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Thermoregulatory differences between phenotypes in the speckled wood butterfly: hot perchers and cold patrollers?

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Abstract Males of the speckled wood butterfly *Pararge aegeria* L. (Satyrinae), actively search for females (“patrolling”) or wait for them at particular places (“perching”). Darker males are more likely to patrol than pale ones, which are mainly territorial perchers. We studied whether this morphological variation relates to thermoregulatory differences. The relationship between thoracic temperature and ambient temperature differed between the colour types under natural conditions: darker males had on average lower body temperatures than paler males. Different activities (e.g. resting, flying) and behavioural strategies (perching or patrolling) were associated with differences in thoracic temperature: patrolling males which mainly engaged in long flights and periods of basking afterwards, had lower thoracic temperatures than perching males which engaged in very short flights, fights and basking. When resting for a while thoracic temperatures did not differ between males practising different strategies. Under laboratory conditions, darker males heated up faster than pale males but there was no difference in the thoracic temperature at which they started to fly. These results indicate that thermal requirements (or general conditions) differ between the behavioural strategies, and that behavioural differences between phenotypes (colour types) relate to differences in thermal ecology. This supports the idea that darker males are better adapted to patrolling. There is no evidence that one mate-locating strategy is always superior to the other, which coincides with the observation that both strategies co-exist. More generally, this study shows that relatively small differences in colour can have a considerable effect on thermoregulation and hence on the behavioural strategies a heliothermic insect will adopt.

Key words Butterflies · Mate-locating strategies · *Pararge aegeria* · Thermoregulation · Wing colour

Introduction

Since physiological rates are temperature-sensitive, an ectotherm’s behavioural and ecological performance, and thus its fitness, are likely to be influenced by body temperature (Huey and Kingsolver 1989). Active flight in particular, requires the maintenance of a high muscle temperature (Josephson 1981; Heinrich 1993), 34–37°C in several temperate butterflies (Vielmetter 1958; Douwes 1976; Rutowski et al. 1994; Dreisig 1995), although some species are able to fly at much lower thoracic temperatures. Therefore, not only in arctic but also in temperate regions, flying ectotherms have to increase their body temperature considerably above air temperature to reach values that allow flight activity. Since flight muscles are mainly located in the thorax, thoracic temperature is the most relevant body temperature to be studied in relation to flight. Internal heat production by shivering is rare in butterflies, which are mainly heliothermic, i.e. they raise their body temperature by basking in sunlight (Casey 1988; Dennis 1993). When flying, myogenic heat is produced, but only large insects generate sufficient heat that is not quickly dissipated by convection (Shelly and Ludwig 1985; Heinrich 1993).

Size is known to be a crucial factor in thermoregulation because it has a strong influence on convective cooling (during flight) and heating (when basking). Smaller butterflies heat up slightly faster than larger ones, but they are also more constrained by temperature in their activity (Heinrich 1986b). But other aspects of a butterfly’s morphology will also influence its thermoregulation. Wings play an important role since heat energy can be transferred from the basal part of the wings to the thorax by conduction and convection of trapped warm air (Digby 1955; Clench 1966; Wasserthal 1975). The thermal characteristics, particularly absorption, of butterfly wings vary between species, according

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to type of basking behaviour, colour and surface structure (Schmitz 1994). Several studies have shown that wing colour can be important in butterflies of cooler environments. For instance, basal melanism increases with altitude and latitude (Watt 1968; Guppy 1986; Kingsolver and Wiernasz 1987). The small increases in preflight warm-up rates owing to melanization appear to have been a strong selective pressure on some alpine and high-latitude butterflies (Watt 1968).

In male butterflies, most of the flight activity is associated with mate-locating behaviour. Two mate-locating strategies are generally distinguished (Scott 1974): males can actively search for females ("patrolling") or wait for them at particular sites ("perching"). In either male strategy, thermoregulation is of prime importance (Heinrich 1986a). Essentially, a territorial percher has to maximize rapid responses to passing females or males challenging territory ownership, and flight efficiency (e.g. flight frequency, manoeuvrability), not flying time. Patrollers are probably true shuttling ectotherms if they must maximize both flight efficiency and the time spent searching for females (Pivnick and McNeil 1986; Dennis 1993; Rutowski et al. 1994; Dreisig 1995). In species where the two strategies co-occur, it has been shown that more individuals patrol when ambient temperature and solar radiation are higher (Dennis 1982; Shreeve 1984; Wickman 1985). The speckled wood butterfly, *Pararge aegeria* (L.), is such a species where the males either defend a sunlit patch on the forest floor waiting for females, or patrol by visiting several patches and searching for females on and between those patches (Wickman and Wiklund 1983; Shreeve 1984, 1987; Van Dyck et al. 1997a). For both males and females these sunlit patches primarily offer basking locations. A high body temperature (32–34.5°C) is required to initiate spontaneous flight (Shreeve 1984).

Previously we have shown (Van Dyck et al. 1997a) that darker males of the speckled wood butterfly *Pararge aegeria* L. are more likely to patrol than pale ones, which are mainly territorial perchers. It was hypothesized that this difference between phenotypes could be explained by variation in thermoregulation. Darker males are expected to be "better designed" for patrolling since they may be better able to recover from temperature loss in flight and to quickly attain the necessary body temperature to continue their flight through shaded conditions. Perching males, particularly if they are too dark, may suffer from overheating in permanent sunlight on a perch. We therefore predict: (1) differences in body temperature with phenotype – since darker males are more often patrolling (flying) and hence faced with convective cooling they will have on average lower body temperatures; (2) differences in body temperature with behaviour; and (3) differences between phenotypes in heating rates – darker males will heat up faster. Here we test these predictions with field measurements and a warming-up experiment in laboratory conditions.

Materials and methods

The study species

The speckled wood is a temperate, satyrine butterfly of average size and mass (male wing length: *c.* 22 mm; fresh male body weight: *c.* 50 mg). In Belgium, it is a common species that occurs in different types of woodland. It is bivoltine (sometimes three generations) and the flight period lasts from April to September (first generation: from April to the end of June; second generation: from July to September).

Field measurements

Fieldwork was conducted in a set of five deciduous woodland fragments, called Lankem, in Herentals (Belgium, 51°08'N, 4°49'E) between 5 May and 15 August 1994 and between 2 May and 17 August 1995 (between 1100 and 1630 hours). Fieldwork was not spread throughout this period but concentrated on sets of successive days with similar, fine weather conditions (sunny, no strong clouds and no rain). A detailed description of the study area is given by Van Dyck et al. (1997a). We collected data on individual phenotype (dorsal wing colour and size), body temperature and two types of behavioural data. Before an individual male was captured to be measured, we followed its activities for at least 5 min and its behaviour was characterized as perching or patrolling (cf. Van Dyck et al. 1997b). The activity immediately before capture was also recorded as: short flight (a flight of a few seconds within a sunlit patch); long flight (more than a few seconds and beyond a single patch); basking (sitting with open wings, i.e. dorsal basking *sensu* Clench 1966); resting (sitting with closed wings); and interacting (fighting with conspecific male).

Thoracic temperatures were measured by stabbing the thorax with a copper-constantan thermocouple (type MT-29/1B, time constant = 0.025 s, Physitemp Instruments) in a hypodermic needle microprobe (needle diameter 29 ga). Temperatures were read to the nearest 0.1°C on a Bailey Bat-12 thermocouple thermometer. The butterflies were captured with a hand net and the needle was inserted directly through the net into the mesopleural part of the thorax (without any direct contact between the human hand and the butterfly's body). The body temperature (i.e., thoracic temperature) was measured within 5 s after capture. From our mark-recapture studies on individuals from which we did measure body temperature and from which we did not, we found no significant differences in recapture rates. Ambient temperature was also measured at the spot of capture at a height of 1 m with a digital thermometer (Flash thermotron) with a shaded probe (reading to 0.1°C).

We scored dorsal wing colour of each individual by visual assessment into one of four categories from pale to dark brown, with high repeatability (Van Dyck et al. 1997a). Because the sample size of the darkest colour category ($n = 8$) was small in this study, categories 3 and 4 were lumped. We measured right forewing length with digital callipers (reading to 0.01 mm, but accurate to 0.40 mm, Van Dyck et al. 1997a) from the wing joint to the wing tip. Butterflies were released at the spot of capture immediately after measuring. Repeated measurements from recapture data were not included.

On 5 June 1995 we recorded solar radiation in full sunlight on 21 sunlit patches and directly beside each patch in shaded conditions between 1100 and 1600 hours. Measurements were done at a height of 1 m with a "black" and a "white" thermometer. The black thermometer (with a dark painted probe) indicated temperature when radiation was absorbed, while the white one (with a probe shaded by a small screen) indicated air temperature. The difference between the two temperatures is a measure of temperature gain by solar radiation (cf. pyranometer; Rosenberg 1974).

Warming experiment

We performed a warming experiment on male speckled woods in the laboratory. Each individual, kept in a plastic box, was cooled in a refrigerator (9–10°C) for 25 min. Next, the individual was able to warm up in an experimental set-up while its body temperature was measured permanently with a microprobe. Temperatures were read every 5 s. The observation was stopped when the butterfly started to flap its wings (i.e., when the threshold for flying was reached). We used a set-up of four mercury vapour lamps (Höhnle, SOL 500 type) which are especially produced (Dr. K. Hönle GmbH, Martinsried, Germany) to approach the solar radiation spectrum and intensity (900 W m⁻² at 25 cm distance). Air temperature in the experimental room was kept constant at 21 ± 2°C with an air-conditioning system. Each lamp had a box-shape and irradiated light through the bottom surface (27 by 22 cm). The four lamps were connected to each other so that they produced a square field of light. The distance (height) between these lamps and the substrate on which the butterfly was placed was 1.15 m.

Since basking posture and wing position may influence heating (Polcyn and Chappell 1986), heating rates were calculated only for those individuals ($n = 23$) that maintained a dorsal basking posture with fully spread wings (*c.* 180°). We expressed the heating rate as the slope of the thoracic temperature-time graph, between 26 and 32°C (see Results for justification), when time was log transformed. We determined basal dorsal wing colour (in the discal forewing cell) by measuring grey values (between 0 = black and 255 = white) with an image-analysing system containing a video camera connected to a computer through a frame grabber (Windig 1991). We used the OPTIMAS image-analysis software (Optimas 1995). We measured colour at the wing base because this is most relevant to thermoregulation (Wasserthal 1975), but in 33 males there was a good correlation between colour measurements on basal and distal part (at 3th, 4th and 5th wing cell, Schwanwitsch 1935) of the forewing ($r = 0.663$, $n = 33$, $P < 0.0001$). The measured grey values corresponded well to the visually scored colour classes as used in the field. Colour type 1 (pale) had scores between 70.9 and 60.7, type 2 between 61.1 and 53.1 and type 3 (dark) between 54.0 and 39.1.

Statistics

We collected data in two years, but since we assume no year effect on the relations between behaviour, phenotype and body temperature, year was not considered as a factor. Generation was also not used as a variable because temporal variation was mainly reflected by ambient temperature: first generation 22.8 ± 0.2°C and second 26.8 ± 0.2°C (ANOVA, $F_{1,210} = 117.4$, $P = 0.0001$). A similar reasoning is valid for date since fieldwork was done within sets of successive, similar days and not spread throughout the season. Therefore, for the full analysis (i.e., complete data set) the differences in date by themselves are not important and differences in ambient temperature are included. Moreover, in each of the sets of fieldwork days, we had data on individuals of all colour classes (when class 3 and 4 were gathered). Additionally, we performed an analysis similar to that based on the total dataset, on a smaller subset of data collected on one day to check explicitly whether the patterns from the total dataset apply regardless of date itself.

We mainly used generalized linear models (procedure GLM in SAS, using type III Sum of Squares; SAS 1990) to analyse the variation of thoracic temperature in relation to ambient temperature, the phenotypic and behavioural parameters. First, we analysed the effects of the variables univariately, and second, we performed a multivariate analysis by fitting the variables and all interaction terms step-wise in a final regression model. The step-wise procedure does not rank variables by their importance (James and McCulloch 1990), but can be used to analyse whether variables explain the same part of the variation in the response variables. Therefore, we did both the univariate and multivariate (i.e. final model) analyses. Final model selection was done by backward

elimination of the least significant factors, including all interaction terms, from the full model. Terms were not eliminated as long as they were incorporated in interactions. Differences in thoracic temperature between males engaged in different activities were evaluated with analysis of variance and Scheffé tests. Means are given ± SE.

Results

Field measurements

Weather conditions

During the day, when weather conditions allowed activity, most speckled wood males engaged in mate locating behaviours (perching or patrolling) on the level of the forest floor (i.e., the flight space of *c.* 1.5 m height), where sunlit patches are scattered throughout the forest. From a thermoregulatory point of view, sunlit patches and shaded areas are very different, notably in the input of solar radiation. This was quantified by the black and white thermometer measurements: temperature excess was up to 10 times higher on a sunlit patch compared to nearby shaded conditions (mean: 3.9 ± 0.5°C and 0.37 ± 0.03°C, respectively).

Mate-locating behaviour

Out of the 212 males, 154 (72.6%) were considered perchers and 27.4% were patrollers. Of the perchers 17.5% were pale, 53.3% intermediately coloured and 29.2% dark, while the proportions of patrollers were 5.1%, 41.4% and 53.5% respectively. So, patrolling males were on average darker than perching males ($F_{1,210} = 13.11$, $P = 0.0004$), but did not differ in wing length ($F_{1,210} = 1.57$, $P > 0.21$). Perching males occurred on average at warmer sites than patrolling males ($F_{1,210} = 6.52$, $P = 0.011$). The activity recorded immediately before capture offered a more detailed comparison of the behaviours between males that used different mate locating strategies (Fig. 1; cf. Van Dyck et al. 1997a). Half of the patrollers were captured in long flight while perchers were mainly sitting on bare ground, dead leaves or vegetation of a sunlit patch with closed wings (34.4%), making short looping flights within a single patch (20.8%) or were fighting with other males or insects (9.1%). Similar proportions of both types of males (about 33%) were basking, which indicates the importance of thermoregulation for both strategies. Basking can be done either with partially or fully spread wings and we found a significantly larger proportion of patrolling males that basked with fully spread wings (15/19 = 79%) than of perching males (25/51 = 49%) (Fisher's exact test, $P = 0.031$). Individuals which were in long flight were on average darker than individuals in short flight, or than individuals that were resting or basking ($F_{4,207} = 3.43$, $P = 0.0097$). Wing length was not correlated with the different activities ($P > 0.91$).

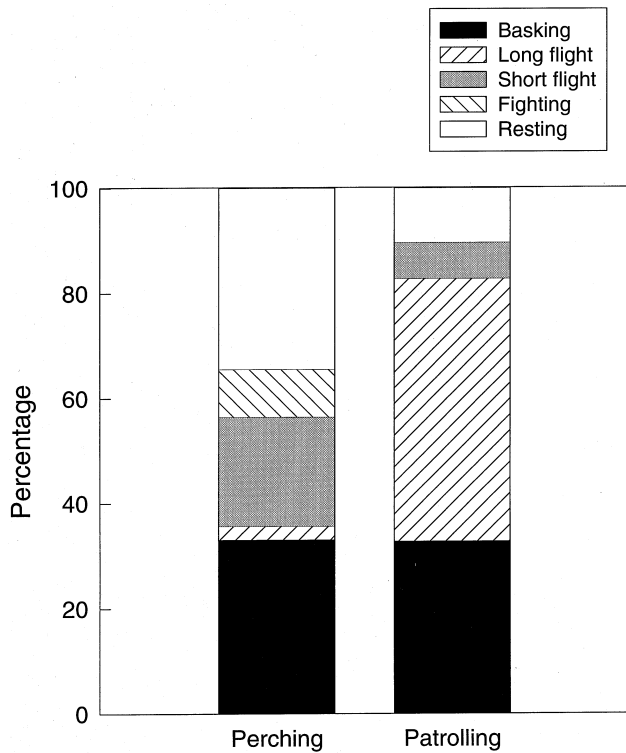


Fig. 1 Behavioural activities of males classified as perchers or patrollers ($n = 154$ and $n = 58$ respectively) immediately before the measurement of thoracic temperature

Thoracic temperature

The overall mean thoracic temperature of males was $32.3 \pm 0.2^\circ\text{C}$ ($n = 212$). With one exception of 22.8°C , all thoracic temperatures were between 25 and 37°C and 66.5% had temperatures within a more narrow range of 30 – 35°C (Fig. 2). Thoracic temperatures were always above the isothermal line (Fig. 3) with a maximum excess of 13.4°C . The mean temperature excess (i.e. thoracic temperature – ambient temperature) was $7.4 \pm 0.2^\circ\text{C}$ ($n = 212$), and the variation in thoracic temperature was much lower than that of ambient temperature (coefficients of variation 6.86% and 11.49% respectively).

When analysed separately, wing colour, ambient temperature, behavioural strategy and activity immediately before the measurement were all significantly related to thoracic temperature (colour: $F_{2,209} = 3.84$, $P = 0.035$; ambient temperature: $F_{1,210} = 83.68$, $P = 0.0001$; strategy: $F_{1,210} = 18.67$, $P = 0.0001$ and activity: $F_{4,207} = 22.19$, $P = 0.0001$), while there was no significant effect of wing length ($P = 0.65$): (1) darker males had lower thoracic temperatures, (2) ambient temperature was positively correlated with thoracic temperature (Fig. 3), (3) patrollers had lower thoracic temperatures than perchers and (4) thoracic temperature was different between males engaging in different activities (Table 1). Scheffé tests showed for which activities the mean thoracic temperatures were

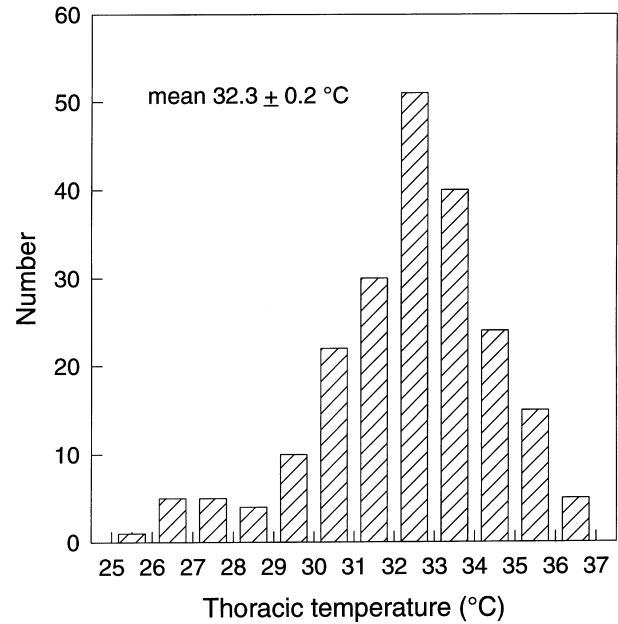


Fig. 2 Frequency distribution of thoracic temperature of males measured in the field ($n = 212$)

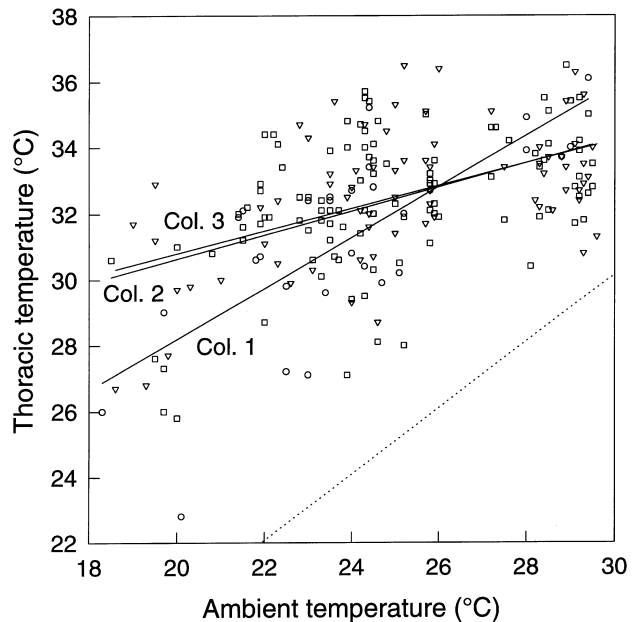


Fig. 3 Relation between thoracic and ambient temperature for the three colour types. Sample sizes were 30, 106 and 76 for colour type 1 (Col. 1, circles): pale, type 2 (Col. 2, squares): intermediate and type 3 (Col. 3, triangles): dark, respectively. The dotted line refers to the isothermal condition

significantly different: butterflies in short flight, long flight or basking had lower temperatures than resting ones, and butterflies in long flight or basking had lower temperatures than fighting ones. Basking temperatures were the most variable, probably because the duration of basking prior to measurement had not been measured.

Next, we tested the effects of wing colour, wing length, ambient temperature, behavioural strategy, ac-

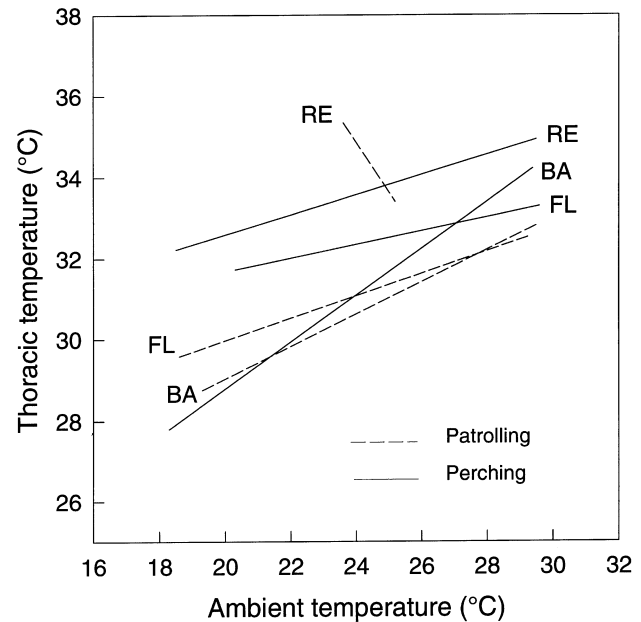
Table 1 Mean thoracic temperature of males engaged in different activities under field conditions

Activity	<i>n</i>	Thoracic temperature (°C)
Long flight	33	31.20 ± 0.26
Basking	70	31.22 ± 0.31
Short flight	36	32.24 ± 0.19
Fighting	14	33.36 ± 0.36
Resting	59	34.03 ± 0.16

tivity before capture and all interaction terms multivariately. In the full model the five-way interaction term turned out to be significant ($P = 0.029$), which is a statistical point due to the interrelations between colour, behavioural strategy and activity, and lacks a reasonable biological interpretation. Therefore the further analysis had to be split up. We did two multivariate analyses for thoracic temperature: (1) with wing colour, wing length, ambient temperature and strategy (thus activity was excluded) and (2) with wing length, ambient temperature, behavioural strategy and activity (thus colour was excluded). The latter gave the opportunity to examine the effects of behavioural strategy (i.e. behaviour over a longer term) and activity (i.e. snapshot of a behaviour) together. For that analysis, we also reduced the number of statistical cells by not taking colour (which was correlated with strategy which was in the analysis) into account and by reducing the behavioural activities from five to three classes: flight, resting and basking. By lumping the different forms of flight activity we also reduced the interdependence of behavioural strategy and activity (see Fig. 1). In other words, the differences between types of flight are now partly reflected by behavioural strategy.

The final model of the first analysis kept wing colour, ambient temperature, behavioural strategy and the interaction between wing colour and ambient temperature as significant terms ($F_{6,205} = 22.22$, $P = 0.0001$; colour: $F_2 = 8.45$, $P = 0.0003$; ambient temperature: $F_1 = 81.44$, $P = 0.0001$; strategy: $F_1 = 20.86$, $P = 0.0001$; colour × ambient temperature: $F_2 = 7.15$, $P = 0.001$). Perchers had higher thoracic temperatures than patrollers. The interaction is shown in Fig. 3: at low ambient temperature pale individuals (colour type 1) had lower thoracic temperatures than the intermediate and dark butterflies (colour types 2 and 3 respectively), but at high ambient temperature they had higher thoracic temperatures than the two darker colour types. In other words, thoracic temperature of darker butterflies varied less with ambient temperature.

The final model of the second analysis kept ambient temperature, activity and two two-way interaction terms, ambient temperature × activity and behavioural strategy × activity as significant terms ($F_{8,203} = 30.21$, $P = 0.0001$; ambient temperature: $F_1 = 64.24$, $P = 0.0001$; activity: $F_2 = 12.90$, $P = 0.0001$; strategy: $F_1 = 1.09$, $P = 0.29$; ambient temperature × activity: $F_2 = 9.62$, $P = 0.0001$ and activity × strategy: $F_2 = 3.07$, $P = 0.048$). The effects are shown in Fig. 4.

**Fig. 4** Relation between thoracic temperature and ambient temperature for males of different activities and behavioural strategies. Sample size for basking (BA), flying (FL) and resting (RE) were 51, 50 and 53 for perching males and 18, 34 and 6 for patrolling males, respectively. The regression lines only are shown

As expected, the main effect of ambient temperature on thoracic temperature is positive. Figure. 4 gives the impression that this is not the case for resting patrollers, but this is based on a very small sample ($n = 6$) in a small range of ambient temperature. If the slopes had really been different, this would be expressed by a significant three-way interaction (ambient temperature × activity × behavioural strategy), which is not the case. What do the two-way interactions mean? At low ambient temperature, thoracic temperature of basking butterflies is lower than those of resting and flying butterflies, but thoracic temperature in the former increased faster with ambient temperature (cf. slopes in Fig. 4). Therefore there was a significant ambient temperature × activity interaction. Perchers had higher thoracic temperatures than patrollers when basking and flying, but not when resting. In other words, the thoracic temperature difference between resting and flying/basking individuals, was more pronounced in patrollers than in perchers. Therefore we found a significant behavioural strategy × activity interaction.

So far, we assumed that date itself had no effect. Although we believe this is justified since ambient temperature was included, we additionally analysed the effects of wing colour, length, ambient temperature and behavioural strategy within a data sample collected at the same day ($n = 25$). In order to reduce the number of statistical cells we did not take the activity classes into account. The model kept wing colour and behavioural strategy as significant terms ($F_{3,21} = 9.40$, $P = 0.0004$; colour: $F_2 = 4.35$, $P = 0.026$ and strategy: $F_1 = 22.97$,

$P = 0.0001$). Thus within 1 day, colour type and mate locating strategy also related to similar differences in thoracic temperatures. This agrees with the results of the total dataset, except for the differences in ambient temperature. Since we collected only data under suitable conditions on some sets of successive days (see Materials and methods), the variation in ambient temperature is smaller within such a day compared to the variation between different days during the season.

Warming experiment

When brought under the warming lamps, the previously cooled butterflies immediately heated up until they started to show flight activity. This heating process typically followed a curvilinear relationship. We analysed four warming parameters in relation to phenotype: (1) initial thoracic temperature (T_i), (2) thoracic temperature at which the butterfly started to fly (T_f), (3) time before flight and (4) heating rate (as explained further). Since strong correlations between these parameters were lacking (all $P > 0.08$), we examined them all (first with separate analyses and then with multivariate GLM models). However, two tendencies were apparent: individuals with a higher T_i tended to initiate flight earlier ($r = 0.37$, $P = 0.087$) and individuals that heated up faster tended to start flight at a higher thoracic temperature ($r = 0.37$, $P = 0.085$).

During the few seconds that were needed to take the butterfly from the plastic box (in the refrigerator at 10°C) and connect it to the thermocouple, the butterfly already heated up considerably (mean T_i : $23.2 \pm 0.2^\circ\text{C}$). Darker individuals had higher T_i ($F_{1,21} = 7.76$, $P = 0.011$) and that was also the case for smaller individuals ($F_{1,21} = 4.79$, $P = 0.04$). Neither T_f nor time before flight were significantly influenced by wing colour or wing length (all $P > 0.2$). T_f was on average $32.3 \pm 0.3^\circ\text{C}$ (range: 30.3 – 33.9°C). Time before flight lasted on average 259 ± 33 s (minimum: 58 s). From the previous section we know that once speckled woods were active under suitable conditions in the field, their temperature rarely dropped below 25°C . Therefore heating rates in the range of 26 – 32°C are considered of particular relevance to male behaviour, and were calculated here as the slopes of the regressions of thoracic temperature against logarithm of time (higher slopes reflected faster heating). We found that darker individuals heated up faster ($F_{1,21} = 4.67$, $P = 0.042$; Fig. 5), but wing length had no effect on heating rate ($P > 0.99$).

The next step was to study the effects of wing colour, wing length, warming parameters and all interaction terms with three GLM models: (1) T_i in relation to wing colour and length; (2) T_f in relation to wing colour, length and T_i ; and (3) heating rate in relation to wing colour, length, T_i and T_f . The first final model kept both wing colour and length, but not their interaction, as significant terms ($F_{2,20} = 12.66$, $P = 0.0003$, colour: $F_1 = 16.90$, $P = 0.0005$ and wing length: $F_1 = 13.09$,

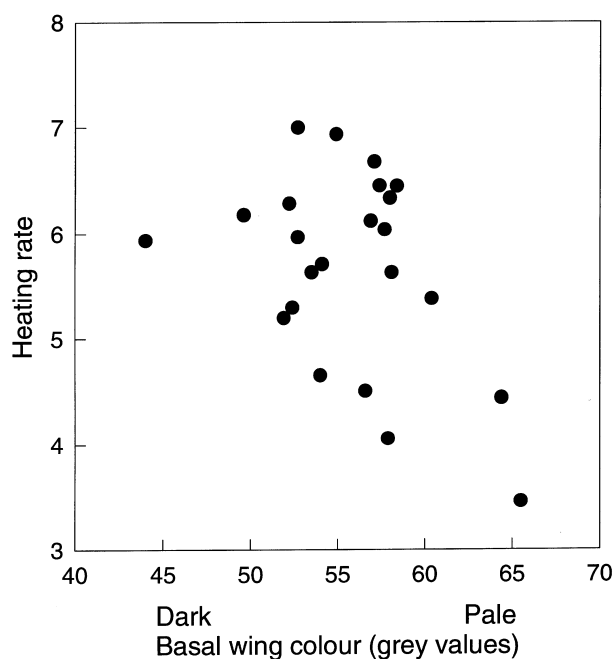


Fig. 5 Relation between heating rate and dorsal basal wing colour in males with fully dorsal basking posture ($n = 23$). Heating rate is expressed as the slope of the thoracic temperature-log time regression in the interval 26 – 32°C .

$P = 0.0017$). So, wing colour and wing length both affected pre-experimental heating. For the second analysis, we did not find a significant model ($P > 0.25$): T_f did not vary with any of the studied parameters. Finally, the final model for heating rate only kept colour as significant term ($F_{1,21} = 4.67$, $P = 0.042$) and therefore reduced to the same result as for the separate analysis of heating rate with wing colour.

Discussion

Dark males of the speckled wood butterfly were more likely to patrol than pale ones which were mainly territorial perchers (Van Dyck et al. 1997a; this study). It was hypothesized that this relationship can be explained by differences in thermoregulatory abilities. Our study has shown evidence for the three predictions we made in this context. First, thoracic temperature, which obviously correlates with ambient temperature, also differed between the colour types. This supports the idea that behavioural differences between phenotypes are based on differences in thermal ecology. Second, different activities before measurement (basking, resting, etc.) and behavioural strategies (perching or patrolling) were associated with differences in thoracic temperature, independently of phenotypic variation. This implies that the thermal requirements indeed differ between strategies. Third, heating rates differed between colour types: darker males heated up faster. This is in accordance with our hypothesis that darker males are better adapted to patrolling, i.e. flying for longer periods in shaded con-

ditions. But since there is no evidence that one of the mate locating strategies is always superior in terms of fitness to the other (see discussion in Van Dyck et al. 1997a), there is no general tendency towards darker speckled woods by means of directional selection.

Patrollers spent more time flying and basking than perchers which were more often resting, fighting and engaging in short flights. We found, as predicted, that the activities of a patroller are associated with lower thoracic temperatures. However, even when engaged in the same activity, patrollers had lower thoracic temperatures than perchers (except when resting). This shows that flight results in a relatively greater amount of heat loss in patrollers. The explanation is that whereas the flights of perchers are mainly short, looping flights within a sunlit patch, those of patrollers are long flights through the woodland, where irradiance is heterogeneously distributed at the forest floor and flying implies passing through shade. Our radiation measurements with black and white thermometers indicated that the temperature excess on a sunlit patch compared to the shade can be profound. Most studies on thermoregulation in butterflies have been conducted on species from more open habitats (e.g., Douwes 1976; Dreisig 1995), but in another woodland satyrine butterfly, *Calisto nubila*, it has been suggested that the high proportion of shade in the habitat and the small body size have a limiting effect on flight through the frequent basking intervals necessary to maintain an elevated thoracic temperature (Shelly and Ludwig 1985). It follows from the previous that patrollers benefit more from a higher heating rate, and hence from darker wings. Patrollers also basked more frequently with their wings completely spread, compared to perchers. This makes sense since body temperature drops more seriously in a patroller, thus it needs a higher heat load. Perchers frequently adopted a minimal shadow position (i.e. resting with closed wings parallel to the solar radiation), which suggests that they try to avoid overheating. On the other hand, body temperatures of resting individuals (closed wings) did not vary with colour, suggesting that behavioural adjustments allow different phenotypes to maintain the same optimal temperature. Besides sit-and-wait, perchers also engage in short, often looping, flights within a sunlit patch. Such behaviour has been interpreted in other butterfly species as a sexual signal (i.e. attracting the attention of passing females; Dreisig 1995), but short flights may have a thermoregulatory function as well: cooling by convection and thus avoiding overheating.

Our warming experiment confirmed the prediction that darker males heated up faster, both in the short period during transfer and in the 26–32°C range. Under laboratory conditions, melanic forms (spring) of the pierid butterfly *Nathalis iole* also heated more quickly than immaculate forms (summer) (Douglas and Grula 1978). But under natural conditions, variation in heating rates may be more complicated since heating rates also vary depending on basking substrate (Heinrich 1993).

Microsite choice may then be crucial and may differ between phenotypes, but this has not yet been investigated. With respect to flight threshold temperature the warming experiment showed no relationship with wing colour or size. Guppy (1986) also found in the butterfly *Parnassius phoebus*, another dorsal basker, that wing melanization did not affect the flight threshold, even though melanization was correlated with body temperature and flight duration in cool conditions.

Regardless of their mate-locating strategy, speckled wood males appear to be active at lower thoracic temperatures than several other butterfly species (Vielmetter 1958; Douwes 1976; Rutowski et al. 1994; Dreisig 1995): the required temperature for flight is only about 32°C and less than 10% of the butterflies observed in the field had temperatures above 35°C. This suggests that the species is adapted to slightly cooler conditions in woodlands. Additional evidence to support this hypothesis is offered by egg-laying experiments; speckled wood females reach their peak in eggoutput at lower temperatures than other satyrine butterfly species (B. Karlsson, personal communication).

Since both this study and the previous ones (Van Dyck et al. 1997a, b) were done under suitable conditions for speckled wood activity, it might be worth comparing the behaviour of different phenotypes under more marginal conditions (cloudy and cool or extremely hot conditions). Since mate locating behaviour is influenced by the environment (i.e. weather and habitat structure, Dennis and Shreeve 1988), the phenotype-behaviour relationship may also shift; for instance pale individuals may more often engage in patrolling when conditions become warmer. In real hot, sunny conditions or areas, *P. aegeria* males may in fact patrol and perch in shaded areas and even defend shaded patches if these are rare, as it was observed in Algarve (Portugal) by Dennis (1986).

But what about size? Heinrich (1986a,b) showed for some butterfly species that large size imposed a slight disadvantage in warm-up time during basking, but nevertheless conferred an enormous advantage in prolonging flight duration before convective heat loss necessitated another bout of basking. The present results showed little effect of size in both the field observations and the warming-up experiment, but we have no data on cooling rates and flight duration. We only found an effect of size on the initial thoracic temperature of the butterflies from the warming experiment. To reduce the influence of convective cooling in flight, a patroller should be large. But, depending on the habitat structure (frequency and configuration of sunlit patches), there may be a trade-off for a patroller between being small (reduce heating time) and large (delay cooling). On the other hand, if competitive ability of a territorial percher also increases with size, it could confound the issue even more and make size-differences between perchers and patrollers even more unpredictable. Perching speckled wood males have been shown to have relatively larger thoraxes compared to patrolling males, which was sug-

gested to be related to power output and manoeuvrability (Van Dyck et al. 1997b). Moreover, in butterflies, there are no indications so far of variation in body mass being correlated with thermal balance in different thermal environments, presumably because of other constraints on body size, but behavioural means of thermoregulation have correspondingly diversified (Heinrich 1986b, 1993).

The results of Shreeve and Smith (1992) strongly suggest that relations between morphology, thermoregulation and mate locating behaviour, similar to the ones we showed, exist at the species level as well. Shreeve and Smith studied the endemic Madeiran speckled wood, *Pararge xiphia*, and the European speckled wood, *P. aegeria*, which was introduced to Madeira in 1976 (Higgins 1977), and found that (1) *P. xiphia* was larger and much darker on the dorsal wing surface, with more hair on body and wing than *P. aegeria*; (2) *P. xiphia* flew for longer periods and was active at lower temperatures and in cooler microclimates than *P. aegeria*; and (3) both species engaged in perching and patrolling, but *P. xiphia* engaged more often in patrolling than did *P. aegeria*. Similar results on these species were independently shown by Jones and Lacey (1992).

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