

Koen Berwaerts · Hans Van Dyck

Take-off performance under optimal and suboptimal thermal conditions in the butterfly *Pararge aegeria*

Received: 21 January 2004 / Accepted: 22 June 2004 / Published online: 7 August 2004
© Springer-Verlag 2004

Abstract Realized fitness in a fluctuating environment depends on the capacity of an ectothermic organism to function at different temperatures. Flying heliotherms like butterflies use flight for almost all activities like mate location, foraging and host plant searching and oviposition. Several studies tested the importance of ambient temperature, thermoregulation and butterfly activity. Here, we test the influence of variation in flight morphology in interaction with differences in body temperature on locomotor performance, which has not been thoroughly examined so far. Take-off free flight performance was tested at two different body temperatures in males and females of the speckled wood butterfly *Pararge aegeria*. We found that both males and females accelerated faster at the optimal body temperature compared to the suboptimal one. The multivariate analyses showed significant sex-specific contributions of flight morphology, body temperature treatment and feeding load to explain variation in acceleration performance. Female and male butterflies with a large relative thorax (i.e. flight muscle investment) mass and large, slender wings (i.e. aspect ratio) accelerated fast at optimal temperature. However, high aspect ratio individuals accelerated slowly at suboptimal temperature. Females of low body mass accelerated fast at optimal, but slowly at suboptimal body temperature. In males, there was an interaction effect between body and relative thorax mass: light males with high relative thorax mass had higher performance than males with a low relative thorax mass. In addition, relative distance to the centre of forewing area was positively related to acceleration at both temperatures in males. Males and females with higher feeding loads had lower levels of acceleration. Finally, males that were able to accelerate fast under both temperatures, had a highly significantly heavier relative

thorax, lower body and abdomen mass. More generally, this study shows that the significance of butterfly flight morphology in terms of flight performance is at least partially dependent on body temperature.

Keywords Body shape · Flight · Sexual dimorphism · Thermoregulation · Feeding

Introduction

Any factor that affects body temperature in ectotherms may have a strong impact on individual fitness since many aspects of physiology and behaviour are temperature dependent (e.g. Gilchrist 1996; Forsman 1999). Realized fitness in a fluctuating environment depends on both tolerance and performance or the capacity of an organism to function at various temperatures (Huey and Kingsolver 1989; Gilchrist 1995). Flying heliotherms like butterflies use flight for almost all adult activities including mate location, foraging, host plant searching and oviposition, predator escape and dispersal (Shreeve 1992). Different types of flight may vary with respect to their biomechanical demands and, hence, with the underlying morphological and physiological variation (Dudley 2000). For instance, male butterflies adopting different behavioural strategies and associated flight types to locate mates have been shown to differ in morphological design at both the interspecific (Wickman 1992) and intraspecific level (Van Dyck 2003 and references therein). Several studies have focused on the importance of ambient temperature, thermoregulation and butterfly activity (e.g. Heinrich 1986; Dennis 1993; Watt 2003), including also thermoregulation postures and relationships with morphology when basking (e.g. Kingsolver 1985; Dennis and Shreeve 1989; Schmitz 1994; Berwaerts et al. 2001). We are, however, particularly interested in testing and quantifying the influence of variation in flight morphology in interaction with differences in body temperature on locomotor performance, which has not been comprehensively examined so far.

K. Berwaerts (✉) · H. Van Dyck
Department of Biology, University of Antwerp,
Universiteitsplein 1,
2610 Antwerp, Belgium
e-mail: koen.berwaerts@ua.ac.be
Tel.: +32-3-8202293
Fax: +32-3-8202271

Here, we report on free flight take-off experiments in the laboratory at optimal and suboptimal body temperature using the speckled wood butterfly (*Pararge aegeria* L.) as a study model. Take-off, or accelerating vertical ascent, is recognized to be one of the most demanding forms of aerial locomotion (Dudley 2000). Both *P. aegeria* males and females rely on sunlit patches at the forest floor to thermoregulate behaviourally by dorsal basking (sensu Clench 1966). When the butterfly starts to fly voluntarily after a period of basking, body temperature usually is within a range of 30–34°C (Shreeve 1984; Van Dyck and Matthysen 1998). During flight body temperature typically decreases by convective cooling, which forces the butterfly to bask again (Van Dyck and Matthysen 1998). Hence, the duration of a single flight bout increases with ambient temperature (Shreeve 1984). Most males are found perching (i.e. typical sit-and-wait behaviour) in a territorial, aggressive way as they try to monopolize a sunlit patch. Acceleration capacity and take-off angle can be expected to be functionally significant components of their locomotor performance as they need to approach and inspect each passer-by as quickly as possible and eventually initiate aerial conflicts with conspecific males which also requires high levels of acceleration and manoeuvrability. Females oviposit on isolated grasses that grow on rather shaded, wet sites (Wiklund and Persson 1983). Hence, their locomotor performance repertoire does clearly not restrict them to the warm sunlit patches that are used for basking.

A series of *P. aegeria* males and females were tested repeatedly under two body temperature treatments. From videotaped sequences, take-off acceleration and take-off angle could be measured. As our earlier work has shown that variation in flight morphology contributes significantly to an explanation of flight performance [under tethered flight conditions with constant ambient temperature (Berwaerts et al. 2002)], we took into account variation in flight morphology (size, mass allocation and wing shape measures) for the analyses of take-off performance. When awaiting their turn to be tested, freshly emerged individuals were kept at low ambient temperature (8°C) to exclude activity and to reduce energy use. Although this was necessary for practical reasons to spread the availability of individuals over time, such conditions mimic the effect of poor weather conditions. In case of dull or rainy weather, adult temperate zone butterflies may have to rest in the vegetation for several hours or even days. After such a period, butterflies typically forage or drink to restore their water balance. As such extended feeding may influence flight performance, we took the factor of feeding load into account for our analyses.

We in particular tested the following predictions:

1. *P. aegeria* males and females accelerate faster under optimal thermal conditions than under suboptimal conditions.
2. As males allocate relatively more material to their flight muscles (i.e. larger relative thorax; Berwaerts et

- al. 2002), take-off performance is expected to be higher in males compared to females (Marden 1987).
3. Acceleration capacity during take-off depends on wing shape with higher performances for individuals with slender forewings (i.e. higher aspect ratio; Betts and Wootton 1988) and a more distant position of the centre of the wing area (i.e. centroid; Berwaerts et al. 2002; Dudley 2000). As sexes differ in this respect, we expect sexual differences; take-off angle increases with higher relative thorax mass (Marden 1987).
4. Acceleration capacity is negatively related to the amount of sugar water a butterfly drinks prior to the experiment.

Materials and methods

Study species

The speckled wood butterfly *P. aegeria* (L.) is a temperate satyrine butterfly (Lepidoptera: Nymphalidae: Satyrinae) that mainly occurs in and near different types of woodland throughout Europe. They feed mainly on honeydew, but also to a lesser extent on fluids of trees, rotten berries, and nectar. Eggs are deposited on different host grass species within a relatively narrow thermal window (Shreeve 1986). The dorsal wing surface is light to dark brown with small yellow patches. In the submarginal zone of the dorsal hindwings, a variable number of black eyespots is present (Shreeve 1987; Van Dyck et al. 1997a). *P. aegeria* has a variable life cycle, with different possible developmental pathways and complex reaction norms with strong plasticity to environmental factors like photoperiod, and secondarily temperature (Wiklund et al. 1983; Nylin et al. 1995). The three major developmental pathways can be induced in the laboratory by manipulating photoperiod (Wiklund et al. 1983), and result in different average flight morphology traits (Van Dyck and Wiklund 2002).

Flight performance tests

Flight performance measurements were done with directly developed adult butterflies (cf. Wiklund et al. 1983) from generation F3 of a recently established laboratory breeding stock consisting of a large number (i.e. 77) of families (23°C; light:dark 16:8 h; host plant *Poa trivialis*). The stock originated from 52 wild caught females (Walenbos, Belgium) retaining relatively large levels of genetic variation. During their caterpillar stages, individuals were reared in groups of four on a potted tuft of grass enclosed by fine-mesh netting.

Before the flight performance of an individual was tested, body mass was determined using an electronic microbalance (MT5 Mettler). Butterflies had ad libitum access to 20% sugar solution for 1 h. They were subsequently placed in an incubator (in little, individual envelopes) for 20 min at one of two temperatures: 22 or 32°C. Individuals were placed one by one at the bottom of

an experimental flight cage (length×breadth×height 0.4×0.4×0.8 m) in the laboratory. Two sides consisted of transparent glass and on top there was a halogen lamp (Massive, 74909/21/30, 500 W). The distance between the lamp and the position of a butterfly at the start of each take-off experiment was 0.85 m. Individuals were always positioned at the same spot and faced with their head in the same direction. Individuals were stimulated to fly (i.e. take-off from resting position with closed wings) by means of tapping the abdomen with a mechanical exposure lever (cf. hand-held pencil in Forsman 1999). Take-off was videotaped by means of two lateral and perpendicular positioned Sony DCR-TRV 130E cameras (25 Hz) (cf. Lee et al. 1996; Nudds and Bryant 2000). Average ambient temperature at the release spot in the cage was 31.1 ±0.2°C; ambient temperature in the room was 22.8±0.1°C (measured at 2 m distance from the cage at 1 m from the floor).

Thorax temperature of a subsample of butterflies was measured to validate the heating treatment. Measurement was done by stabbing the central, lateral part of the thorax with a copper–constantan thermocouple in a hypodermic needle microprobe (MT-29/1B, needle diameter 29 ga, time constant=0.025 s, Physitemp Instruments, cf. Van Dyck and Matthysen 1998). The temperature was read within 12 s (11.9±0.9 s). In the 22°C treatment, thorax temperature after 20 min in the incubator was 22.4±0.1°C ($n=18$). In the 32°C treatment, thorax temperature after 20 min in the incubator was 29.6±0.1°C ($n=18$). The latter value is within the range of optimal body temperature as studied in the field and in the laboratory, while the first can be considered suboptimal (Van Dyck and Matthysen 1998).

After the fixed period of heating, an individual was taken from the incubator and placed in the experimental cage, which took on average 13.7±0.7 s ($n=49$). Individuals were tested in an alternate order with a 20-min interval between consecutive trials. All trials of a particular individual (i.e. three trials for each temperature treatment) were performed on one day. Individuals were weighed afterwards on the same day. Feeding load was calculated as the difference between body mass before and after sugar intake. Individuals were killed by freezing (−18°C) and stored for morphological measurements. The age of the individuals in our experiment was 7.0±0.3 days and 6.2±0.2 days for females and males, respectively.

Measurement of flight traits

All video-sequences were screened in order to select only those sequences where only a single stimulation was required to induce take-off. Next, every sequence was digitized (NAC XY-coordinator) until the individual was near the side of the flight cage or went off screen. The two, two-dimensional (2D) flight trajectories were merged into one 3D trajectory. Scaling was done using the midposition of the individual in the flight cage. A comparison with a scale based on the extreme front or back position in the

cage, as determined by filming a static, carefully calibrated object, gave an maximum error of 12%. Next, 3D cumulative distance was calculated (using Pythagoras's rule), filtered (using a zero phase-shift Butterworth filter according to Winter 1990) and acceleration was calculated using a fourth-order central difference (Biewener and Full 1990). Acceleration was extracted from the first six frames (time period of 6×0.04 s) (cf. Crompton et al. 2003). The direction of take-off was quantified as the take-off angle that was calculated between the first and the third frame. The angle between the sides formed by the 3D distance and 2D distance (projected in the horizontal plane), respectively, was therefore used in combination with the cosine rule. Only individuals for whom data of all three trials were available (i.e. 55 and 45% for the suboptimal and optimal temperature test, respectively) were retained in the dataset of this paper. As a consequence, sample sizes for morphology are larger than for flight performance. Maximal acceleration and maximal take-off angle were independently extracted, so they are not necessarily from the same trial. There was no significant relationship between trial number and acceleration or with take-off angle. Hence, no systematic training or exhausting effect could be detected. We calculated repeatabilities using ANOVA according to Lessells and Boag (1987). Repeatabilities of acceleration performance ranged from 32% under suboptimal ($F_{55,112}=2.42$, $P<0.001$) to 43% under optimal thermal conditions ($F_{44,90}=3.29$, $P<0.001$) in females and from 21% under suboptimal ($F_{107,216}=1.78$, $P<0.001$) to 35% under optimal thermal conditions ($F_{87,176}=2.60$, $P<0.001$) in males. As the repeatability of take-off angle was both under the suboptimal and optimal thermal conditions <0.1 ($P>0.05$), we decided not to use take-off angle any further in the analyses.

Measurement of morphological traits

Frozen butterflies were dried to constant mass in an incubator at 60°C for 24 h. Total dry body mass was measured (electronic microbalance MT5 Mettler). Next, the thorax was carefully separated from legs, wings, abdomen and head. Body parts were also weighed separately. Wing length, wing area and position of the centre of wing area (i.e. centroid) were measured with an image-analysing system (OPTIMAS 6.51 software; Optimas 1999) from digital photos (Olympus Camedia C-3030 zoom camera) of dorsal forewing surfaces (Berwaerts et al. 2002). This technique allows reliable, high-resolution measurements. Forewing aspect ratio was calculated as 4×forewing length²/forewing area using untransformed data.

Statistics

Prior to analyses outliers were removed and all data, except feeding load, were log₁₀ transformed to improve normality. Coefficients of variation were calculated as the

ratio of SD on the mean. Means are given \pm SE, and analyses were split by sex unless stated otherwise. Relative thorax mass and relative forewing centroid were calculated as residuals on total body mass. Relative feeding load was calculated as residual on total body mass, relative thorax mass and forewing centroid and forewing aspect ratio. We used two different sets of performance traits: the first one included only the maximal values of acceleration, the second included the values of all trials and was therefore an overall measure. Analyses were mainly multivariate regression models. In both cases, individual was included as a random factor since we have repeated measurements (trials) for each temperature and, for the overall measure, for each individual. Jar—referring to the jar in which an individual grew up with three other caterpillars—was also included as a controlling random factor for a common larval environment (Littell et al. 1996). Only uncorrelated independent variables were included in the same model (James and McCulloch 1990; Neter et al. 1996). As a consequence, multiple regression analyses were done for two different sets of independent variables: (1) temperature treatment, body mass, relative thorax mass, forewing aspect ratio and relative feeding load, and (2) temperature treatment, body mass, relative centroid and relative feeding load. Final model selection was done by stepwise backward elimination of factors with the highest non-significant *P*-values starting from a full mixed model (including all two-way interaction terms). Factors were not eliminated as long as they were incorporated in interaction terms. We checked for the normality of the residuals of each regression model. All analyses were performed using SAS 8.01 software.

Results

Table 1 summarizes sexual differences in flight performance and flight morphology. At suboptimal body temperature, maximal acceleration was faster in males than in females. Considering overall instead of maximal

acceleration, there was a similar, but non-significant trend (Table 1). At optimal body temperature, however, acceleration performance did not differ between the sexes. Females had on average larger total dry body mass than males, which is mainly due to their heavier abdomen mass. Females had larger, but not longer, forewings than males, so aspect ratio is higher in males. Forewing centroid was more distally located in females (Table 1). When adjusting for differences in overall size (i.e. body mass), males allocated relatively more mass to their thorax (32 vs. 23%) and their forewing centroid was relatively more distally located from the wing base than in females (calculated from data in Table 1). After free access to sugar water before take-off measurements, females drunk more than males; feeding load was on average 42 and 31% of fresh body mass in females and males, respectively.

Both males and females accelerated faster in the optimal body temperature treatment compared to the suboptimal one, and this was the case for both overall and maximal acceleration (Table 2). Overall acceleration increased from suboptimal to optimal temperature, on average 13.5% and 15.5% in males and females, respectively. For maximal acceleration, the increase was on average 16 and 19.5%, respectively. Acceleration performance at suboptimal temperature was strongly positively related to acceleration performance at optimal temperature in both males and females ($F_{1,78}=16.60$; $P=0.0001$; no significant sex effect).

The multivariate analyses showed significant sex-specific contributions of flight morphology, body temperature treatment and feeding load to explain variation in acceleration performance. At both temperatures, males and females with high feeding loads had lower levels of overall and maximal acceleration (Table 2). Maximal acceleration in males belonging to the top quartile for feeding load was 5.22 ± 0.14 m/s² and 6.33 ± 0.79 m/s² under the suboptimal and optimal body temperature treatment, respectively, while it was, respectively, 6.15 ± 0.18 m/s² and 7.29 ± 0.41 m/s² for males of the lowest quartile. In females, these values were 4.63 ± 0.18 m/s² and

Table 1 Descriptive statistics of morphological and flight performance traits. Means are calculated without effect of jar included. Statistical tests for sex differences on log10-transformed data with random effects of jar and individual (only for overall traits) included. *CV* Coefficient of variation, *acc* overall acceleration capacity, *macc* maximal acceleration capacity, *subopt* suboptimal temperature, *opt* optimal temperature

	Females				Males				Sex
	Mean	SE	<i>n</i>	CV (%)	Mean	SE	<i>n</i>	CV (%)	
Body mass ^a (mg)	36.12	0.75	76	18.12	30.04	0.52	140	20.32	***
Body mass ^b (mg)	51.31	1.09	75	18.43	39.09	0.59	140	17.84	***
Body mass ^c (mg)	17.90	0.38	69	17.63	13.23	0.20	133	17.68	***
Feeding load (mg)	15.11	0.82	75	47.07	9.31	0.66	140	57.94	***
Thorax mass (mg)	4.16	0.09	69	17.34	4.18	0.07	133	18.75	–
Abdomen mass (mg)	9.95	0.29	74	25.30	5.40	0.14	137	30.97	***
Forewing area (cm ²)	1.44	0.02	74	9.65	1.36	0.01	138	12.13	***
Forewing length (cm)	1.90	0.01	74	4.81	1.88	0.01	138	6.33	–
Aspect ratio (–)	10.06	0.04	75	3.09	10.42	0.03	138	3.26	***
Centroid (cm)	1.01	0.01	74	5.27	0.98	0.01	138	6.54	**
Acc–subopt (m/s ²)	4.53	0.10	56	16.52	4.73	0.08	108	17.58	<i>P</i> =0.079
Macc–subopt (m/s ²)	5.19	0.13	56	18.74	5.60	0.10	108	18.56	**
Acc–opt (m/s ²)	5.23	0.19	45	24.37	5.36	0.14	88	24.50	–
Macc–opt (m/s ²)	6.20	0.20	45	21.64	6.51	0.17	88	24.50	–

P*<0.01; *P*<0.001

^aBefore experiment

^bAfter experiment

^cTotal dry body mass

Table 2 Multivariate relationships between acceleration performance and flight morphology, temperature and feeding load for overall acceleration performance and maximal acceleration performance. Jar and individual were used as random effects. For the class trait temperature, regression analysis renders only estimates for one class. In (A) body mass, relative thorax mass, aspect ratio, relative feeding load and temperature were used as independent traits in the model, whereas in (B) body mass, relative centroid, relative feeding load and temperature were used. Only final models are shown

	<i>n</i>	<i>df</i>	<i>F</i>	<i>P</i>	Estimate	SE
	<i>(df)</i>					
Overall acceleration performance						
Females						
A						
Intercept				NS	-0.40	0.95
Feeding load	1	49.6	11.97	**	-0.01	0.002
Relative thorax mass	1	41.2	6.36	*	0.44	0.18
Aspect ratio	1	59.3	0.00	NS	1.10	0.94
Temperature	1	260	4.21	*	2.13	1.04
Aspect ratio×temperature	1	260	4.47	*	-2.19	1.03
B						
Intercept				***	0.71	0.01
Feeding load	1	49.7	8.00	**	-0.005	0.002
Temperature	1	254	22.43	***	-0.07	0.01
Males						
A						
Intercept				NS	-0.09	0.58
Feeding load	1	132	13.75	***	-0.006	0.002
Relative thorax mass	1	139	62.89	***	0.79	0.10
Aspect ratio	1	122	0.15	NS	0.80	0.57
Temperature	1	535	2.83	NS	1.23	0.73
Aspect ratio×temperature	1	534	3.12	0.08	-1.28	0.72
B						
Intercept				***	0.72	0.01
Feeding load	1	122	12.18	***	-0.006	0.002
Centroid	1	128	51.91	***	1.97	0.27
Temperature	1	540	34.68	***	-0.06	0.01
Maximal acceleration performance						
Females						
A						
Intercept				NS	-1.02	0.93
Feeding load	1	47	14.58	***	-0.006	0.002
Relative thorax mass	1	41.5	6.99	*	0.40	0.15
Temperature	1	39	3.70	0.06	2.01	1.05
Total dry body mass	1	60.1	0.00	NS	-0.17	0.15
Aspect ratio	1	59.4	1.47	NS	2.02	0.86
Total dry body mass×tem-	1	41.7	3.64	0.06	0.32	0.17
perature						
Aspect ratio×temperature	1	37.7	6.56	*	-2.49	0.97
B						
Intercept				***	1.09	0.19
Feeding load	1	46.4	10.26	**	-0.005	0.002
Temperature	1	43.2	8.54	**	-0.61	0.21
Total dry body mass	1	65.5	0.07	NS	-0.24	0.15
Total dry body mass×tem-	1	43.5	6.32	*	0.42	0.17
perature						

Table 2 (continued)

	<i>n</i>	<i>df</i>	<i>F</i>	<i>P</i>	Estimate	SE
	<i>(df)</i>					
Males						
A						
Intercept				***	0.88	0.09
Feeding load	1	117	24.84	***	-0.008	0.002
Total dry body mass	1	102	0.55	NS	-0.06	0.08
Relative thorax mass	1	142	8.72	**	3.78	1.28
Temperature	1	119	49.75	***	-0.08	0.01
Total dry body mass×Re-	1	144	5.57	*	-2.70	1.14
lative thorax mass						
B						
Intercept				***	0.81	0.009
Feeding load	1	143	20.50	***	-0.008	0.002
Centroid	1	143	43.90	***	1.87	0.28
Temperature	1	120	47.92	***	-0.08	0.01

P*<0.05; *P*<0.01; ****P*<0.001

5.90±0.27 m/s² for the top quartile for feeding load and 5.69±0.28 m/s² and 7.05±0.43 m/s² for the lowest quartile.

Female butterflies with a large relative thorax mass accelerated faster—both overall and maximal acceleration—than females with a small relative thorax (Table 2). For both overall and maximal acceleration, there was also an additional interaction effect of body temperature treatment and forewing aspect ratio: females with large, slender wings (i.e. high aspect ratio) accelerated fast at optimal, but slowly at suboptimal body temperature (Figs. 1, 2). For maximal acceleration, there was an interaction effect of dry body mass with temperature treatment: females of low body mass accelerated fast at optimal, but slowly at suboptimal body temperature (Fig. 3).

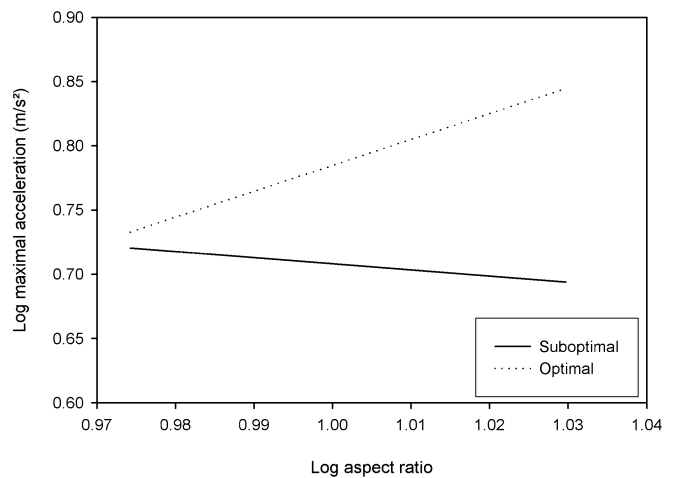


Fig. 1 Regression of aspect ratio in a function of maximal acceleration capacity in females for suboptimal and optimal temperature. The regression lines only are shown

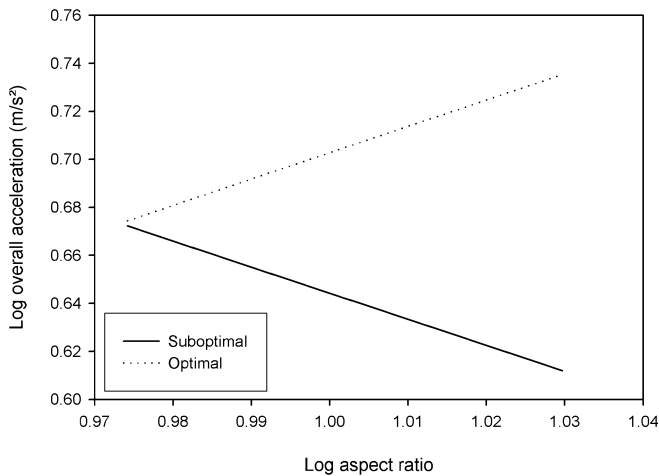


Fig. 2 Regression of aspect ratio in a function of overall acceleration capacity in females for suboptimal and optimal temperature. The regression lines only are shown

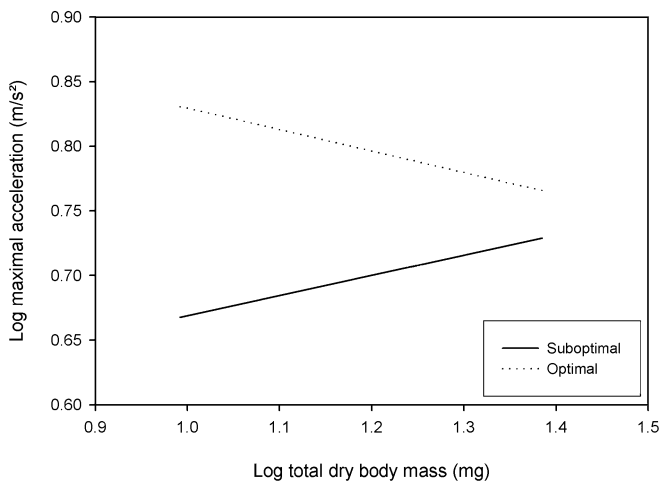


Fig. 3 Regression of total dry body mass in a function of maximal acceleration capacity in females for suboptimal and optimal temperature. The regression lines only are shown

In male butterflies, both overall and maximal acceleration increased with larger relative thorax mass and with a more distant forewing centroid from the wing base (Table 2). However, at the level of maximal acceleration, there was an interaction effect of relative thorax mass with body mass: light males but with high relative thorax mass had a higher performance than males with a low relative thorax mass, while heavy males had average performances regardless of their relative thorax mass (Fig. 4). For males there was also a similar forewing aspect ratio \times body temperature treatment interaction effect as was the case in females: individuals with large, slender wings accelerated fast at optimal, but slow at suboptimal body temperature (Fig. 5).

In males, we noticed a highly interesting subsample of individuals that were able to accelerate fast under both the optimal and suboptimal temperature treatment. From the flight morphology point of view, these males were not a random sample as they had a highly significantly heavier

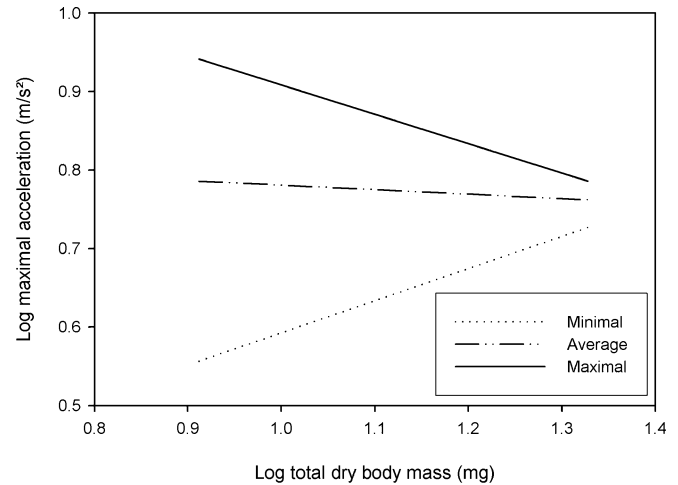


Fig. 4 Regression of total dry body mass in a function of maximal acceleration capacity in males for minimal, average and maximal relative thorax mass. The regression lines only are shown

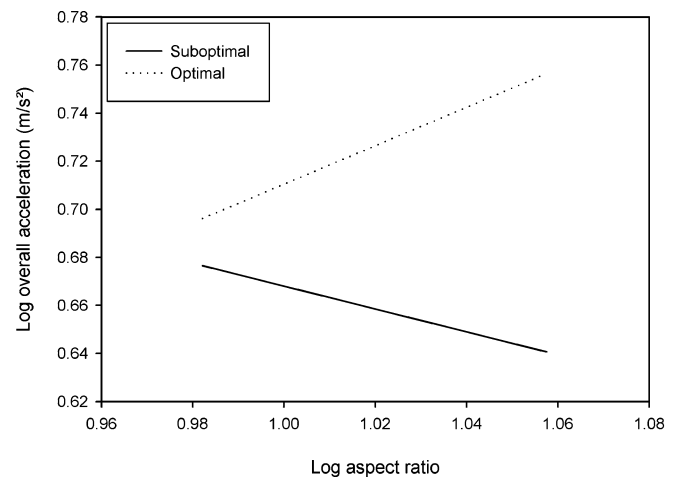


Fig. 5 Regression of aspect ratio in a function of overall acceleration capacity in males for suboptimal and optimal temperature. The regression lines only are shown

relative thorax ($t=7.47$, $df=25.1$, $P<0.0001$), a low total body mass ($t=-2.06$, $df=111$, $P=0.042$) and thus a low abdomen mass ($t=-2.68$, $df=108$, $P=0.0085$) ($n=5$).

Discussion

Accelerating vertical ascent represents one of the most energetically demanding forms of aerial locomotion (Dudley 2000). Males and females of the butterfly *P. aegeria* accelerated faster during take-off in the optimal body temperature treatment (32°C) compared to the suboptimal temperature treatment (22°C), as predicted. Maximal acceleration was 16–20% lower at suboptimal body temperature. Mechanical power output of flight muscles increases with body temperature, and is maximal near species-specific muscle temperatures characteristic of free flight (Stevenson and Josephson 1990; Lehmann 1999; Dudley 2000). For several temperate zone butter-

flies, these temperatures are often $>30^{\circ}\text{C}$ [34–37°C (Douwes 1976; Rutowski et al. 1994; Dreisig 1995)]. In *Colias* butterflies (Tsuiji et al. 1986) and honeybees (Esch 1976), flight speed and wing beat frequency increased with increasing thoracic temperature. In our *P. aegeria* experiment, in the optimal body temperature treatment the temperature may not be the optimal one *sensu stricto* as the body temperatures we checked were “only” close to 30°C , and *P. aegeria* has been observed to fly at $30\text{--}34^{\circ}\text{C}$ (Shreeve 1984; Van Dyck and Matthysen 1998). Nevertheless, the gain in terms of acceleration performance with body temperature clearly follows from our experiment.

Fast acceleration during take-off is a typical behavioural trait of a territorial perching male (Wickman 1992). Chasing and aerial spiralling flights during conflicts with conspecific males also require high levels of acceleration and manoeuvrability (Van Dyck 2003). Hence, our observation of increased acceleration performance at a higher body temperature—well above average ambient air temperature in temperate zone regions—can be related to the thermal value of a sunlit patch as a territory for *P. aegeria*. Knowing that occupied individual sunlit patches vary considerably in thermal properties (H. Van Dyck, K. Berwaerts, T. Merckx, K. Lauwers, unpublished data), one may predict that the careful selection of a thermally appropriate sunlit patch may contribute significantly to territorial performance of the resident male. However, the pattern is more complex, as the thermal value of a patch should be evaluated relative to the morphology of the territory holder.

Additional to the effect of body temperature, we found flight morphology to contribute significantly to an explanation of variation in take-off acceleration performance. Here, there is a clear sex-specific component. At suboptimal body temperature, males were able to accelerate faster than females. This was particularly true when considering maximal acceleration values and is in agreement with our prediction. Sexual differences in flight design, including size, allocation to thorax (i.e. to flight muscles) and wing shape, correspond to a less powerful design for take-off (or generally acceleration and likely manoeuvrability) in females compared to males. This is also in line with our earlier experiments comparing relative flight force among *P. aegeria* males and females under tethered flight conditions (Berwaerts et al. 2002). The heaviest females were only able to increase their take-off acceleration by 7% from suboptimal to optimal body temperature, whereas the lightest females increased their acceleration considerably more (Fig. 3). In females, acceleration and particular levels of manoeuvrability are also important [like in hovering types of flight in order to alight on specific parts of host grasses (Shreeve 1986)], but we argue that selection on acceleration capacity and manoeuvrability is much more pronounced among competing males. Females typically select rather wet, shaded parts of the forest to lay eggs singly on isolated host grasses (Wiklund and Persson 1983) and will, hence, experience convective cooling and thus lower body temperatures. However, oviposition flights are altered by

extensive bouts of basking and resting (Shreeve 1986). For territorial perching males, it can be essential to keep on chasing or fighting at suboptimal body temperatures. When a territorial male alights after a period of convective cooling during a chase or aerial fight in order to increase thoracic temperature again by basking (Van Dyck and Matthysen 1998), he may frequently need to take-off again before reaching an optimal temperature. Observations by Van Dyck et al. (1997a) showed that a territorial male took-off on average ca. 30 times h^{-1} to engage in inter- and intraspecific interactions. Males of the skipper butterfly *Thymelicus lineola* are better able to fly at a lower thoracic temperature than females who are larger (Pivnick and McNeil 1986). In the same vein but at the interspecific level, larger species of bees and bumblebees require higher minimum thoracic temperatures to initiate flight (Bishop and Armbruster 1999). Males and females of *P. aegeria* are the largest in the late-spring brood when intensive solar radiation at the sunlit patches is highest (Van Dyck and Wiklund 2002), which is an interesting correlation in this respect, but it clearly needs further testing.

As predicted (cf. Marden 1987), relative thorax mass, and hence allocation to flight muscles, was strongly correlated with acceleration performance in both males and females. So, our free-flight take-off experiment contributes to the evidence from tethered flight experiments (Berwaerts et al. 2002) that males with larger thoraxes relative to their total body mass accelerate more powerful. This further supports the correlative pattern of differences in relative thorax mass between territorial perching and non-territorial patrolling males (Van Dyck et al. 1997b; Van Dyck 2003). The effect of relative thorax mass on acceleration performance was additive to the variation explained by body temperature. Interestingly, we found that males that were able to perform at the top level under both body temperature treatments had a larger relative thorax mass than the other males in our sample. They also had a low total body mass and small abdomen. This suggests that a particular flight morphology—most likely underpinned by associated physiological differences—may buffer a *P. aegeria* male's flight performance against variation in temperature. This observation is in line with recent evidence from manipulative experiments (Kemp and Wiklund 2004) showing that contests among *P. aegeria* males are not settled due to resource-correlated asymmetries in thoracic temperature, as was concluded from experiments by Stutt and Willmer (1998). To what extent flight morphology contributes to intrinsic territorial contest capacity remains to be evaluated (Kemp and Wiklund 2001). In females, there is another aspect of flight performance that needs to be dissected. During a female's lifetime, her egg load and thus abdomen mass will decrease considerably as eggs are laid. Hence, relative thorax size will increase (e.g. Fischer and Kutsch 2000), which may have strong impact on flight performance, including take-off and manoeuvrability. However, through histolysis females may be able to re-allocate material from the flight muscles to egg production as they age (Karlsson 1994) which means that predictions are not straightfor-

ward (see Berwaerts et al. 2002). In several other insects, egg load has been found to constrain flight performance (Berrigan 1991; Isaacs and Byrne 1998) and in birds, egg load can cause a detrimental effect on aerial manoeuvrability (Witter et al. 1994; Lee et al. 1996).

Wing shape was also related to take-off performance in both males and in females; aspect ratio is a measure of the narrowness of the wing (Dudley 2000) and affected both sexes similarly. Additionally, there was an effect of relative centroid (i.e. the spanwise distribution of wing area) in males only. Moreover, in males the effect of wing centroid was much stronger than of aspect ratio. Both aspect ratio and centroid have been found to attribute to an overall butterfly wing shape trait that functionally relates to flight kinematics (Srygley 1999). The individuals we used were fairly intact, but under natural conditions, butterfly wings may experience high levels of wing wear. This may lead to a reduction in wing area which in turn affects aspect ratio and the position of the wing centroid. A couple of studies in other insects did not find clear effects of wing wear on flight performance measures (e.g. Orthoptera: Fischer and Kutsch 2000; bumblebees: Hedenström et al. 2001). Kingsolver (1999) was able to manipulate the kinematics of *Colias* butterflies (e.g. wing beat frequency) by clipping parts of their wings, but survival rates in the field did not differ among manipulated and intact individuals.

In agreement with the fourth prediction, feeding load had always a strong, negative effect on take-off performance in both males and females. Since most of the digestive system is located in the abdomen (Srygley and Chai 1990; Marden and Chai 1991), feeding load will particularly increase the mass of the abdomen. As a consequence, the centre of mass shifts away from the thorax which in turn causes an increase in the radial inertial moment of the body and hence a decrease in acceleration and manoeuvrability (Srygley and Chai 1990; Srygley 1994; Norberg 1995; Marden 2000). This effect is likely to be larger in females as they feed more, both in absolute and relative terms. Moreover, under natural conditions the maturation of the eggs in the abdomen will further contribute to the effect. The amount of sugar water the butterflies took in our experiments may not be comparable with an average feeding turn under “normal” natural conditions, but rather with conditions after a period of evaporation without compensation when butterflies have been inactive for a couple of days. Such conditions are not exceptional in temperate zone regions, like in Belgium. However, to the best of our knowledge little is known on variation in butterfly feeding quantities. A significant body mass increase related to foraging has been noticed in several other insects: nectar in bumblebees (Heinrich 1975; Wolf and Schmid-Hempel 1989), prey-carrying in beewolves (Strohm and Linsenmair 1997) and in cicada killers *Sphecius speciosus* (Coelho 1997), and nuptial gifts in dance flies (Marden 1989). In birds like robins (Lind et al. 1999) and blackcaps (Kullberg et al. 1996), the ability to take-off fast was constrained by high migratory fuel load. In contrast to females, *P. aegeria*

males have only rarely been seen foraging when they hold their territory (H. Van Dyck, personal observation). Either they forage on honeydew in the canopy before ascending to sunlit patches at the forest floor, or they effectively forage much less than females do.

Acceleration and flight-angle can describe 3D-flight patterns during take-off. The acceleration measure in our experiment was repeatable, while take-off angle was not. Therefore the latter measure was not used in the analyses. Calculated repeatabilities of animal flight performance are apparently relatively rare in the literature, particularly in insects. Bonser and Rayner (1996) report a 60% repeatability of ground reaction forces in starlings' take-off. According to Nudds and Bryant (2000), free flight acceleration performance had a repeatability of 59% in zebra finches. We have also measured flight performance in *P. aegeria* using a tethered flight set-up (Berwaerts et al. 2002). Then, individual repeatability (when testing each individual 10 times) was considerably higher in both females (77%, $n=59$, $F_{60,404}=29.83$, $P<0.0001$) and males (89%, $n=69$, $F_{67,477}=73.77$, $P<0.0001$) compared to this take-off experiment. The type of stimulus to induce flight was, however, also different among both studies and the choice of a reliable locomotor stimulus is not always straightforward. In our tethered flight experiments we used tarsal reflex, while here we used abdomen tapping. In the bird studies, attacks by artificial predators were used as visual cues to evoke a repeatable flight performance (e.g. Kullberg et al. 1996, 1998; Lind et al. 1999). Abdomen tapping as a stimulus may have contributed to the absence of a repeatable take-off angle. Tapping when sitting at rest is probably more similar to a predator attack. Hence, a random take-off angle would make sense in adaptive terms of predator escape. Use of a visual cue like an artificial predator could be worth testing in butterflies as well. However, we would predict that it is not necessarily a suitable alternative in *P. aegeria*. The main anti-predation mechanism of this butterfly is not flying away from a predator—territorial males may even approach passing birds in an aggressive way—but rather crypsis when sitting. We argue that the most sensitive period for predation is when *P. aegeria* rests in the canopy overnight, but also during days with poor weather. Additionally, stimulus reaction and tracking and interception abilities could be sex-specific (Dudley 2000). In *P. aegeria*, sexes considerably differ in behaviour and probably also in visual cues. This is supported by the fact that eyes are significantly larger in males than in females (Rutowski 2000).

Acknowledgements We thank two anonymous referees for valuable comments on an earlier version of the manuscript, Peter Aerts, Kristiaan D'Août, Anthony Herrel and Tom Van Dooren for helpful discussions, Willem Talloen for writing a macro to measure wing traits using Optimas, Kristien Berwaerts, Koen Borghgraef, Joeri Cortens, Kristin De Ceuster, Maarten Jacobs, Jan Scholliers and Els Vints for technical assistance and Els Prinsen and Reinhart Ceulemans for the use of their climate rooms. K. Berwaerts is supported by a PhD grant from the Flemish Institute for the Promotion of Scientific and Technological Research (IWT). H. Van Dyck is a postdoctoral fellow with the Fund for Scientific Research in Flanders-Belgium (FWO). This work is supported by a GOA project of the University of Antwerp (15R/3942).

References

- Berrigan D (1991) Lift production in the flesh fly, *Neobellera*–*Sarcophaga* *bullata* Parker. *Funct Ecol* 5:448–456
- 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.). *J Zool* 255:261–267
- Berwaerts K, Van Dyck H, Aerts P (2002) Does flight morphology relate to flight performance? An experimental test with the butterfly *Pararge aegeria*. *Funct Ecol* 16:484–491
- Betts CR, Wootton RJ (1988) Wing shape and flight behaviour in butterflies (Lepidoptera: Papilionoidea and Hesperioidea): a preliminary analysis. *J Exp Biol* 138:271–288
- Biewener AA, Full RJ (1990) Force platform and kinematic analysis. In: Biewener AA (ed) *Biomechanics—structures and systems: a practical approach*. Oxford University Press, Oxford, pp 45–73
- Bishop JA, Armbruster WS (1999) Thermoregulatory abilities of Alaskan bees: effects of size, phylogeny and ecology. *Funct Ecol* 13:711–724
- Bonsler RHC, Rayner JMV (1996) Measuring leg thrust forces in the common starling. *J Exp Biol* 199:435–439
- Clench HK (1966) Behavioral thermoregulation in butterflies. *Ecology* 47:1021–1034
- Coelho JR (1997) Sexual size dimorphism and flight behavior in cicada killers, *Sphecius speciosus*. *Oikos* 79:371–375
- Crompton B, Thomason JC, McLachlan A (2003) Mating in a viscous universe: the race is to the agile, not to the swift. *Proc R Soc Lond Ser B* 270:1991–1995
- Dennis RLH (1993) *Butterflies and climate change*. Manchester University Press, Manchester
- Dennis RLH, Shreeve TG (1989) Butterfly wing morphology variation in the British Isles: the influence of climate, behavioural posture and the hostplant-habitat. *Biol J Linn Soc* 38:323–348
- Douwes P (1976) Activity in *Heodes virgaureae* (Lep., Lycaenidae) in relation to air temperature, solar radiation, and time of day. *Oecologia* 22:287–298
- Dreisig H (1995) Thermoregulation and flight activity in territorial male graylings, *Hipparchia semele* (Satyridae), and large skippers, *Ochlodes venata* (Hesperiidae). *Oecologia* 101:169–176
- Dudley R (2000) *The biomechanics of insect flight: form, function, evolution*. Princeton University Press, Princeton, N.J.
- Esch H (1976) Body temperature and flight performance of honey bees in a servo-mechanically controlled wind tunnel. *J Comp Physiol* 190:265–277
- Fischer H, Kutsch W (2000) Relationships between body mass, motor output and flight variables during free flight of juvenile and mature adult locations, *Schistocerca gregaria*. *J Exp Biol* 203:2723–2735
- Forsman A (1999) Temperature influence on escape behaviour in two species of pygmy grasshoppers. *Ecoscience* 6:35–40
- Gilchrist GW (1995) Specialists and generalists in changing environments: I. Fitness landscapes of thermal sensitivity. *Am Nat* 146:252–270
- Gilchrist GW (1996) A quantitative genetic analysis of thermal sensitivity in the locomotor performance curve of *Aphidius ervi*. *Evolution* 50:1560–1572
- Hedenström A, Ellington CP, Wolf T (2001) Wing wear, aerodynamics and flight energetics in bumblebees (*Bombus terrestris*): an experimental study. *Funct Ecol* 15:417–422
- Heinrich B (1975) Thermoregulation in bumblebees. II. Energetics of warm-up and free flight. *J Comp Physiol* 166:155–166
- Heinrich B (1986) Thermoregulation and flight activity of a satyrine, *Coenonympha inornata* (Lepidoptera: Satyridae). *Ecology* 67:593–597
- Huey RB, Kingsolver JG (1989) Evolution of thermal sensitivity of ectotherm performance. *Trends Ecol Evol* 4:131–135
- Isaacs R, Byrne DN (1998) Aerial distribution, flight behaviour and eggload: their inter-relationship during dispersal by the sweetpotato whitefly. *J Anim Ecol* 67:741–750
- James FC, McCulloch CE (1990) Multivariate analysis in ecology and systematics: panacea or Pandora's box? *Annu Rev Ecol Syst* 21:129–166
- Karlsson B (1994) Feeding habits and change of body composition with age in three nymphalid butterfly species. *Oikos* 69:224–230
- Kemp DJ, Wiklund C (2001) Fighting without weaponry: a review of male-male contest competition in butterflies. *Behav Ecol Sociobiol* 49:429–442
- Kemp DJ, Wiklund C (2004) Residency effects in animal contests. *Proc R Soc Lond Ser B* (in press)
- Kingsolver JG (1985) Thermoregulatory significance of wing melanization in *Pieris* butterflies (Lepidoptera: Pieridae): physics, posture, and pattern. *Oecologia* 66:546–553
- Kingsolver JG (1999) Experimental analyses of wing size, flight, and survival in the western white butterfly. *Evolution* 53:1479–1490
- Kullberg C, Fransson T, Jakobsson S (1996) Impaired predator evasion in fat blackcaps (*Sylvia atricapilla*). *Proc R Soc Lond Ser B* 263:1671–1675
- Kullberg C, Jakobsson S, Fransson T (1998) Predator-induced take-off strategy in great tits (*Parus major*). *Proc R Soc Lond Ser B* 265:1659–1664
- Lee SJ, Witter MS, Cuthill IC, Goldsmith AR (1996) Reduction in escape performance as a cost of reproduction in gravid starlings, *Sturnus vulgaris*. *Proc R Soc Lond Ser B* 263:619–624
- Lehmann F-O (1999) Ambient temperature affects free-flight performance in the fruit fly *Drosophila melanogaster*. *J Comp Physiol B* 169:165–171
- Lessells CM, Boag PT (1987) Unrepeatable repeatabilities: a common mistake. *Auk* 104:116–121
- Lind J, Fransson T, Jakobsson S, Kullberg C (1999) Reduced take-off ability in robins (*Erithacus rubecula*) due to migratory fuel load. *Behav Ecol Sociobiol* 46:65–70
- Littell RC, Milliken GA, Stroup WW, Wolfinger RD (1996) SAS system for mixed models. SAS Institute, Cary, N.C.
- Marden JH (1987) Maximum lift production during takeoff in flying animals. *J Exp Biol* 130:235–258
- Marden JH (1989) Effects of load-lifting constraints on the mating system of a dance fly. *Ecology* 70:496–502
- Marden JH (2000) Variability in the size, composition, and function of insect flight muscles. *Annu Rev Physiol* 62:157–178
- Marden JH, Chai P (1991) Aerial predation and butterfly design: how palatability, mimicry, and the need for evasive flight constrain mass allocation. *Am Nat* 138:15–36
- Neter J, Kutner MH, Nachtsheim C, Wasserman W (1996) *Applied linear statistical models*. Irwin, Chicago, Ill.
- Norberg UM (1995) How a long tail and changes in mass and wing shape affect the cost for flight in animals. *Funct Ecol* 9:48–54
- Nudds RL, Bryant DM (2000) The energetic cost of short flights in birds. *J Exp Biol* 203:1561–1572

- Nylin S, Wickman P-O, Wiklund C (1995) Life-cycle regulation and life history plasticity in the speckled wood butterfly: Are reaction norms predictable? *Biol J Linn Soc* 55:143–157
- Optimas (1999) *Optimas 6.51*. User's guide and technical reference, 9th edn. Optimas, Washington, D.C.
- Pivnick KA, McNeil JN (1986) Sexual differences in the thermoregulation of *Thymelicus lineola* adults (Lepidoptera: Hesperidae). *Ecology* 67:1024–1035
- Rutowski RL (2000) Variation of eye size in butterflies: inter- and intraspecific patterns. *J Zool* 252:187–195
- Rutowski RL, Demlong MJ, Leffingwell T (1994) Behavioural thermoregulation at mate encounter sites by male butterflies (*Asterocampa leilia*, Nymphalidae). *Anim Behav* 48:833–841
- Schmitz H (1994) Thermal characterization of butterfly wings: I. Absorption in relation to different color, surface structure and basking type. *J Therm Biol* 19:403–412
- Shreeve TG (1984) Habitat selection, mate location, and microclimatic constraints on the activity of the speckled wood butterfly, *Pararge aegeria*. *Oikos* 42:371–377
- Shreeve TG (1986) Egg-laying by the speckled wood butterfly (*Pararge aegeria*): the role of female behaviour, host plant abundance and temperature. *Ecol Entomol* 11:229–236
- Shreeve TG (1987) The mate-location behaviour of the male speckled wood butterfly *Pararge aegeria*, and the effect of phenotypic differences in hind-wing spotting. *Anim Behav* 35:682–690
- Shreeve TG (1992) Adult behaviour. In: Dennis RLH (ed) *The ecology of butterflies in Britain*. Oxford University Press, Oxford, pp 22–45
- Srygley RB (1994) Locomotor mimicry in butterflies? The associations of positions of centres of mass among groups of mimetic, unprofitable prey. *Phil Trans R Soc Lond B* 343:145–155
- Srygley RB (1999) Locomotor mimicry in *Heliconius* butterflies: contrast analyses of flight morphology and kinematics. *Phil Trans R Soc Lond B* 354:203–214
- Srygley RB, Chai P (1990) Flight morphology of Neotropical butterflies: palatability and distribution of mass to the thorax and abdomen. *Oecologia* 84:491–499
- Stevenson RD, Josephson RK (1990) Effects of operating frequency and temperature on mechanical power output from moth flight muscle. *J Exp Biol* 149:61–78
- Strohm E, Linsenmair KE (1997) Female size affects provisioning and sex allocation in a digger wasp. *Anim Behav* 54:23–34
- Stutt AD, Willmer P (1998) Territorial defense in speckled wood butterflies: do the hottest males always win? *Anim Behav* 55:1341–1347
- Tsuji JS, Kingsolver JG, Watt WB (1986) Thermal physiological ecology of *Colias* butterflies in flight. *Oecologia* 69:161–170
- Van Dyck H (2003) Mate-location, a matter of design? Adaptive morphological variation in the speckled wood butterfly. In: Boggs CL, Watt WB, Ehrlich PR (eds) *Butterflies: evolution and ecology taking flight*. University of Chicago Press, Chicago, Ill., pp 353–366
- Van Dyck H, Matthysen E (1998) Thermoregulatory differences between phenotypes of 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. *J Evol Biol* 15:216–225
- Van Dyck H, Matthysen E, Dhondt AA (1997a) The effect of wing colour on male behavioural strategies in the speckled wood butterfly. *Anim Behav* 53:39–51
- Van Dyck H, Matthysen E, Dhondt AA (1997b) Mate-locating strategies are related to relative body length and wing colour in speckled wood butterfly *Pararge aegeria*. *Ecol Entomol* 22:116–120
- Watt W (2003) Mechanistic studies of butterfly adaptations. In: Boggs CL, Watt WB, Ehrlich PR (eds) *Butterflies: evolution and ecology taking flight*. University of Chicago Press, Chicago, Ill., pp 319–352
- Wickman P-O (1992) Sexual selection and butterfly design—a comparative study. *Evolution* 46:1525–1536
- Wiklund C, Persson A (1983) Fecundity, and the relation of egg weight variation to offspring fitness in the speckled wood butterfly *Pararge aegeria*, or why don't butterfly females lay more eggs? *Oikos* 40:53–63
- Wiklund C, Persson A, Wickman P-O (1983) Larval aestivation and direct development as alternative strategies in the speckled wood butterfly, *Pararge aegeria*, in Sweden. *Ecol Entomol* 8:233–238
- Winter DA (1990) *Biomechanics and motor control of human movement*. Wiley, Waterloo
- Witter MS, Cuthill IC, Bonser RHC (1994) Experimental investigations of mass-dependent predation risk in the European starling, *Sturnus vulgaris*. *Anim Behav* 48:201–222
- Wolf TJ, Schmid-Hempel P (1989) Extra loads and foraging life span in honeybee workers. *J Anim Ecol* 58:943–954