawlins 1980). Basically, three species-specific basking mechanisms are proposed for butterflies. Dorsal and lateral basking are most widespread and involve orientation of the body so that either dorsal or ventral wing surfaces are perpendicular to the plane of incident solar radiation (Shreeve & Dennis 1992, Rutowski et al. 1994). The thorax is warmed by direct exposure to the sun, and from heat conducted from basal wing regions. A third posture involves angling the wings downward so that the distal margins are appressed to the substrate. This appression posture could be viewed of as an extension of dorsal basking most often adopted under relatively cool conditions (Kemp & Krockenberger 2002). These mechanisms imply that large areas of wing surfaces bear limited thermal consequences. In the absence of genetic correlations between the colour of basal versus more distal wing parts (but see Kingsolver &
Wiernasz 1991), the outer wing parts are isolated from selection for thermoregulatory performance (Wasserthal 1975). Then, they may freely function adaptively in other contexts, such as aposematic signalling and mate recognition (Vane-Wright & Bopper 1993, Jiggins et al. 2001, Kapan 2001, Ellers & Boggs 2003).

Since both thermoregulation and convective cooling are strongly influenced by microclimatic parameters (e.g. Shipp et al. 1987, Peng et al. 1994), flight performance and mobility of insects are dependent on the physical structure of the local environment, and, hence, it is dependent on microclimate (Vielmetter 1958, Kingsolver & Watt 1983, 1984). Habitat fragmentation goes along with altered landscape structures. Such changed structures cause alterations in microclimatic conditions, such as ambient temperature, ground temperature, wind speed and the amount of solar radiation that penetrates the habitat. Altered microclimatic conditions may select for adaptations in optimal thoracic temperature for flight in ectothermic insects. In the same vein, fragmentation may influence thermoregulation behaviour and/or thermoregulation-related morphology in order to counteract effects on thoracic thermal budget (Meyer & Sisk 2001).

### 1.3.3 Sexual Differences

Effects of habitat fragmentation, that cause microclimates to change also, may be different for males versus females, since both sexes differ in many respects (e.g. mobility, flight-morphology, thermoregulation-related morphology). Consequently, trade-offs in one sex may be differently affected by fragmentation in the other sex. In particular, females are limited by the number of zygotes they can produce and, therefore, will maximise offspring quality through resource acquisition or mate choice. Males are limited by the number of receptive mates (Andersson 1994). In most butterfly species, females mate soon after eclosion and often mate only once (Wedell et al. 2001). Hence, from a male's perspective, females are rare resources. As a result, and as predicted from sexual selection theory, males will favour early emergence (i.e. protandry) over large size under most circumstances (Wiklund & Fagerström 1977, Fischer & Fiedler 2000, 2001). Male and female behaviour during courtship and mating agrees well with sexual selection theory (Rutowski 1984, Silberglied 1984, Anderson 1994). Males are aggressive to rivals and persistent in locating and courting females. Females are more ‘reserved’ and effective at rejecting males (Wiklund & Forsberg 1991). Such decisions seem to be dominated by olfactory cues. Although butterfly sexes differ in coloration pattern, and though male coloration is in general more brilliant (Darwin 1859), female choice is not governed by stimuli from the coloration pattern of males. Females use visual cues primarily in the UV (Rutowski 1982). Hence, the major selective agent responsible for brilliant male coloration is intra-sexual communication between males (Silberglied 1984). Butterfly sexes differ considerably in flight behaviour relating to their different ecological functions (Shreeve 1992a, Van Dyck 2003). Males spend most of their active time to mate location (Shreeve 1992a), but this mainly covers local movements. Females have been suggested to be the more dispersive sex in butterflies (Baker 1984, Hill et al. 1999a, Merckx et al. 2003, Hanski et al. 2004). While females spend most of their active time searching for host plants, male behaviour and distribution is completely dependent on female behaviour and distribution (Wiklund 2003). As a result of the ample sexual differences, and hence of different reactions and sensitivities, it is important to look at both sexes when it comes to study effects of habitat fragmentation.

### 1.4 Model species: Pararge aegeria

We chose *P. aegeria* as a model to study effects of habitat fragmentation on the evolutionary ecology of movement behaviour. *P. aegeria* is a temperate-zone satyrine butterfly (Lepidoptera: Nymphalidae) that primarily occurs in woodland with dappled sunlight (Tolman & Lewington 1997). The butterfly also occurs in more fragmented agricultural landscape and other habitat types with some kind of woodland aspect, including parks, gardens and along hedgerows in the more central and southern parts of its distribution (Dover & Sparks 2000). Throughout Europe it is a non-threatened, widespread species and it has recently expanded in several countries, meanwhile extending its range northwards (Maes & Van Dyck 1999, Asher et al. 2001). These changes in distribution are associated with two factors: climate and habitat change. In many parts of Europe, woodland habitats have substantially changed: the decline of coppicing during the twentieth century affected butterflies of shady woodland in a less detrimental way than it affected early successional species, such as fritillaries (a/o Gorissen et al. 2004). The spread of *P. aegeria* also suggests the strong influence of recent climate changes. However, the ability of *P. aegeria* to colonise areas as they become climatically suitable is likely to be constrained by the availability of potential habitat (Hill et al. 1999c, 2001).

Eggs are laid singly on leaves of various grasses. In spring and autumn, females tend to lay eggs in warm, sheltered spots on the edges of woodland, but in summer they choose more shady positions within woodland (Wiklund & Persson 1983, Shreeve 1986a, b). The cryptic larvae rest under the grass blades, and feed on the edges of the leaves. The pupae are formed on the host plant or on other vegetation nearby. The life style is unusually complex: this species hibernates at either the larval or pupal stage and three developmental pathways are possible (a/o Lees & Tilley 1986, Wiklund et al. 1983, Van Dyck & Wiklund 2002). These pathways are characterised by complex reaction norms cued by photoperiod and temperature (Nylin et al. 1989, 1993, 1995, Gotthard et al. 1994, Silby et al. 1997, Nylin & Gothard 1998, Van Dyck & Wiklund 2002). As a result of the different pathways, there are usually three generations each year in Belgium. However, the flight periods of each generation tend to overlap. Consequently, this species can be seen at almost any time from March to October.

Both *P. aegeria* males and females rely on sunlit patches at the forest floor to thermoregulate behaviourally by dorsal basking (sensu Clench 1966). When the butterfly...
starts to fly voluntarily after a period of basking, body temperature is usually within a range of 30-34°C (Shreeve 1984, Van Dyck & Matthysen 1998). During flight, body temperature decreases by convective cooling, which forces the butterfly to bask again (Van Dyck & Matthysen 1998). Hence, the duration of a single flight bout increases with ambient temperature (Shreeve 1984). Both sexes mainly feed on honeydew and are rarely seen feeding on flowers, except early and late in the year when aphid activity is low (Asher et al. 2001). The behaviour of males makes them more conspicuous than females, which spend more time basking or feeding (Shreeve 1992). Males adopt one of two strategies for locating mates: perching in a territory (i.e. a small spot of sunlight, from where they rise rapidly to intercept any intruder) or patrolling through the habitat. Van Dyck et al. (1997a) have shown that males with paler wing colour are predominantly territorial and darker males are predominantly patrollers. This is thought to be because darker males warm up more quickly when basking, and, therefore, are able to spend longer periods in flight (Van Dyck & Matthysen 1998). So, thermal requirements differ between the behavioural strategies, and behavioural differences between phenotypes relate to differences in thermal ecology (Van Dyck et al. 1997a, Van Dyck & Matthysen 1998). Perchers also have a relatively large thorax, a difference which is explained to be adaptive. It facilitates individuals to accelerate at a higher level, which could be of advantage in escalated fights (Van Dyck 2003 and references therein).

We chose the speckled wood (Pararge aegeria L.) as a model to investigate several aspects of evolutionary ecology of movement behaviour in the context of habitat fragmentation. A preliminary analysis revealed differences in morphological design among differently fragmented landscapes (Berwaerts et al. 1998). Moreover, several morphological traits in males and females have been found to possess a genetic basis (Van Dyck et al. 1998, Berwaerts 2004), and morphological design has been interpreted in terms of performance (Berwaerts et al. 2002). Furthermore, P. aegeria is one of the most studied butterfly species; extensive background knowledge made it easier to test the research questions.

1.5 Study areas

The research described in the following chapters is based upon observations during field studies as well as upon data collected by means of experiments. Experimental designs included reciprocal transplant experiments and observations in common garden set-ups. Observed individuals originated from five study sites. Those were situated in central Belgium in three contrasting types of landscape that reflect different degrees of fragmentation: (i) large continuous oak woodlands (‘Meerdaalwoud’ (1255 ha) and ‘Bos ter Rijst’ (280 ha)), (ii) a set of small (1.3 to 19.0 ha) oak woodland fragments, covering 8% of the study area (‘Boshoek’), with relatively small inter-patch distances (85 to 865 m), and (iii) highly fragmented, agricultural landscapes composed of intensively used fields and pastures (the large majority of the area) and to a lesser extent houses and farms, orchards, tiny woodlots and several sunken roads with hedgerows (‘Hoegaarden’ and ‘Rillaar’). The ‘Hoegaarden’ study area (555 ha) consisted of 33 sunken roads with hedgerows that made up a distance of 8.9 km in total. Only 3% of the study area was covered by six small (0.4 to 11.2 ha) and mainly poplar woodlots. The ‘Rillaar’ study area (401 ha) consisted of 15 sunken roads with hedgerows (3.9 km) and six small oak woodlots (3% of the study area; 0.3 ha to 6.3 ha). The depth of the sunken roads – eroded agricultural tracks bordered by steep grassy and woody banks – varied from 0.5 m to several meters; their width from 5 to 25 m and their lengths from 80 to 1370 m (but sometimes with gaps of some meters). Distances between study landscapes range from 7 to 60 km and are situated within regions with similar topography, soil and macroclimate (Figure 1.1).

Figure 1.1: Distribution of the five study sites on a map covering parts of the provinces Vlaams-Brabant and Antwerpen (Flanders, Belgium) and that distinguishes between optimal and suboptimal habitat for P. aegeria. Optimal habitat is oak woodland; Suboptimal habitat is all other habitat (e.g. deciduous woodland, structure rich conifer woodland, orchards, hedgerows, sunken roads) (Based upon a biological validation map of Flanders: version 1.0-2.0-2.1, Institute of Nature Conservation, Brussels).
Within the context of habitat fragmentation we address evolutionary ecological questions related to movement behaviour in the butterfly P. aegeria. Habitat fragmentation is considered to be the major cause of the current mega-extinction crisis (Pimm & Raven 2000). However, fragmentation may also affect species in more subtle ways. Our study species copes with highly fragmented, agricultural landscapes. Nevertheless, P. aegeria is a common and widespread butterfly species (Maes & Van Dyck 1999, Asher et al. 2001).

In order to examine how P. aegeria deals with habitat fragmentation, we observed and experimentally compared populations from differently fragmented landscapes. Since phenotypic plasticity is a manner to deal with varying environments (West-Eberhard 2003), we first examined whether butterflies show landscape-related plasticity in flight morphology. Altered landscape structures imply altered distributions of resources, that may select for changed abilities to cross distances among resources. Hence, it may be adaptive to respond with changes in flight morphology (a/o Dempster et al. 1976, Van Dyck & Matthysen 1999). In the case of ectotherms, microclimate is another factor that directly affects the cost of flying (Kingolver & Huey 1998, Dudley 2000, Chown & Nicolson 2004). Therefore, we first examined to what extent woodland landscapes differed from highly fragmented agricultural landscapes in terms of microclimate. Next, we tested whether these contrasting microclimates had an impact on thermal budgets for flight. Finally, we addressed how P. aegeria could compensate behaviourally and morphologically for microclimatic differences. Given these morphological and micro-climatic differences among both contrasting types of landscape, we experimentally tested whether individuals originating from both types of landscape differ in their ability to fly at suboptimal to even critically low temperatures, using large climate chambers. Additionally, we experimentally tested whether flight ability was differently affected with changing temperatures in both sexes. Since differences in thermal properties and in the distribution and frequency of resources among landscapes may directly affect the cost of flying, the level of movements (e.g. mate location behaviour, dispersal propensity, habitat-detection capacity) may differ among differently fragmented landscapes.

Phenotypic plasticity in flight morphological traits:
- We tested for landscape-related plasticity in flight morphology using a reciprocal transplant experiment with offspring of females from differently fragmented landscapes (Chapter 2)
- We examined whether this plasticity is able to respond to natural selection (Chapter 2)

Thermoregulatory needs for flight:
- We compared microclimate among contrasting types of landscape (Chapter 3)
- We investigated differences in thermal budgets for flight (i.e. cooling distance, heating rate) among these landscapes, and we estimated the impact of microsite selection (i.e. vegetation structures, flight height) on thermal budgets, using P. aegeria dummies as models (Chapter 3)
- We tested for differences in thermoregulation-related morphology among populations from these contrasting types of landscape (Chapter 3)
- We looked for differences in flight ability between types of landscape and sex, by means of testing laboratory-reared offspring from both types of landscape in flight cabinets at several low temperatures (Chapter 4)

Movements:
- We observed and compared mate location behaviour in the two contrasting types of landscape (Chapter 5)
- We studied movements and habitat boundary crossing of laboratory-reared individuals that originated from these landscapes, using an experimental landscape as a common environment (Chapter 6)
- We performed a release-experiment, with a reciprocal transplant set-up, to test for differences in habitat-detection capacity between individuals from these contrasting types of landscape (Chapter 7)

This dissertation is presented as a compilation of published, accepted and submitted articles. It presents the results in different chapters. At the moment of writing, chapter 6 is published in the Proceedings of the Royal Society of London series B, chapter 5 is accepted for publication in Animal Behaviour, chapter 2 is submitted to Oecologia, chapter 3 to Functional Ecology, chapter 4 to Biology Letters and chapter 7 to Journal of Animal Ecology. Chapter 8 concerns a general discussion in which we discuss the results in a larger framework and in which we formulate future directions.
Landscape structure and phenotypic plasticity in flight morphology in the butterfly Pararge aegeria

[Thomas Merckx & Hans Van Dyck]

In evolutionary time, varying environments may lead to different morphs as a result of genetic adaptation and divergence or of phenotypic plasticity. Landscapes that differ in the extent of habitat fragmentation may provide different selection regimes for dispersal, but also for other ecological functions. Several studies on flying insects have shown differences in flight morphology between landscapes, but whether such differences result from plastic responses have rarely been tested. We did a reciprocal transplant experiment with offspring of speckled wood butterfly females (Pararge aegeria) from three types of landscape differing in fragmentation: woodland landscape, landscape with woodland fragments and agricultural landscape with only hedgerows. Young caterpillars were allowed to grow individually on potted host grasses in small enclosures under the three landscape conditions (split-brood design). Mortality in caterpillars was much higher in agricultural landscape compared to the other landscapes. Additive to the effect of landscape of development, landscape of origin also affected mortality rate in a similar way. Flight morphology of the adults resulting from the experiment differed significantly with landscape. Independent of the landscape of origin, males and females that developed in agricultural landscape were the heaviest and had the greatest wing loadings. Females that developed in agricultural landscape had heavier thoraces (i.e. greater flight muscle allocation) in line with adaptive predictions on altered dispersal behaviour with type of landscape. In males, relative thorax mass did not respond significantly relative to landscape of development, but males originating from landscape with woodland fragments allocated more into their thorax compared to males from the other types. We found significant G x E interactions for total dry mass and wing loading. Our results suggest the existence of landscape-related phenotypic plasticity in butterfly flight morphology.
Introduction

In evolutionary terms, organisms have in principle two alternatives to deal with varying environments. They can either follow the pathway of genetic adaptation and divergence, or phenotypic plasticity. For instance, seasonal morphological variation can result from differential success of different genetic forms (e.g. Cepaea nemoralis — Cain & Sheppard 1954), or, of plastic responses of the genotype based on environmental cues producing different phenotypic forms (e.g. seasonal polyphenism — Shapiro 1976). The reaction norm or the degree to which a genotype responds in a plastic way to environmental variation can be under genetic control, and hence, be subject to selection (Price et al. 2003).

Habitat fragmentation is a widely recognised phenomenon that alters the environment in multiple ways for several organisms (Meffe & Carroll 1997, Hanski 1999, Fahrig 2003) providing different selection regimes. In this vein, several studies using flying insects as study models have shown differences in flight morphology between landscapes (e.g. Dempster 1991, Taylor & Merriam 1995, Thomas et al. 1998, Hill et al. 1999a, Norberg & Leimar 2002). Morphological patterns have typically been interpreted in terms of assumed changes in dispersal rates with habitat fragmentation, such as selection against mobility with increased isolation in line with Dempster et al. (1976).

Several of those flight-morphological traits (including total body mass, relative thorax mass, wing loading and forewing aspect ratio) have a heritable basis (Hill et al. 1999b, Berwaerts et al. 2004), which is a prerequisite for evolutionary change. In seasonal environments, however, there is evidence for adaptive phenotypic plasticity in the same morphological and life history traits in multivoltine butterflies (Fric & Konvicka 2002, Van Dyck & Wiklund 2002). Reaction norms for morphological traits (and life history traits) may differ — ultimately in adaptive terms — between geographic regions (Nylin et al. 1995) or even between populations at close proximity (Sibly et al. 1997). We are not aware of studies testing adaptive morphological plasticity in relation to landscape structure in a context of habitat fragmentation. Moreover, plasticity studies have only rarely been done under field conditions (Lorenzon et al. 2001). In the laboratory, environmental regimes with changed means without changes in variance appear to be the rule (but see Miner & Vonesh 2004). Besides practical constraints, the obvious reason to avoid field experiments relates to the fact that field conditions are typically too complex and variable. However, to test associations between phenotypic plasticity and landscape structure, reciprocal transplant experiments provide an interesting scope. Such experiments are of course limited because they do not allow insight in the environmental cues and causal factors that are operating.

In this paper, we evaluate to what extent a particular genotype produces different phenotypes in different landscapes using the speckled wood butterfly (Pararge aegeria L.) as a model. This butterfly is primarily a woodland species (Tolman & Lewington 1997), but it also occurs in fragmented, agricultural landscape with only small and scattered pieces of ‘woodland’ habitat like woodlots and hedgerows (Dover & Sparks 2000, Merckx et al. 2003). There is considerable knowledge on the functional flight morphology in this species (Berwaerts et al. 2002, Van Dyck 2003 and references therein), and earlier work has suggested morphological differences between landscapes (Berwaerts et al. 1998). Here, we performed a reciprocal transplant experiment with offspring of females from three landscapes differing in the degree of fragmentation: continuous woodland landscape, agricultural landscape with several nearby woodland fragments, and intensively used agricultural landscape with only hedgerows and a few woodlots (hereafter referred to as woodland landscape, landscape with woodland fragments, and agricultural landscape, respectively). Following a split-brood approach, offspring of each individual female was distributed evenly among these three landscapes. Young caterpillars were allowed to grow individually on potted host plants in small enclosures under local field conditions. Hence, it is also possible to monitor mortality during development and to explore survival rate relative to landscape of origin and of development. After eclosion, adults were collected to analyse their morphology.

We test for adaptive differences in flight morphology assuming that dispersal rate has changed with landscape structure. Flight morphology includes relative thorax mass (i.e. allocation to flight muscles), wing loading and forewing aspect ratio (i.e. wing shape). For a detailed discussion of the functional significance see Dudley (2000), Berwaerts et al. (2002) and Van Dyck (2003). However, butterfly flight is multifunctional (Shreeve 1992b, Watt 2003) and is not exclusively related to dispersal ability, which may confound interpretations (Van Dyck & Matthysen 1999). P. aegeria males and females differ considerably in their flight behaviour relating to their different ecological roles (Van Dyck 2003). Males spend most of their active time in mate location. They either adopt an aggressive territorial sit-and-wait strategy on a sunlit patch (‘perching’) or a search strategy in which a male explores a wider area looking for receptive females (‘patrolling’) (Wickman & Wiklund 1985, Shreeve 1987). In either case, mate location mainly covers local movements. On the other hand, there is evidence that females are the more dispersive sex. At considerable distance outside woodland habitat, Baker (1984) only observed P. aegeria females traversing the landscape. This is anecdotal evidence, but stronger support comes from comparative work on flight morphology between recently colonised sites (at the edge of the distribution) versus permanently populated sites (at the core area of the distribution). Hill et al. (1999b) found significant differences in P. aegeria females only; females from newly established populations were larger, had larger thoraces and had lower aspect ratios. In our earlier work testing variation in dispersal propensity in experimental cages, we found only clear responses in P. aegeria females (Merckx et al. 2003). So, it makes sense to restrict a test on dispersal-related variation in flight morphology to females only. As a result of altered costs and benefits of dispersal, fragmented landscapes are expected to select for higher mobility since resources are no longer concentrated but scattered over different fragments (e.g. Olivieri & Gosoyin 1997, Van Dyck & Matthysen 1999). Based on the literature of functional flight-morphology in...
butterflies (Dudley 2000, Van Dyck 2003) and as applied by Hill et al. (1999b), we predict females to have a larger size (larger total body mass), to have higher wing loading, to allocate more mass to flight muscles (higher relative thorax mass), and to possess lower aspect ratio with increasing degree of fragmentation. Elsewhere we have shown that mate location is more frequently of an intermediate type between perching and patrolling in agricultural landscape compared to woodland landscape (Merckx & Van Dyck 2005), but this may have little impact when average values of flight morphology are tested among landscapes. So, we predict no differences in male flight morphology.

We can also test a prediction on size that follows from a constraint hypothesis and contrasts with the earlier predictions. We know that fragmented, agricultural landscape is on average warmer than woodland landscape (Merckx, Van Dongen, Matthysen & Van Dyck, unpublished). Higher ambient temperature makes larvae growing faster which leads to smaller adults (Silby et al. 1997, Nylin & Gotthard 1998). Hence, from this constraint hypothesis, we predict smaller individuals in more fragmented landscape in males and females, which is in contrast with our earlier predictions.

Finally, we also use the experimental data on flight morphology to test for significant genotype × environment interactions as families (or genotypes) may differ in their plastic responses. Such variability is a prerequisite for phenotypic plasticity to respond to natural selection and hence to be adaptive (West-Eberhard 2003).

Methods

STUDY SPECIES
The speckled wood (Parnassius aegeria L.) is primarily a woodland butterfly (Tolman & Lewington 1997), but it also breeds in other habitat types with some kind of woodland aspect, including hedgerows (Dover & Sparks 2000). This butterfly can use three developmental pathways corresponding to different seasonal cohorts: (i) development with a pupal winter diapause resulting in early-spring adults, (ii) development with a larval winter diapause resulting in late-spring adults and (iii) direct development resulting in summer generation adults (Wiklund et al. 1983, Nylin et al. 1989). Honeydew is the main adult feeding resource and several grasses can be used as larval host plant (Shreeve 1986a). Eggs are deposited on isolated grass plants that grow under shaded, rather wet conditions surrounded by some bare ground (Wiklund & Persson 1983). Our recent work has pointed to several differences between speckled woods living in woodland landscape versus fragmented agricultural landscape, including dispersal propensity (Merckx et al. 2003), temperature-related female fecundity (Karlsson & Van Dyck 2005), mate location (Merckx & Van Dyck 2005) and habitat finding ability (Merckx & Van Dyck, unpublished).

RECIPROCAL TRANSPLANT EXPERIMENT
In summer 2001, we caught females in three different landscapes in central Belgium: (i) a landscape dominated by deciduous oak woodland (‘Meerdaalwoud’, 1255 ha), (ii) a set of small oak woodland fragments (‘Boshoeck’, 1 to 9.0 ha) with relatively small interpatch distances (85 to 865 m), and (iii) a highly fragmented, agricultural landscape composed of intensively used fields and pastures (the large majority of the area) and to a lesser extent houses and farms, orchards, tiny woodlots and several sunken roads with hedgerows (‘Hoegaarden’). This hedgerow landscape consisted of 35 sunken roads with hedgerows that made up a total distance of 8.9 km. Only three percent of the study area was covered by six small (0.4 to 1.2 ha) and mainly poplar woodlots (Table 2.1). The caught females were transported to the laboratory and allowed to lay eggs on potted tufts of grass (Poa trivialis) in individual cages. Offspring of two females of each landscape was used in the experimental set-up: thirty second instar larvae of each female were placed in individual enclosures (11 cm wide x 11 cm long x 35 cm high) containing a fresh tuft of potted Poa trivialis. Grasses had been reared in the laboratory under standardised conditions for light, ambient temperature, nutrition, water and soil substrate (Wiklund et al. 1983). 180 enclosures were equally spread among the three types of landscape where females were collected. They were placed at sites in the landscape where larvae of P. aegeria also naturally occur (TM, pers. obs.). Cages were inspected frequently and we recorded when larvae or pupae were dead or when adults were not fully eclosed. Freshly eclosed adults were collected, killed and stored by freezing (-20°C) for morphological measurements. The experiment covered a period from May 16 to July 26. Next, we repeated the experiment from July 24 to September 25 (n=180). Given summertime conditions, all individuals followed a direct development to the adult stage (see Wiklund et al. 1983).

Table 2.1. Overview of percentages optimal and suboptimal habitat surface area for given study areas with three landscapes: (i) woodland landscape, (ii) landscape with woodland fragments, and (iii) agricultural landscape. Optimal habitat is oak woodland; Suboptimal habitat is all other habitat (deciduous woodland, structure rich conifer woodland, orchards, hedgerows, sunken rural roads). Based upon a biological validation map of Flanders (version 1.0-2.0-2.1, Institute of Nature Conservation, Brussels).

<table>
<thead>
<tr>
<th>landscape type</th>
<th>study area</th>
<th>optimal habitat</th>
<th>suboptimal habitat</th>
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<tbody>
<tr>
<td>woodland</td>
<td>138 ha</td>
<td>95%</td>
<td>5%</td>
</tr>
<tr>
<td>woodland fragments</td>
<td>753 ha</td>
<td>9%</td>
<td>2%</td>
</tr>
<tr>
<td>agricultural</td>
<td>555 ha</td>
<td>0%</td>
<td>5% * 8.9 km sunken roads</td>
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MORPHOLOGICAL MEASUREMENTS
Morphological measurements were according to the methods of Van Dyck & Wiklund (2002). Prior to the measurement, specimens were dried in an incubator until constant mass during 24 h at 60°C. Total dry mass was measured with a microbalance (Mettler...
Toledo). Next, specimens were dissected by separating head, thorax, abdomen, legs and wings. Thorax mass was also weighed. Forewings were photographed (Olympus Camedia C-3030) to measure forewing length and forewing area using an image analyser system (Optimas 1999). Morphological characteristics were grouped into three measures: (1) total dry mass, (2) wing shape: aspect ratio (4*forewing length/forewing area) and (3) relative allocation: relative thorax mass and wing loading (total dry mass/forewing area). Both forewing length and forewing area was correlated with total dry mass (Pearson correlation coefficients: \( r < 0.0001 \)). Repeated measurements for mass and wing traits on 20 individuals showed high repeatabilities (between 0.99 and 1.00). Individuals with substantial wing wear were excluded from the analyses.

**ANALYSES AND STATISTICS**

Counts of dead larvae and pupae and counts of not fully eclosed adults were summed and contrasted with counts of full-grown individuals. Differential survival with ‘landscape of development’ and ‘landscape of origin’ was tested by means of \( \chi^2 \)-tests. Flight-morphological traits were analysed in function of ‘landscape of origin’ and ‘landscape of development’. Main effects were tested using mixed regression models (Proc Mixed, SAS 2001). In line with the predictions, models were run separately for males and females. Models also included ‘family’ (i.e. ‘genotype’) (nested within ‘landscape of origin’) and ‘session’ as random effects. For each trait it was tested by log-likelihood ratio whether the interaction of the ‘family x landscape of origin’ random parameter with ‘landscape of development’ made a significant contribution to the model. If so, it meant that a significant \( G \times E \) interaction was present. After removing outliers of two males, original data were normally distributed. Final statistical models were obtained by backward elimination of non-significant factors (highest \( P \)-values). Residuals of the final models were normally distributed.

**Results**

The proportion of individuals that survived to the adult stage was significantly smaller in agricultural landscape (58%) compared to woodland landscape (74%) and landscape with woodland fragments (76%) \( (n=346, \chi^2=10.59, P=0.005) \). Independent of landscape of development, there was also a significant effect of landscape of origin on survival rate \( (n=346, \chi^2=13.42, P=0.001) \). Fewer individuals that originated from the agricultural landscape survived to the adult stage (57%) than did individuals that originated from woodland landscape (72%) \( (n=229, \chi^2=5.44, P=0.02) \). Individuals that originated from woodland landscape had the highest survival (79%), although this proportion was not significantly different from landscape with woodland fragments.

Independent of their landscape of origin, males and females that developed as larvae in the agricultural landscape were heavier and had higher wing loadings than individuals that developed in woodland landscape and landscape with woodland fragments (Figure 2.1). Females that developed in the agricultural landscape had heavier thoraces than females that developed in the other two types of landscape. In males, relative thorax mass did not respond significantly in relation to landscape of development, but a landscape of origin effect was present. Males originating from the landscape with woodland fragments allocated more mass to the thorax than individuals from woodland landscape and agricultural landscape. Forewing aspect ratio did not differ with landscape of development, or with landscape of origin (Table 2.2). Hence, we observed landscape-related phenotypic plasticity for total dry mass, wing loading and relative thorax mass. For the latter trait, however, plasticity was only present in females.

In both sexes, individuals from different families had different responses to the same environmental conditions for total dry mass and wing loading. These differences in the observed phenotypic plasticity among genotypes imply that \( G \times E \) interactions were present for these traits. With regard to relative thorax mass, a \( G \times E \) interaction was only present in males. No \( G \times E \) interaction could be detected for aspect ratio.

<table>
<thead>
<tr>
<th>Table 2.2. Summary of the analyses of different flight-morphological traits of <em>P. aegeria</em> offspring from a reciprocal transplant enclosure experiment in relation to landscape of origin (woodland landscape, landscape with woodland fragments or agricultural landscape) and landscape of development (idem) (see Methods). Mixed procedures (SAS) were run for both sexes separately.</th>
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<tr>
<td>variable</td>
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<td>total dry mass</td>
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<td>relative thorax mass</td>
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**Figure 2.1:** Total dry mass of female *P. aegeria* offspring from different landscape origin that developed under woodland landscape, landscape with woodland fragments and agricultural landscape conditions in a reciprocal transplant enclosures experiment. We used means ± SE.
Discussion

Our reciprocal transplant experiment in different landscapes, following a split-brood approach, showed that adult offspring of *P. aegeria* females differed significantly in adult flight morphology. This suggests the existence of landscape-related phenotypic plasticity as the same genotype produced a different morphology in, for instance, a woodland landscape versus a highly fragmented agricultural landscape. Interestingly, the observed phenotypic plasticity for body size contrasts with the constraint hypothesis, which predicts smaller individuals with fragmentation in both males and females due to higher growth rates with higher ambient temperatures (Stiby et al. 1997, Nylin & Gotthard 1998). Our results demonstrated the opposite. Hence, they point to adaptive differences. In line with the assumption of changed dispersal rates with landscape structure, we showed that individuals that developed in the agricultural landscape were heavier and had higher wing loading than those that developed in woodland landscape and landscape with woodland fragments, irrespective of their landscape of origin. Although these effects were similar among males and females, phenotypic plasticity for relative thorax mass was only present in the female sex. This is in line with the prediction that an adaptive response of variation in flight morphology is easier to detect in females. As predicted according to the adaptive hypothesis, females that developed in the agricultural landscape allocated more mass to flight muscles than did females that developed in woodland landscape and landscape with woodland fragments. However, effects were not consistent for all flight-morphological traits: in both sexes no significant difference biases the variation in phenotypic expression in adults between the landscapes. This leads to the hypothesis that species with high levels of plasticity in morphology and life history have a greater capacity to deal with changing and highly variable landscapes than other species. Contrary to several other butterfly species that show clear to dramatic declines (Maes & Van Dyck 2001), *P. aegeria* is one of the few species that increased highly significant in distribution and abundance, at least in NW-Europe (e.g. UK: Asher et al. 2001, The Netherlands: van Swaay & Groenendijk 2004).

Although our experimental set-up principally aimed to evaluate plastic responses in flight morphology, it also revealed significant differences in juvenile mortality among types of landscape. A much higher mortality in agricultural landscape for caterpillars of this woodland butterfly points to a novel aspect of habitat fragmentation that requires further research. Besides the direct effect of higher mortality under agricultural landscape conditions, the additive significant effect of landscape of origin suggests some divergence among the populations and types of landscape with respect to the capacity of larvae to deal with (or tolerate) different microclimatic conditions. It shows that evolutionary ecological studies in a context of habitat fragmentation should not be limited to adult ecology alone. Moreover, it needs to be evaluated to what extent increased mortality biases the variation in phenotypic expression in adults between the landscapes.

Acknowledgements

Thanks to S. Van Dongen for valuable comments on statistics and to K. Borghgraef for practical assistance. The University of Antwerp (GOA 15R/942) and the Belgian Federal Office of Scientific, Technical and Cultural Affairs (OSTC-PADD II EV/06/168) funded the research. TM was research aspirant with the Fund of Scientific Research Flanders-Belgium (F.W.O.). The experiments comply with the current laws of Belgium.
Chapter 3

Habitat fragmentation and thermoregulation

Summary

1. We assessed the impact of vegetation structure and type of landscape on microclimatic conditions for flight activity, using the heliothermic butterfly Pararge aegeria as a model.
2. Dummies that initially were warmed up to similar thorax temperatures were able to cover larger distances, and they heated up faster, with higher ambient temperature and with lower wind speed.
3. Convective cooling decreased with increasing degree of shelter, and with lower flight height. The smaller impact is reflected in lower values of SE’s. Flight bouts bridged 140 ± 23 m under unsheltered, 81 ± 13 m under partly sheltered and 29 ± 6 m under sheltered conditions.
4. Ambient temperature and wind force were higher in highly fragmented, agricultural landscapes than in continuous woodland landscapes.
5. Only males had a higher amount of fur in agricultural landscape. This sexual difference is interpreted in terms of differences in life history among the sexes: from an adaptive viewpoint, selection pressure to extend flight activity during periods with less favourable microclimatic conditions, as is more often the case in agricultural landscape, should be higher in males, since males are predicted to maximise the time spent to mate location, while females should allocate energy toward egg-laying rather than flight-activity.
Introduction

In ectotherms, the relationship between body temperature and performance typically shows an optimum bounded by critical thermal limits (Dudley 2000, Chown & Nicolson 2004). The thermal sensitivity of performance, especially locomotor performance, has become an important research focus in evolutionary physiology and ecology, and flight performance and thermoregulation of insects are inextricably linked (Kingsolver & Huey 1998, Chown & Nicolson 2004). In many flying insects (including butterflies), flight is a multifunctional trait relating to different behaviours with direct fitness consequences like predator escape, mate location, foraging, and oviposition (Dudley 2000, Watt 2003). Their flight performance directly depends on body temperature of the thorax where all flight muscles are located (Dudley 2000). Thoracic temperature is the net result of heat gained by behavioural thermoregulation and by physiological processes associated with muscle contraction and of heat lost by convective cooling (Clench 1966). Convective cooling is proportional to the difference between body temperature and air temperature and it is proportional to the velocity of the airflow over the insect body (Kingsolver 1983, Watanabe & Imoto 2003). Since both thermoregulation and convective cooling are influenced by variation in microclimate (e.g. Shipp et al. 1987, Peng et al. 1994), insect flight performance may depend on the physical structure of the local environment (Kingsolver & Watt 1984). Habitat fragmentation results in altered landscape structure and composition providing different microclimatic conditions (e.g. ambient temperature, ground temperature, wind speed, amount of solar radiation that penetrates the habitat; Mallock 1993, Malcolm 1998, Honnay et al. 2001). In ectothermic insects, altered thermal environmental profiles may lead to evolutionary change in thermal properties of local populations. So, habitat fragmentation may influence thermoregulation behaviour and thermoregulation-related morphology in order to counteract effects of altered microclimatic conditions that would take flight muscle temperature out of the optimal range for flight activity. However, so far only few studies have addressed the potential link between changing landscape structures and microclimate for heliothermic insects like butterflies (Meyer & Sisk 2001). We are not aware of studies evaluating variation in thermal flight budgets among landscapes differing in degree of fragmentation.

In this article, we use adults of the butterfly Pararge aegeria (L.) to test for differences in thermoregulation-related morphology and to assess the relative value of a thermal budget in terms of flight distance in relation to landscape structure in two contrasting types of landscape: woodland landscape and highly fragmented, agricultural landscape. When fully active, thoracic temperature in P. aegeria is within the range of 30-34°C (Shreeve 1984, Van Dyck & Matthysen 1998). As in other flying heliotherms, this butterfly interrupts flight bouts with periods of basking since flight typically imply convective cooling towards suboptimal body temperatures (Van Dyck & Matthysen 1998). Basking is done by spreading the wings dorsally (Clench 1966). Butterfly dummies (i.e. dead specimens, with spread wings, on a hypodermic needle with a thermocouple) have been shown to be useful instruments to study – at least some – aspects of their thermal ecology (e.g. Berwaerts et al. 2001, Kemp & Krockenberger 2002). Dummies have largely similar thermal properties as living butterflies (Heinrich 1986). Obviously, living butterflies influence or regulate their body temperature by adjusting their posture and by microclimate selection. So, dummies are no full equivalent to living butterflies, but – as it is generally the case with models – tools that simplify complexity in order to dissect sources of variation, in this case operational thermal differences among different landscapes.

First, we compared two thermoregulation-related traits of adult butterfly morphology between samples of woodland and agricultural landscapes: degree of dorsal melanisation of basal wing parts and amount of fur on thorax and basal wings. Higher levels of dorsal melanisation of basal wing parts result in higher heating rate during basking (Van Dyck & Matthysen 1998, Berwaerts et al. 2001). Body fur has been shown to be functionally significant for thermal isolation by reducing convective cooling (e.g. Kingsolver 1983). So, we predict agricultural landscape butterflies to have darker dorsal wings and more fur than woodland butterflies, in order to counteract associated higher levels of convective cooling. Berwaerts et al. (1998) have shown preliminary evidence for such an effect, at least for wing melanisation.

Next, we did two experiments with butterfly dummies. In a first experiment we estimated the distance that can be covered by a dummy until thorax temperature is cooled down from optimal temperature for flight activity (33°C) to a suboptimal temperature (25°C). This is what we call a thermal budget for flight. It allows testing for differential effects of general vegetation structure and type of landscape on convective cooling, and hence on the value of a thermal budget in terms of flight distance. In a second experiment we examined heating rate of the thorax from suboptimal (25°C) to optimal temperature for flight (33°C) under the same environmental conditions as in the first experiment. Measurements of the two experiments were done close to the substrate (0.1 m) and at 1.0 m height because other studies have suggested the importance of shelter in this respect (Willmer 1986, Dover & Sparks 2000). To test differential effects of vegetation structure we distinguished between three structural categories: (i) unsheltered: open field (only in agricultural landscape), (ii) partly sheltered: along hedgerow (agricultural landscape) or along woodland fringe (woodland landscape), and (iii) sheltered: in sunken roads (agricultural landscape) or inside woodland (woodland landscape). Thermal budget for flight is expected to be larger under warmer weather conditions but to be smaller under conditions of strong wind (convective cooling). So, we predict larger variation in cooling distances and heating rates in agricultural landscape than in woodland landscape. Similarly, we predict more variation when shelter providing vegetation is lacking compared to (partly) sheltered conditions in the two types of landscape (Dover 1996, Noss & Csuti 1997, Morecroft et al. 1998), and we predict lower impact of convective cooling (i.e. less variation) close to the substrate (Willmer 1986, Dover & Sparks 2000).
**Methods**

**STUDY SPECIES**
P. aegeria is a satyrine butterfly primarily of woodland throughout Europe, but it also occurs in open, fragmented landscapes with some woodland aspect, like agricultural landscapes with hedgerows and small patches of woodland (Dover & Sparks 2000, Merckx et al. 2003). Males either defend a sunlit spot at the forest floor as a territory or they patrol between several sunlit spots to find mates (Van Dyck 2005 and references therein). This bivoltine species is characterised by adaptive seasonal plasticity for flight- and thermoregulation-related morphology (Van Dyck & Wiklund 2002).

**STUDY LANDSCAPES**

For morphological measurements, we sampled spring and summer individuals in four Belgian areas representing two types of landscape: (a) two landscapes dominated by deciduous oak woodland: Meerdaalwoud (1255 ha) and Bos ter Rijst (280 ha), and (b) two highly fragmented, agricultural landscapes composed of intensively used fields and pastures (the large majority of the area) and to a lesser extent houses and farms, commercial orchards, tiny patches of woodland and several sunken roads with hedgerows: Rillaar and Hoegaarden (see Merckx et al. 2001). In both woodland landscapes, butterflies were collected within study plots of c. 135 ha of 100% woodland habitat. The Hoegaarden study area (555 ha) consists of 33 sunken roads with hedgerows (8.9 km) and six small patches of woodland. The Rillaar study area (401 ha) consists of 15 sunken roads with hedgerows (3.9 km) and six small patches of woodland. In both areas, small patches of woodland (0.3 to 11.2 ha) cover 3% of the area. Study landscapes are characterised by similar topography, soil type and macroclimate. Distances ranging from 7 to 60 km separate these four landscapes from one another.

Experimental dummy measurements were done in Hoegaarden (agricultural landscape) and Meerdaalwoud (woodland landscape). They were performed simultaneously in both areas during periods when climatic conditions allow butterfly activity: sunny weather and wind speed maximally 3 Beaufort (BF). We performed experiments during three periods (1st: April, 14-24; 2nd: May, 28 - July, 8; 3rd: July, 11 - August, 2). The heating rate experiment was only performed during the first and second period.

**MORPHOLOGICAL MEASUREMENTS**

In spring and summer of 2000 and 2001, P. aegeria individuals (n=650) were captured at the four study landscapes and stored at -20°C. Prior to measurement, specimens were dried in an incubator during 24 h at 60°C. Next, wings were carefully separated from the thorax. Forewings were photographed (Olympus Camedia C-3050) in order to measure basal darkness (grey values) using an image analyser (Optimas 6.5). From all captured females (n=133), and from a separate set of males (n=283), we additionally photographed a close-up (binocular microscope Leica MZ25: magnification = c. 46) of the forewings’ basal part to allow assessment of the amount of fur. The amount of fur on these basal wing parts was scored into three classes: (1) few short hairs, (2) medium amount of fur, the wing itself is clearly visible, and (3) lot of fur covering most of the basal wing surface. Repeated measurements for basal wing colour on a sample of 20 individuals showed a high repeatability (0.94). Only one out of 20 individuals was scored differently for fur in an independent assessment. For 35 females we checked the relationship between amount of fur on the basal forewings and on the thorax (three classes as well). We could only show a weak relationship: 15 individuals were scored to the same class, 16 individuals to an adjacent class and four individuals were scored to an opposite class. Individuals with substantial wing wear were excluded from the analyses.

**AMBIENT TEMPERATURE**

During almost three months (May, 3 2003, 05.00 hrs. - July, 29 2003, 16.00 hrs.), 12 thermoprobes connected to dataloggers (Hobo Pro Series) measured ambient air temperature each five minutes at a height of 1 m above the substrate. In each of four study sites we placed three thermoprobes. In total, we distinguished between six vegetation/landscape classes: (i) inside woodland (‘core’), (ii) at the edge of clearings within woodland (‘clearing’), (iii) at the outer fringe of woodland (‘Fringe’), (iv) between both woody sides of sunken roads (‘inner sunken road’), (v) outer edges of woody sunken roads (‘outer sunken road’), and (vi) salient, exposed locations within agricultural landscape (open’). BoxCar Pro 4 software was used to calculate average ambient temperatures for each hour and maximal ambient temperature of each day for different thermoprobes.

**COOLING DISTANCE EXPERIMENT**

By placing a butterfly dummy in a sunlit patch, it was heated to a thoracic temperature of 39°C. At this temperature, the observer started to walk with the dummy at a constant speed of c. 1 m/s. This is a relevant velocity in free flying P. aegeria (T. Merckx, unpublished data, see also Bunker 1993). The head of the dummy was always positioned forward and the spread wings were held horizontal. We recorded time and associated distance until dummy thoracic temperature dropped to 25°C (with a maximum of 300 m or c. 5 min). For each trial, we also recorded ambient temperature and wind speed (BF). Experiments were performed under three categories of vegetation structure: (i) unsheltered: open field, at least within a 50 m radius (only in agricultural landscape), (ii) partly sheltered: along tall hedgerow (1 4 m) (agricultural landscape) or along woodland fringe (woodland landscape), within 1 and 2 m sideways, and (iii) sheltered: in sunken roads with tall hedgerows along both sides (agricultural landscape) or inside woodland, at least 50 m from a clearing or the woodland fringe (woodland landscape). For each specific location (n=98), the experiment was repeated three times, both at 1 m and 0.1 m height above the substrate. We used 11 specimens of an independent population of intermediate landscape structure (CDE-campus, University of Antwerp, which
represents a half-open park type of landscape). This selection of specimens showed little inter-individual variation in basal wing colour and fur and the use of specific dummy individuals was randomised over the different measurements.

HEATING RATE EXPERIMENT
A dummy was brought to a thoracic temperature of 25°C by placing it in the shade. At 25°C, the dummy was placed with its wings parallel to the ground in a sunspot and time was recorded. The experiment was stopped when thoracic temperature of the dummy reached 37°C. For each trial, we also recorded ambient temperature and wind speed. Experiments were performed under three conditions of shelter (see above). We repeated the experiment three times for each specific location (n=109), and both at 1 m and at 0.1 m above the substrate. In total, six dummies were used in a random order. Again, they originated from the same independent population. Dummy specimens did not show substantial inter-individual variation in basal wing colour and amount of fur.

STATISTICAL ANALYSES
Basal wing colour was compared between seasons and types of landscape. Main effects and their interaction were tested via mixed regression models (Proc Mixed, SAS 2001), which were run for both sexes separately. In order to control for possible effects of year and study area, we added year (2 levels), study area (nested within type of landscape) (2 levels) and the interaction of study area and season as random effects. To obtain final models, we applied backward elimination of non-significant factors (highest P-values).

Model residuals had normal distributions. Log-likelihood tests were performed to check how this incorporation was done. The linear mixed model is traditionally formulated according to Laird and Ware (1982) as:

\[ Y = X\beta + Zb + \epsilon \]

where \(X\beta\) reflects the fixed effects part and \(Zb\) the random effects part of the model, and \(\epsilon\) the residual error assumed to be normally distributed with zero mean and variance \(\sigma^2\). If we set the fixed effects and random effects equal to a linear predictor \(\theta = X\beta + Zb\), then this model can be easily reformulated as:

\[ Y \sim N(\theta, \sigma^2) \]

These two formulations are generally and interchangeably used in linear mixed models (Verbeke & Molenberghs 2001). In order to avoid having to replace the censored observations by an arbitrary score, we adapted the ‘traditional’ mixed model developed above in order to allow explicit estimation of these missing censored datapoints. Censored data were set as missing and assumed to result from a distribution without any mass below the upper limit of the data. Formally, in order to incorporate the censoring, this model was slightly adapted as:

\[ Y \sim N(\theta, \sigma^2) \text{ if } Y \text{ observable} \]
\[ Y_{\text{censored}} \sim N(\theta, \sigma^2) \text{ with mass above the upper limit of the data if } Y \text{ censored} \]

The above model allows making inferences about the means through the linear predictor \(\theta\). In addition, we were interested in the variability in cooling distances and heating rates in relation to landscape, shelter, and height. We therefore fitted heterogeneous variance models to allow separating the residual variance (\(\sigma^2\)) components to be estimated for the different levels of these three factors. This was achieved by a multiplicative model at the level of \(\sigma^2\):
A ratio (R) of two residual variance components equal to 1 hereby indicates that the vari-
ances did not differ significantly. This type of complex models can be easily fitted in a
Bayesian framework using Monte Carlo Markov Chain (MCMC) simulation techniques.
We performed all analyses in the computer package WINBUGS (version 1.4) which is
freely available at http://www.mrc-bsu.cam.ac.uk/bugs. Bayesian analyses are based on the
likelihood principle but also involve making prior assumptions about the parameters in
the model in the form of a prior distribution. As we had no prior knowledge about the
parameters, we used weak prior distributions. For all fixed effects parameters, a normal
prior distribution with zero mean and a variance of 1000 was applied. For variance com-
ponents, a uniform prior on the standard deviation was used with mass between zero
and 1000. The prior distributions of the ratios R were truncated normal distributions
with mean equal to 1 and a variance of 1000, and no mass for negative values. In each
analysis we ran five independent chains of 10000 iterations after discarding the first
4000 iterations to avoid dependence on the initial values. Convergence was checked by
visual inspection of the MCMC’s and by the Gelman and Rubin shrink factor. Posterior
distributions were summarised by their mean and 95% credibility interval (CI). For
more details we refer to Gelman et al. (1995) and Carlin & Louis (2000). Model selection
was performed in two steps. First, heterogeneity in residual variance was investigated
in a model containing all fixed and random effects. Next, a backward elimination proc-
ess was performed on the factors landscape, shelter and height and their interactions.
All other covariates were kept in the model.

Results

THERMOREGULATION-RELATED MORPHOLOGY
Males had more fur than females (\( \chi^2 = 14.40; P = 0.0007; \text{df} = 4 \)), and they had more fur
in agricultural than in woodland landscapes (Fisher’s exact, \( P = 0.0001 \)); within both
types of landscape there was no difference between study sites. Females showed a trend
to have more fur during summer than during spring (Fisher’s exact, \( P = 0.066 \)), but showed
no difference between the two types of landscape. Basal wing colour did not
differ between agricultural and woodland landscapes. Males were darker in spring than
in summer (\( F_{1,227} = 22.07, P = 0.036 \)), but there was no such a pattern in females.

AMBIENT TEMPERATURE
We observed differences in average and maximal ambient temperature among the six
vegetation/landscape classes (average temperature: \( F_{5,407} = 15.95, P = 0.0042 \); maximal
temperature: \( F_{5,276} = 27.76, P = 0.0081 \) (Table 3.1). There was no significant difference in
variance among the classes. Regardless of interclass variation, average and maximal
temperatures were higher in agricultural landscape than in woodland landscape (ave-
erage temperature: \( F_{5,414} = 10.07, P = 0.031 \) – agricultural: 17.0 \( \pm \ 0.20^\circ \text{C} \) versus woodland: 16.1
\( \pm \ 0.20^\circ \text{C} \); maximal temperature: \( F_{5,412} = 12.23, P = 0.031 \) – agricultural: 23.8 \( \pm \ 0.57^\circ \text{C} \)
versus woodland: 21.3 \( \pm \ 0.50^\circ \text{C} \)).

COOLING DISTANCE EXPERIMENT
Larger distances could be covered by moving butterfly dummies when ambient tempera-
ture was higher (\( \beta_{\text{temp}} = 3.50, 95\% \text{ CI: } 1.36 \pm 5.60 \)), and smaller distances with increasing
wind speed (differences in cooling distances after log-transformation using wind class
4 (relatively strong wind) as reference: \( \beta_{\text{wind}} = 3.66, 95\% \text{ CI: } 16.8 \pm 44.0; \beta_{\text{wind}} = 3.09, 95\%
\text{ CI: } 4.0 \pm 28.5; \beta_{\text{wind}} = 0.93, 95\% \text{ CI: } -1.4 \pm 22.3 \)). There was a two-way interaction between
vegetation structure (shelter) and flight height: cooling distances were shorter at 1 m
than at 0.1 m height, but this effect was smaller under more sheltered conditions (dif-
ference between high and low height: \( \beta_{\text{height}} = -36.5, 95\% \text{ CI: } -53.3 \pm 19.4 \); partly sheltered: \( \beta_{\text{height}} = -12.8, 95\%
\text{ CI: } -19.2 \pm 6.54 \); sheltered: \( \beta_{\text{height}} = -4.4, 95\% \text{ CI: } 7.69 \pm 1.17 \) (Figure 3.1a, b). From the viewpoint of differences in cooling distances after log-
transformation using wind class 4 (relatively strong wind) we can interpret these
results as showing a stronger effect of shelter (larger cooling distances in sheltered versus
unsheltered conditions) at lower height (0.1 m) (lower height: \( \beta_{\text{shelter}} = -34.4, 95\% \text{ CI: } -32.4 \pm 2.2 \);
\beta_{\text{shelter}} = -9.5, 95\% \text{ CI: } -13.7 \pm 5.7; \beta_{\text{shelter}} = -33.8, 95\% \text{ CI: } -76.1 \pm 11.8 \); higher height: \( \beta_{\text{shelter}} = -17.7, 95\%
\text{ CI: } -21.4 \pm 6.4; \beta_{\text{shelter}} = -61.1, 95\% \text{ CI: } -104 \pm 26.3; \beta_{\text{shelter}} = -45.4, 95\% \text{ CI: } -69.3 \pm 23.6 \) (Figure 3.1a, b). A two-
way interaction between flight height and type of landscape was found as the dif-
fERENCE between high and low height (i.e. shorter cooling distances at high than at low
height) was observed in woodland (\( \beta_{\text{height}} = -4.4, 95\% \text{ CI: } -7.69 \pm 1.17 \), but not in the
agricultural landscape (\( \beta_{\text{height}} = 1.82, 95\% \text{ CI: } -2.39 \pm 6.04 \)) (Figure 3.1a, b). There was no

Table 3.1: Summary of least squares means of average and maximal ambient temperature during adult
flight activity in suitable P. aegeria habitats for six vegetation/landscape classes in two types of landscape
(woodland versus agricultural landscape).

<table>
<thead>
<tr>
<th>landscape</th>
<th>vegetation/landscape class</th>
<th>average temperature (°C ± SE)</th>
<th>max temperature (°C ± SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>woodland</td>
<td>core</td>
<td>15.9 ± 0.3</td>
<td>21.3 ± 0.43</td>
</tr>
<tr>
<td></td>
<td>clearing</td>
<td>15.9 ± 0.3</td>
<td>21.3 ± 0.43</td>
</tr>
<tr>
<td></td>
<td>fringe</td>
<td>16.5 ± 0.23</td>
<td>21.5 ± 0.43</td>
</tr>
<tr>
<td>agricultural</td>
<td>inner sunken road</td>
<td>16.7 ± 0.33</td>
<td>23.3 ± 0.43</td>
</tr>
<tr>
<td></td>
<td>outer sunken road</td>
<td>17.3 ± 0.33</td>
<td>23.4 ± 0.43</td>
</tr>
<tr>
<td></td>
<td>open</td>
<td>17.4 ± 0.25</td>
<td>25.7 ± 0.57</td>
</tr>
</tbody>
</table>
three-way interaction between type of landscape, flight height and vegetation structure, nor a two-way interaction between type of landscape and vegetation structure. The period in which observations were done, did not contribute significantly to the model. Residual variances did not differ between the two flight height classes ($R_{flight}=1.27$, 95% CI: 0.95 – 1.74) nor between the two types of landscape ($R_{landscape}=0.95$, 95% CI: 0.74 – 1.27). Residual variance was smaller in partly sheltered conditions relative to unsheltered conditions ($R_{shelter}=0.39$, 95% CI: 0.24 – 0.65) and even stronger when comparing sheltered and unsheltered conditions ($R_{shelter}=0.15$, 95% CI: 0.10 – 0.24). Thus, variability in cooling distances was highest under unsheltered conditions and decreased (up to a factor 6) when vegetation structure provided more shelter (Figure 3.1a, b).

Figure 3.1: Cooling distances of *P. aegeria* dummies in relation to vegetation structure and type of landscape (open circles: highly fragmented, agricultural landscapes; filled circles: woodland landscapes). We used means ± SE’s. Figure 3.1a: high flight height (1 m). Figure 3.1b: low flight height (0.1 m).

**HEATING RATE EXPERIMENT**

There was an increased heating rate between 25 and 33°C when ambient temperature was higher ($\beta_{temp}=-0.0085$, 95% CI: -0.010 – -0.0068). Effects of wind speed on heating were complex and non-linear: albeit there was no significant difference in heating rate between relatively high wind speed and windless conditions ($\beta_{wind}=0.0024$, 95% CI: -0.005 – 0.000), heating rate was slower with increasing wind speed ($\beta_{wind}=0.0020$, 95% CI: 0.001 – 0.002; $\beta_{wind}=0.0039$, 95% CI: 0.025 – 0.052). There was a three-way interaction between type of landscape, vegetation structure and flight height. From the viewpoint of the effect of flight height (smaller heating rates at high versus low height), this interaction can be interpreted as being a consequence of the fact that the difference in heating rates between high and low height increased with degree of shelter in the agricultural landscape (difference between high and low height: unsheltered: $\beta_{high-low}=0.025$, 95% CI: 0.015 – 0.035; partly sheltered: $\beta_{high-low}=0.017$, 95% CI: 0.002 – 0.037; sheltered: $\beta_{high-low}=0.009$, 95% CI: 0.001 – 0.017) whereas the opposite was true for the woodland landscape (difference between high and low height: unsheltered: no data available; partly sheltered: $\beta_{high-low}=0.019$, 95% CI: 0.014 – 0.025; sheltered: $\beta_{high-low}=0.028$, 95% CI: 0.017 – 0.040) (Figure 3.2a, b). Looking at the main effect of vegetation structure, heating rates did not differ between partly sheltered and unsheltered conditions, but heating rates were higher in sheltered relative to unsheltered conditions ($R_{shelter}=0.027$, 95% CI: 0.011 – 0.044) (Figure 3.2a, b). The period in which observations were done, made no significant contribution to the model. Residual variances did not differ between the two classes of flight height ($R_{height}=0.85$, 95% CI: 0.65 – 1.20) nor between the two types of landscape ($R_{landscape}=0.85$, 95% CI: 0.65 – 1.20). Residual variance was smaller in partly sheltered relative to unsheltered conditions ($R_{shelter}=0.52$, 95% CI: 0.34 – 0.81) but not so when comparing sheltered and unsheltered conditions ($R_{shelter}=1.04$, 95% CI: 0.66 – 1.72) (Figure 3.2a, b).

Figure 3.2: Heating times of *P. aegeria* dummies in relation to vegetation structure and type of landscape (open circles: highly fragmented, agricultural landscapes; filled circles: woodland landscapes). We used means ± SE’s. Figure 3.2a: high flight height (1 m). Figure 3.2b: low flight height (0.1 m).

**Discussion**

Fragmented landscapes may provide different thermal environments for flying heliotherms like butterflies. Our parallel measurements of ambient temperature at sites where the speckled wood butterfly *Pararge aegeria* occurs in continuous woodland versus corresponding sites in fragmented agricultural landscape indicated different thermal profiles. Ambient temperature in and along hedgerows and small patches of woodland in agricultural landscape was on average higher than in woodland landscape. Woodland landscape provides much shadier, cooler conditions that also buffer ambient temperature. Typical sunlit patches that *P. aegeria* uses to bask and as rendezvous sites (Shreeve 1984), are better sheltered against convective cooling. In agricultural landscape, the tiny patches of woodland and narrow hedgerows do proportionally have larger amounts...
of irradiated surface, but more sites are exposed to the cooling effect of higher wind speed in the surrounding open land. So, an agricultural landscape appears to be more variable in operational temperature profile for butterflies. We experimentally tested the potential impact of the different types of landscape for *P. aegeria* flight activity and performance using butterfly dummies (i.e. dead butterfly on a thermoprobe). In butterflies, dummies have largely similar thermal properties as living butterflies (Heinrich 1986). The distance or time that we could walk with a dummy before it was cooled from optimal (i.e. 33 °C) to the threshold of basking (i.e. 25 °C) under woodland conditions at 1 m height (27 ± 4.5; n=102) corresponded well with values on voluntarily flight bouts in a woodland population of *P. aegeria* studied by Shreeve (1984) (26 ± 4 s in males and 18 ± 6 s in females). This suggests that the technique provides relevant estimates (see also Berwaerts et al. 2001, Kemp & Krockenberger 2002). Dummies were found to cover larger distances – and they also heated up faster - with higher ambient temperature and lower wind speed. In the same vein, we observed that variation in cooling distance and in heating rate was higher in agricultural landscape compared to woodland landscape. Heating rates were significantly higher in sheltered relative to unsheltered conditions of vegetation structure. Although we could cover on average longer distances before dummies were cooled down under unsheltered conditions, variation in flight distance was highest under such conditions (i.e. open agricultural land) and was reduced by a factor up to six when dummies were moved under more sheltered conditions. Earlier, Merckx & Van Dyck (2002) have shown for two grassland butterflies that occur in agricultural land that they also were more confined to the vicinity of hedgerows when risk of convective cooling was high (i.e. relatively high wind speed). Additionally, moving close to the ground or substrate, particularly at places with sheltered conditions, had also a significant effect to lower the impact of convective cooling on flight distance or duration.

In the same vein, Rutowski et al. (1994) have shown that male butterflies adjusted perch height in relation to ambient temperature. We have found based on independent behavioural observation data that *P. aegeria* males flew more frequently at low height in agricultural landscape than in woodland landscape (Merckx & Van Dyck, 2005).

Although butterflies have some opportunities to avoid the problem of convective cooling by seeking sheltered vegetation structures in open agricultural landscape, the physiological cost of daily life may still be different in highly fragmented, agricultural landscapes versus sheltered woodland. From an evolutionary viewpoint, this may result in different life styles among different landscapes. In another experiment where real butterflies were induced to fly under standardised conditions at low suboptimal body temperatures, individuals of woodland landscape origin flew for longer than did agricultural landscape butterflies (Merckx, Karlsson & Van Dyck, unpublished). Within the range of temperatures of voluntary flight, Merckx et al. (2003) have shown in a common arena test in outdoor cages that *P. aegeria* of woodland landscape origin were more at flight than those of agricultural landscape. The combined results suggest physiological adaptations among differently fragmented landscapes that provide different thermal environments.

Our results also showed evidence for morphological adaptation in line with predicted thermal variation. Males of agricultural landscapes had more fur on thorax and basal wings than did woodland males. More fur is known to promote thermal isolation like has been observed in lowland versus highland populations of Colias butterflies (Kingsolver 1983). So, in a next step comparative experiments with dummies should take this variable into account to estimate the benefit in terms of gained flight distance or duration with and without fur. Another test would be to release woodland males into agricultural landscape and quantify to what extent they are more restricted in their flight time budget or more restricted to sheltered micro-sites in the agricultural landscape than local males. Experiments in which fur is removed from the thorax provide additional tools to estimate the benefit of this trait relative to landscape structure.

Variation in the degree of wing melanisation also affects thermoregulation and, hence, flight activity (Kingsolver & Watt 1984, Guppy 1986, Watt 2003, Ellers & Boggs 2004). Earlier work on males of *P. aegeria* has suggested darker individuals in agricultural landscape compared to woodland (Berwaerts et al. 1998); darker wings allow faster heating (Van Dyck & Mattysen 1998) which would be in particular an advantage in environments with high risk of convective cooling. Elsewhere we have shown using a field experiment that *P. aegeria* males which developed in agricultural landscape had indeed darker basal wings than males that developed in woodland (Merckx & Van Dyck, unpublished). However, there was no significant relationship with basal melanisation in the wild caught specimens of our current study. But it has recently been shown that the degree of melanisation is affected by environmental stress (like desiccation of host grasses) (Talloen et al. 2004). Such factors may to some extent confound the melanisation patterns between landscapes, at least in some years.

Effects on the thermoregulation-related morphological variation appear to be limited to males. This makes sense relative to the different behavioural repertoires of both sexes in butterflies (Pivnick & McNeil 1986). In males, extended flight during periods with less favourable microclimatic conditions increase the probability of locating virgin females; virgin females may emerge even under relatively poor weather. Knowing that there is a trade-off in females between allocations for reproduction versus flight (e.g. Hughes et al. 2003), females need to be more economical in terms of flight activity. So, butterfly females typically spend less time flying than males (Shreeve 1984). Hence, thermal isolation to extend flight bouts under relatively poor conditions (high convective cooling) is most frequently important to males. This may result in differential selection regimes for such traits between the sexes.

At the landscape level, our results attract the attention to the importance of vegetation structures as potential corridors for movement across fragmented landscapes. Moreover, the importance or significance of such structural characteristics will be
weather or climate dependent. If butterflies are only able to make relative short flights, a finer grained ecological network of corridors would be required. Moreover, for ectotherms like butterflies hedgerows are likely to be rather stepping stones for thermal reasons than really continuous conduits which they may be for endotherms. Alternatively, it may be that the types of ‘daily’ or ‘routine’ butterfly movements we have focused on are not necessarily representative for real dispersal movements (Van Dyck & Baguette, 2005).

Finally, our study has focused on thermal differences for adults only. Of course, thermal profiles of the landscapes may have profound effects on the larval stages as well. In this context, variation in oviposition site choice among landscapes provides, for instance, an interesting perspective of further research as female behaviour and morphological design may be affected by selection on oviposition site choice.

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Sex, landscape and butterfly flight ability

Sex- and landscape-related differences in thermal under-limit for flight ability in a woodland butterfly

[Thomas Merckx, Bengt Karlsson & Hans Van Dyck]

Summary

Active time budgets of flying ectotherms depend on body temperature. Thermal knowledge of flight ability and performance is currently, however, heavily biased towards the range of voluntary flight activity. However, flight under suboptimal conditions also may be important in terms of fitness. Here, we studied flight ability and duration of laboratory-reared Pararge aegeria (L.) butterflies that originated from a woodland and an agricultural landscape, under low temperatures (10-21°C) in a common-garden set-up. We predict that males are able to fly at lower temperatures than females as males have lower wing loading. Since woodland is on average cooler than agricultural landscape we also predict that flight ability at low temperature is better developed in woodland individuals. Individuals showed flight activity at all tested temperatures and flights were longer with increasing temperature. Males flew for longer than did females. However, there was no difference with sex at the lowest temperature, but an increasing difference with increasing temperature. We showed that woodland individuals flew for longer and had higher wing loading than agricultural landscape butterflies. Our results, that shed new light on the thermal ecology of flight at suboptimal temperatures, are discussed from both proximate and ultimate points of view.
Introduction

The idea of economic analysis of costs and benefits is central to the evolutionary ecological understanding of behaviour (Krebs & Davies 1997). Active flight has, for instance, clear benefits to track resources and to escape from predators, but it is energetically costly (Dudley 2000). Flying heliotherms (including butterflies) use solar radiation by basking behaviour as an external heat source, which is energetically less costly than physiological heat production (Dennis 1993). But the reverse side to this cheap option is that their active time budget can be severely constrained by poor weather.

In butterflies, voluntary flight activity is typically within a range of body temperatures of 28-38°C (e.g. Dennis 1993). In temperate-zone environments this is usually well above ambient temperature. Therefore, they thermoregulate behaviourally by basking and microsite selection to keep body temperature optimal (Shreeve 1992a). Thermal knowledge of flight ability and performance is currently biased towards the range of voluntary flight activity, but is far more incomplete for flight under suboptimal conditions, including the under-limit for flight activity. This has lead to the view that temperature is ‘dictating’ a butterfly’s behaviour. But recent evidence has contested this view. Although Pararge aegeria butterflies accelerated faster during take-off under optimal than under suboptimal thermal conditions, some individuals were able to perform at top level under both conditions (Berwaerts & Van Dyck 2004). Experiments by Kemp et al. (2006) have shown that P. aegeria males won territorial conflicts independent of thermal asymmetries among the contestants. This contrasts with earlier views that disputes were settled on the base of differential deviance from optimal body temperature (Hardy 1998).

Even if they are short, flights at low temperature outside the range of voluntary flight activity, but is far more incomplete for flight under suboptimal conditions, including the under-limit for flight activity. This has lead to the view that temperature is ‘dictating’ a butterfly’s behaviour. But recent evidence has contested this view. Although Pararge aegeria butterflies accelerated faster during take-off under optimal than under suboptimal thermal conditions, some individuals were able to perform at top level under both conditions (Berwaerts & Van Dyck 2004). Experiments by Kemp et al. (2006) have shown that P. aegeria males won territorial conflicts independent of thermal asymmetries among the contestants. This contrasts with earlier views that disputes were settled on the base of differential deviance from optimal body temperature (Hardy 1998).

Methods

STUDY SPECIES

Throughout Europe, P. aegeria is primarily a woodland butterfly, but also occurs in fragmented agricultural landscape with patches of woodland and hedgerows. Voluntary flights after basking are associated with thorax temperatures of 30-34°C (Shreeve 1984, Van Dyck & Mattheysen 1998). The average duration of spontaneous flight bouts in the field increases with ambient temperature from 10 s at 17°C to 90 s at 25°C (Shreeve 1984).

SAMPLED LANDSCAPES AND BREEDING PROCEDURE

In May 2004, females were captured at several sites in a woodland landscape (Meerdaalwoud) and in an agricultural landscape (Rillaar) in Belgium. Intersite distance of captures within each type was 0.5-5.0 km. Meerdaalwoud is a closed landscape dominated by deciduous oak woodland. Rillaar is composed of intensively used fields and pastures (80% of the area), houses and farms (10%), commercial orchards (5%), small patches of woodland (2.5%) and several sunken rural roads with hedgerows (2.5%). Females were allowed to oviposit on tufts of the grass Poa annua in small cages. Larvae were further reared under controlled climatic conditions that allow direct development (22°C, LD 12:12 h). After emergence, adults from five agricultural and three woodland landscape families were used for the experiment. Prior to observations, individuals were stored at 8°C and LD 12:12 h and they were not fed or mated. Finally, butterflies were killed and stored at -20°C. Next, they were dried during 24 h at 60°C and weighed (Sauter microbalance AR204). Forewing area was measured from digital pictures (Olympus Camedia C-5030) using an image analyser system (Optimas 1999).

TESTING FLIGHT ABILITY

Individual flight ability and duration was measured for males and females originating from the two types of landscape in experimental flight cabinets (height x length x
width: 2.4 m x 4 m x 2 ml. We used five identical flight cabinets differing in ambient temperature: 10, 13, 15, 17 and 21°C. Prior to the experiment, there was an acclimatization period of 30 min. After this period, thorax temperature was at the prevailing ambient temperature in the cabinet (thorax thermoprobe measurement in 10 individuals; see Van Dyck & Matthysen 1998). Flight ability was tested for each individual under each temperature, but the order was randomised. The test was similar to Pivnick & McNeil (1996): a butterfly sitting with closed wings was picked up from its plastic box with a pair of tweezers and was released from a standard height of 2 m. The behaviour was observed and timed using a stopwatch. Observation lasted until the butterfly was alighted. An individual was tested three times for each of the five treatments. Between trials individuals rested in plastic boxes while others were tested. In total, we tested 88 individuals: 22 males and 22 females of woodland landscape origin and 28 males and 16 females of agricultural landscape origin. Since *P. aegeria* is protandrous (Nylin et al. 1993), tested males were significantly older than females (X ± SE=5.1 ± 0.11 versus 4.2 ± 0.31 days since eclosion; Student T test: t_{1,131}=19.63, P<0.0001). However, there was no age difference between individuals from the two types of landscape.

**Statistical Analysis**

We used mixed regression models (Proc Mixed, SAS 2001) to analyse flight duration in relation to the factors of interest: ambient temperature (5 classes), landscape of origin (2 classes), age (i.e. days since eclosion), wing loading (total dry mass/forewing area) and sex. Flight duration was log-transformed and wing loading was transformed by taking the arc sine of the square root to obtain normally distributed model residuals. Because wing loading covaried strongly with sex, the analysis was done separately for each sex. Model selection starting from a full model (all factors of interest and interaction terms) was done by stepwise backward elimination of nonsignificant factors. Factors were not eliminated as long as they were incorporated in interaction terms. As individuals were tested repeatedly, the model took into account ‘individual’ and the interaction ‘individual x ‘trial’ as random effects.

**Results**

We observed some to considerable flight activity at all tested temperatures (range of flight duration 1.1-600.8 s). At 10°C flights lasted very short but on average at least three times as long as the falling of a dead specimen and butterflies always alighted in a controlled manner on the legs. Butterflies flew for longer when ambient temperature was higher and males flew for longer than females (F_{1,132}=19.53, P<0.0001). However, there was no difference with sex at the lowest temperature, but the increasing difference with temperature explains the highly significant temperature x sex interaction (F_{2,132}=17.39, P<0.0001; Figure 4.1). At 21°C males flew on average five times as long as females. In females, there was a tendency for a similar effect of landscape of origin as in males. Age was significant only in interaction with temperature: younger individuals flew for longer than did older ones, especially at higher temperatures. Females

**Table 4.1. Summary of the analyses of flight endurance of (i) *P. aegeria* males and (ii) *P. aegeria* females in relation to the wing loading of the butterfly, air temperature of the flight cabinet, age and landscape of origin (see Material & Methods) using Mixed procedure (SAS).**

<table>
<thead>
<tr>
<th>Effect</th>
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<th>j</th>
<th>F</th>
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<td>0.001</td>
</tr>
</tbody>
</table>

Figure 4.1. Temperature x sex interaction effect on flight duration
flew for longer when they had lower wing loading but this relationship was much steeper at low ambient condition than at the relatively higher temperatures (10°C: \( \beta = -2.9; 13°C: \beta = -3.0; 15°C: \beta = -0.5; 17°C: \beta = -0.5 \)). At 21°C only, we observed a reversed effect (\( \beta = 7.1 \)).

**Discussion**

Our experiment showed that the butterfly *P. aegeria* is able to show flight activity at much lower temperatures (10-21°C) than the voluntary flight range (30-34°C – Shreeve 1984). So, the threshold for flight activity must be even below 10°C. This is much lower than in several other butterfly species (e.g. Pivnick & McNeil 1986, Masters et al. 1988, Norberg 2003). *P. aegeria* is able to fly at low ambient temperature, but this is generally avoided (i.e. short flights after experimental induction only) suggesting a considerable physiological cost. In the same vein, older butterflies that have already used much of their reserves (Karlsson 1994) had shorter flights. In line with our key predictions, males and females responded differently and the same was true for butterflies of woodland versus agricultural landscape origin.

Since males were significantly older than females, the result of males flying for longer than females should be considered a conservative result. Ultimately, the observed sexual difference of a lower willingness to fly at suboptimal temperature in females relates to the fundamental differences in their life history relative to males. For males extended periods of flight increase the probability of locating virgin females. Even under relatively poor weather virgin females may emerge. Knowing that there is a trade-off in females between allocations for reproduction versus flight (e.g. Hughes et al. 2003), females need to be more economical in terms of flight activity. So, butterfly females typically spend less time flying than males (Shreeve 1984), which most likely contributes to the explanation of male-biased samples in mark-release-recapture studies (e.g. Wahlberg et al. 2002).

At the proximate level, the difference in flight duration at low temperature can be attributed to lower wing loading in males (Gilchrist 1990). It allows flight at lower wingbeat frequency (Casey 1981) and while this wingbeat frequency is temperature-dependent (Stevenson & Josephson 1990). Minimum temperature for flight in *Thymelicus lineola* increased with an experimental increase in wing loading (Pivnick & McNeil 1986). Sexual difference in wing loading in *P. aegeria* is mainly caused by differences in body mass rather than forewing area (Berwaerts et al. 2002). So, the smaller male size can be seen as an adaptation to extend flight activity.

*P. aegeria* of woodland landscape origin flew for longer at low ambient temperatures than did agricultural landscape butterflies. Within the range of temperatures of voluntary flight, Merckx et al. (2003) have already shown that *P. aegeria* of woodland landscape origin are more at flight than agricultural landscape butterflies. Karlsson & Van Dyck (2005) have experimentally shown that females of woodland landscape origin laid more eggs at low ambient temperature than did agricultural landscape females, but the pattern reversed at high ambient temperature. Hence, all these results point at temperature-related adaptation in the cooler environment of a continuous woodland landscape versus a warmer, but thermally more variable fragmented agricultural landscape. At the proximate level, the observed differences in wing loading among landscape types (higher wing loading in a woodland than in an agricultural landscape) are likely to contribute to the effect, since high wing loading was associated with longer flights at 21°C in females and at all but one temperature tested in males. Conversely, in the temperature range of voluntary flight a lower wing loading allows flying with lower wingbeat frequency (Casey 1981) and, hence, it allows longer flights.

Our study provides a novel perspective to the examination of flight ability and performance below optimal body temperature and to what extent it contributes differently in males and females to landscape-related patterns of adaptation. It is timely now to quantify experimentally the physiological cost of flight under suboptimal thermal conditions in terms of fitness in both sexes.

**Acknowledgements**

Thanks are due to K. Lauwers and M. Jacobs for assistance and to S. Jakobsson for providing facilities at Tovetorp research station. This research was funded by the University of Antwerp (GOA 15R/3942), the Belgian Federal Office of Scientific, Technical and Cultural Affairs (OSTC-PADD II EV/06/16B) and a grant to R.K. from the Swedish Research Council for Environment, Agricultural Sciences and Spatial Planning. T.M. had a PhD-grant of Scientific Research Flanders-Belgium (FWO-research aspirant).
Chapter 5

Variation in mate location

Mate location behaviour of the butterfly Pararge aegeria in woodland and fragmented landscapes

[Thomas Merckx & Hans Van Dyck]

Summary

Visually cued mate location behaviour in insects such as butterflies is typically classified by ‘wait or seek’ dichotomies. Perching males adopt a sit-and-wait strategy at a particular spot rising to intercept passing females (which is often done in an aggressive territorial way), whereas patrolling males are permanently on the wing searching for females. The potential influence of changes in landscape caused by habitat fragmentation on mate location behaviour has only rarely been addressed. We investigated this behaviour among populations of the speckled wood butterfly, Pararge aegeria, living in continuous woodland versus highly fragmented agricultural landscape with hedgerows and small patches of woodland. In the latter landscape males showed higher levels of aggressive fast take-offs (an indicator of territorial perching), but also higher levels of displacement (an indicator of patrolling). In an independent census, behaviour intermediate between perching and patrolling was much more frequent in the agricultural landscape than in the woodland landscape. Our results suggest that the dichotomy of perching versus patrolling as typically observed in woodland fades away in highly fragmented agricultural landscape. We discuss our results in relation to differences in densities and thermal properties of both types of landscape.
Introduction

Variation in visually cued mate location behaviour in insects has widely been classified using ‘wait or seek’ dichotomies (e.g. Odonata: Heinrich & Casey 1976; Thysanoptera: Crespi 1988; Hymenoptera: Alcock & Houston 1996). In butterflies, Scott (1974) defined the variants as perching and patrolling, respectively. Perching males adopt a sit-and-wait strategy at a particular spot rising to intercept passing females, whereas patrolling males are permanently on the wing searching for females. As male butterflies spend most of their active time locating mates (Shreeve 1992a), different mate location strategies may have consequences for their local distribution and daily movements. Some butterfly species are exclusive patrollers and others predominantly perchers, whereas in some other species both behavioural strategies occur (Dennis & Williams 1987, Wickman 1988, 1992, Shreeve 1992a, Van Dyck 2003). Dennis & Shreeve (1988) argued that patrolling is the ancestral behaviour; the evolution of perching depends on habitat structure, because the presence of distinct landmarks (e.g. hills, host plant clumps and sunlit patches) is essential for a perching strategy. Male butterflies can also show aggressive, territorial behaviour. Although territoriality is most often connected to perching, they are not synonymous. Some species show perching without any aggression towards conspecific males (Shreeve 1992a). Assuming that territorial behaviour is costly, the territory economics hypothesis predicts that monopolizing a perch with aggressive behaviour is viable only under low to moderate male density (Parker 1978, Thornhill & Alcock 1983, Rutowski 1991). In other words, high population density would favour nonterritorial patrolling (Scott 1974, Willmer 1991).

Although alternative mate location strategies have attracted much attention (Thornhill & Alcock 1983, Rutowski 1991, Wiklund 2003 and references therein), the potential influence of changing landscape structures on mate location behaviour has only rarely been addressed. Habitat fragmentation provides a typical framework for studies on changes in dispersal behaviour (e.g. Hill et al. 1996, Thomas et al. 1998), but other behaviours, including mate location, may also be changed (Van Dyck & Matthysen 1999).

Habitat fragmentation may simply affect densities and, hence, frequencies of territorial perching and nonterritorial patrolling males in systems where both strategies occur. However, things can be more complicated in fragmented landscapes when conditions favouring one of the strategies change as well, but not necessarily in parallel with changes in density. Hence, habitat fragmentation may alter cost-benefit balances of alternative mate location strategies.

We tested for differences in mate location behaviour among populations of the speckled wood butterfly, Pteroura egeria L., living in continuous woodland versus highly fragmented agricultural landscape with some small patches of woodland and hedgerows. Males either perch in an aggressive territorial way on a sunlit patch on the forest floor, or they patrol by flying from one sunlit patch to the other through more shady conditions (Wickman & Wiklund 1983, Shreeve 1987, Van Dyck 2003 and references therein). Under relatively cool ambient conditions, as in spring or in closed shaded coniferous woodland, the value of defending a sunlit patch is high and territorial perching is then observed as the dominant strategy (Wickman & Wiklund 1983, Shreeve 1984, 1987). Furthermore, in these heliotherms patrolling flights are constrained by local climatic conditions, which are the result of both the weather and the habitat structure (Dennis & Shreeve 1988, Wickman 1988). Flight duration increases in relation to air temperature, convective body cooling being slower at high temperatures when the difference between optimal body temperature and air temperature is less (Shreeve 1984, Van Dyck & Matthysen 1998).

From a speckled wood’s perspective, highly fragmented landscapes are likely to provide a different template for mate location behaviour than continuous woodland, for three reasons that we investigated in this study. First, densities per unit of habitat are likely to be higher in fragmented landscapes. Woodland represents a continuous area in terms of resources in general and for mate location sites (e.g. sunlit patches) in particular. In highly fragmented landscapes, however, resources are restricted to ‘lines’ (e.g. hedgerows) and ‘bits’ (e.g. patches of woodland) which may lead to a concentration of individuals (Chardon et al. 2003). In other words, densities per unit of habitat are expected to be higher in fragmented landscapes. Hence, in line with the territory economics hypothesis, we predicted less perching but more patrolling for fragmented landscapes. Second, thermal conditions differ considerably between woodland and hedgerows (or patches of woodland) in highly fragmented agricultural landscapes. Measurements of ambient temperature at suitable P. egeria habitat during flight activity showed that average and maximal daily temperatures are higher in fragmented agricultural landscapes than in woodland (Mercx, Van Dongen, Matthysen & Van Dyck, unpublished). So, from a thermal constraint hypothesis, we predicted a shift towards higher levels of local mobility with fragmentation. To reduce the impact of convective cooling in open fragmented landscapes, butterflies should seek shelter and fly closer to the vegetation than in woodland. Finally, the occurrence of landmarks for mate location may vary with landscape structure. Sunlit patches are landmarks where territorial perching males settle and patrolling males fly from one sunlit patch to the other. There are no oviposition or feeding sources in such a patch: it is only a spot clearly delineated by solar radiation penetrating through holes in the canopy. In fragmented agricultural landscapes, a considerable proportion of P. egeria habitat occurs as relatively small lines such as hedgerows and sunken roads. Clearly delineated sunspots are rare and a large part of the hedgerow is irradiated. Under such conditions males are predicted to make longer flights than typical territorial perchers do in woodland. In woodland, the majority of males show either a clear perching or patrolling behaviour, although a minority may have an intermediate behaviour (Van Dyck et al. 1997b). We tested these predictions using data from behavioural observations and mark-release-recapture programmes in woodland and a highly fragmented agricultural landscape in central Belgium.
Methods

STUDY SPECIES

The speckled wood is a temperate-zone satyrine butterfly (Lepidoptera: Nymphalidae). Throughout Europe, it is primarily a woodland species (Tolman & Lewington 1997). In the northern parts of the European distribution it is confined to woodland, whereas more to the south it also occurs in more fragmented landscapes with some woodland aspects such as agricultural landscapes with hedgerows and small patches of woodland (Dover & Sparks 2000, Mercx et al. 2003). Honeydew is the main adult feeding resource and several grasses are used as larval host plants (Shreeve 1986a). In woodlands, females lay eggs singly on isolated grass plants that grow under shaded, rather wet conditions (Wilkund & Persson 1983). In Belgium, P. aegeria is a common and widespread species (Maes & Van Dyck 1999).

STUDY AREAS

We studied male mate location behaviour of P. aegeria in central Belgium at four different sites representing the two contrasting landscapes: woodland and a highly fragmented agricultural landscape. Meerdaalwoud (1255 ha) and Bos ter Rijst (280 ha) represent closed landscapes dominated by oak woodland. Hoegaarden (603 ha) and Rillaar (361 ha) represent open, agricultural landscapes. The latter type of landscape was composed mainly of intensively used fields and pastures (Hoegaarden: 85%; Rillaar: 67%) of the total area/landscape, urban areas (4% and 20%, respectively), tiny patches of woodland (3%) and sunken roads, which are eroded agricultural tracks, bordered by steep grassy and woody banks (7% and 3%, respectively). The sunken roads are 0.5 m to several m deep, 5 - 25 m wide (Gulinck et al. 1991) and from 80 (but sometimes with gaps of some m) to 1370 m long.

BEHAVIOURAL OBSERVATIONS

In spring and summer 2002, we observed the behaviour of 62 P. aegeria males under fine weather conditions that allowed butterfly activity: 26 in the woodland landscape (Meerdaalwoud: 17; Bos ter Rijst: 9) and 36 in the agricultural landscape (Hoegaarden: 22; Rillaar: 14). During an observation session, an individual male was followed for 5 min. Different activities (flying, basking and resting) were recorded with a minidisk (Sony MZ-N710). When the male took flight, we also recorded take-off speed (slow versus fast) and flight altitude (0.5 m). Labelled sticks were placed at positions where individuals rested or changed flight direction. After the observation session, we measured the positions of all sticks and distances between sticks, with a laser instrument (Leica DISTO classic3, Leica Geosystems AG, Heerbrugg St. Gallen, Switzerland). Measurements were stored on schematic drawings of the flight tracks. We calculated the following measures for each observation session: proportion of time spent flying, number of displacements, (proportional) number of fast take-offs, mean altitude of flight and mean and total distance moved. For each observation session, we also recorded ambient temperature (± 0.1°C) and relative wind velocity (Beaufort scale). After the observation session, individuals were captured by hand net and killed and stored at -18°C. Individuals that did not adopt mate location behaviour (but exclusively foraged or rested) were excluded from analyses. As a result, analyses were run on 50 individuals. During behavioural observations, ambient temperature was lower in the agricultural than in the woodland landscape (19.6 ± 0.3°C versus 23.1 ± 0.7°C; Student T test: t = -4.74, P < 0.001), and mean wind force was higher in the agricultural than in the woodland landscape (Fisher’s exact test: P = 0.006).

MARK - RELEASE - RECAPTURE DATA

We collected mark - release - recapture (MRR) data during six surveys (8 - 13 August 2000) at one agricultural landscape area (Rillaar) and during five surveys (23 - 29 August 2000) at one woodland landscape area (Meerdaalwoud). Surveys were conducted only during periods with fine weather conditions. To obtain comparative sampled areas, we did MRR experiments in a part of each study landscape: 81 ha/4.9 km and 138 ha/7.4 km (area/perimeter ratios of Rillaar and Meerdaalwoud 165.3 and 186.5, respectively). The largest recordable movements were 1550 m and 2200 m, respectively. During each survey, we checked the whole area for males and females, and altered the trajectory regularly. We captured butterflies by hand net and marked them individually at first capture with unique numbers on the ventral side of the left hindwing with fine, nontoxic, permanent markers (Staedtler Lumocolor Permanent marker 313, Staedtler, Nürnberg, Germany). On first capture, butterflies were released at the site of capture. For second captures, butterflies were collected and stored in the laboratory (-18°C). For each capture we recorded data, time, exact position and mark number. Before each capture, we observed the mate location behaviour for several minutes, to classify a male as (1) percher, (2) patroller or (3) showing a behaviour intermediate between perching and patrolling (see method applied by Van Dyck et al. 1997b). Distances of movements between capture and first recapture were measured on detailed maps of the study sites. Distances were log transformed prior to analyses.

STATISTICAL ANALYSES

We used mixed regression models (Proc Mixed, SAS 2001) to analyse the behavioural data. All time, length and number variables were expressed as proportions of total observation time. Since we were mainly interested in the effects of landscape (open versus closed) and of ambient temperature, we used these variables and their interaction as fixed effects in the models. As two study sites represent each landscape structure, ‘site’ nested within ‘landscape’ was used as a random effect in each model. As data were collected during two periods (period 1: end of April - start of June; period 2: mid August), we also included period as a random effect. Backward selection of nonsignificant factors was done to obtain
Densities of both sexes, expressed as encounter rate within suitable habitat, were significantly higher in the agricultural landscape than in the woodland landscape (0.19 versus 0.13 individuals/min; \( t_{13} = -3.26, P = 0.006 \)). In the agricultural landscape, males performed significantly more fast take-offs (\( X \pm SE = 2.9 \pm 0.4, \) range 0 - 8 versus \( 1.5 \pm 0.3, \) range 0 - 5 per 5 min) but also more displacements (5.2 \( \pm \) 0.6, range 1 - 16 versus \( 3.2 \pm 0.5, \) range 1 - 7 per 5 min) than did males in the woodland landscape (Figure 5.1a, b, Table 5.1). The frequency of fast take-offs also tended to increase with increasing ambient temperature. Males from the agricultural landscape covered larger total distances during the behavioural observation session than conspecifics from the woodland landscape (\( X \pm SE = 48.2 \pm 6.9 \) m, range 3.5 - 173.5 m versus \( 32.4 \pm 5.1 \) m, range 3.3 - 103.4 m). However, this difference switched at relatively high ambient temperatures (Figure 5.1c, Table 5.1). Similarly, the mean displacement or step length was larger for males in the agricultural landscape than for woodland males at low ambient temperatures, but the relation reversed at relatively high ambient temperatures (Figure 5.1d, Table 5.1). Landscape had no effect on the proportion of time spent flying among males, but males flew more frequently at low heights in the agricultural landscape than in the woodland landscape (Fisher’s exact test, \( P = 0.008 \)).

**Table 5.1. Results of mixed regression models (Mixed procedure, SAS) for the proportional number of fast take-offs and displacements and total and mean displacement length in relation to the landscape of origin of male *P. aegeria* butterflies (woodland or fragmented agricultural landscape) and ambient temperature at the start of the observation**

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</tr>
<tr>
<td></td>
<td>temperature</td>
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<td>1.15</td>
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<tr>
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<td>landscape x temperature</td>
<td>1.45</td>
<td>8.57</td>
<td>0.005</td>
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</table>

Figure 5.1. (a) Number of fast take-offs (\( X \pm SE \)), (b) number of displacements (\( X \pm SE \)), (c) total displacement length and (d) mean displacement length of male *P. aegeria* butterflies in a woodland landscape (\( n = 20 \)) and in a fragmented agricultural landscape (\( n = 30 \)) under natural conditions, proportional to observation time.
At the agricultural landscape site, we captured 158 males and 47 females; 30.4% of the males were recaptured, but no females were. At the woodland landscape site, we captured 105 males and five females; 37.1% of the males and one female were recaptured. Females therefore had lower recapture frequencies than males, especially in the agricultural landscape site ($\chi^2_{1} = 8.64, P < 0.0001$). Overall recapture percentages (i.e. for both sexes) and the proportion of mobile (>50 m) to sedentary (<50 m) males did not differ with landscape structure. Within the subsample of mobile males, tracked distances did also not differ between the landscape types.

The frequency of males showing pure territorial perching behaviour was significantly lower in the agricultural landscape than in the woodland landscape (based on first capture data; 42 versus 80%; $\chi^2_{1} = 37.05, P < 0.0001$). Mate location behaviour intermediate between perching and patrolling was relatively rare in the woodland landscape, but much more common in the agricultural landscape (10 versus 48%; $\chi^2_{1} = 40.82, P < 0.0001$).

### Discussion

Our study showed significant differences in the mate location behaviour of male *P. aegeria* butterflies living in a woodland landscape versus a highly fragmented agricultural landscape. In the latter landscape, males more frequently performed fast take-offs from a resting posture than in the woodland landscape. Such take-offs are typically associated with territorial perching (see Rutowski 1991 and references therein). Since densities per unit of habitat were higher in the agricultural landscape, this observation is in contrast to the territory economics hypothesis and our first prediction of more patrolling rather than perching in the fragmented landscape. On the other hand, agricultural landscape males made more and wider displacements, associated with patrolling, than woodland males. This result is in line with the thermal constraint hypothesis and the second prediction of higher mobility in the fragmented landscape; during observations, however, ambient temperature was lower in the agricultural landscape, so that the assumed higher average ambient temperature is not likely to explain the difference. In the independent census, we found in the same vein a much higher frequency of behaviour intermediate between perching and patrolling in the agricultural landscape than in the woodland. This suggests that the clear dichotomy of perching versus patrolling as typically observed in woodland (Van Dyck 2003 and references therein) fades away to some extent in a highly fragmented, agricultural landscape.

In earlier studies on *P. aegeria* the vast majority of males could be classified as either percher or patroller (Davies 1978, Wickman & Wiklund 1983, Shreeve 1987, Van Dyck et al. 1997a, b). Males are consistent in adopting one of the two strategies, although switches may occur (Van Dyck et al. 1997b). In addition to males switching, we observed that some males also combine traits of both strategies into intermediate mate location behaviour. Fischer & Fiedler (2001) showed in another butterfly that aggressive, territorial males also used a flexible combination of perching and patrolling. Our study shows that the degree to which *P. aegeria* males do so depends on the type of landscape.

Although we found clear differences in mate location behaviour with landscape, the underlying mechanism requires further analysis. Mate location strategies have been described in relation to demography but in many ectotherms (including butterflies) mate location behaviour is strongly affected by thermal conditions (Willmer 1991). Wickman (1985, 1988) concluded for two other butterflies that population density is of small, often negligible, importance for determination of mate location behaviour.

Fast take-offs from a resting posture are facilitated at higher temperatures (Berwaerts & Van Dyck 2004). We found a trend towards increased frequency of fast take-offs with increasing ambient temperature. In an open agricultural landscape, ambient temperature is typically higher than in deciduous woodland, where there is a cooler, more buffered microclimate (Merckx, Van Dongen, Matthesyn & Van Dyck, unpublished). However, the influence of wind by convective cooling can be much more severe along a hedgerow in open landscape than on a sunlit patch in woodland (Merckx, Van Dongen, Matthesyn & Van Dyck, unpublished). In two other butterflies, individuals compensated for higher levels of wind speed, and hence convective cooling, by flying closer to structures that provided shelter (Merckx & Van Dyck 2002).

The higher frequency of intermediate mate location behaviour in the agricultural versus woodland landscape can be explained from an adaptive point of view. The combination of aggressive territoriality and movements could permit males in more linear, relatively narrow landscape structures to adopt a kind of ‘customs strategy’ towards conspecific males and females. Males would thus be able to keep an entire section of the linear habitat structures completely irradiated. Under such conditions, males in an agricultural landscape fly especially along or in hedgerows and sunken roads rather than through the open space. Circumstantial evidence of this comes from censuses where we collected females in this landscape (TM, pers. obs.). Furthermore we have experimentally shown that females originating from an agricultural landscape have lower propensities to cross woodland - open space boundaries than do females from a woodland landscape (Merckx et al. 2003).

On the other hand, the higher frequency of intermediate mate location behaviour in the agricultural than in the woodland landscape could be nonadaptive and related to physical, optical differences in the occurrence of sunlit patches with landscape structure. These landmarks for mate location are more clearly delineated in woodland, where solar radiation penetrates through holes in the canopy; in a fragmented agricultural landscape such clearly delineated sunspots are scarcer and a considerable proportion of the linear habitat structures are completely irradiated. Under such conditions, males cannot use the contrast of sunshine and shade as a physical border of a territory. In woodland, males follow sunlit patches when patches move during the day, indicating
that only the simple light-shade contrast is used rather than particular vegetation structures per se. This nonadaptive, constraint explanation is obviously not mutually exclusive with the former explanation.

In conclusion, our study illustrates that behavioural consequences of habitat fragmentation are not limited to, for instance, dispersal, but behaviours related to reproduction such as mate location may also change. There are no reasons to believe that such changes are unique to butterflies.

**Acknowledgements**

Thanks are due to E. Janssens, K. Borghgraef and J. Cortens who assisted with fieldwork. The University of Antwerp (GOA 15R/3942) and the Belgian Federal Office of Scientific, Technical and Cultural Affairs (OSTC-PADD II EV/06/168) funded this research. T.M. is research aspirant with the Fund of Scientific Research Flanders-Belgium (F.W.O.).
As landscapes change, mobility patterns of species may alter. Different mechanistic scenarios may, however, lead to particular patterns. Here, we tested conflicting predictions from two hypotheses on butterfly movements in relation to habitat fragmentation. According to the resource distribution hypothesis, butterflies in more fragmented landscapes would have higher levels of mobility as resources are more scattered. On the other hand, these butterflies could have lower levels of mobility as they experience more frequently ‘hard’ habitat boundaries (i.e. higher crossing costs) compared to butterflies in landscapes with continuous habitat; i.e. the behaviour-at-boundaries hypothesis. We studied movements, habitat boundary crossing and habitat preference of laboratory reared individuals of *Pararge aegeria* that originated from woodland and agricultural landscapes, using an experimental landscape as a common environment (outdoor cages) to test the predictions, taking into account sexual differences and weather. Woodland butterflies covered longer distances, were more prone to cross open-shade boundaries, travelled more frequently between woodland parts of the cages and were more at flight than agricultural butterflies. Our results support the behaviour-at-boundaries hypothesis, with ‘softer’ boundaries for woodland landscapes. Since the butterflies were reared in a common environment, the observed behavioural differences rely on heritable variation between populations from woodland and agricultural landscapes.
Introduction

As habitats become more fragmented, changes in habitat patch geometry and quality may affect the costs and benefits of dispersal, resulting in changed dispersal rates among differently fragmented landscapes (Thomas 2000). If a continuous distribution of resources has broken up to a scattered distribution, responses towards higher or lower mobility can be expected depending on the spatial scale of fragmentation, or the spread of resources, relative to the maximum range of exploration and dispersal of a species (Van Dyck & Matthysen 1999). Both theoretical models (e.g. Leimar & Norberg 1997, Oliivi & Gouyon 1997) and indirect empirical evidence from changes in flight morphology (e.g. Dempster 1991, Thomas et al. 1998) have suggested evolutionary responses as a result of selection on mobility in relation to landscape structure.

More recently, behavioural aspects of dispersal have received more attention (e.g. Jonsen & Taylor 2000a, Conradt et al. 2001, Bowman et al. 2002, Hanski et al. 2002) and several studies suggest a direct relationship between variation in behavioural responses at habitat boundaries and variation in dispersal (e.g. Haddad 1999, Schultz & Crone 2001, Berggreen et al. 2002). Ries & Debinski (2000) showed that a less mobile habitat specialist butterfly returned more frequently at habitat boundaries than did a more mobile habitat generalist. Moreover, the specialist responded strongly to different types of boundaries, while the generalist responded only to boundaries of high contrast. At the intraspecific level, returning behaviour at boundaries occurred more frequently in a highly fragmented habitat network compared to a more aggregated network in the localized butterfly Prolossiana eunomia (Schtickzelle & Baguette 2003).

In this paper, we compare movements and behaviour of speckled wood butterflies (Pararge aegeria L.) from differently fragmented landscapes – woodland versus agricultural landscape – in an experimental common environment consisting of large outdoor cages. Our main purpose is to test predictions on adult butterfly mobility that follow from a resource distribution hypothesis versus a behaviour-at-boundaries hypothesis. For butterflies, the presence of suitable egg-laying sites is an important habitat characteristic. For P. aegeria in agricultural landscapes, habitat occurs in the form of hedgerows and tiny woodlots, and will be much more fragmented than the corresponding habitat in woodlands. One might then predict higher mobility in agricultural landscapes, for instance a higher incidence of long flights, as an adaptation to the scattered resource distribution (Taylor & Merriam 1995). On the other hand, one can make the opposite prediction when considering differential behavioural responses at habitat boundaries with type of landscape. For butterflies from a woodland landscape, most boundaries are soft boundaries in the sense that crossing them will bring a butterfly to another piece of woodland habitat after a few to tens of meters of open, but mostly sheltered, areas such as rides or clearings. In contrast, butterflies from agricultural landscape would rather use boundaries, like hedgerows, as their habitat per se, and leaving them can be interpreted as crossing hard boundaries in the sense of encountering open, unsheltered areas for tens to hundreds of meters (e.g. fields and pastures). Hence, as boundaries mean on average different things in the two landscapes, one may predict higher incidence of boundary crossing, or higher mobility at this local scale, in woodland butterflies compared to agricultural landscape butterflies.

Comparative field studies between landscapes may point at relevant behavioural differences, but are less powerful to test for selection on mobility as they cannot rule out behavioural flexibility. We are particularly interested in selection on behavioural responses and movements relative to landscape structure, which has often been assumed – for instance to interpret morphological variation among landscapes (e.g. Hill et al. 1999) – but has far less been directly tested. Therefore, we adopted an experimental approach by releasing butterflies from woodland and agricultural landscapes simultaneously in a common environment. We used naive butterflies that were reared under controlled conditions in the laboratory from females collected in both types of landscape. Hence, any behavioural differences would be a consequence of heritable variation.

Besides the effect of landscape of origin, we also examined sexual differences in mobility and how movements and behaviour were affected by weather conditions. In several butterflies (including P. aegeria), females have been considered the more dispersive sex (Baker 1984). However, males are likely to be more frequently observed flying as they spend most of their time locating females. Two strategies of mate location behaviour co-occur in P. aegeria: territorial perching (i.e. aggressive sit-and-wait behaviour on a sunlit patch) and patrolling (i.e. searching behaviour in more or less permanent flight over a wider area) (Davies 1978, Wickman & Wiklund 1983). Being flying heliotherms, weather conditions may have a severe impact on several aspects of butterfly biology, in particular on activity and movements (Dennis 1993). As ambient temperature increases, both males and females of P. aegeria fly for longer and they change microhabitat selection within woodland habitats (Shreeve 1984). So, in this paper we test the conflicting predictions in relation to landscape structure, as well as sexual and weather related differences and their interaction effects.

Methods

STUDY SPECIES

Throughout Europe, the speckled wood (Pararge aegeria L.) is primarily a woodland butterfly (Tolman & Lewington 1997), but may also occur in other habitat types with some kind of woodland aspect, including parks, gardens and along hedgerows. Adults are rarely seen on flowers, but mainly feed on other resources like honeydew. Several grasses can be used as larval host plant (Shreeve 1986a). Eggs are deposited on isolated grass plants, surrounded by some bare ground, that grow under shaded, rather wet conditions (Wiklund & Persson 1983).
SAMPLED LANDSCAPES AND BREEDING PROCEDURE

In August 2002, females of *P. aegeria* were captured at several sites in two different types of landscape in Belgium. The first was a closed landscape dominated by deciduous woodland with differently sized clearings, pastures and rides (Meerdaalwoud). The other was an open, agricultural landscape composed of intensively used fields and pastures (c. 80% of the area), houses and farms (c. 10%), commercial orchards (c. 5%), tiny woodlots (c. 2.5%) and several sunken roads with hedgerows (c. 2.5%) (Rillaar). Hence, from a speckled wood’s perspective, Meerdaalwoud is a rather continuous landscape as far as egg laying sites and sheltered sunlit patches for mate location are concerned, while in Rillaar such sites can only be found along or in hedgerows and tiny woodlots that are scattered in the landscape. Collected females were allowed to oviposit on tufts of grass (*Poa annua*) in small cages in the laboratory. Next, eggs were transported to Stockholm where the individuals were rear on *Poa annua* in climate rooms under controlled conditions (22 °C, LD 22 : 2 h). After emergence, adults – from 6 woodland and 12 agricultural landscapes families – were sexed and individually marked by writing a unique number on the ventral side of both hindwings with a fine permanent, non-toxic and waterproof pen with silver ink. Until release in the outdoor cages, adults were stored in climate chambers (8 °C, LD 21 : 3 h).

OBSERVATIONS IN OUTDOOR CAGES

Behavioural observations were done by one person between September 11-18 2002 in a manipulated environment in two identical, oblong outdoor cages with half-circle shaped cross sections (Manufacturer: Serres de France S.A., w x l x h: 8 x 30 x 4 m – Norberg et al. 2002). Both cages were located side by side with their longitudinal axes positioned in EW orientation in a sheltered pasture near Tovetorp field station (<100 km SW of Stockholm, Sweden). The first cage was somewhat more exposed than the second cage that was positioned between the first cage and a third cage that was not used. The cage roof and walls consisted of fine-meshed netting. The experimental cage set-up was similar to Leimar et al. (2003): both ends – 8 m each – had a closed, rather shady habitat condition (i.e. mimic for woodland structure) and the central zone – 14 m – was an open meadow. The closed habitat, providing shade with scattered sunlit patches, was created by the use of camouflage netting and non-transparant tarpaulin in which holes of varying size were cut. In addition, several sheets of camouflage netting were hanging down from the roof and two artificial christmas trees were placed in each end part. In each cage we provided six adult feeding stations (i.e. sugar solution on sponge). In each of the two cages five individuals of each sex and origin (woodland or agricultural landscape) – hence 20 individuals per cage in total – were placed at random positions. Densities were kept constant over time; if an individual was not observed during an entire day, it was replaced by another one of corresponding sex and origin. After five days of observations, all individuals were collected and replaced by a new sample of individuals according to the same experimental design. In total, 92 individuals were released.

A first group of individuals was observed during a three day period that was significantly warmer than the period of the second group of individuals (mean ambient temperature at noon: 20 ± 1 °C versus 14 ± 1 °C, U=0, n=3 and 5, P=0.036). Hence, weather condition was taken into account for the analyses as a categorical variable (warm versus cold weather).

During one-hour-long observation sessions, the observer walked slowly through the cage and binoculars were used to read individual marks on the wings from a distance without disturbing the butterflies. The location of each butterfly (using a 2 x 2 m grid system) and its behaviour (three categories: flying, resting and basking) were recorded. A subsample of flying individuals (n=75) was also followed until they were alighted; individual tracks and behaviours were recorded. From the observations (i.e. locations and behaviours) we inferred the following variables: (a) traversal rate (i.e. the number of movements from one shady end of the cage to the other divided by the time between the first and last observation, including only those parts of the day when weather conditions would allow butterfly activity; available time for active flight was assessed for each day separately), (b) moved distance rate within an observation session (i.e. the summed moved distance divided by the summed time between the first and last observations within the session), (c) tracked distance (i.e. the summed moved distance during the times the individual was followed divided by the number of these tracking events), (d) crossing tendency (i.e. the number of open-shade boundary crossings per tracking event), (e) proportion of observations in the open (the middle third, not including the 2 m wide boundary sections of the cage), and (f) proportions of observations an individual was flying, basking or resting.

STATISTICAL ANALYSES

Preliminary tests (including variable transformation choice, tests for variance homogeneity and detecting outliers) were done using R software version 1.6 (Ihaka & Gentleman 1996). The individual butterfly was used as statistical unit. Data were analysed in relation to landscape of origin (woodland or agricultural landscape), weather condition (warm or cold), and sex. We also included cage as a factor, as well as interaction terms, but we dropped such terms from the final models if they neither improved the fit of a model nor gave rise to P-values smaller than 0.1.

To analyse the data on traversals, we used SAS version 8 (SAS 2001; GenMod procedure). Assuming that an individual has a given probability per time unit to traverse the open middle part of a cage, the number of traversals over a period of time will follow a Poisson distribution. For this reason, we used generalized linear models with Poisson response (and log link function). Effects of variation in the time available for traversal were taken into account by using the logarithm of this time as so-called offset variable
in the models (this is analogous to a covariate). We judged the fit of a model by noting how close the scaled deviance per degree of freedom was to one.

For the other analyses we applied general linear models using the same SAS software (GLM procedure). We used the so-called Akaike Information Criterion (AIC) to judge the fit of a model. In the analysis of moved distance rate within an observation session, we excluded individuals with less than 15 min summed within-session time, since the random variation in the rate becomes very high for short times. For the measures based on proportions of observations (measures (e) and (f) above) we excluded individuals with five or less observations in total, to reduce random variation in the proportions.

By suitable choice of transformations for the dependent variables, and in some analyses removal of outliers, we achieved homogeneous variances in the different tests (we also checked that any statistical significances did not depend on the removal of outliers). Means are given ± SE.

**Results**

**MOVEMENTS BETWEEN SHADED PARTS**

Males seemed to be somewhat more variable in their traversal rate between the shaded ends of the cage than females. This could reflect variation in mate location strategies, in which case the model assumption of Poisson distribution of the number of traversal might be violated. The best fitting model that included both sexes showed a higher traversal rate in woodland butterflies compared to agricultural butterflies, and some non-significant tendencies for the other factors and interaction terms (Table 6.1a). If sexes were analysed separately, we found a very good fit for the females (scaled deviance per df of 1.006). Woodland females moved more frequently from one shaded part to the other than did agricultural females and traversal rate was higher under warmer weather conditions (Table 6.1b, Figure 6.1). On the other hand, the analysis for males showed a less good fit (scaled deviance per df of 1.124) and no significant effect of either landscape of origin or weather conditions (Table 6.1c, Figure 6.1).

**Table 6.1. Results of a generalized linear model for the traversal rate between shaded ’woodland’ parts in the outdoor cage in relation to the landscape of origin of the speckled wood butterflies (woodland or agricultural landscape) and weather condition (see Methods) when taking into account both sexes (a), females (b) and males only (c). The GenMod procedure was applied (SAS) using type III sums of squares.**

<table>
<thead>
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<th>effect</th>
<th>( \chi^2 )</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
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<td>0.002</td>
</tr>
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<td>sex</td>
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<td>0.75</td>
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<td>weather</td>
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<tr>
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<tr>
<td>c) traversal rate (movements/h) males</td>
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<tr>
<td></td>
<td>weather</td>
<td>0.06</td>
<td>0.81</td>
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</table>

**Figure 6.1. Traversal rate of female (N = 43) and male (N = 45) speckled woods from woodland landscape origin and agricultural landscape origin under warm (white bars) and cooler weather conditions (black bars) in large outdoor cages. Traversal rate is expressed as the number of movements from one shady end of the cage to the other plotted against time between first and last observation, including only those parts of the day when weather conditions allow butterfly activity. We used weighted means (with corresponding time present as weight) ± SE (by bootstrapping).**

**MOVEMENTS AND BOUNDARY CROSSING WITHIN OBSERVATION SESSIONS**

Flight distances per time unit within observation sessions were longer in woodland butterflies than in agricultural butterflies and were longer under cooler weather conditions (Table 6.2a, Figure 6.2a). There was a non-significant tendency for a difference in those distances between the two cages as distance moved tended to be longer in the second cage. Within the subsample of followed individuals, there was no significant effect on distance covered per tracking event (Table 6.2b). However, woodland butterflies were found to cross open-shade boundaries more often than did agricultural butterflies (Table 6.2c, Figure 6.2b).

**HABITAT PREFERENCE**

Only 20% of all butterfly observations were in the open middle part of the cage. The open part was, however, more used under cooler conditions than under warm conditions, with no significant differences between sexes or landscape of origin (Table 6.2d, Figure 6.2c). Considering only the boundary stripes, butterflies were observed predominantly along the open irradiated site of the boundary rather than in the shaded site (\( t_{1, 673} = 7.73, \ d.f. = 74, \ P < 0.0001 \)).
Flight Activity

Woodland butterflies were more often observed flying than agricultural butterflies, but this effect of landscape was larger for females than for males. Nonetheless, males were more observed at flight compared to females. The proportion of observations of flying butterflies differed between the cages as butterflies were more often at flight in the second cage. There was no significant effect of weather condition (Table 6.2e, Figure 6.2d). As there were no significant differences for basking, the results for resting largely represent the inverse of those for flying; woodland butterflies rested less than agricultural butterflies, and females more than males. There was an interaction effect of landscape of origin with weather: under relative cooler conditions butterflies were observed less frequently resting, and this effect was larger for woodland than for agricultural butterflies (Table 6.2f).

Our experimental approach to compare behavioural responses and movements among speckled wood butterflies in a large cage set-up revealed differences with landscape of origin. Woodland butterflies covered longer distances, were more prone to cross the open-shade boundaries, travelled more frequently between the shaded cage ends and were more frequently at flight than agricultural butterflies. Hence, the results support the prediction of the behaviour-at-boundaries hypothesis rather than the contrasting variable effect.
prediction of the resource distribution hypothesis. Particularly for the traversal rate between the shaded ends, the differences between landscapes were clearly present in females. In a similar cage set-up, Leimar et al. (2003) found higher traversal rates in speckled woods compared to another woodland satyrine Lopinga аchne (both from Sweden) which agrees with their differences in habitat exploration and dispersive nature. Within P. aegeria, a Swedish woodland population (N-European subspecies tircis) was found to be more mobile than a Madeiran population (S-European subspecies aegeria) living in more open habitats (Leimar et al. 2003). Our results suggest similar behavioural differences at a much smaller geographic scale within Belgian populations occurring in woodland versus agricultural landscapes.

The naive butterflies used in our experiment were the F1-generation (reared under common garden conditions in the laboratory) of wild-caught females from both landscape types. Hence, this study provides evidence for heritable behavioural differences – a prerequisite for selection – and suggests that differences among landscapes do not follow from behavioural flexibility alone. So, in addition to environmental differences among the landscape types that may directly mediate differences in behaviour, there are also intrinsic differences in responses.

Although there are no empirical studies yet available on long-distance dispersal movements and capacity in speckled woods (Hill et al. 1999a), one may argue whether the observed differences have any relevance for dispersal. Observed movements in the outdoor cage mainly reflect explorative or ‘daily’ movements (Leimar et al. 2003, Norberg et al. 2002). In a study by Van Dyck et al. (1997a) recapture distance of males within a complex of woodland fragments was on average less than 40 m within woodland vegetation, but some males moved between fragments covering up to 450 m. The spatial dimensions of the used cages do not allow a direct comparison of dispersal capacity per se. However, several recent studies have pointed out the significance of behaviour at boundaries to emigration decisions (Haddad 1999, Ries & Debinski 2001, Schultz & Cran 2001, Morales 2002, Schtickzelle & Baguette 2003). It is important to realise that behaviour at boundaries relates to dispersal propensity and not necessarily to dispersal capacity (in terms of covered distance), although both could be interrelated as well. Variation in the behaviour at boundaries can be studied at relatively small scales, like in our cages. Therefore, our results are at least relevant in terms of habitat exploration and dispersal propensity. Lower traversal rates and boundary crossing for speckled woods in agricultural landscapes do make sense as resources are much scarcer and travelling in open, unsheltered land is likely to be more costly for this primarily woodland butterfly. So, our study suggests that behavioural responses that affect dispersal in speckled woods have been changed by selection as woodland landscapes change to agricultural landscapes (i.e. increasing degree of habitat fragmentation).

Only little is known on landscape perception distances in butterflies (Conradt et al. 2000). However, the two separated ‘woodland parts’ in the experiment are within the perceptive – most likely visual – detection range of speckled woods (Merckx & Van Dyck, unpublished). Therefore, it would be highly relevant to test their movements in a much longer cage (e.g. length of 100 m or so). The potential role of visual stimuli (like another piece of woodland within a few meters of open area) relative to boundary crossing decisions requires further research. We hypothesize that this may contribute significantly to traversal rates (particularly in woodland species), and that its role varies among populations from woodland or agricultural landscapes. Hence, we predict that, in a much longer cage, woodland butterflies would move less between the woodland parts compared to agricultural butterflies, and that the overall mobility will be lower than in the current experiment, assuming all other factors (e.g. weather conditions, densities) being equal.

Speckled wood males were more frequently observed flying than females. This is in agreement with basic behavioural differences between the sexes in butterflies as males spend most of their active time budget to mate location, while females rest and bask during considerable time periods between oviposition, foraging and dispersal flights (Shreeve 1992a). This sexual difference in time budget use probably caused that the effect of landscape on flight activity – woodland butterflies were more often observed flying than agricultural butterflies – was larger in females than in males (i.e. significant landscape x sex interaction). Higher flight incidence is not always related to higher levels of net displacement (Merckx & Van Dyck 2002). The two distinct types of mate location behaviour that co-occur in speckled woods and also in our cages – territorial perching versus patrolling – provide an explanation for the less good fit to Poisson distribution of traversals among males compared to females. Territorial perchers are more likely to stay in one part compared to patrolers who will move on.

In contrast to movement rates and crossing tendency, we did not find differences in habitat preference among the woodland and agricultural butterflies; all preferred the shady, ‘woodland’ parts. However, there was a significant effect of weather condition. Under cooler weather, more butterflies were observed in the open, more irradiated part of the cage. This is in agreement with thermal microhabitat selection under natural conditions; in a British woodland population Shreeve (1984) observed that speckled woods were more frequently present on rides and clearings (i.e. higher levels of irradiance and ambient temperature) than on small sunlit patches in the woodland when ambient temperature was low, but the use of the latter type of microhabitat increased with temperature. Weather condition also affected female traversal rate in the predicted way; flight duration increased with ambient temperature (Shreeve 1984), which resulted in higher traversal rates under warmer weather conditions. There was no effect of weather on flight incidence, but as habitat preference changed towards warmer, irradiated sites under cooler conditions (see above), the butterflies were capable to compensate and keep the time budget for flight unchanged (at least within the weather conditions of the experiment). Proportions of butterflies seen at flight or at rest are obviously not entirely independent (see Results), but besides the effect of sex, there were highly significant
differences with landscape of origin for both variables. Moreover, in case of the analysis of proportion of resting, it is intriguing to notice that the effect of weather was larger in woodland than in agricultural butterflies (i.e. significant weather x landscape interaction). Being flying heliotherms, both landscape types have quite different thermal properties for butterflies and the degree of variation is likely to interact with weather conditions. In our common arena approach, both populations were faced with the same environmental set-up, and so the differences point at intrinsic differences how woodland and agricultural speckled woods deal with the environment. Such behavioural differences are likely to be underpinned by thermal physiological differences.

So far, the majority of research on dispersal has in particular focused on movement patterns (e.g. frequency distributions of distances, maximum distance, etc.). However, mechanistic research on movements is, as a complementary field, also required for a better understanding of dispersal and how this is affected in fragmented landscapes. Hill et al. (2001) observed a c. 45% slower expansion rate in speckled woods in a landscape that had 24% less woodland. However, different mechanistic scenarios could lead to such a pattern, and remediating conservation strategies in fragmented landscapes would benefit considerably from a better understanding of the mechanisms. Hedgerows have often been assumed to be corridors for woodland species, but for butterflies (among several other taxa) there is hardly empirical evidence for this (Dover & Sparks 2000). From our experiments and preliminary work on woodland perception from varying distances (Merckx & Van Dyck, unpublished), we hypothesize that woodland butterflies may be encouraged to cross a woodland boundary if there is a landscape feature that is perceived as woodland (e.g. a hedgerow). Hence, networks of hedgerows would rather function according to a stepping stone principle than to corridors sensu stricto. This requires careful testing, and we, more generally, believe that further experiments on behaviour at habitat boundaries and on landscape perception will contribute significantly to conservation strategies at the landscape level.

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Chapter 7

Landscape and habitat-finding ability

Landscape-related differences in the ability to find habitat between populations of a woodland butterfly

[Thomas Merckx & Hans Van Dyck]

Summary

1. The ability of an organism to find new habitat is likely to contribute to dispersal success in different landscapes.
2. We compared the ability of speckled wood butterflies (*Pararge aegeria* L.) from two types of landscape to orient towards forested habitat as a behavioural assay to the ability to perceive forested habitat at a distance (25-200 m).
3. This field release-experiment showed that butterflies of fragmented agricultural landscape origin were better able to orient towards habitat from a wider distance than butterflies of woodland landscape origin. They followed more linear tracks, displayed flight angles that deviated less from orientation towards habitat, and showed orientations that were significantly directed towards habitat from wider distances than butterflies of woodland origin. If target habitat was more apparent, attraction was facilitated.
4. Our behavioural experiment provides novel insights into landscape connectivity and points at inter-population differences in the ability to find habitat associated with landscape type.
Introduction

Although dispersal is widely recognised to be a process with important ecological and evolutionary implications, there is yet much to learn about behavioural mechanisms, like search strategies, that dispersing animals employ to detect new habitat (Clobert et al. 2004). There is an increased interest to dissect the behavioural variation behind dispersal patterns, but so far most studies have particularly focused on dispersal propensity when comparing the tendencies to cross habitat boundaries between species or populations (e.g. Berggren et al. 2002, Ries & Debinski 2001, Schtickzelle & Baguette 2003, Schultz & Crone 2001). However, dispersal is a three-stage process including emigration (crossing of habitat boundary), traversing through a landscape matrix and settlement (immigration or colonisation) (Ims & Yoccoz 1997). Dispersal propensity is only one part of the chain to understand the process of dispersal. The ability of animals to perceive habitat at a distance once they move through the landscape is also a relevant behavioural mechanism that may contribute to dispersal success in fragmented landscapes (Lima & Zollner 1996, Zollner 2000). However, empirical information on the perceptual abilities of animals in real landscapes is still limited (Desrochers & Hannon 1997, Gillis & Nams 1998, Zollner 2000, Zollner & Lima 1999, Bonte et al. 2004, Schooley & Wiens 2003).

In this article, we report on orientation behaviour in a woodland butterfly – the speckled wood Pararge aegeria (L.) – to find woodland habitat and we test the hypothesis of differential abilities among populations from highly fragmented agricultural landscape versus woodland landscape. In the former landscape the habitat of P. aegeria is confined to scattered bits and pieces like hedgerows and small woodlots. From an adaptive point of view the time spent searching for forested habitat in a hostile agricultural matrix could be reduced by an increased ability to find habitat (e.g. increased perceptual range) compared to populations living in a woodland landscape with continuous habitat. Hence, we predict that butterflies from agricultural landscape have better perceptual abilities than woodland butterflies. We test the responses of the butterflies at two sites to evaluate additionally the role of the dimension of the target habitat using a pasture with a high woodland fringe and a pasture with a hedgerow. A high woodland fringe is expected to facilitate habitat attraction more than does a hedgerow.

We used, in a manner similar to Zollner (2000), the ability of P. aegeria from both types of landscape to orient towards forested habitat from a distance as a behavioural assay to the ability to perceive forested habitat at a distance. These abilities were assessed by capturing P. aegeria at distant woodland and agricultural landscape and releasing them at an unfamiliar pasture at different distances from forested habitat in order to follow and quantify their flight trajectories. Critical to this approach is the assumption that movement oriented towards the forested habitat is equivalent to the perception of habitat. In an earlier experiment in outdoor cages, Merckx et al. (2003) have shown evidence that P. aegera originating from both types of landscape preferred shady, sheltered woodland conditions to open conditions like pasture. Therefore, it is reasonable to assume that the released butterflies are motivated to search immediately for habitat. Moreover, open, relatively short-turf pasture is a hostile environment for P. aegeria which is essentially a woodland butterfly (Chardon et al. 2003).

Methods

P. aegeria is a satyrine butterfly (Lepidoptera: Nymphalidae). Throughout Europe the prime habitat is woodland, but the species also occurs in fragmented landscapes with some kind of woodland aspect like along hedgerows and in woodlots in agricultural landscape (Merckx et al. 2003). Adults mainly feed on honeydew and to a lesser extent on nectar.

Equal numbers of P. aegeria males were captured at two different types of landscape in central Belgium (n = 98). The first landscape of origin was closed landscape dominated by deciduous woodland with differently sized clearings and rides (Meerdaalwoud). The second was open, agricultural landscape composed of intensively used fields and pastures (85% of the area), houses and farms (4%), tiny woodlots (3%) and several hedgerows or woody sunken rural roads (Hoegaarden). Hence, the landscapes represent a contrast between rather continuous habitat for P. aegeria versus highly fragmented landscape (habitat <5% of the area, and scattered across the landscape). Butterflies were captured from different sites in each landscape (see Merckx et al. 2003). Hence they most likely belonged to separated local populations in each landscape as the distance between individual captures was 0.5 to 5.0 km. They were captured by hand net, temporarily stored in individual plastic jars and transported in a cool box (ca. 15°C) to one of the release sites.

The release-experiment was done during nine days with fine weather conditions that allow butterfly activity between the end of July and the beginning of September 2002. Butterflies were released – one at the time – at two different sites representing open agricultural landscape (mainly pastures under active agricultural use) with forested habitat at one side only. None of the butterflies was familiar with the site of release. At one of the release sites, the forested habitat was a ca. 40 m high woodland fringe. At the other site, it was a tall hedgerow with a height of ca. 5 m. Release distances from the habitat edge were 25, 50, 100 and 200 m. Releases were randomised between release distances and landscapes of origin. Before release, an individual was transferred from the cool jar into a butterfly net without any direct handling by the observer. The butterfly typically crawled out the net, started to bask by spreading the wings dorsally and took off spontaneously after a while. At that moment observations started. Two observers tracked the butterfly; one placed tagged sticks to register the flight track and the other timed – using a stopwatch – and recorded the observed behaviours. Butterflies were never followed too close (<0 m) to avoid interference from the observers. An observation session lasted until the butterfly either (i) reached the forested habitat, (ii) did not reach habitat but crossed a 250 m distance threshold and continued to fly into the open, agricultural landscape away from the forested habitat, or (iii) interrupted its track and rested (>5 minute).
The latter category (n=7) was obviously not used in the analyses. After each release, the XY-co-ordinates of the placed sticks were determined by measuring the distances to reference points (i.e. triangulation) using a laser instrument (Leica Disto® classic®).

From the flight trajectories (including positions and timing) and behavioural observations we derived several parameters that typified the movement pattern. For each individual it was recorded whether or not it reached the forested habitat. Total covered distance, total flight time and mean flight velocity were measured. Flight trajectories were classified as one of four classes: (i) straight flight track, (ii) mainly straight flight track, but with one curve, (iii) flight track with several curves, without returning to the point of release and (iv) petal-like loop pattern (i.e. an at least almost complete ellipse or more ellipses from and back to the point of release). We imposed 25 m-sections from and parallel with the habitat edge on the open landscape matrix. If a flight track crossed such a 25-m line, the angle between the flight track and the straight line to the habitat (0-360°) was determined. The linearity of flights between two successive 25 m-section lines was expressed as Euclidean distance / real covered distance. Measures of linearity were arcsine root transformed prior to analysis to reach normality. For each release event, average wind speed and ambient temperature were measured.

Mixed regression models were used to analyse the movement parameters [movement angle (0-360°) and linearity] in relation to landscape of origin, distance to habitat and their interaction as fixed effects. Only four classes (25, 50-75, 100-150 and 175-225 m) were used for the parameter distance to habitat based on the 25 m line sections because a larger amount of classes reduced goodness of fit of the models. Since the flight track of an individual may cross several 25 m line sections, ‘individual’ was included in the model as a random effect. Due to significant three-way interaction effects (landscape of origin x distance to habitat x release site), analyses were done separately for the two release sites as landscape of origin and the interaction with distance to habitat are the factors of highest interest. Mean velocity was analysed as a function of landscape of origin, release distance, release site, and all mutual interactions as fixed effects and residual ambient temperature as a covariate (Proc Mixed, SAS 2001). The response variable ‘reaching habitat or not’ was analysed with a model with a binomial error structure using landscape of origin, release site and distance to habitat as fixed effects (Glimmix macro, SAS 2001). In a separate analysis for reaching habitat, the effect of type of flight track was tested. The flight track class ‘straight with one curve’ was omitted from that analysis since it could not be estimated (n=11). Wind speed was always included as a random effect. Backward selection of non-significant factors (highest P-values) was done to obtain the final statistical model. Differences of Least Squares Means were used as a posteriori tests among groups. Normality of model residuals was always checked.

Directedness towards the forested habitat was analysed using circular statistics (Oriana version 1.01, Kovach Computing Services, Anglesey, Wales). As this could not be done in a multivariate way, three separate tests were done: 1) distance to habitat for both release sites together, 2) distance to habitat for both landscapes of origin separately, and 3) distance to habitat for both release sites separately. Here, five classes could be used for the distance to habitat (25, 50, 75, 100 and 125-225 m). Rayleigh tests were used to assess whether flight angle distribution differed significantly from randomness. Mean vector length (r) and 95% confidence intervals were calculated as measures of the variation around mean direction (Batschelet 1981).

**Results**

**REACHING TARGET HABITAT, TRAJECTORIES AND VELOCITY**

The probability that a released speckled wood butterfly reached the target habitat decreased with distance from habitat (F_{1,7}=6.25, P<0.001). Success rate in reaching habitat was higher at the site with a high woodland fringe as target than at the site with a hedgerow as target (F_{1,7}=7.10, P=0.012).

A flight track with several curves, but without returning to the point of release, was shown by most butterflies (45.1%). One quarter of the observed flight tracks were straight (25.3%). A straight flight track with only one curve was followed by 13.2% of the individuals, and a petal-like loop pattern was shown by 16.5% of the individuals.

Mean velocity increased with release distance from habitat (at 25 m: 2.04±0.2 m/s; at 50 m: 2.30±0.3 m/s; at 100 m: 2.50±0.2 m/s), but was considerably lower in the butterflies released at 200 m: 0.95±1.0 m/s (F_{1,3}=4.24, P=0.0077). Velocity did not differ with landscape of origin, release site or ambient temperature.

**LINEARITY OF FLIGHT TRAJECTORIES**

At the woodland fringe site, flight tracks of butterflies of agricultural landscape origin had a significantly higher level of linearity than flight tracks of butterflies of woodland landscape origin (0.910±0.02 versus 0.840±0.03; F_{1,4}=6.16, P=0.017). This effect could not be detected at the hedgerow site. Whereas there was no effect of distance present at the woodland fringe site, we observed a trend for higher linearity at 25 m from habitat than at distances from 100 m on at the hedgerow site (t_{11}=1.95, P=0.054).

**FLIGHT ANGLE RELATIVE TO THE TARGET HABITAT**

At the woodland fringe site, flight angles of butterflies of agricultural landscape origin deviated significantly less from straight direction to the habitat than flight angles of butterflies of woodland landscape origin (51.9°±16.3 versus 75.9°±17.1; F_{1,4}=4.53, P=0.039). There was an additive effect of distance from habitat as flight angles deviated more from straight direction to the habitat with distance from habitat (F_{1,7}=2.81, P=0.043; Figure 7). At the hedgerow site, there was no significant effect of landscape of origin, nor of release distance. However, angles deviated more from straight direction to the habitat at distances from 100 m on than they did at 25 m from habitat (t_{11}=2.04, P=0.044).
flight directedness

In the overall test, the distribution of the orientation of the butterflies differed significantly from non-random until 100 m from the habitat. In case of a non-random pattern, the orientation was always towards the habitat as reflected by the mean vector directions close to 0°. Vector directions became more scattered around the mean vector with increasing distance from the habitat (Table 7.1a; Figure 7.2a).

When testing for the effect of the landscape of origin, different thresholds for the distance at which flights were orientated towards the habitat were found. Butterflies of agricultural landscape origin showed significant differences from randomness until 100 m, whereas butterflies of woodland landscape origin did so up to 50 m only. So, mean vector lengths at 75 m and 100 m were larger in butterflies of agricultural landscape than of woodland landscape (Table 7.1b; Figure 7.2b). Comparing the effect of release site, butterflies at 75 m from habitat possessed orientations that differ significantly from randomness at the woodland fringe site, whereas orientations were only marginally significant directed at the hedgerow site (hedgerow: 37°). The mean vector length was also larger at the woodland fringe site (woodland fringe: 0.57 versus hedgerow: 0.33 – Table 7.1c; Figure 7.2c).

Our release-experiment showed that speckled wood butterflies were less likely to orient towards forested habitat from a wider distance in open agricultural land within a range of release distances of 25-200 m. If the target habitat was more apparent (high woodland fringe versus lower hedgerow), attraction towards the forested habitat was facilitated. In line with our key prediction, butterflies of agricultural landscape populations were better able to orient towards habitat from a wider distance than butterflies of woodland landscape populations. They followed tracks with a higher degree of linearity, displayed...
flight angles that deviated less from orientation towards habitat, and showed orientations that were significantly directed towards habitat from wider distances than butterflies of woodland origin. Selection for directed flight in fragmented, agricultural landscape populations is in line with an adaptive response in such an environment as it reduces the time budget spent within the hostile matrix; it is likely to lower associated costs in terms of energy, predation risk and loss of active time for reproduction (Zollner & Lima 1999).

Our results add to the diverse and highly significant ecological and evolutionary effects that habitat fragmentation may have on life history traits of organisms, including dispersal. Earlier, our colleagues and we have detected differences among P. aegeria populations of woodland versus fragmented agricultural landscape in dispersal propensity (Merckx et al. 2003) and in temperature-related fecundity and longevity (Karlsson & Van Dyck 2005). In both studies the butterflies that were tested were reared in a common environment after parental stock was collected in both types of landscape. Here, we directly compared captured adults of both types of landscape as was done in a similar study with small mammals (Zollner 2000). So, from a causal point of view, we cannot discriminate at this stage between intrinsic differences versus differences in experience between the butterflies of different landscape of origin. This requires further testing, but for habitat crossing behaviour we earlier showed evidence that both groups differ intrinsically, most likely relying on heritable differences (Merckx et al. 2003).

The observation of insect dispersers that need to approach potential habitat relatively close to be attracted has been made a few times before. Coyne et al. (1987), for instance, showed that Drosophila flies moved distances of several kilometres between oases, but they were only able to detect an oasis from <10 m distance. Euphydryas butterflies also moved up to 5 km between habitat patches, but they only oriented towards a serpentine grassland habitat at a distance of <50 m (Harrison 1989). But the differences between P. aegeria populations of different landscapes suggest that detection ability may vary considerably at the intraspecific level. Butterflies from agricultural landscape oriented towards forested habitat from twice as wide than butterflies from woodland landscape (100 versus 50 m). Besides the clear average differences with landscape of origin, there was considerable inter-individual variation. For instance, two individuals released at 200 m from habitat successfully reached habitat adopting a flight track of high degree of linearity (values of 0.98 and 0.93).

Although our study does not provide insight into the sensory mechanism of habitat perception, butterfly movements are most likely to be visually cued. However, considering the relatively poor acuity of the butterfly eye, Rutowski (2003) predicts that even large objects such as trees several meters high may not be resolvable at distances of more than 20 to 30 meters. However, empirical data on landmark detection at the landscape level are – to our knowledge – lacking in butterflies (among several other taxa). The size of the compound eyes relative to total body size did at least not differ between specimens of both types of landscape (T. Merckx, unpublished data), but there are several

Figure 7.2: Angular orientation of released butterflies, defined relative to the point of release and the place at which following was stopped. The dots on each unit circle represent the angular orientations of individual butterflies. The part of the habitat closest to the point of release is defined as 0° for each release. Vectors indicate average angle (mean vectors (m), Batschelet 1981) and are surrounded by 95% CIs. Data are given for (a) butterflies of both release sites together at five distance classes from forested habitat (25, 50, 75, 100, 125-225 m), (b) butterflies of both landscape origins separately at distances from forested habitat of 75 and 100 m, and (c) butterflies of both release sites separately at a distance from forested habitat of 75 m.
other traits that may relate to visual detection capacity (Rutowski 2003 and references therein). We cannot exclude an additional, or even to some extent alternative, role of olfactory cues (Baguette 2003, Fownes & Roland 2002). Hansson (1995) suggested plant-produced odours to be potentially important cues for locating and evaluating foraging sites in Lepidoptera, but direct evidence has yet to be experimentally shown in butterflies.

The presence of habitat across the landscape has been shown to affect expansion rates in *P. aegeria* in different areas in the U.K. (Hill et al. 2001). At a smaller spatial scale, the incorporation of habitat elements into a landscape connectivity measure significantly improved modelling patterns of presence/absence for *P. aegeria* in woodland fragments compared to the use of Euclidean distance in two Belgian landscapes (Chardon et al. 2003). Merckx et al. (2003) showed that *P. aegeria* from woodland landscape had higher propensity to cross habitat boundaries than did conspecifics from agricultural landscape in a common arena test. In combination with our current results on differential perceptual ability, we hypothesise that *P. aegeria* butterflies of agricultural landscape are better able to deal with a coarse-grained ecological infrastructure (e.g. less compact network of hedgerows or woodlot stepping stones) to move through fragmented landscapes than are butterflies of woodland landscape populations. Relatively low perceptual ability is likely to be associated with more time spent searching in the hostile matrix. This in turn may increase mortality risks that are considered important costs associated with dispersal (Waser et al. 1994, Mennechez et al. 2004). The idea of differential optimal or minimally required geometry of corridor or stepping stone networks for the same species in different types of landscape is valuable to test in several other species, particularly those of high conservation interest. The importance of strong contrasts to facilitate orientation to forested habitat is also a significant observation in this respect. The woodland fringe was eight times higher than was the hedgerow at our release sites. So, hedgerows and woodlots with much taller dimensions – particularly higher trees – are predicted to be landmarks of wider attraction across the landscape for this type of organism. Such insights can be taken into account in modelling connectivity using cost distance approaches (Chardon et al. 2003). They further contribute to an improved understanding of how structural landscape connectivity is translated into functional connectivity for a particular target species (Bowman & Fahrig 2002, Schooley & Wiens 2003, Stevens et al. 2004). More generally, it illustrates that a better understanding of perceptual ranges and the effects on movements will contribute to the analyses of population dynamics in fragmented landscapes and hence to conservation strategies (Lima & Zollner 1996).

Our study also raises questions about behavioural mechanisms of movements through the landscape matrix that are interpreted as being dispersal. Conradt et al. (2000) and Conradt et al. (2001) concluded from release-experiments with two other satyrine butterfly species that dispersers search systematically for habitat by flying in a succession of progressively larger ellipsoidal loops away from and back to their starting point. However, only 21.6% of the released butterflies engaged in this pattern of petal-like searching loops (Conradt et al. 2001). One could argue that their sites of capture and release were too nearby relative to the spatial scale of movements and to the perceptual range in these species to exclude home-range like movements or real homing. It has been noticed before that homing movements differ behaviourally from dispersal movements in several taxa (Zollner & Lima 1999, Van Dyck & Baguette 2003). Moreover, it has been recognised that the petal-like loop movements are typical for homing (e.g. Hoffmann 1983) and for movements to exploit local resources (e.g. Müller & Wehner 1994). In our study, all tested individuals were unfamiliar with the release sites. The propotion of released butterflies that engaged in the pattern of petal-like searching loops in our study was even slightly lower (16.5%) than the proportion reported in Conradt et al. (2001). Behaviourally different types of movement (like homing, and foraging as routine movements on the one hand and oriented linear movements as special movements to displace on the other) should be recognised more explicitly in dispersal studies as they have different assumptions and consequences on how animals move through the landscape (Van Dyck & Baguette 2003). Animals may extend explorative movements like during foraging beyond one physical patch of resources (e.g. Fraser & Stutchbury 2004). In our experiments, one particular butterfly that was released at 100 m took off with a strong vertical component and flew at high level (30 m versus typically 1-2 m) and very fast (6.2 m/s versus average of 2.14 ±0.13 m/s) in a straight line to the woodland fringe. In contrast with Conradt et al. (2000, 2001), fast, directed movements have been associated with dispersal by several other authors (e.g. Johnson et al. 2002, Mate et al. 1998). Both slow, explorative movements with lots of curves in the trajectories and fast, directed movements may contribute to net displacement, although the latter are likely to represent dispersal behaviour (Van Dyck & Baguette 2003). The role of sexual differences may need further attention as well, particularly if the dispersive nature is more pronounced in only one of the sexes. Here we have tested males only. Returning to visual perceptual abilities, it is known in several butterflies (including *P. aegeria*) that females have much smaller compound eyes than do males (Rutowski 2003). Yet, we are only at the beginning of understanding the behavioural mechanisms of dispersal and particular of how organisms perceive and respond to landscape elements and target habitat once they move through the landscape matrix.

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8.1 Habitat fragmentation, movement behaviour and thermoregulation

Especially the process of habitat fragmentation brings about the current world-wide extinction crisis (Pimm & Raven 2000, Wilson et al. 2004). However, despite an intense degree of habitat fragmentation, the speckled wood butterfly (P. aegeria) succeeds to remain a common and widespread butterfly species (Maes & Van Dyck 1999, Asher et al. 2001); its occurrence is not restricted to large patches of habitat (woodland) but also includes highly fragmented, agricultural landscapes where habitat is restricted to ‘bits’ and ‘pieces’ (e.g. hedgerows, small patches of woodland, woody sunken rural roads). Nonetheless, habitat fragmentation may have less visible evolutionary implications (reviewed in Debinski & Holt 2000, Fahrig 2003). In order to reveal this evolutionary impact, we observed and experimentally compared populations from differently fragmented landscapes.

Landscape structures that are altered by fragmentation often imply larger distances between resources. Furthermore, fragmentation may cause microclimates to change. This was shown for sites where P. aegeria occurs: open, highly fragmented, agricultural landscapes were on average warmer, but also more windy and more variable in temperature than corresponding sites of the butterfly in closed, continuous woodland (Chapter 3). This is of high relevance, especially for ectotherms like butterflies as their activity patterns are directly influenced by microclimatic conditions. This was, for example, observed in the higher traversal rate within our outdoor cage set-ups under warmer weather conditions and in the changed habitat preference for warmer, irradiat-
ed sites under cooler conditions (Chapter 6). In the same vein, we showed that P. aegeria dummies were able to cover larger distances given a fixed thermal budget, and that they heated up faster, under conditions of higher ambient temperature and lower wind speed (Chapter 3). The larger variation observed in cooling distance and heating rate within fragmented, agricultural landscapes, compared to woodland landscapes, is in line with the co-occurrence of higher ambient temperatures and higher wind speeds in agricultural landscapes (Chapter 3). Therefore, when ectotherms cross a boundary of an habitat patch in an unfragmented landscape versus a similar boundary in a fragmented configuration, this may mean a different cost: (i) the probability to encounter resources is lower in fragmented landscapes due to the scarceness of and the higher distances in between resources, and (ii) the probability to experience an open, unsheltered environment for a longer time is higher, and so are most likely the associated costs in terms of energy, time and mortality risk (exhaustion, predation) (Zollner & Lima 1999). These effects of altered landscape structures on boundary hardness, via combined effects on resource distribution and microclimate, may cause strong selection with regard to movement behaviour, such as selection against mobility with increased isolation (e.g. Dempster et al. 1976). The figure below illustrates the relationships and interactions between the different components involved in this study by means of a flowchart (Figure 8.1).

Figure 8.1. Overview of the relationships, at both the level of the environment and the organism, between the major components involved within this study of the evolutionary ecology of movement behaviour of P. aegeria butterflies within a context of habitat fragmentation. Numbers on arrows relate to corresponding chapters that treat specific relationships.

In the following paragraph we summarise the evidence for an ecologically-based divergent selection (Rundle & Nosil 2005) between P. aegeria populations from differently fragmented types of landscape:

(I) In line with the assumption of higher dispersal rates with fragmented landscape structure, due to larger inter-resource distances (Taylor & Merriam 1995), we showed by means of a reciprocal transplant experiment with split-brood design that individuals that developed in agricultural landscape were heavier and had higher wing loading than individuals that developed in woodland landscape and landscape with woodland fragments, irrespective of their landscape of origin (Chapter 2) [for a detailed discussion of the functional significance of these flight-related morphological traits see Dudley (2000), Berwaerts et al. (2002) and Van Dyck (2003)]. This landscape-related phenotypic plasticity strongly points to adaptive differences in flight-related morphology since our observations contrasted with a constraint hypothesis, which predicted smaller individuals with fragmentation due to higher growth rates with higher ambient temperatures (Sibly et al. 1997, Nylin & Gotthard 1998).

(II) In line with a more frequent experience of ‘hard’ (i.e. higher crossing cost) boundaries in butterflies of fragmented landscapes than in butterflies of unfragmented landscapes, we observed in a common arena test that P. aegeria F1-individuals originating from a continuous woodland landscape had a higher propensity to cross habitat boundaries than had conspecifics from highly fragmented, agricultural landscape (Chapter 6).

(III) In line with selection for directed flight in fragmented landscape populations to reduce the time budget spent within the hostile matrix, our release-experiment is among the firsts to show evidence for intra-specific differences in the ability to find habitat relative to the landscape of origin of the tested individuals. As a key result, P. aegeria butterflies of agricultural landscape origin were able to orient towards habitat from a distance twice as wide than butterflies of woodland landscape origin (Chapter 7).

(IV) Besides behavioural compensations like flying low to the ground and close to shelter-providing vegetation (Chapter 3 and 5), tests with adult offspring from both landscape origins in flight cabinets at several low ambient temperatures pointed further to temperature-related physiological adaptation relative to type of landscape: individuals of woodland landscape origin flew for longer than did agricultural landscape butterflies (Chapter 4). Also within the range of voluntary flight, we have shown in a common arena test that P. aegeria of woodland landscape origin were more at flight than conspecifics from agricultural landscape (Chapter 6). In the same vein, Karlsson & Van Dyck (2005) have experimentally shown that females of woodland landscape origin laid more eggs at low ambient temperature than did agricultural landscape females, but the pattern reversed at higher ambient temperatures.
In line with the observed microclimatic differences between differently fragment- ed landscapes (Chapter 3 and 5), we demonstrated that individuals of agricultural landscapes compensated for higher amounts of convective cooling as they were covered with more fur than woodland individuals (Chapter 3). We interpret this to be a morphological adaptation since fur helps to reduce the impact of the typi- cally higher wind speeds that often leads to higher levels of convective cooling in agricultural landscape (Kingsolver 1983).

Although the underlying mechanism requires further analysis, male butterflies were observed to show a higher frequency of mate location behaviour intermediate between perching and patrolling in agricultural landscapes (Chapter 5). This illustrates that behavioural consequences of habitat fragmentation are not limited to dispersal – which has been the major issue in this field – but behaviours related to reproduction such as mate location may also change. As male butterflies spend most of their active time locating mates (Shreeve 1992a), different mate location strategies may have different consequences for rates of movements as well. Our results suggest that the dichotomy of perching versus patrolling as typically observed in P. aegeria males of woodland (Van Dyck 2003) fades away in fragmented agricul- tural landscape (Chapter 5).

The studied flight- and thermoregulation related morphological traits are known to have a heritable basis (Hill et al. 1999a, b, Berwaerts 2004), which is a prerequisite for evolution- ary change. Furthermore, differences observed via common garden and reciprocal transplant approaches may indeed point at intrinsic, heritable differences. Hence, the differences we observed may indicate evolutionary consequences of habitat fragmenta- tion. An important, accelerating factor in such processes of micro-evolution (relatively fast local adaptation) is phenotypic accommodation (i.e. evolutionary change without genet- ic change), which results from phenotypic plasticity (i.e. environmental respon- siveness) (West-Eberhard 2003). To our best knowledge, we are among the firsts to show results that suggest the existence of landscape-related, adaptive phenotypic plasticity in butterfly flight morphology (Chapter 2). Other key results are the significant genotype-by-environment (G x E) interactions that we found for some of these traits. In other words, we demonstrated that different genotypes produced a different flight-morphol- ogy in different microclimatic environments (i.e. differently fragmented landscapes) (Chapter 2). These interactions, that may be seen as genetic variation for plasticity (Pigliucci et al. 1999), strengthen the hypothesis that the observed landscape-related phenotypic plasticity is adaptive and that it contributes to the observed evolutionary changes in thermoregulation and movement behaviour (e.g. dispersal) in differently fragmented landscapes.

Throughout our study on the evolutionary ecology of P. aegeria’s movement behaviour in a context of habitat fragmentation, we observed that evolutionary changes in thermoregulation and movement behaviour (see 8.1.) differed relative to sex. These sexual differences can be understood in relation to the multifunctionality of butterfly flight (Shreeve 1992a, Watt 2003), which differs between males and females relating to their different behavioural repertoires and ecological roles (Van Dyck 2003). Males are the more ‘wasting’ sex. They spend most of their active time budget to mate location and, hence, mainly move locally but are more subject to convective cooling. In order to in- crease the probability of locating virgin females, males extend flight even during peri- ods with less favourable microclimatic conditions, because under relatively poor weath- er also virgin females may emerge. Females, on the other hand, are characterised by a trade-off between allocation towards reproduction versus flight (i.e. the oogenesis-flight syndrome – Hughes et al. 2003). Hence, females need to be more economical in terms of flight activity. So, butterfly females typically spend less time flying than males (Shreeve 1984) and they will preferentially fly during periods with favourable weather condi- tions: they rest and bask during considerable time periods between oviposition, forag- ing and dispersal flights (Shreeve 1992a). However, despite their lower flight incidence, females are considered to be the more dispersive sex (Baker 1984). This illustrates that general flight activity is not necessarily a good indicator of dispersal ability (Merckx & Van Dyck 2002). As a result of these different behavioural repertoires and ecological functions between the sexes, the two following selective regimes for some traits will be different between males and females. The selection pressure on thermoregulation- related morphological traits in order to maximise the time period that flight muscle temperature is within the optimal range for powerful flight, will be higher in males. On the other hand, since males spend most of their active time to mate location and, hence, mainly move locally, the selection pressure on flight-related morphology for enhanced dispersal ability will be higher in females.

In line with the predicted higher selection on some aspects of thermoregulation in males, we showed that the pattern of darker (i.e. faster heating (Van Dyck & Matthysen 1998, Berwaerts et al. 2001)) wings in springtime compared to the warmer summertime was only present in males; it was lacking in females (Chapter 3). In the same vein, we showed that females have less fur (i.e. less thermal isolation, higher impact of convective cooling – Kingsolver 1983) than males (Chapter 3) and had a lower willingness to fly at temperatures within the range of voluntary flight activity in the experimental cages (Chapter 6). The latter observation most likely contributes to the explanation of male-biased samples in mark-release-recapture studies (e.g. Wahlberg et al. 2002). In line with the key predictions of the indoor experiment on flight ability at suboptimal temperatures, males and females responded differently: again, males were more will- ing to fly than females (Chapter 4). Ultimately, the latter observed sexual difference
relates to the fundamental differences in life history between the sexes. From a proximate viewpoint, this difference can be attributed to lower wing loading (i.e. ratio of total body mass to wing area) in males from a proximate point of view (Gilchrist 1990). It allows flight at lower wingbeat frequency (Casey 1981) and wingbeat frequency is temperature-dependent (Stevenson & Josephson 1990). For example, minimum temperature for flight in the butterfly Thymelicus lineola increased with an experimental increase in wing loading (Pivnick & McNeil 1986). The sexual difference in wing loading in P. aegeria is mainly caused by differences in body mass rather than forewing area (Berwaerts et al. 2002). So, the smaller male size can be seen as an adaptation to extend flight activity. Furthermore, we showed that only males differed adaptively in the amount of fur between types of landscape (Chapter 3). These sexual differences may result from differential selection regimes for thermoregulation-related morphological traits that allow maximisation of the time period that flight muscle temperature is within the optimal range for powerful flight. Comparative experiments with individuals that are exposed to different treatments to remove fur could test the hypothesis that males are confronted with higher selection on this aspect of thermoregulation. We predict that the negative effects of shaved thoraces on flight activity (duration of flight bouts, power…) will be more obvious in males than in females.

The predicted higher selection on the dispersal process in females is supported by comparative work on flight morphology between recently colonised sites versus permanently occupied sites: Hill et al. (1999b) found significant differences in P. aegeria females only; females from newly established populations were larger, had larger thoraces and had lower aspect ratios than females from old populations. In the same vein, Hanski et al. (2004) found significant effects – relating to energy metabolism – in females only in a study on variation in dispersal propensity among populations of the butterfly Melitaea cinxia. When testing for variation in dispersal propensity and flight activity among landscapes of origin, we also found the clearest responses in P. aegeria females (Chapter 6). Furthermore, landscape-related phenotypic plasticity for relative thorax mass was only present in females (Chapter 2). The direction of this effect was in line with the adaptive prediction about dispersal ability. In our release experiment on differential ability to detect habitat among butterflies from differently fragmented landscapes, we have, however, tested males only for practical reasons. Thus, the role of sexual differences in this context requires further testing. Nevertheless, it is known in several butterflies (including P. aegeria) that females have much smaller compound eyes than do males (Rutowski 2003). However, the size of the compound eyes relative to total body size did not differ between the tested males of both types of landscape, despite the clear effect of landscape of origin (Chapter 7). But there are several other traits that may relate to visual detection capacity (Rutowski 2003 and references therein). Only an experimental approach will be able to tell whether the effect of landscape of origin, as observed in males, is also present in females. Furthermore, this approach will allow detecting whether perceptual abilities in females, the more dispersive sex, differ from males’ abilities to detect habitat. All results on differential effects of habitat fragmentation on thermoregulation and movement behaviour for both sexes of P. aegeria add significantly to the diverse ecological and evolutionary effects of habitat fragmentation on life history traits of organisms.

8.3 Landscape, conservation and variation in functional connectivity

We take the view that P. aegeria individuals pay a higher cost when they leave a habitat patch within highly fragmented, agricultural landscapes, relative to the same action in a more continuous woodland landscape (see also 8.1.). In fragmented landscapes, boundary crossing may imply a higher chance to experience larger distances of open, unsheltered environment. Our common garden experiment with large outdoor cages showed that P. aegeria butterflies prefer forested spots over open environments: irrespective of the landscape of origin, butterflies preferred the shaded cage ends (forested habitat) over the open middle part of the cages (open matrix) (Chapter 6). This result supports the view that open landscape matrices are hostile environments for this butterfly species of forested habitat. The chances to get predated are probably higher in an open environment. Furthermore, resources are scarcer and inter-resource distances are larger in highly fragmented, agricultural landscapes. This may imply higher probabilities to lose ‘costly’ time and energy (Waser et al. 1994, Mnenchez et al. 2004). Our experiment with common garden cage set-up revealed that P. aegeria from highly fragmented, agricultural landscape had lower propensity to cross open-shade boundaries than did conspecifics from woodland landscape (Chapter 6). This result in combination with the higher ability to detect habitat in individuals from agricultural landscape (Chapter 7) leads to the following hypothesis: P. aegeria butterflies of agricultural landscape may be better able to deal with a more coarse-grained ecological infrastructure (i.e. a less compact network of corridors and stepping stones) to move through fragmented landscapes than are butterflies of woodland landscapes. This idea of a differential optimal or minimally required geometry of corridor or stepping stone networks for the same species in different landscape types is valuable to test in several other species, particularly those of high conservation interest. It may be significant to make use of such knowledge when restoring landscapes with regard to the conservation of particular target species. More specifically, knowledge of the optimal geometry of habitat networks may be useful when it comes to create environments that allow functional displacements of a sufficient number of individuals from particular focal species.

We hypothesised that P. aegeria from highly fragmented, agricultural landscapes may be better able to deal with a more coarse-grained ecological infrastructure than conspecifics from continuous woodland landscapes. Furthermore, we showed evidence
for locally adapted thermoregulation- and flight-related morphology (Chapter 2 and 3). This may further facilitate individuals from agricultural landscapes to undertake larger displacements, all the more given the on average higher ambient temperatures in agricultural landscapes (Chapter 3). At the same time, however, our results attract the attention to the importance of vegetation structures as potential corridors for movement across fragmented landscapes: we observed higher heating rates and a less variable thermal budget for flight in the vicinity of such structures, especially when wind speed was higher (Chapter 3). In the same vein, Mercilx & Van Dyck (2003) have shown earlier in two other butterfly species that they flew more in the vicinity of hedgerows, particularly during more windy conditions. So, the importance or significance of such landscape elements may be weather or climate dependent (see also Dover 1996, Dover & Sparks 2000). If butterflies are only able to make relative short flights, a more fine-grained ecological network of corridors would be required. Furthermore, we showed by means of P. aegeria dummies that flying more closely to the substrate may be an additional way to behaviourally lower the impact of convective cooling resulting from habitat fragmentation (Chapter 3). Accordingly, the study of Hill et al. (2001) indicated that the availability of breeding habitat is important: P. aegeria range expansion in the UK was c. 45% slower in an area that had 24% less woodland. Nevertheless, this result may largely depend on the nature and density of the network of greenveins within the intervening matrix, which have been shown to be major players in the lagging behind of species expansions to current, warmer climates (Hill et al. 1999c, 2002). So, a network of shelter providing structures in the landscape matrix may at least mitigate effects of habitat fragmentation as flying at low height in the vicinity of these structures significantly increases the thermal budget for flight (Chapter 3) and, hence, may increase landscape connectivity and extend flight distances for butterflies traversing through fragmented landscapes.

So far, the majority of research on movements has in particular focused on patterns (e.g. frequency distributions of distances, maximum distance, etc. – e.g. Clobert et al. 2001). But different mechanistic scenarios could lead to the same pattern. Therefore, mechanistic research on movements is required for a better understanding of movements (e.g. dispersal) and how this is affected in fragmented landscapes. As a consequence, remediation conservation strategies in fragmentated landscapes would benefit from a better understanding of the mechanisms. For example, hedgerows have often been assumed to be corridors for woodland species, but for butterflies (among several other taxa) there is hardly empirical evidence for this (but see Dover & Sparks 2000, Dover & Fry 2001, Cant et al. 2003). From our experiments, we hypothesise that woodland butterflies may be encouraged to cross a woodland boundary if there is a landscape feature that is perceived as woodland (e.g. a hedgerow). Hence, networks of hedgerows would rather function according to a stepping stone principle than to corridors per se. Moreover, for ectotherms such as butterflies hedgerows are likely to be rather stepping stones for thermal reasons than really continuous conduits which they may be for endotherms. Of course, this requires further and careful testing. Furthermore, the importance of strong contrasts to facilitate orientation to forested habitat is a significant observation in this respect (Chapter 7). From our experiment in which we compared the effect of an eight-fold difference in height of the target habitat on released butterflies, we predict that hedgerows and patches of woodland with much taller dimensions – particularly higher trees – may function as landmarks of wider attraction across the landscape for this type of organism. Such insights can be taken into account in modeling connectivity using cost distance approaches (Chardon et al. 2003). They further contribute to an improved understanding of how structural landscape connectivity is translated into functional connectivity for a particular target species (Bowman & Fahrig 2002, Schooley & Wiens 2003, Stevens et al. 2004).

More generally, we believe that further experiments on behaviour at habitat boundaries will give us a better understanding of the decision-making process with regard to movements at patch boundaries. Furthermore, examining the scale at which individuals orient and respond to landscape geometry may give a better understanding of perceptual ranges and their effects on movements. The new methodology in which also butterfly flight paths are tracked across the landscape with harmonic radar (Cant et al. 2005) promises to be of significant value for future investigations in this direction. The strategy used to orient within the landscape and the scale at which the landscape is perceived restrict the sustainability of populations within habitat fragments (Dover & Fry 2001). Consequently, this experimental approach will contribute significantly to understanding the role played by landscape in population dynamics. It is this kind of knowledge which is vital to evidence-based conservation strategies at the landscape level.
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This thesis has an evolutionary ecological focus, investigating how organisms cope with changing environments. More specifically, this work addresses the intriguing and timely issue of differing life history traits (including functional morphology and behaviour) in landscapes where an organism’s habitat gets fragmented. Habitat fragmentation is widely recognised as a key problem for biodiversity at the global and local scale. The speckled wood butterfly (*Pararge aegeria* L.) was chosen as a model of a flying ectotherm (more precisely a heliotherm). Although this temperate-zone satyrid is primarily a woodland butterfly, it also occurs in more fragmented landscapes which include woodland aspects; such as agricultural landscapes with hedgerows and/or small patches of woodland. A primary interest is to evaluate to what extent landscape structure influences movement behaviour, such as tracking resources and dispersal between populations. Movements are closely linked with the behaviour and properties of butterfly thermoregulation. In order to test a series of hypotheses, we compared the behaviour, morphology and thermal properties of individuals from continuous woodland landscapes to those from highly fragmented, agricultural landscapes. In the latter, the environment between suitable habitat fragments is an intensively managed and hostile landscape matrix. Using several experimental approaches – including reciprocal transplant experiments and observations in ‘common garden’ designs – we provide evidence for land-
scape-related differences in behaviour, functional morphology and eco-physiology. This suggests fast micro-evolutionary changes. From an adaptive point of view, these differences are in line with the assumption of different dispersal rates that may result from altered microclimatic conditions and from changed distributions (and hence encounter rates) of resources between landscape types.

In evolutionary time, varying environments may lead to different morphs as a result of genetic adaptation and divergence, or phenotypic plasticity; the latter allows individuals to deal with varying or changing environments more rapidly than the former. Results of the first experiment (i.e. reciprocal transplant with offspring of females from differently fragmented landscapes), suggest the existence of landscape-related phenotypic plasticity in butterfly flight morphology. To the best of our knowledge, this is the first evidence for this kind of plasticity. The observed change in flight morphology can be interpreted in terms of altered ability to cover distances among resources, in line with altered distributions and encounter rates of resources in the landscape associated with habitat fragmentation. In addition, we found significant genotype-by-environment (G x E) interactions for some of the traits. This suggests that the observed landscape-related phenotypic plasticity can respond to natural selection, and, hence, that it may contribute to evolutionary changes in dispersal (and probably in other flight-related behaviours that need further research) with changed landscape structure. The cues causing these responses have not yet been studied, but microclimatic parameters (such as temperature) are most likely involved. In this context, we hypothesised that species with high levels of plasticity in morphology and life history have a greater capacity to deal with changing and variable landscapes than other species. The experiment also revealed new data on differential larval mortality between the different landscapes, and showed an additional role of landscape of origin to this mortality. This demonstrates that evolutionary ecological studies on habitat fragmentation should not be exclusively limited to adult ecology.

Altered microclimates may cause direct effects on flight and costs of flight in small ectotherms such as butterflies, independent of changes in the distribution of resources (e.g. as host plants or feeding sources). Agricultural landscapes were typified by higher ambient temperatures and wind speeds compared to woodland landscapes. This was found to lead to a larger variation in heating rate of *P. aegeria* in agricultural landscapes, using butterfly dummies (i.e. dead butterfly specimens) on thermoprobes. We simulated flight also using these dummies to measure the distance required to cool thorax flight temperatures from optimal (33°C) to a given (25°C) suboptimal temperature under different environmental/landscape conditions. Butterflies may compensate for the higher level of convective cooling in agricultural landscapes by flying more frequently in the vicinity of shelter providing vegetation structures, especially when they do so close to the substrate. We were then able to prove that living individuals fly more frequently at lower heights in agricultural landscapes than in woodlands. Besides these behavioural responses among adult offspring of field-collected females in large, outdoor cages, revealed that woodland butterflies were more mobile than those from agricultural landscapes compared to woodland. Similarly, in an independent census, there was a much higher frequency of behaviour intermediate between perching and patrolling in agricultural landscapes than in woodland. These results suggest that the clear dichotomy of perching versus patrolling as typically observed in woodland, fades away in highly fragmented, agricultural landscapes. Although alternative mate location strategies have attracted much attention among behavioural ecologists, and habitat fragmentation provides a typical framework for studies on changes in dispersal behaviour, the study of mate location in the context of habitat fragmentation has only rarely been addressed. Our experimental approach to compare behavioural responses among adult offspring of field-collected females in large, outdoor cages, revealed that woodland butterflies were more mobile than those from agricultural landscapes: more frequently at flight, covered longer distances, were more prone to cross open-shade boundaries, and travelled more frequently between 'woodland' parts in the cages. In another field experiment, we released butterflies form both types of landscape at several distances from suitable habitat to evaluate differences in detection ability. Butterflies from the agricultural landscape oriented themselves towards forested habitat

**Summary**

Altered microclimates may cause direct effects on flight and costs of flight in small ectotherms such as butterflies, independent of changes in the distribution of resources (e.g. as host plants or feeding sources). Agricultural landscapes were typified by higher ambient temperatures and wind speeds compared to woodland landscapes. This was found to lead to a larger variation in heating rate of *P. aegeria* in agricultural landscapes, using butterfly dummies (i.e. dead butterfly specimens) on thermoprobes. We simulated flight also using these dummies to measure the distance required to cool thorax flight temperatures from optimal (33°C) to a given (25°C) suboptimal temperature under different environmental/landscape conditions. Butterflies may compensate for the higher level of convective cooling in agricultural landscapes by flying more frequently in the vicinity of shelter providing vegetation structures, especially when they do so close to the substrate. We were then able to prove that living individuals fly more frequently at lower heights in agricultural landscapes than in woodlands. Besides these behavioural responses among adult offspring of field-collected females in large, outdoor cages, revealed that woodland butterflies were more mobile than those from agricultural landscapes compared to woodland. Similarly, in an independent census, there was a much higher frequency of behaviour intermediate between perching and patrolling in agricultural landscapes than in woodland. These results suggest that the clear dichotomy of perching versus patrolling as typically observed in woodland, fades away in highly fragmented, agricultural landscapes. Although alternative mate location strategies have attracted much attention among behavioural ecologists, and habitat fragmentation provides a typical framework for studies on changes in dispersal behaviour, the study of mate location in the context of habitat fragmentation has only rarely been addressed. Our experimental approach to compare behavioural responses among adult offspring of field-collected females in large, outdoor cages, revealed that woodland butterflies were more mobile than those from agricultural landscapes: more frequently at flight, covered longer distances, were more prone to cross open-shade boundaries, and travelled more frequently between 'woodland' parts in the cages. In another field experiment, we released butterflies form both types of landscape at several distances from suitable habitat to evaluate differences in detection ability. Butterflies from the agricultural landscape oriented themselves towards forested habitat...
from a distance twice as far as did conspecifics from woodland. These results agree with heritable differences in flight activity, dispersal propensity and habitat-finding ability among the landscapes pointing to evolutionary consequences of habitat fragmentation. We discuss several new ideas that emerge from the results, such as about the variation between populations from different landscapes to deal with coarse-grained ecological infrastructures. It may be valuable to test in other species, particularly those of high conservation interest, for different optima in the geometry of corridor or stepping stone networks for the same species in different landscape types.

In addition to the several lines of evidence for evolutionary changes in thermoregulation and movement behaviour with landscape, we found that the effects were often sex-specific. These sexual differences can be understood relative to the multifunctional nature of butterfly flight, which differs between males and females as they differ in behavioural repertoires and ecological roles. This study on the evolutionary ecology of movement, behaviour, functional morphology and thermoregulation in *P. aegeria* adds significantly to the diverse ecological and evolutionary effects that habitat fragmentation may have on life history traits of organisms dealing with our man-made landscapes.
Samenvatting

Vanuit een evolutionair ecologisch gezichtspunt focust dit doctoraatsproefschrift op de vraag hoe organismen omgaan met veranderende omgevingen. Dit werk behandelt meer bepaald het intrigerende en actuele punt van veranderende ‘life history’ aspecten (zoals functionele morfologie en gedrag) in landschappen waarin de habitat van een soort gefragmenteerd raakt. Habitatfragmentatie wordt algemeen erkend als een belangrijk probleem voor biodiversiteit, en dat zowel op lokale als op meer globale schaal. Als studiemodel voor een vliegend ectotherm organisme (en meer specifiek een heliotherm organisme) kozen we het Bont zandoogje (Pararge aegeria L.). Hoewel dit zandoogje van gematigde streken in hoofdzaak een bosvlinder is, komt het ook voor in meer gefragmenteerde landschappen met een zeker bosaspect, zoals in landbouwlandschappen met hagen en houtkanten en/of lapjes bos. Onze interesse gaat in hoofdzaak uit naar het bepalen van de mate waarin de structuur van het landschap een invloed uitoefent op verplaatsingsgedrag (bv. hulpbronnen zoeken, dispersie tussen populaties). Verplaatsingen bij vlinders zijn sterk gelinkt met thermoregulatie. Met het oog op het testen van een reeks hypotheses vergeleken we het gedrag, de morfologie en de thermale eigenschappen van individuen uit grote boscomplexen met die uit sterk gefragmenteerde landbouwlandschappen. In deze laatste is de hoeveelheid geschikt habitat beperkt tot snippers in een intensief beheerde, vijandige landschapsmatrix. Aan de hand van ver-
scheidene experimentele benaderingen – waaronder wederkerige transplantatie experimenten en observaties in ‘common garden’ opstellingen – leveren we bewijsmateriaal voor landschapsgerelateerde verschillen in gedrag, functionele morfologie en eco-fysiologie. Dat wijst op snelle micro-evolutionaire veranderingen. Vanuit een adaptief gezichtspunt komen die verschillen overeen met de assumptie van een andere mate van dispersie, die mogelijk resulteert uit veranderde microklimatologische omstandigheden en uit een andere spreiding (en dus een andere trefkans) van de hulpbronnen tussen landschapstypes.

In een evolutionair perspectief kunnen variërende omgevingen leiden tot verschillende morfologische varianten die een gevolg zijn van genetische adaptatie en divergentie, of van fenotypische plasticiteit; dit laatste laat individuen toe sneller om te gaan met variërende of veranderende omgevingen. De resultaten van een eerste experiment (i.e., wederkerige transplantatie met nakomelingen van wijfjes uit verschillend gefragmenteerde landbouwlandschappen) suggereren het bestaan van landschapsgerelateerde fenotypische plasticiteit in vliegerelateerde morfologie bij vlinders. Voor zover we weten, is dit de eerste maal dat zo’n plasticiteit aangetoond wordt. De waargenomen verschillen in vliegerelateerde morfologie kunnen geïnterpreteerd worden vanuit een veranderd vermogen om afstanden tussen hulpbronnen te overbruggen. Dat is in overeenstemming met veranderde spreidingen en trefkansen van hulpbronnen in het landschap als een gevolg van habitatfragmentatie. Bovendien vonden we significante genotype-omgevinginteracties bij enkele morfologische kenmerken. Dat wijst erop dat de waargenomen landschapsgerelateerde fenotypische plasticiteit in staat is op natuurlijke selectie te reageren. Ze draagt op die manier bij tot evolutionaire veranderingen in dispersie (en waarschijnlijk ook in ander vliegerelateerd gedrag dat verder onderzoek vereist) met een veranderde structuur van het landschap. Welke prikkels die reacties veroorzaken, is nog niet bestudeerd, maar microklimatologische parameters (zoals temperatuur) zijn zeer waarschijnlijk betrokken partij. In dat verband formulieren we een hypothese die stelt dat soorten met een hoge mate aan plasticiteit in morfologie en ‘life history’ meer troeven bezitten om het hoofd te bieden aan veranderende en variabele landbouwlandscappen. Het experiment onthulde ook nieuwe gegevens over differentiële larvale mortaliteit tussen de verschillende landbouwlandscappen en toonde aan dat het landschap als oorsprong een bijkomende rol vervult in deze mortaliteit. Dat laat zien dat evolutionair ecologische studies rond habitatfragmentatie best niet beperkt blijven tot ecologie van adulte individuen.

Veranderde microklimaten kunnen onafhankelijk van veranderingen in de configuratie van hulpbronnen, zoals waardplanten of voedselbronnen, ook rechtstreekse effecten sorteren op het vliegen (en de kosten ervan) in kleine ectotherme organismen zoals vlinders. Landbouwlandschappen werden gekenmerkt door een hogere omgevingstemperatuur en windsterkte vergeleken met boslandschappen. Dat verschil vertaalt zich in een grotere variatie in opwarmingsnelheid van P. aegeria in landbouwlandscappen, wat we bestudeerden met dummy’s (i.e., dode vlinders) op een thermosonde. Met die dummy’s simuleerden we ook vluchten om de afstand te schatten die kan overbrugd worden om de thorax van een optimale vliegtemperatuur (33°C) af te koelen tot een bepaalde suboptimale temperatuur (25°C), en dat onder verschillende omgevings-/landschapscondities. Vlinders zouden kunnen compenseren voor de hogere graad van convectieve koeling in landbouwlandschappen door frequenter in de nabijheid van beschuttingverlenende vegetatiestructuren te vliegen, vooral wanneer ze dat op lage vlieghoogte doen. We toonden aan dat levende individuen inderdaad frequenter op lage hoogte vliegen in landbouwlandschappen dan in bos. Behalve gedragsverschillen vonden we ook morfologische verschillen: individuen in landbouwlandschappen hadden meer beharing dan bosindividuen. Omdat beharing isoëlt, kan dat geïnterpreteerd worden als een morfologische adaptatie om de impact van convectieve koeling te verminderen. Die resultaten vestigen de aandacht op het potentiële belang van vegetatiestructuren als corridors voor verplaatsingen doorheen gefragmenteerde landbouwlandschappen. Bovendien voorspellen we dat het belang van die structuren weers- of klimaatsafhankelijk is. Mochten vlinders enkel in staat zijn om relatief korte vluchten te maken, zou een meer fijnkorrelig ecologisch netwerk van corridors vereist zijn. Daarnaast testten we op een experimentele manier of het vliegen vermogen verschillt tussen in het laboratorium gekweekte nakomelingen van wijfjes uit een boslandschap versus een landbouwlandschap. Los van de observatie dat P. aegeria in staat is te vliegen bij veel lagere omgevingstemperaturen dan andere dagvlindersoorten die tot nu toe werden bestudeerd, vlogen vlinders van bossoorsprong langer bij suboptimale en optimale temperaturen dan nakomelingen van wijfjes uit een landbouwlandschap. Samengevat kunnen we stellen dat alle resultaten temperatuurgerelateerde adaptieve verschillen suggereren tussen de koelere, maar beschutte omgevingen van grote boscomplexen en de warmere, maar meer open omgevingen van gefragmenteerde landbouwlandschappen. De selectieregimes op verplaatsingsgedrag, door de aan vliegactiviteit verbonden kosten, zouden gerelateerd kunnen zijn aan de graad van habitatfragmentatie en dat zowel als een gevolg van microklimatologische eigenschappen als van de configuratie van de hulpbronnen. Hier bestudeerden we of en in welke mate P. aegeria vlinders van contrasterende landschapsverschillen verschillen in verplaatsingsgedrag of in ermom samenhangende kenmerken, zoals partnerzoekgedrag, vliegactiviteit, dispersie, en het vermogen om beoost habitat te vinden. We vonden een significant effect van landschap op partnerzoekgedrag; mannetjes vertoonden grotere aantallen agressieve ‘take-offs’ (i.e., indicator van territoriaal ‘perching’) en ze maakten meer verplaatsingen (i.e., indicator van ‘patrolling’) in landbouwlandschappen dan in boscomplexen. Evenzo toonde een onafhankelijke census aan dat er in landbouwlandschappen een veel hogere frequentie voorkomt van een gedrag intermediair tussen ‘perching’ en ‘patrolling’. Die resultaten suggereren dat de duidelijke grens tussen ‘perching’ en ‘patrolling’, zoals die typisch terug te vinden is in een boslandschap, vervaagt in sterk gefragmenteerde

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landbouwlandschappen. Hoewel alternatieve partnerzoekstrategieën heel wat aandacht kregen bij gedragsecologen, en hoewel habitatfragmentatie een typisch kader biedt voor studies over veranderingen in dispersiegedrag, is er slechts zelden onderzoek gedaan naar partnerzoekgedrag in de context van habitatfragmentatie. Onze experimentele benadering om buiten, in grote kooien gedragsresponsen te vergelijken tussen volwassen nakomelingen van in het veld verzamelde wijfjes, bracht aan het licht dat vlinders van het bos mobieler waren dan deze van het landbouwlandschap; ze waren vaker aan het vliegen, ze legden grotere afstanden af, ze waren sneller geneigd om open-schaduw grenzen over te steken, en ze verplaatsten zich frequenter tussen de ‘bos’delen in de kooien. In een ander veldexperiment lieten we vlinders van beide landschaps types los op meerdere afstanden van geschikt habitat om verschillen in detectiecapaciteit na te gaan. Vlinders van het landbouwlandschap waren in staat om zich te oriënteren naar bebossed habitat vanaf een dubbel zo grote afstand dan hun soortgenoten van het bos. Die verschillen tussen individuen van andere landschaps types stemmen overeen met erfelijke verschillen in vliegactiviteit, dispersieneiging en vermogen om habitat te detecteren; ze wijzen op evolutieva lne gevolgen van habitatfragmentatie. We bespreken een aantal nieuwe ideeën die uit de resultaten ontspruiten. Bijvoorbeeld de idee over de variatie tussen populaties van verschillende landschappen om het hoofd te bieden aan een grofkorrelige ecologische infrastructuur. Het kan nuttig zijn om bij andere, en dan vooral bedreigde soorten, na te gaan of er verschillende optima bestaan inzake de geometrie van corridor- en stapsteen netwerken voor eenzelfde soort in verschillende landschaps types.

Aansluitend bij al die argumenten voor evolutionaire veranderingen in thermo regulatie en verplaatsingsgedrag als gevolg van de structuur van het landschap merkten we dat de effecten vaak geslachtsspecifiek zijn. Die seksuele verschillen kunnen begrepen worden in het licht van het multifunctionele karakter van het vlieggedrag bij vlinders. De functie van vliegen verschilt tussen mannetjes en wijfjes, in overeenstemming met hun verschillende gedragsrepertoires en ecologische functies. Dit onderzoek naar de evolutionaire ecologie van verplaatsingen, gedrag, functionele morfologie en thermoregulatie in P. aegeria levert een betekenisvolle bijdrage tot de diverse ecologische en evolutionaire effecten die habitatfragmentatie kan hebben op ‘life history’ kenmerken van organismen in cultuurlandschappen.
Het doctoraat zit er bijna op. En dat vind ik erg spijtig! Weet je, het geeft veel voldoening aan natuurstudie te doen. Bovendien heeft evolutionaire biologie iets van ‘een openbaring’, die je toelaat een omvattend zicht te krijgen op de mechanismes achter geobserveerde patronen. Een echte ‘waarheidservaring’ noem ik het, zonder opium, zonder geloof… Werken aan dit doctoraat was dan ook enorm deugdoend! In een ongedwongen en ontspannen werksfeer kon ik van het ene experiment in het andere duiken. En alle hadden ze iets te vertellen. Naast een al dan niet duidelijk antwoord op de geteste hypotheses, riepen ze steeds een heleboel nieuwe vragen op. Aan onderzoeksvragen was er dus nooit een gebrek!

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“Nothing in biology makes sense, except in the light of evolution” (T. Dobzhansky, 1973)

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