

Sex- and landscape-related differences in flight ability under suboptimal temperatures in a woodland butterfly

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Summary

1. Active time budgets of flying ectotherms depend on body temperature. Knowledge of flight ability and performance is currently heavily biased towards the range of temperatures favourable for voluntary flight activity. Flight under suboptimal temperature conditions may also be important, however, in terms of fitness.
2. Here we study the ability of organisms to fly at body temperatures colder than those at which flight is initiated voluntarily, and the duration of such flights. Laboratory-reared *Pararge aegeria* (L.) butterflies that originated from woodland and agricultural landscape were tested at five temperatures (range: 10–21 °C) in a common-garden set-up.
3. We predict that males are able to fly at lower temperatures than females as males have lower wing loading (i.e. body mass/wing area). Since woodland is on average cooler than agricultural landscape we also predict that flight ability at low temperature is better developed in woodland individuals.
4. Individuals showed an ability to fly at all tested temperatures and flights were longer with increasing temperature. Males flew for longer than females. There was no difference in flight duration between sexes at the lowest temperature, but an increasing difference with increasing temperature. We showed that woodland butterflies flew for longer and had higher wing loading than agricultural landscape butterflies.
5. Our results shed new light on the thermal ecology of flight at suboptimal temperatures and are discussed from both proximate and ultimate points of view.

Key-words: Body temperature; flight performance; habitat fragmentation; *Pararge aegeria*; sexual dimorphism

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Introduction

Active flying organisms benefit from being able to track resources and to escape from predators, but flight is energetically costly (Kohane & Watt 1999; Dudley 2000). Flying heliotherms (including butterflies) use solar radiation as an external heat source, which is energetically less costly than physiological heat production (Dennis 1993). The disadvantage of using solar radiation is that their active time budget can be severely constrained by poor weather or when inhabiting cooler (e.g. shadier) vegetation types.

In butterflies, flight is initiated voluntarily only when body temperature is within a given range of body temperatures. These optimal temperatures typically vary between 28 and 38 °C (e.g. Dennis 1993). In temperate-zone environments this is usually well above ambient temperature. Therefore, they thermoregulate behaviourally by basking and micro-site selection to keep body temperature optimal (Shreeve 1992). Thermal knowledge of flight ability and performance is currently biased towards the range of temperatures favourable for voluntary flight activity, but is incomplete with regards to flight under suboptimal temperatures (i.e. body temperatures at which flight is not voluntarily initiated and which are lower than optimal temperatures). This lack of knowledge has led to the view that temperature ‘dictates’ 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

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conditions, some individuals were able to perform at top level under both conditions (Berwaerts & Van Dyck 2004). Experiments by Kemp & Wiklund (2004) have shown that *P. aegeria* males won territorial conflicts independent of thermal asymmetries among the contestants. This finding 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 can be significant in terms of fitness (e.g. escaping a predator attack from a position of rest). Here, we test flight ability and duration in *P. aegeria* under low ambient temperature in an experimental set-up. Males and females may show different behavioural responses at low temperature, in line with their different behavioural repertoires and ecological functions. Females typically mate only once soon after eclosion and, hence, are a rare resource for males. Since male reproductive success is limited by the number of receptive females (Andersson 1994), we can predict selection on early emergence (protandry – Wiklund, Wickman & Nylin 1992) and early activity or activity under suboptimal conditions in males (Pivnick & McNeil 1986) to maximize time spent locating mates (Shreeve 1992). Females however, are limited by the number of zygotes produced (Parker 1978). Assuming that the cost of flight can be reduced by flying under optimal thermal conditions (cf. trade-off between flying and egg-laying), females' flight activity can be expected to be much more limited than males' (Pivnick & McNeil 1986).

At the proximate level, males and females also differ in flight morphology (for example, wing loading, i.e. body mass/wing area). Flight morphology interacts with ambient temperature to realize a particular level of flight performance (Berwaerts & Van Dyck 2004). Lower wing loading in males (Berwaerts, Van Dyck & Aerts 2002) allows them to fly with lower wing beat frequency to generate the same amount of lift (Casey 1981). Since wing beat frequency depends on temperature in general (Stevenson & Josephson 1990), we predict that males are able to fly at lower temperatures than females because males are able to generate higher lift at low temperatures than females.

Besides this sexual aspect, we are particularly interested in testing evolutionary differences in temperature-related flight ability between landscapes. We test landscapes that differ significantly in degree of habitat fragmentation by testing butterflies originating from a continuous woodland landscape *vs* open agricultural landscape with hedgerows and some woodlots (cf. Merckx *et al.* 2003). Woodland landscapes are considered to be the original, natural setting of this butterfly species. Males mainly engage in using sunlit spots at the forest floor for mate-location; females mainly search for isolated grass plants that grow under shaded, wet conditions surrounded by some bare ground for egg deposition (Wiklund & Persson 1983). Woodlands are typically cooler and better buffered

environments than more open land (Noss & Csuti 1997). So, from an adaptive viewpoint we predict that there is stronger selection for flight ability at low temperature in woodland populations compared with open agricultural landscape populations.

Materials and 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. These landscapes have different temperature profiles (T. Merckx, S. Van Dongen, E. Matthysen & H. Van Dyck, unpublished data), which create differential selection regimes for *P. aegeria* populations (Karlsson & Van Dyck 2005). Voluntary flights after basking are associated with thorax temperatures of 30–34 °C (Shreeve 1984; Van Dyck & Matthysen 1998). The average duration of flights 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 (cf. Merckx *et al.* 2003). Captures within each landscape probably represent unrelated individuals as the distance between individual captures was 0.5–5.0 km. Hill *et al.* (2003) estimated maximum dispersal rate of *P. aegeria* to be 1.03–2.7 km year⁻¹, but the dispersal range of the average individual is likely to be considerably less than such values at an invasion front (Hill *et al.* 1999). 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, 22:2 h light : dark). After emergence, adults reared from five agricultural and three woodland landscape females were used for the experiment. They were directly stored at 8 °C (in order to have the metabolism on low activity, and more specifically to suppress oviposition) and 12:12 h L:D and they were not fed or mated prior to observations. Finally, butterflies were killed and stored at –20 °C. Next, they were dried during 24 h at 60 °C and weighed (Sauter microbalance AR1014). Forewing area was measured using digital pictures (Olympus Camedia C-3030, Tokyo, 163-0314, Japan) and an image analyser system (Optimas 1999, Wokingham, RG41 2PX, UK).

TESTING FLIGHT ABILITY

Individual flight ability and duration were measured for males and females originating from the two types of landscape in experimental flight cabinets (height \times length \times width: 2.4 m \times 4 m \times 2 m). We used five identical flight cabinets differing in ambient temperature: 10, 13, 15, 17 and 21 °C. 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. 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; cf. Van Dyck & Matthysen 1998). Flight ability was tested for each individual under each temperature, but the order was randomized. The test, in which butterflies are forced to fly by dropping them, was similar to Pivnick & McNeil (1986): 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. Flight duration was timed from the moment at which the butterfly was released to the point at which it alighted. An individual was tested three times for each of the five treatments. Between trials individuals rested in plastic boxes while others were tested. Since *P. aegeria* is protandrous (Nylin *et al.* 1993), tested males were significantly older than females ($X \pm SE = 5.1 \pm 0.11$ vs 4.2 ± 0.31 days since eclosion; Student *T*-test: $t_{45.8} = -2.75$, $P = 0.009$). There was no age difference between individuals from the two types of landscape ($X \pm SE = 4.5 \pm 0.24$ vs 5.0 ± 0.19 days since eclosion; Meerdaalwoud vs Rillaar, respectively; Student *T*-test: $t_{82.1} = -1.64$, $P = 0.10$).

STATISTICAL ANALYSIS

We used mixed regression models (Proc Mixed, SAS 2001) to analyse flight duration in relation to the factors of interest (i.e. fixed effects): ambient temperature (five classes), landscape of origin (two 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 final 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 non-significant factors. Factors were not eliminated as long as they were incorporated in interaction terms. As individuals were tested repeatedly, the model took into account the 'individual' and the interaction 'individual' \times 'trial' as random effects. Degrees of freedom were calculated using the Satterthwaite option (Littell *et al.* 1996).

Results

We observed varied flight activity at all temperatures (range of flight duration 1.1–600.8 s). At 10 °C, flights were very short, but lasted on average almost three times as long as the falling of a dead specimen ($X \pm SE = 3.3 \pm 0.1$ vs 1.2 ± 0.04 s) and butterflies always alighted in a controlled manner on the legs. Butterflies flew for longer when ambient temperature was higher ($F_{4,1011} = 24.01$, $P < 0.0001$). Males flew for longer than females ($F_{1,131} = 19.63$, $P < 0.0001$). However, there was no difference with sex at the lowest temperature, but the increasing difference with temperature explains the highly significant temperature–sex interaction ($F_{4,1012} = 17.39$, $P < 0.0001$). At 21 °C, males flew on average five times as long as females (Fig. 1).

Table 1 shows the statistical analyses of flight duration relative to all factors of interest (temperature, age, wing loading and landscape of origin) and their interactions in males and in females. We first deal with

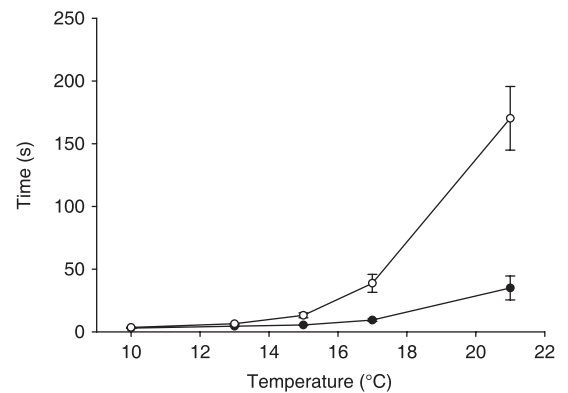


Fig. 1. The association of flight duration with temperature for female (●) and male (○) butterflies.

Table 1. Summary of the analyses of flight endurance of *P. aegeria* males and females in relation to the wing loading of the butterfly, air temperature of the flight cabinet, age and landscape of origin (see Material and methods) using Mixed procedure (SAS)

Effect	<i>F</i>	<i>P</i>
Males		
Temperature	$F_{4,525}$	11.74 < 0.0001
Age	$F_{1,128}$	15.84 0.0001
Landscape	$F_{1,114}$	4.55 0.035
Wing loading	$F_{1,42.2}$	2.39 0.13
Wing loading \times Temperature	$F_{4,524}$	11.20 < 0.0001
Age \times Temperature	$F_{4,536}$	4.48 0.0014
Landscape \times Temperature	$F_{4,533}$	3.94 0.0037
Landscape \times Age	$F_{1,120}$	5.69 0.019
Females		
Temperature	$F_{4,195}$	3.38 0.011
Age	$F_{1,21.5}$	0.44 0.51
Landscape	$F_{1,16.7}$	3.94 0.064
Wing loading	$F_{1,18.8}$	0.02 0.89
Wing loading \times Temperature	$F_{4,196}$	4.86 0.0010
Age \times Temperature	$F_{4,196}$	4.84 0.0010

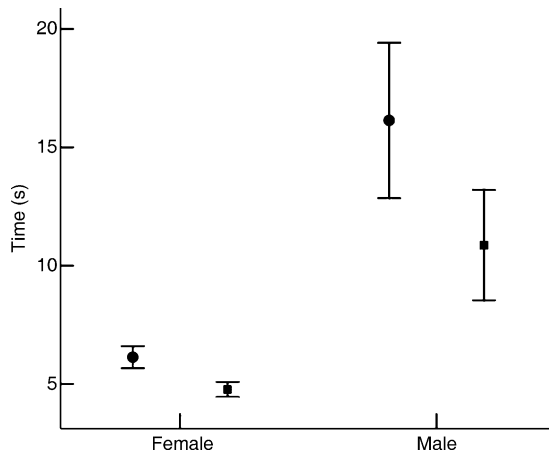


Fig. 2. The effect of landscape of origin on flight duration for female and male butterflies for flights at 15 °C (woodland: circles; agricultural: squares).

the results of males and then females, which were not completely identical. To give an indication what the data themselves look like, Figs 1 and 2 and Table 2 show the means \pm SE of flight duration for the groups of interest in both male and female butterflies.

In males, flight duration increased highly significantly with ambient temperature (Fig. 1). Males originating from the woodland landscape flew for longer than did agricultural landscape butterflies (e.g. for flights at 15 °C: Fig. 2). This effect of landscape of origin appears to have had a strong effect on its own, but the landscape effect also interacted with temperature and with age. The effect of landscape of origin increased gradually with temperature. The highest temperature, however, was an exception to this trend, since the effect of landscape of origin was smallest at 21 °C. Younger males flew for longer, but especially at higher temperatures. The effect of age was stronger in males of woodland landscape origin. High male wing loading was associated with longer flights at all temperatures except for the treatment of 17 °C (slopes: 2.4 (10 °C); 4.0 (13 °C); 3.6 (15 °C); -10.2 (17 °C); 14.0 (21 °C); $< 0.0001 < P < 0.01$ (reference: 21 °C)).

In females, the final model for flight endurance contained fewer factors and also fewer significant main effects (Table 1). Temperature had a similar effect in females as in males, although it was much more significant in males (Fig. 1). There was a tendency for a similar effect of landscape of origin as in males (e.g. for flights at 15 °C: Fig. 2). Age was significant only in interaction with temperature: younger females flew for longer than did older female butterflies, especially at higher temperatures. Females flew for longer when they had lower wing loading but this relationship was steeper at low ambient condition than at the relatively higher temperatures (slopes: -2.9 (10 °C); -3.9 (13 °C); -0.5 (15 °C); -0.5 (17 °C)). At 21 °C we observed a reversed effect (slope: 7.1), which differed from the slopes at the other temperatures tested ($< 0.0001 < P < 0.004$).

Table 2. Summary of mean flight duration in seconds (\pm SE) relative to the combined effects of landscape of origin and ambient temperature, of landscape of origin and age, of age and ambient temperature, and of wing loading and ambient temperature, separately in males and females. In the analyses age and wing loading were treated as continuous variables, but for clarity reasons we cut down the variation in two levels each here ('young' (4–5 days in males; 1–5 days in females) and 'old' (6–8 days in males; 6–7 days in females); 'low' (< 0.093 in males; < 0.119 in females) and 'high' (> 0.093 in males; > 0.119 in females))

		Continuous woodland	Fragmented agricultural
Landscape \times Temperature			
Males	10 °C	3.53 \pm 0.19	3.59 \pm 0.20
	13 °C	7.12 \pm 1.04	5.98 \pm 0.42
	15 °C	16.14 \pm 3.28	10.87 \pm 2.33
	17 °C	31.69 \pm 7.79	44.14 \pm 11.18
	21 °C	193.58 \pm 39.28	151.91 \pm 33.36
Landscape \times Age			
Males	Young	29.39 \pm 4.95	29.27 \pm 5.22
	Old	15.86 \pm 6.82	18.95 \pm 6.15
		Young	Old
Age \times Temperature			
Males	10 °C	3.56 \pm 0.14	3.61 \pm 0.64
	13 °C	6.67 \pm 0.56	4.79 \pm 0.65
	15 °C	13.63 \pm 1.98	11.93 \pm 5.36
	17 °C	43.74 \pm 8.92	19.92 \pm 5.39
	21 °C	203.77 \pm 29.41	64.07 \pm 37.06
Females	10 °C	2.91 \pm 0.18	3.25 \pm 0.21
	13 °C	4.50 \pm 0.31	4.53 \pm 0.35
	15 °C	5.87 \pm 0.39	4.91 \pm 0.46
	17 °C	10.00 \pm 1.31	6.88 \pm 1.08
	21 °C	41.81 \pm 13.29	21.97 \pm 11.28
		Low	High
Wing loading \times Temperature			
Males	10 °C	3.42 \pm 0.18	3.78 \pm 0.23
	13 °C	6.25 \pm 0.52	7.15 \pm 1.04
	15 °C	11.66 \pm 2.65	15.95 \pm 3.26
	17 °C	58.31 \pm 13.99	19.01 \pm 4.59
	21 °C	143.30 \pm 32.75	232.25 \pm 41.78
Females	10 °C	3.47 \pm 0.40	2.87 \pm 0.24
	13 °C	5.48 \pm 0.62	4.17 \pm 0.43
	15 °C	5.73 \pm 0.73	6.03 \pm 0.49
	17 °C	6.73 \pm 1.17	9.11 \pm 1.32
	21 °C	12.72 \pm 4.40	26.64 \pm 9.18

Discussion

Our experiment showed that the butterfly *P. aegeria* is able to fly at much lower body temperatures (10–21 °C) than the voluntary flight range (30–34 °C – Shreeve 1984). Because we observed flight at 10 °C, the body temperature threshold for flight activity must be lower than 10 °C, which is much lower than in several other butterfly species (e.g. Pivnick & McNeil 1986; Masters, Malcolm & Brower 1988; Norberg 2003). This result agrees with the shade-dwelling nature of the species (Karlsson & Wiklund 2005). *P. aegeria* is able to fly at

low ambient temperature, but this is generally avoided (i.e. short flights after experimental induction only), which may suggest a considerable physiological cost. In the same vein, older butterflies that have already used much of their reserves (cf. Karlsson 1994) had shorter flights. In line with our key predictions, males and females responded differently and butterflies of landscapes that differ in thermal profile (woodland vs open agricultural landscape origin) also responded differently. The latter suggests strong, but landscape-related differential selection by ability to fly at below optimal temperatures.

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 relative to males, relates to fundamental differences in their life history. Males benefit in terms of their reproductive success by extended periods of activity to locate mates, including early arrivals at sunlit patches on the forest floor. As they spend the night in the canopy, short flights at suboptimal temperature in the canopy to adjust their position to more sunny sites where they can heat up more easily are expected to be important in this respect. Moreover, as there is intensive fighting behaviour among perching males and extended flights through shade in case of patrolling males (Wickman & Wiklund 1983; Shreeve 1984), they are more frequently subjected to flight at suboptimal body temperatures (Van Dyck & Matthysen 1998; Berwaerts & Van Dyck 2004). In the latter case this would correspond to our treatment of 21 °C rather than that at 10 °C. Assuming that there is a trade-off in females between allocations for reproduction vs flight (e.g. Hughes, Hill & Dytham 2003), females need to be more economical in terms of flight activity. Butterfly females typically spend less time flying than males (Shreeve 1984), which probably 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 wing beat frequency (Casey 1981) and wing beat 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). It would be interesting to test whether or not the smaller size of males is an adaptation in order to extend flight activity.

Pararge 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 often on the wing 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 vs a warmer, but thermally more variable fragmented agricultural landscape. However, the interpretation in adaptive and mechanistic terms requires careful and further evaluation. First, the landscape effect in females was not as strong as in males. Secondly, although several distant locations were sampled in each landscape – well beyond the range of dispersal of the average individual butterfly (cf. Methods) – we only used butterflies from a single agricultural landscape and a single woodland landscape. Therefore, owing to practical limitations, there was no replication of the landscape-of-origin effect *per se* in the current analyses. Comparisons among landscape types would appear to be a more general limitation of evolutionary ecological studies (e.g. Partecke *et al.* 2004; Karlsson & Van Dyck 2005). Moreover, we have implicitly made the assumption that voluntary flight occurs in the same temperature range for butterflies from both landscape and also for males and females. However, this is not necessarily the case. Watt and colleagues have shown for *Colias* butterflies that males have a broader temperature range for voluntary flight than females in colder habitat taxa, but not in lowland taxa (Watt 2003 and references therein). Therefore, in order to have a more complete understanding of the thermal ecology of *P. aegeria* additional work is required to test for similar patterns with landscape and sex and how this may affect the interpretation of flight at suboptimal temperatures.

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 wing beat 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.

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