

Thermal flight budget of a woodland butterfly in woodland versus agricultural landscapes: An experimental assessment

Thomas Merckx^{a,*}, Stefan Van Dongen^b, Erik Matthysen^a, Hans Van Dyck^c

^aLaboratory of Animal Ecology, Department of Biology, University of Antwerp, Belgium

^bEvolutionary Biology Group, Department of Biology, University of Antwerp, Belgium

^cBiodiversity Research Centre, Behavioural Ecology & Conservation Group, University of Louvain (UCL), Belgium

Received 3 October 2006; accepted 17 March 2007

Abstract

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. Dummies (i.e., dead specimens with spread wings, on a hypodermic needle with a thermocouple) 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. Convective cooling decreased with increasing degree of shelter, and with lower flight height. Simulated flight bouts bridged 140 ± 23 m under unsheltered, 81 ± 13 m under partly sheltered and 29 ± 6 m under sheltered conditions. Ambient temperature and wind force were higher in highly fragmented, agricultural landscapes than in continuous woodland landscapes. Males, and not females, were furrer in agricultural landscapes. This sexual difference is interpreted in terms of differences in life history among the sexes.

© 2007 Gesellschaft für Ökologie. Published by Elsevier GmbH. All rights reserved.

Zusammenfassung

Wir schätzten den Einfluss der Vegetationsstruktur und des Landschaftstyps auf die mikroklimatischen Bedingungen für die Flugaktivität ab, indem wir den heliothermischen Schmetterling *Pararge aegeria* als Model benutzten. Attrappen (d.h. tote Individuen mit ausgebreiteten Flügeln auf einer Injektionsnadel mit einem Thermofühler), die zu Beginn auf die gleiche Thoraxtemperatur aufgewärmt wurden, konnten größere Distanzen zurücklegen und erwärmten sich schneller bei höherer Umgebungstemperatur und geringerer Windstärke. Die Abkühlung durch Konvektion nahm mit zunehmendem Windschutz und geringerer Flughöhe ab. Die simulierten Flüge erreichten eine Strecke von 140 ± 23 m unter ungeschützten, von 81 ± 13 m unter teilweise geschützten und 29 ± 6 m unter geschützten Bedingungen. Die Umgebungstemperaturen und die Windstärken waren in fragmentierten Agrarlandschaften höher als in durchgängigen Waldlandschaften. Die Männchen und nicht die Weibchen waren in den

*Corresponding author. Wildlife Conservation Research Unit, Department of Zoology, University of Oxford, Tubney House, Abingdon Road, Tubney, Abingdon OX13 5QL, UK. Tel.: +440 1865393104; fax: +440 1865393101.

E-mail address: thomas.merckx@zoology.oxford.ac.uk (T. Merckx).

Agrarlandschaften pelziger. Die Unterschiede der Geschlechter können in Bezug auf Unterschiede in der Lebensstrategie zwischen den Geschlechtern interpretiert werden.

© 2007 Gesellschaft für Ökologie. Published by Elsevier GmbH. All rights reserved.

Keywords: Adaptation; Flight activity; Flight morphology; Habitat fragmentation; Landscape structure; Microclimate; *Pararge aegeria*; Thermoregulation; Vegetation structure

Introduction

In ectotherms, the relationship between body temperature and performance typically shows an optimum bounded by critical thermal limits (Chown & Nicolson, 2004; Dudley, 2000). 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 (Chown & Nicolson, 2004; Kingsolver & Huey, 1998). In many flying insects (including butterflies), flight is a multifunctional trait relating to different behaviours with direct fitness consequences such as predator escape, mate-location, foraging, and oviposition (Dudley, 2000; Watt, 2003). Their flight performance directly depends on the temperature of the thorax where all flight muscles are located (Dudley, 2000). Thoracic temperature is the net result of heat lost by convective cooling, and of heat gained by behavioural thermoregulation and physiological processes associated with muscle contraction (Clench, 1966). Convective cooling is proportional to the difference between body and air temperature, and 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., Peng, Sutton, & Fletcher, 1994; Shipp, Grace, & Schallje, 1987), insect flight performance may depend on the physical structure of the local environment (Kingsolver & Watt, 1984).

Habitat fragmentation results in altered landscape structures and compositions, providing different microclimatic conditions (e.g., ambient temperature, ground temperature, wind speed, amount of solar radiation that penetrates the habitat Honnay, Verheyen, & Hermy, 2001; Malcolm, 1998; Matlack, 1993). Habitats with different thermal environmental profiles may lead to evolutionary change in thermal properties of ectothermic insects (Karlsson & Wiklund, 2005). Hence, habitat fragmentation may cause changes in thermoregulation behaviour and morphology so as to keep flight muscle temperature within the optimal range for flight activity (Berwaerts & Van Dyck, 2004). 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 the degree of habitat fragmentation.

Here, we use adult *Pararge aegeria* (L.) butterflies to test whether landscape structure affects thermoregulation-related morphology. Tests and experiments are performed in two contrasting types of landscape: woodland landscapes versus highly fragmented, agricultural landscapes. We also assess the relative value of a thermal budget in terms of flight distance. Thoracic temperature of a fully active *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 implies convective cooling towards suboptimal body temperatures (Van Dyck & Matthysen, 1998). Basking is done by spreading the wings dorsally (Clench, 1966). Butterfly dummies have been shown to be useful instruments to study – at least some – aspects of their thermal ecology (e.g., Berwaerts, Van Dyck, Vints, & Matthysen, 2001; Kemp & Krockenberger, 2002) as they have largely similar thermal properties to living butterflies (Heinrich, 1986). However, while dummies give a useful measure of steady-state thoracic temperatures of live butterflies at rest, living butterflies obviously influence or regulate their body temperature by adjusting their posture and by microclimate selection. Cooling and heating times will be longer than in dummies since water loss after death reduces body mass and heat storage capacity. So, dummies are not fully equivalent to living butterflies, but they are tools that simplify complexity in order to identify sources of variation, in this case operational and relative thermal differences among different landscapes.

We compared both the degree of dorsal melanisation as well as the furriness of basal wing parts between samples from woodland and agricultural landscapes. Since darker wings result in shorter heating times (Berwaerts et al., 2001; Van Dyck & Matthysen, 1998), and fur increases thermal isolation (e.g., Kingsolver, 1983), we predict that butterflies from agricultural landscapes have darker wings and are furrier than woodland butterflies, so as to counteract the high levels of convective cooling in agricultural landscapes. Next, we did two experiments with butterfly dummies, in which we tested for differential effects of vegetation structure and type of landscape (i) on the thermal budget for flight, and (ii) on the heating time of the

thorax. We additionally contrasted measurements at 0.1 and 1.0 m above the substrate.

Thermal budgets, which translate into the length of flight bouts, are expected to be larger under warm weather conditions, but they are expected to be smaller under conditions of strong wind due to convective cooling. So, we predict the variation in cooling distances and heating times to be larger within agricultural landscapes than within woodland landscapes. Similarly, we predict more variation in cooling distances and heating times when vegetation providing shelter is lacking, compared to (partly) sheltered conditions in the two types of landscape (Dover, 1996; Morecroft, Taylor, & Oliver, 1998; Noss & Csuti, 1997), and we predict a lower impact of convective cooling (i.e., less variation in cooling distances and heating times) close to the substrate (Dover & Sparks, 2000; Willmer, 1986).

Materials and methods

Study species

The Speckled Wood (*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, Van Dyck, Karlsson, & Leimar, 2003). Males either defend a sunlit spot on the forest floor as a territory or they patrol between several sunlit spots to find mates (Van Dyck, 2003 and references therein). The spring generation of this bivoltine species is darker than the summer generation (Van Dyck & Wiklund, 2002).

Study landscapes

For morphological measurements, we sampled spring and summer individuals in four areas, all situated within Flanders (Belgium) and 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 farmed fields and pastures (the large majority of the area) and to a lesser extent buildings, orchards, copses and several sunken roads with hedgerows: Rillaar and Hoegaarden (cf. Merckx et al., 2003). In both woodland landscapes, butterflies were collected within study plots of ca. 135 ha of 100% woodland habitat. The Hoegaarden (555 ha) and Rillaar (401 ha) study areas each contain six copses (0.3–11.2 ha) covering 3% of the areas, and 33 (8.9 km) and 15 (3.9 km) sunken roads

with hedgerows, respectively. Study landscapes are characterised by a similar topography, soil type and macroclimate. Distances ranging from 7 to 60 km separate these four landscapes.

The study was performed in Hoegaarden (agricultural landscape) and Meerdaalwoud (woodland landscape). They were carried out simultaneously in both areas during periods when climatic conditions allowed for butterfly activity: sunny weather and wind speeds ≤ 3 Beaufort (BF). We performed experiments during three periods (first: 14–24 April; second: 28 May–8 July; third: 11 July–2 August). The heating time 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 in the four study landscapes and stored at -20°C . Prior to measurement, specimens were dried in an incubator for 24 h at 60°C . Next, wings were carefully separated from the thorax. Forewings were photographed (Olympus Camedia C-3030) in order to measure basal darkness (grey values) using an image analyser (Optimas 6.5). From all captured females ($n = 56$), and from a separate set of males ($n = 283$), we took close-ups (binocular microscope Leica MZ125: magnification = ca. $46\times$) of the forewings' basal parts to allow assessment of the furriness, scored into three classes: (1) bald: a few short hairs, (2) furry: a medium degree of furriness with the wing surface clearly visible, and (3) very furry: a lot of fur covering most of the wing surface. Repeated measurements for basal wing colour on a sample of 20 individuals showed a high repeatability (0.94). Fur estimates had a similar accuracy.

Ambient temperature

For almost 3 months (3 May 2003, 05:00 h–29 July 2003, 16:00 h), 12 thermo probes connected to data loggers (Hobo Pro Series) measured ambient air temperature every 5 min at a height of 1 m above the substrate. In each of four study sites, we placed three thermo probes. In total, we distinguished between six vegetation/landscape classes: (1) inside woodland ('core'), (2) at the edge of clearings within woodland ('clearing'), (3) at the outer fringe of woodland ('fringe'), (4) between both woody sides of sunken roads ('inner sunken road'), (5) outer edges of woody sunken roads ('outer sunken road'), and (6) salient, exposed locations within agricultural landscape ('open'). BoxCar Pro 4 software was used to calculate each hour's average ambient temperature and each day's maximal ambient temperature for every thermo probe.

Cooling distance experiment

By placing a butterfly dummy in a sunlit patch, it was heated to a thoracic temperature of 33 °C. At this temperature, the observer started to walk with the dummy at a constant speed of ca. 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 the time and the distance moved when the dummy's thoracic temperature dropped to 25 °C (with a maximum of 300 m or ca. 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 within a ≥ 50 m radius (only in agricultural landscapes), (ii) partly sheltered: along tall hedgerow (± 4 m) (agricultural landscapes) or along woodland fringe (woodland landscapes), within 1 and 2 m sideways, and (iii) sheltered: in sunken roads with tall hedgerows on both sides (agricultural landscapes) or inside woodland, ≥ 50 m from a clearing or the woodland fringe (woodland landscapes). For each specific location ($n = 98$), the experiment was repeated three times, both at 1 and 0.1 m height above the substrate. We used 11 specimens of an independent population from a half-open park type of landscape (i.e., representing an intermediate landscape structure, CDE-campus, University of Antwerp). This selection of specimens showed little inter-individual variation in basal wing colour and furriness. The use of specific dummy individuals was randomised over the different measurements.

Heating time experiment

Dummies were brought to a thoracic temperature of 25 °C by placing them in the shade. At 25 °C, a 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 33 °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 and 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 furriness.

Statistical analyses

Basal wing colour was compared between seasons and types of landscape. Main effects and their interaction

were tested using 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 performed backward elimination of non-significant factors (highest p -values). Final model residuals were normally distributed. The frequency of different fur classes was analysed using Fisher's exact tests (Proc Freq, SAS 2001).

Ambient temperature profiles (average temperature for each hour and maximal day temperature) were compared between vegetation/landscape classes (6 levels), and tested using mixed regression models (Proc Mixed, SAS 2001). In order to control for possible effects of study area, we added study area (4 levels) as a random effect in both models. Model residuals were normally distributed. Log-likelihood tests were performed to check for interclass differences in variance. In addition, and using mixed regression models as well, average and maximal temperatures were compared between types of landscape (agricultural versus woodland landscapes) with vegetation/landscape class as a random effect. Model residuals were normally distributed.

We performed survival analyses to handle the right-censored cooling distance and heating time data. However, since we were confronted with several fixed and random effects, we opted for an adapted linear mixed model approach in order to estimate censored data points. Cooling distances and heating times were compared between landscapes, vegetation structure classes (shelter), and height classes. All main effects and interactions were tested. In order to control for possible confounding factors, wind speed [4 classes: 1 = 0 BF (no wind), 2 = 1 BF, 3 = 2 BF, 4 = 3 BF (relatively strong wind)], ambient temperature, walking velocity (after log transformation, and for the cooling distance experiment only), and period were added as fixed effects. The specific location (98 and 110 levels in the cooling distance and heating time experiment, respectively), dummy (11 and 6 levels in the cooling distance and heating time experiment, respectively), and person (3 levels in both experiments) were added as random effects. Cooling distances were log transformed and, as indicated above, if distances were larger than 300 m (= 2.477 after log transformation) the experiment was stopped. Heating times were arcsin transformed and the experiment was terminated when the temperature of 33 °C was not reached after 180 s. Therefore, in both experiments data were right-censored (8% and 25% of data points in the cooling distance and heating time experiment, respectively). This was incorporated in the analysis by treating these censored observations as missing data points originating from a truncated normal distribution. Details on this incorporation, on the estimated variability in cooling distances and heating

times in relation to landscape, shelter, and height, and on model selection, are provided as supplementary material (see Supplementary Appendix A).

Results

Thermoregulation-related morphology

Males had more fur than females ($\chi^2_2 = 14.40$; $p = 0.0007$), and they had more fur in agricultural than in woodland landscapes (Fisher's exact, $p < 0.0001$) (Table 1); 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 (Table 1). Basal wing colour did not differ between agricultural and woodland landscapes. Males were darker in spring than in summer ($F_{1,2.17} = 22.07$, $p = 0.036$), 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: $F_{5,5.04} = 15.95$,

Table 1. Counts of fur categories of basal wing parts in males and females from two agricultural and two woodland landscapes: bald: a few short hairs; furry: a medium degree of furriness with the wing surface clearly visible; very furry: a lot of fur covering most of the wing surface

Furriness	Agricultural	Woodland	Total
<i>Males</i>			
Bald	12	62	74
Furry	54	51	105
Very furry	82	22	104
<i>Females</i>			
Bald	11	4	15
Furry	26	6	32
Very furry	7	2	9

$p = 0.0042$; maximal temperature: $F_{5,12} = 7.76$, $p = 0.0018$) (Table 2). There was no difference in variance among the classes. Regardless of interclass variation, average and maximal temperatures were higher in agricultural landscapes than in woodland landscapes (average temperature: $F_{1,4.04} = 10.07$, $p = 0.033$ – agricultural: 17.0 ± 0.20 °C versus woodland: 16.1 ± 0.20 °C; maximal temperature: $F_{1,3.53} = 12.23$, $p = 0.031$ – agricultural: 23.8 ± 0.51 °C versus woodland: 21.3 ± 0.50 °C).

Cooling distance experiment

Larger distances could be covered by moving butterfly dummies when ambient temperature was higher ($\beta_{\text{temp}} = 3.50$, 95% CI: 1.36–5.66), and smaller distances with increasing wind speed [differences in cooling distances after log-transformation using wind class 4 (relatively strong wind) as a reference: $\beta_{1-4} = 31.66$, 95% CI: 16.8–44.0; $\beta_{2-4} = 18.0$, 95% CI: 4.0–28.9; $\beta_{3-4} = 11.9$, 95% CI: –1.4 to 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 (difference between high and low height: unsheltered: $\beta_{\text{high-low}} = -36.5$, 95% CI: –53.3 to –19.4; partly sheltered: $\beta_{\text{high-low}} = -12.8$, 95% CI: –19.2 to –6.54; sheltered: $\beta_{\text{high-low}} = -4.4$, 95% CI: –7.69 to –1.17) (Fig. 1A and B). From the viewpoint of differences in cooling distances between the three classes of vegetation structure this interaction can be interpreted as showing a stronger effect of shelter (longer cooling distances in sheltered versus unsheltered conditions) at lower height (0.1 m) (lower height: $\beta_{\text{partly sheltered-unsheltered}} = -41.4$, 95% CI: –82.4 to –3.94; $\beta_{\text{sheltered-unsheltered}} = -95.2$, 95% CI: –137 to –57.2; $\beta_{\text{sheltered-partly sheltered}} = -53.8$, 95% CI: –78.1 to –31.8; higher height: $\beta_{\text{partly sheltered-unsheltered}} = -17.7$, 95% CI: –57.5 to 18.4; $\beta_{\text{sheltered-unsheltered}} = -63.1$, 95% CI: –104 to –26.3; $\beta_{\text{sheltered-partly sheltered}} = -45.4$, 95% CI: –69.3 to –23.6) (Fig. 1A and B). A two-way interaction between flight height and type of landscape was found as the difference

Table 2. Summary of least squares means of average and maximal ambient temperatures during adult flight activity in suitable *P. aegeria* habitats for six vegetation/landscape classes in two types of landscape (woodland versus agricultural landscape)

Landscape	Vegetation/landscape class	Average temperature (°C ± SE)	Maximal temperature (°C ± SE)
Woodland	Core	15.9 ± 0.23	21.0 ± 0.43
	Clearing	15.9 ± 0.23	21.3 ± 0.43
	Fringe	16.5 ± 0.23	21.5 ± 0.43
Agricultural	Inner sunken road	16.7 ± 0.23	22.8 ± 0.43
	Outer sunken road	17.1 ± 0.23	23.4 ± 0.43
	Open	17.4 ± 0.25	25.2 ± 0.57

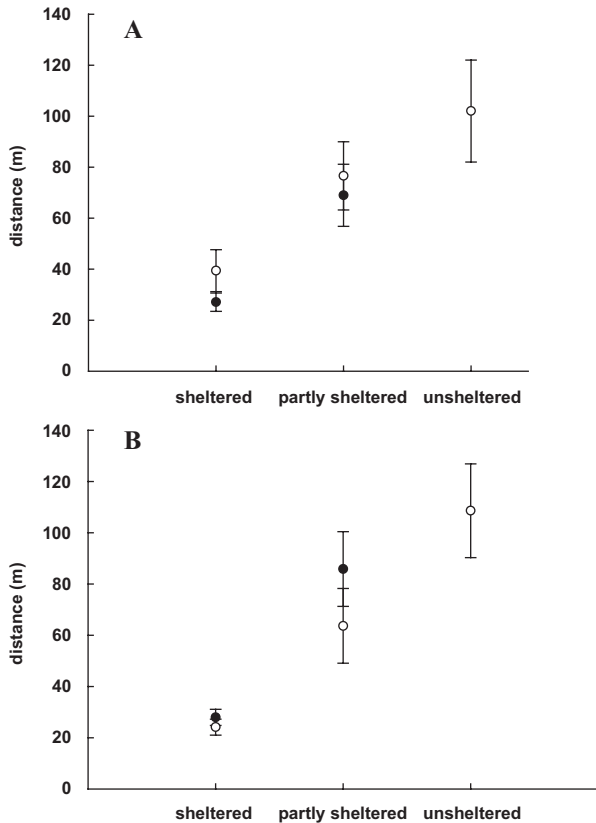


Fig. 1. Cooling distances (means \pm SE) of *P. aegeria* dummies in relation to vegetation structure and type of landscape (open circles: highly fragmented, agricultural landscapes; filled circles: woodland landscapes). (A) Flight height = 1.0 m, (B) flight height = 0.1 m.

between high and low height (i.e., shorter cooling distances at high compared to low height) was observed in woodland ($\beta_{\text{high-low}} = -4.42$, 95% CI: -7.69 to -1.17), but not in the agricultural landscapes ($\beta_{\text{high-low}} = 1.81$, 95% CI: -2.39 to 6.04) (Fig. 1A and B). There was no 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 neither differ between the two flight height classes ($R_{\text{height}} = 1.27$, 95% CI: 0.90 – 1.74) nor between the two types of landscape ($R_{\text{landscape}} = 0.95$, 95% CI: 0.74 – 1.27). Residual variance was smaller in partly sheltered conditions than in to unsheltered conditions ($R_{\text{shelter2}} = 0.39$, 95% CI: 0.24 – 0.65) and even stronger when comparing sheltered and unsheltered conditions ($R_{\text{shelter3}} = 0.15$, 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 (Fig. 1A and B).

Heating time experiment

Heating (from 25 to 33 °C) took a shorter time when ambient temperature was higher ($\beta_{\text{temp}} = -0.0085$, 95% CI: -0.010 to -0.0068). Effects of wind speed on heating were complex and non-linear: albeit there was no significant difference in heating time between relatively high wind speed and windless conditions ($\beta_{1-4} = 0.0024$, 95% CI: -0.005 to 0.010), heating time was longer with increasing wind speed ($\beta_{2-4} = 0.020$, 95% CI: 0.011 – 0.028 ; $\beta_{3-4} = 0.039$, 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 (longer heating time at high versus low height), this interaction can be interpreted as being a consequence of the fact that the difference in heating time between high and low height increased with degree of shelter in the agricultural landscape (difference between high and low height: unsheltered: $\beta_{\text{high-low}} = 0.025$, 95% CI: 0.015 – 0.035 ; partly sheltered: $\beta_{\text{high-low}} = 0.017$, 95% CI: -0.002 to 0.037 ; sheltered: $\beta_{\text{high-low}} = 0.009$, 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: $\beta_{\text{high-low}} = 0.019$, 95% CI: 0.014 – 0.025 ; sheltered: $\beta_{\text{high-low}} = 0.028$, 95% CI: 0.017 – 0.040) (Fig. 2A and B). Looking at the main effect of vegetation structure, heating times did not differ between partly sheltered and unsheltered conditions, but they were shorter in sheltered than in unsheltered conditions ($\beta_{\text{shelter sheltered-unsheltered}} = 0.027$, 95% CI: 0.011 – 0.044) (Fig. 2A and B). The period in which observations were done, made no significant contribution to the model. Residual variances neither differ between the two classes of flight height ($R_{\text{height}} = 0.85$, 95% CI: 0.56 – 1.21) nor between the two types of landscape ($R_{\text{landscape}} = 0.85$, 95% CI: 0.63 – 1.20). Residual variance was smaller in partly sheltered than in unsheltered conditions ($R_{\text{shelter2}} = 0.52$, 95% CI: 0.34 – 0.83) but not so when comparing sheltered and unsheltered conditions ($R_{\text{shelter3}} = 1.04$, 95% CI: 0.66 – 1.72) (Fig. 2A and B).

Discussion

Fragmented landscapes may provide different thermal environments for flying heliotherms like butterflies. Our parallel measurements of ambient temperature at sites where *P. aegeria* occurs in continuous woodland versus corresponding sites in fragmented agricultural landscapes, indicated different thermal profiles. Ambient temperature in and along hedgerows and copses in agricultural landscapes was on average higher than in woodland landscapes. Woodland landscapes provide

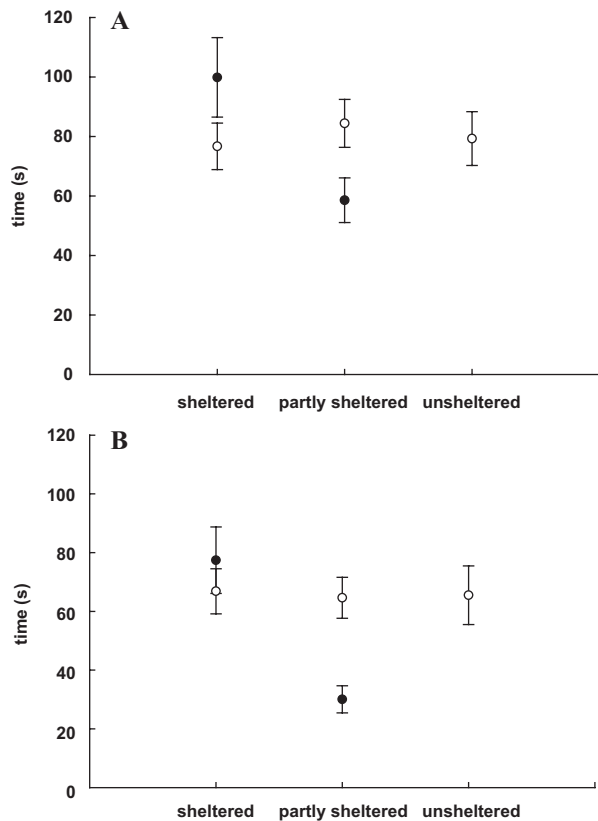


Fig. 2. Heating times (means \pm SE) of *P. aegeria* dummies in relation to vegetation structure and type of landscape (open circles: highly fragmented, agricultural landscapes; filled circles: woodland landscapes). (A) Flight height = 1.0 m; (B) flight height = 0.1 m.

much shadier, cooler conditions, which 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 landscapes, copses and narrow hedgerows have proportionally larger amounts of irradiated surface, but more sites are exposed to the cooling effect of higher wind speed. Hence, agricultural landscapes appear 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*'s flight activity and performance, using butterfly dummies. The distances walked with dummies in woodland under sheltered conditions at 1 m height, as shown in Fig. 1A, correspond to 27 s. This corresponds 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), and 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 time was higher in agricultural landscapes compared to woodland landscapes. Heating times were significantly shorter in sheltered than in unsheltered conditions. Although we could cover 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 6 when dummies were moved under more sheltered conditions. Earlier, Merckx and Van Dyck (2002) have shown for two grassland butterflies, which 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 the significant effect of reducing the impact of convective cooling on flight distance or duration. In the same vein, Rutowski, Demlong, and Leffingwell (1994) have shown that male butterflies adjusted perch height in relation to ambient temperature. Merckx and Van Dyck (2005) showed that *P. aegeria* males flew more frequently at low height in agricultural landscape than in woodland landscape.

Although butterflies have some opportunities to avoid the problem of convective cooling by seeking sheltered vegetation structures in open agricultural landscapes, the physiological cost of daily life may still be different in highly fragmented, agricultural landscapes versus sheltered woodlands. From an evolutionary viewpoint, this may result in different life styles among different landscapes. In another experiment where live butterflies were induced to fly under standardised conditions at suboptimal body temperatures, individuals from woodland flew for longer than their conspecifics from an agricultural landscape (Merckx, Karlsson, & Van Dyck, 2006). 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. These two studies suggest that the contrasting thermal environments of unfragmented versus fragmented landscapes have led to physiological adaptations.

Fragmented, agricultural landscapes are characterised by irradiation at ground level, and hence provide warmer conditions for flight than woodlands. But at the same time, butterflies are more vulnerable to convective cooling in the less sheltered, agricultural landscapes. Therefore, we predict agricultural landscapes to be more variable in operational temperature profile for butterflies. Our observation that males of agricultural landscapes were furrer, i.e., better thermally isolated (Kingsolver, 1983), than woodland males gives evidence of morphological adaptation to this

variability of the thermal environment. Whether these differences result from a genetic adaptation and/or from phenotypic plasticity is a matter for further research. Nevertheless, we predict that they are intrinsic differences. This would be in line with earlier results on landscape-related differences in morphology, behaviour and life-history, as obtained from experiments with a ‘common garden’ approach in the same *P. aegeria*-landscape system (Karlsson & Van Dyck, 2005; Merckx et al., 2003).

Variation in the degree of wing melanisation also affects thermoregulation and, hence, flight activity (Ellers & Boggs, 2004; Guppy, 1986; Kingsolver & Watt, 1984; Watt, 2003). Earlier work on *P. aegeria* males has suggested darker individuals in agricultural landscapes than in woodlands (Berwaerts, Van Dyck, Van Dongen, & Matthysen, 1998); darker wings allow faster heating (Van Dyck & Matthysen, 1998), which would be an advantage in environments with high risk of convective cooling. Elsewhere, using a field experiment, Merckx and Van Dyck (2006) demonstrated that *P. aegeria* males that developed in an agricultural landscape had indeed darker basal wings than males that developed in woodland. However, there was no significant relationship with melanisation in the wild caught specimens of our current study. Nevertheless, the degree of melanisation is affected by environmental stress (e.g., desiccation of host grasses) (Talloon, Van Dyck, & Lens, 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 in view of the different behavioural repertoires of both sexes in butterflies (Pivnick & McNeil, 1986). In males, extended flight during periods with less favourable microclimatic conditions increases the probability of locating virgin females; virgin females may emerge even in poor weather. Therefore, thermal isolation allowing for longer flights at high convective cooling is more important to males. Since there is a trade-off between reproduction and flight in females (Baguette & Schtickzelle, 2006; Hughes, Hill, & Dytham, 2003), they need to economize flight activity and, hence, spend less time flying than males (Shreeve, 1984). This may result in differential selection regimes for such traits between the sexes.

At the landscape level, dispersal is influenced by the spatial distribution of habitat patches, even in butterfly species with high dispersal ability (Vandewoes-tijne, Martin, Liegois, & Baguette, 2004). Our results direct attention to the importance of vegetation structures as potential corridors for movements across fragmented landscapes. Moreover, the significance of such structural characteristics will be weather/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. Alternatively, it may be that the types of ‘daily’ or ‘routine’ butterfly movements we have focused on, are not necessarily representative for displacement or real dispersal movements (Van Dyck & Baguette, 2005).

Acknowledgements

We are grateful to K. Borghgraef, J. Cortens, M. Jacobs, and several students who assisted with experiments and/or taking images of wings and thoraces. TM was research aspirant with the Fund of Scientific Research Flanders-Belgium (F.W.O.). This research was funded by the University of Antwerp (GOA 15R/3942) and the Belgian Federal Office of Scientific, Technical and Cultural Affairs (OSTC-PADD II EV/06/16B).

Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.baae.2007.03.009.

References

- Baguette, M., & Schtickzelle, N. (2006). Negative relationship between dispersal distance and demography in butterfly metapopulations. *Ecology*, *87*, 648–654.
- Berwaerts, K., & Van Dyck, H. (2004). Take-off flight performance under optimal and suboptimal temperature in the butterfly *Pararge aegeria*. *Oecologia*, *141*, 536–545.
- Berwaerts, K., Van Dyck, H., Van Dongen, S., & Matthysen, E. (1998). Morphological and genetic variation in the speckled wood butterfly (*Pararge aegeria* L.) among differently fragmented landscapes. *Netherlands Journal of Zoology*, *48*, 241–253.
- Berwaerts, K., Van Dyck, H., Vints, E., & Matthysen, E. (2001). Effect of manipulated wing characteristics and basking posture on thermal properties of the butterfly *Pararge aegeria* (L.). *Journal of Zoology*, *255*, 261–267.
- Bunker, S.J. (1993). *Form, Flight Pattern and Performance in Butterflies (Lepidoptera: Papilionoidea and Hesperioidea)*. Ph.D. thesis. University of Exeter.
- Chown, S. L., & Nicolson, S. W. (2004). *Insect physiological ecology: Mechanisms and patterns*. Oxford: Oxford University Press.
- Clench, H. K. (1966). Behavioral thermoregulation in butterflies. *Ecology*, *47*, 1021–1034.

- Dover, J. W. (1996). Factors affecting the distribution of satyrid butterflies on arable farmland. *Journal of Applied Ecology*, 33, 723–734.
- Dover, J. W., & Sparks, T. (2000). A review of the ecology of butterflies in British hedgerows. *Journal of Environmental Management*, 60, 51–63.
- Dudley, R. (2000). *The biomechanics of insect flight – Form, function, evolution*. Princeton, NJ: Princeton University Press.
- Ellers, J., & Boggs, C. L. (2004). Functional ecological implications of intraspecific differences in wing melanization in *Colias* butterflies. *Biological Journal of the Linnean Society*, 82, 79–87.
- Guppy, C. S. (1986). The adaptive significance of alpine melanism in the butterfly *Parnassius phoebus* F. (Lepidoptera: Papilionidae). *Oecologia*, 70, 205–213.
- Heinrich, B. (1986). Thermoregulation and flight activity of a satyrine *Coenonympha inornata* (Lep.: Satyridae). *Ecology*, 67, 593–597.
- Honnay, O., Verheyen, K., & Hermy, M. (2001). Permeability of ancient forest edges for weedy plant species invasion. *Forest Ecology and Management*, 5554, 1–14.
- Hughes, C. L., Hill, J. K., & Dytham, C. (2003). Evolutionary trade-offs between reproduction and dispersal in populations at expanding range boundaries. *Proceedings of the Royal Society of London Series B*, 270, S147–S150.
- Karlsson, B., & Van Dyck, H. (2005). Does habitat fragmentation affect temperature-related life-history traits? A laboratory test with a woodland butterfly. *Proceedings of the Royal Society of London Series B*, 272, 1257–1263.
- Karlsson, B., & Wiklund, C. (2005). Butterfly life history and temperature adaptations; dry open habitats select for increased fecundity and longevity. *Journal of Animal Ecology*, 74, 99–104.
- Kemp, D. J., & Krockenberger, A. K. (2002). A novel method of behavioural thermoregulation in butterflies. *Journal of Evolutionary Biology*, 15, 922–929.
- Kingsolver, J. G. (1983). Thermoregulation and flight in *Colias* butterflies: Elevational patterns and mechanistic limitations. *Ecology*, 64, 534–545.
- Kingsolver, J. G., & Huey, R. B. (1998). Evolutionary analyses of morphological and physiological plasticity in thermally variable environments. *American Zoologist*, 38, 545–560.
- Kingsolver, J. G., & Watt, W. B. (1984). Mechanistic constraints and optimality models – Thermoregulatory strategies in *Colias* butterflies. *Ecology*, 65, 1835–1839.
- Malcolm, J. R. (1998). A model of conductive heat flow in forest edges and fragmented landscapes. *Climate Change*, 39, 487–502.
- Matlack, G. R. (1993). Microenvironment variation within and among forest edge sites in the eastern United States. *Biological Conservation*, 66, 185–194.
- Merckx, T., Karlsson, B., & Van Dyck, H. (2006). Sex- and landscape-related differences in flight ability under sub-optimal temperatures in a woodland butterfly. *Functional Ecology*, 20, 436–441.
- Merckx, T., & Van Dyck, H. (2002). Interrelations among habitat use, behavior, and flight-related morphology in two co-occurring satyrine butterflies, *Maniola jurtina* and *Pyronia tithonus*. *Journal of Insect Behavior*, 15, 541–561.
- Merckx, T., & Van Dyck, H. (2005). Mate location behaviour of the butterfly *Pararge aegeria* in woodland and fragmented landscapes. *Animal Behaviour*, 70, 411–416.
- Merckx, T., & Van Dyck, H. (2006). Landscape structure and phenotypic plasticity in flight morphology in the butterfly *Pararge aegeria*. *Oikos*, 113, 226–232.
- Merckx, T., Van Dyck, H., Karlsson, B., & Leimar, O. (2003). The evolution of movements and behaviour at boundaries in different landscapes: A common arena experiment with butterflies. *Proceedings of the Royal Society of London Series B*, 70, 1815–1821.
- Meyer, C. L., & Sisk, T. D. (2001). Butterfly response to microclimatic conditions following Ponderosa pine restoration. *Restoration Ecology*, 9, 453–461.
- Morecroft, M. D., Taylor, M. E., & Oliver, H. R. (1998). Air and soil microclimates of deciduous woodland compared to an open site. *Agricultural and Forest Meteorology*, 90, 141–156.
- Noss, R. F., & Csuti, B. (1997). Habitat fragmentation. In G. K. Meffe, & C. R. Carroll (Eds.), *Principles of conservation biology* (2nd ed, pp. 269–304). Sunderland, MA: Sinauer Associates Inc.
- Peng, R., Sutton, S. L., & Fletcher, C. R. (1994). Distribution patterns of some species of Scatopsidae (Insecta, Diptera) and the effect of microclimate on their flight activity. *Journal of Zoology*, 232, 585–594.
- Pivnick, K. A., & McNeil, J. N. (1986). Sexual differences in the thermoregulation of *Thymelicus lineola* adults (Lepidoptera: Hesperidae). *Ecology*, 67, 1024–1035.
- Rutowski, R. L., Demlong, M. J., & Leffingwell, T. (1994). Behavioural thermoregulation at mate encounter sites by male butterflies (*Asterocampa leilia*, Nymphalidae). *Animal Behaviour*, 48, 833–841.
- Shipp, J. L., Grace, B. W., & Schallje, G. B. (1987). Effects of microclimate on daily flight activity of *Simulium arcticum* Malloch (Diptera, Simuliidae). *International Journal of Biometeorology*, 31, 9–20.
- Shreeve, T. G. (1984). Habitat selection, mate location, and micro-climatic constraints on the activity of the speckled wood butterfly *Pararge aegeria*. *Oikos*, 42, 371–377.
- Talloon, W., Van Dyck, H., & Lens, L. (2004). The cost of melanization: Butterfly wing coloration under environmental stress. *Evolution*, 58, 360–366.
- Vandewoestijne, S., Martin, T., Liegois, S., & Baguette, M. (2004). Dispersal, landscape occupancy and population structure in the butterfly *Melanargia galathea*. *Basic and Applied Ecology*, 5, 581–591.
- Van Dyck, H. (2003). Mate-location: A matter of design? Adaptive morphological variation in the speckled wood butterfly. In C. L. Boggs, W. B. Watt, & P. R. Ehrlich (Eds.), *Butterflies: Ecology and evolution taking flight* (pp. 353–366). Chicago: The University of Chicago Press.
- Van Dyck, H., & Matthysen, E. (1998). Thermoregulatory differences between phenotypes in the speckled wood butterfly: Hot perchers and cold patrollers? *Oecologia*, 114, 326–334.
- Van Dyck, H., & Wiklund, C. (2002). Seasonal butterfly design: Morphological plasticity among three developmental pathways relative to sex, flight and thermoregulation. *Journal of Evolutionary Biology*, 15, 216–225.

- Van Dyck, H., & Baguette, M. (2005). Dispersal behaviour in fragmented landscapes: Routine or special movements? *Basic and Applied Ecology*, 6, 353–545.
- Watanabe, M., & Imoto, T. (2003). Thermoregulation and flying habits of the Japanese sulfur butterfly *Colias erate* (Lepidoptera: Pieridae) in an open habitat. *Entomological Science*, 6, 111–118.
- Watt, W. B. (2003). Mechanistic studies of butterfly adaptations. In C. L. Boggs, W. B. Watt, & P. R. Ehrlich (Eds.), *Butterflies: Ecology and evolution taking flight* (pp. 319–352). Chicago: The University of Chicago Press.
- Willmer, P. G. (1986). Microclimatic effects on insects at the plant surface. In B. E. Juniper, & T. R. E. Southwood (Eds.), *Insects and plant surfaces* (pp. 65–80). London: Edward Arnold.

Available online at www.sciencedirect.com

