Impaired escape flight ability in butterflies due to low flight muscle ratio prior to hibernation

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SUMMARY

For an animal to escape an attacking predator, speed and manoeuvrability are likely to be crucial factors. Previous studies on reptiles and birds have revealed that gaining weight, due to for instance egg load or lipid accumulation, leads to impaired escape ability and possibly increases the risk of being caught by predators. Here we tested whether the flight performance of an insect, the small tortoiseshell butterfly (*Aglais urticae*), is affected by variations in body mass due to feeding by comparing flight performance parameters between individuals that (i) took flight spontaneously or after being subjected to a simulated predator attack and (ii) varied in flight muscle ratio (FMR: thorax mass/total body mass). The results show that butterflies that were subjected to a simulated predator attack flew at higher velocities and straighter than butterflies that were allowed to take off spontaneously. Furthermore, our study suggests, for the first time, that butterflies may experience impaired escape ability after feeding, which directly leads to a decrease in FMR; a reduction from 0.5 to 0.3 reduced escape flight velocity by about 37% at 0.5 m from the start. Finally, this study shows that FMR affects take-off angle and sinuosity, with steeper angles and more turning in butterflies with low FMR.

Key words: predation risk, insect flight, weight, *Aglais urticae*.

INTRODUCTION

The ability of an animal to escape predation strongly determines its future fitness. However, many otherwise beneficial physiological alterations may impair escape ability. For example, weight gain due to lipid accumulation or pregnancy may decrease escape speeds, increasing the risk of being caught by predators. Reduced escape ability due to pregnancy has been shown in a variety of animal groups such as reptiles (Seigel et al., 1987; Miles et al., 2000), birds (Lee et al., 1996; Kullberg et al., 2002a), fish (James and Johnston, 1998) and scorpions (Shaffer and Formanowicz, 1996). Further, migratory birds loaded with fuel (Kullberg et al., 1996; Kullberg et al., 2000), birds that increase body mass during incubation (Kullberg et al., 2002b) and moulting birds with reduced wing areas (Swaddle and Witter, 1997; Swaddle et al., 1999) show similar limitations in escape performance.

Aside from chemical defences and camouflage, adult butterflies rely on their flight ability to avoid predation (Chai and Srygley, 1991), and as for any winged prey animal, take-off ability, flight speed and manoeuvrability are likely to be crucial factors during a predator attack (Marden and Chai, 1991). Whereas chemical defence is a fairly well studied area (reviewed in Brower, 1984), little empirical data exist on evasive flight and its physiological, morphological and behavioural determinants (Berwaerts et al., 2002).

Feeding dramatically and instantaneously affects the butterfly body composition. Adults can increase body weight by 15 to 51% after feeding (Pullin, 1987; Knopp and Krenn, 2003) and, over longer time scales, monarch butterflies (*Danaus plexippus*) accumulate lipids prior to migration such that up to 45% of body weight is fat (Brown and Chippendale, 1974).

Butterflies that hibernate must spend enough time foraging prior to over-wintering to deposit sufficient stores to survive the winter

and reproduce the following spring. However, the extended time spent feeding increases the amount of time the butterfly is exposed to predators. Further, feeding may lower vigilance, increasing attack rates by birds (Morse, 1975), one of the main predators of adult butterflies (Dempster, 1984; Pinheiro, 1996; Burger and Gochfeld, 2001).

The small tortoiseshell (*Aglais urticae*) is a common butterfly throughout Sweden that hibernates in dark, sheltered areas. It is known to require a minimum lipid accumulation of about 20% of body mass to survive the winter (Pullin, 1987), making it a suitable model for studying weight gain and flight behaviour. *Aglais urticae* are eaten by birds when encountered (Vallin et al., 2006) and are therefore likely to experience predator attacks, and should thus depend on their flight to escape.

Along with wing and body morphology, and temperature (Tsuji et al., 1986; Marden and Chai, 1991; Stutt and Willmer, 1998; Van Dyck and Matthysen, 1998; Berwaerts and Van Dyck, 2004), flight muscle ratio [FMR: thorax mass/total body mass (Marden, 1987)] greatly influences butterfly flight performance (Wickman, 1992; Berwaerts et al., 2002). FMR correlates positively with flight speed and manoeuvrability, whereas an increased posterior weight load reduces FMR and changes the centre of body mass, compromising manoeuvrability and decreasing speed in forward flight (Srygley and Chai, 1990; Srygley and Dudley, 1993). As butterflies cannot increase flight muscle mass in the adult stage (Boggs, 1981), extra weight from feeding and lipid accumulation will decrease FMR, potentially compromising flight performance.

No study has, to our knowledge, measured the effects of natural weight increases on free flight behaviour in butterflies (but see Kingsolver and Srygley, 2000; Srygley and Kingsolver, 2000; Berwaerts et al., 2002; Berwaerts et al., 2006). In this study, we

present an experimental procedure with naturally added weight (ingested food), which alters the FMR of *Aglais urticae*. We measured flight performance during normal take off and when subjected to a simulated predator attack and demonstrate that butterfly flight behaviour is indeed affected by FMR and perceived predation risk.

MATERIALS AND METHODS

The adult *Aglais urticae* (Linnaeus 1758) butterflies used in this study were wild caught between the 25th of August and 1st September, 2005, in the vicinity of Tovetorp Zoological Research Station, located in southeast Sweden (58°56′N 17°08′E) and kept in indoor flight cages (0.65 m × 0.65 m × 0.70 m) provided with moist paper towels to prevent dehydration. To achieve variation in food load, half of the butterflies were also provided with a 10% sugar solution. The flight cages were placed 0.4 m from a south-facing window with additional light provided by two Philips Powertone HPI-T Plus 400 W light bulbs (Stockholm, Sweden) in the ceiling. Butterflies were kept in the flight cages for 3 h on the capture day and for 1 h the day after capture, which was also the day that they were subjected to trials. When not in flight or used in experimental trials, butterflies were kept in a dark incubator (Termaks KB8000, Bergen, Germany) at 8±0.1°C to minimize weight loss, reduce activity and stress level, and to facilitate handling. Butterflies spent a minimum of 30 min in the incubator before being used in trials to ensure sufficient cooling. The trials were conducted in an indoor arena (3.0 m × 4.7 m × 2.0 m) illuminated by eight high-frequency natural light fluorescent tubes (Philips TL5 HO 54W) and a spotlight (Philips Broadway MSR 200; high-efficiency hot restrike metal halide lamp with UV light) to encourage forward flight; butterfly activity is known to be affected by UV light (Scherer and Kolb, 1987). White mosquito netting prevented butterflies from flying into the lamps. Room temperature was held at 20±1°C.

To simulate an attack, a model predator (black cardboard box 0.2 m × 0.15 m × 0.15 m) was attached to a cart on a rail at a 14 degree incline and released 2 m from the butterfly perch. Butterfly flights were recorded both with a digital video camera and with a Trackit 3D-camera system (Biobserve Gmbtl, Bonn, Germany) that provided 50 *x*-, *y*- and *z*-coordinates per second (Fig. 1).

For each trial, a butterfly was taken from the incubator and placed on a perch in the arena, where it was allowed to warm up (~5±2 min; indicated by wing quivering and positioning). Then it was either 'attacked' by the model predator, evoking an 'escape flight' or allowed to take off spontaneously ('control flight'). After performing a trial, butterflies were cooled for about 15 min in the incubator and then weighed to the nearest 0.1 mg (Precisa 205A SCS, Dietikon, Switzerland; fresh weight); thereafter the butterflies were killed by freezing (–18°C) and dissected to obtain thorax weight (head, legs and wings excluded) and abdomen weight.

For every individual, velocity, take-off angle and sinuosity were determined using the coordinates provided by the Trackit 3D camera system and Track 3d (a computer software program developed for analysing space–time data by Ulf Norberg, Stockholm University). For each flight, these measurements were calculated at 0.1, 0.3, 0.5, 0.7, 0.9 and 1.1 m distance from the start. Flight velocity (m s^{-1}) was calculated when butterflies passed each of the six distances from the perch by measuring the distance between two successive coordinates and dividing by the time between the two recorded coordinates. The angle of ascent was
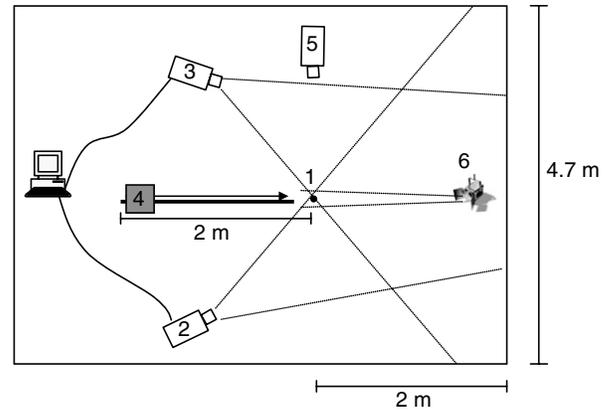

Fig. 1. Experimental setup. A butterfly was placed on a 0.2 m high perch (referred to as start in analyses, 1) in the centre of the room, just on the border of the area viewed by the two CCD Trackit cameras (2 and 3) connected to a computer for storage of coordinates. A model predator (a black cardboard box 0.15 m × 0.15 m × 0.20 m; 4), situated 2 m from the perch, was released by hand to simulate attacks, stopping 0.15 m from the perch. Butterfly take off was also recorded by a digital camera perpendicular to the take off (5). To ensure flight into the area viewed by the cameras, a spotlight was placed in the ceiling (6), illuminating the perch where the butterfly started. Only the flight area of the room was illuminated by fluorescent lamps in the ceiling (the area marked by 2 m × 4.7 m), the rest of the room being unlit.

calculated for each of the six distances as the angle between horizontal and a line drawn between the perch and the height of the butterfly at that distance. Sinuosity was calculated as the total distance flown divided by the length of a straight line from the perch to the location of the butterfly at each of the six distances. Morphological data were only included for butterflies that performed successful flights. In total, 59 butterflies were considered successful at performing flights that could be analysed, i.e. provided coordinates for long enough distances (set to a minimum length of 0.3 m) to be analysed. Butterflies typically took off in a more or less straight line towards the ceiling. Butterflies that were obviously damaged or ill were excluded from trials, as were butterflies that were inactive for more than 10 min in the experimental arena or flew outside of the recording area.

All data, including residuals, were normally distributed and a general linear model (GLM) with repeated measures for the six distances from the start was used (Statistica, version 7.1. StatSoft, Inc. 2005, Uppsala, Sweden). The type of flight (escape or control) was used as a categorical predictor, and flight muscle ratio (FMR: thorax mass/total body mass) as a continuous predictor. We also used linear regression to illustrate the relationship between FMR and flight ability in escape and control flights.

RESULTS

Male and female butterflies in this study did not differ significantly in any of the analysed weight measurements (Student's *t*-test, d.f.=50 and N_{male}=24, N_{female}=28 for all tests; FMR: P=0.808, t=0.244; body mass: P=0.902, t=–0.123; thorax mass: P=0.957, t=0.053; abdomen mass: P=0.881, t=0.150).

Butterfly FMR ranged from 0.30 to 0.50 and did not differ between escape and control butterflies, and nor did any of the other measurements (*t*-test, d.f.=57 and N_{escape}=32, $N_{control}$=27 for all tests; FMR: P=0.588, t=–0.545; body mass: P=0.550, t=0.602; thorax mass: P=0.969, t=–0.545; abdomen mass: P=0.354, t=0.934).

Flight speed

Butterflies that were subjected to a model predator attack flew at significantly higher velocities than did their unattacked conspecifics (Table 1, Fig. 2). Furthermore, there was an effect of FMR on individuals performing flights after a simulated attack; escape fliers with a high FMR managed faster flights at 0.5 m from the start than escape fliers with a low FMR, whereas there was no such effect of FMR on flight speed among individuals performing control flights (linear regression for velocities at 0.5 m from the start; escape flights: N=27, b=0.46, r^2=0.21, P=0.014; control flights: N=32, r^2=0.03, P=0.352; Fig. 3A,B). According to the relationship found, a decrease in FMR from 0.5 to 0.3, which represents the variation in FMR found in our study, reduced escape flight velocity by 37% at 0.5 m from the start.

Table 1. Effect of FMR and type of flight on velocity, angle of ascent and sinuosity

Source (d.f.)	Velocity		Angle of ascent		Sinuosity	
	F	P	F	P	F	P
Between subjects						
FMR (1,56)	0.56	0.46	2.85	0.10	1.01	0.32
Type of flight	5.27	**<0.05**	0.30	0.59	2.70	0.11
Within subjects						
Distance (5,280)	1.67	0.14	3.53	**<0.01**	12.65	**<0.001**
Distance × FMR	0.37	0.87	5.13	**<0.001**	6.39	**<0.001**
Distance × type of flight	0.99	0.43	1.26	0.28	5.14	**<0.001**

Summary statistics of general linear model with FMR as a continuous predictor, treatment as a categorical predictor, and the six measurements at different distances from the start as the dependent variable with repeated measures design. Bold text represents significant P values.

Angle of ascent

The angle of ascent varied with distance from the start, with butterflies flying at lower angles at 0.1 m than at greater distances (Fig. 4, Table 1; Tukey's test: P<0.01 for each distance). There was also an interaction between FMR and distance from the start (Table 1), where high FMR resulted in lower angles of ascent at 0.1 m from the start (Fig. 5), but not at the other distances (GLM with repeated measures followed by sequential Bonferroni correction: at 0.1 m: $F_{1,56}$=7.9, P<0.007; at all the other distances P>0.1). The type of flight (escape/control) was not found to affect the angle of ascent (Table 1).

Sinuosity

Sinuosity varied with distance from the start (Table 1), with butterflies flying less straight at 0.1 m, and reducing sinuosity along the flight path (Fig. 6; Tukey's test revealed that sinuosity at 0.1 and 0.3 m differed significantly from that at all other distances: P<0.0001). Furthermore, there was an interaction between distance from the start and type of flight, where butterflies that performed escape flights flew straighter early during the flight
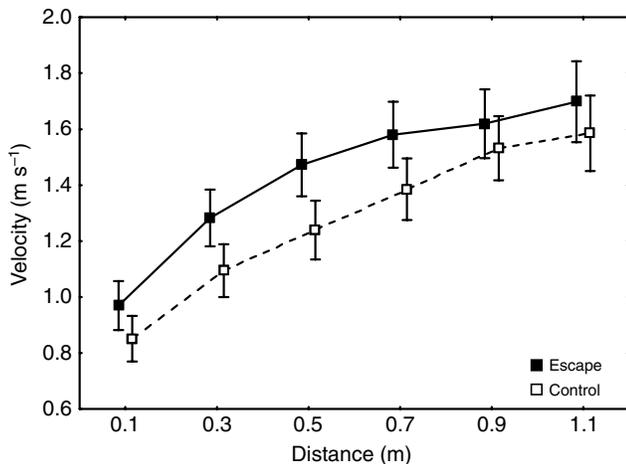
compared with butterflies that took off spontaneously (Fig. 6; Tukey's test: P<0.0001). An interaction between FMR and distance from the start was also reported in the GLM (Table 1); however, it was not significant after a sequential Bonferroni correction (P<0.05).

DISCUSSION

Our results show that butterfly flight speed and sinuosity are affected by simulated predator attacks as these butterflies flew faster, and in a straighter line immediately after take off, than butterflies in the unattacked group. These results indicate that the butterflies interpreted the attack as a threat and tried to outpace the predator. The escape speed that an attacked winged prey can produce is important for survival and consequently fitness. Furthermore, flight speed is proposed to be closely connected to
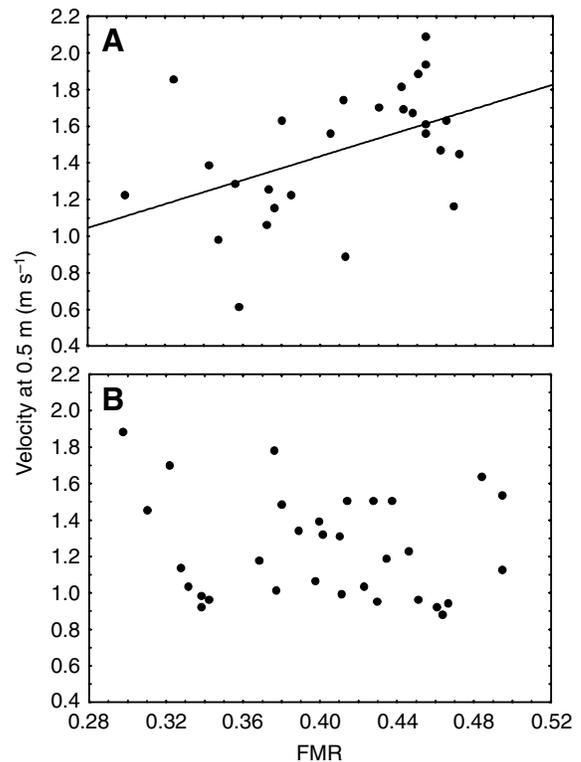

Fig. 2. Mean (±95% confidence interval) velocities at each of the six distances from the start of escape and control flights.

Fig. 3. Flight velocity 0.5 m from the start in relation to FMR of individual butterflies during (A) escape flights (N=27; P=0.014; r^2=0.21, b=0.46) and (B) control flights (N=32, r^2=0.03, P=0.352).
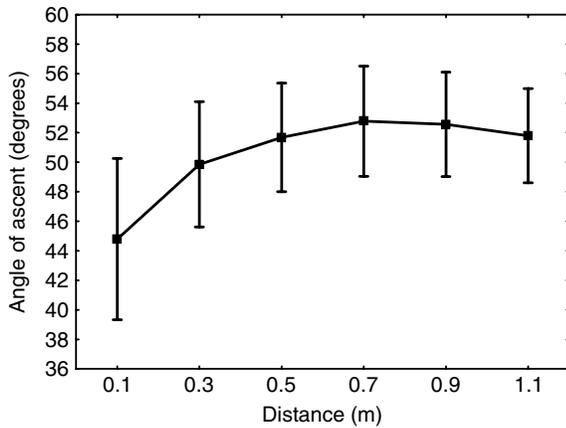
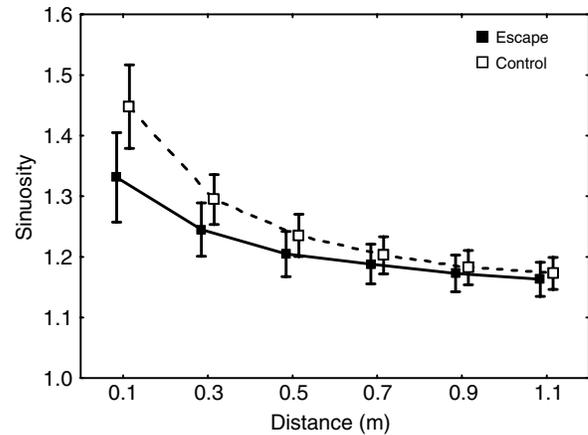Fig. 4. Mean angle of ascent (±95% confidence interval) at each of the six distances from the start.

Fig. 6. Mean sinuosity (±95% confidence interval) at the six distances from the start for escape and control flights.

the amount of flight muscle mass in relation to total body mass that the animal possesses (e.g. Marden, 2000). Supporting this notion, our study on *Aglais urticae* shows that butterflies with higher FMRs flew faster when escaping model predator attacks than butterflies with lower FMRs. The relationship between FMR and flight speed in butterflies has been tested previously, yet the data are inconclusive. For instance, Berwaerts et al. (Berwaerts et al., 2002) reported higher flight capacity with high FMR in tethered *Pararge aegeria*. Moreover, interspecific studies of tropical butterflies indicate that palatable species, with generally high FMR, show faster flights and better manoeuvrability than unpalatable species with generally lower FMR (Marden and Chai, 1991; Srygley and Dudley, 1993; Pinheiro, 1996; Srygley and Kingsolver, 1998; Kingsolver and Srygley, 2000). However, when Srygley and Kingsolver (Srygley and Kingsolver, 2000) subjected the palatable butterfly *Anartia fatima* to added weights amounting to 15% of body mass they did not reveal any relationship between FMR and flight speed. It should be noted that the methods of measuring flight performance have varied among different studies. For example, flight speed has been measured on butterflies being followed flying over a lake, while flight performance was measured as the time until butterflies were captured by a bird inside a small cage, or by
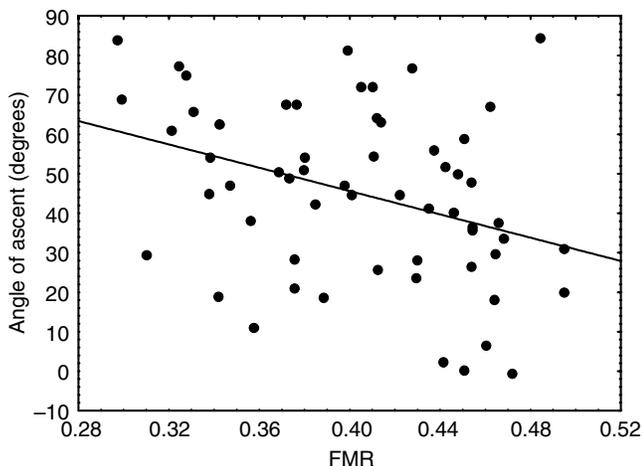

Fig. 5. Angle of ascent in relation to FMR at 0.1 m from the start (N=59, P=0.006, r^2=0.126, b=−0.36).

a human equipped with an insect net. Thus, measurements have been less precise than in our study, and also the nature of the flight has varied in terms of escape responses or natural flight. We suggest that our experimental set up has the ability to give a better estimate and a detailed analysis of the relationship between free flight performance and FMR in butterflies. Consequently, as predicted, but not earlier demonstrated, we revealed a relationship between FMR and flight velocity when studying escape take off, as the butterflies should be expected to fly at speeds nearer to their maximum capacity.

Despite the large increase in weight expected in this species before hibernation (Pullin, 1987), FMR in *Aglais urticae* surpasses the lower limit (0.12–0.16) needed for lift production in still air (Marden, 1987). Indeed, it is fairly high when compared with the scarcely available data for other palatable butterfly species (Marden and Chai, 1991). Still, a reduction in FMR from 0.5 to 0.3, as was the range in this study, markedly decreased flight speed in escaping butterflies by 37%, indicating that gaining weight may well conflict with escape ability. Also, FMRs reported in this study presumably do not represent the lowest occurring naturally since the butterflies were captured during lipid accumulation, not after entering hibernation. Even though most butterflies did fly in the experimental arena, it is possible that the butterflies with very low FMRs were excluded from the study as a result of not flying. Kingsolver and Srygley (Kingsolver and Srygley, 2000) noted a decrease in flight activity in *Colias* and *Pontia* butterflies after an experimental reduction of FMR by 10–17%. Remaining still might be a way of reducing predator encounters and, in the case of *Aglais urticae*, might allow it to rely on its cryptic appearance while resting. The predation pressure on butterflies by birds has been proposed to increase during the autumn (Ide, 2006), and wings from *Aglais urticae* and *Inachis io* were found around bushes (*Buddleja davidii*) visited by these butterflies for nectar feeding (M.A., personal observation), suggesting that they experience attacks whilst foraging.

Our finding that high FMRs gave lower take-off angles immediately after the butterflies took flight could be explained by a change in body and stroke plane angle in heavier butterflies as a result of a posterior shift in the centre of body mass (Srygley and Chai, 1990; Srygley, 1994; Norberg, 1995; Marden, 2000). However, we found no evidence that take-off angles were negatively affected in the long run by a reduction in FMR (Marden,

1987). Most butterflies, regardless of treatment, performed flights with a net upwards movement, suggesting that these ranges of FMR do not curb the ability to take off.

That there was no difference in body mass or FMR between sexes in this study is reasonable since both males and females need to accumulate fat stores for hibernation. However, there is much reason to expect that FMR should vary between the sexes during spring due to reproductive investment, with females carrying a non-trivial egg load (i.e. lower FMR), whereas males should be lighter (i.e. higher FMR) as a result of spermatophore transfer. Possible differences in flight ability between males and females as a result of weight loads thus remain to be examined.

In summary, our study shows that escaping butterflies with a high FMR manage faster and straighter escape flights. This suggests that gaining weight has consequences for a butterfly's ability to evade an attacking predator and should be a major factor affecting behaviour and physiology in most palatable butterfly species.

REFERENCES

Berwaerts, K. and Van Dyck, H. (2004). Take-off performance under optimal and suboptimal thermal conditions in the butterfly *Pararge aegeria. Oecologia* **141**, 536-545.

Berwaerts, K., Van Dyck, H. and Aerts, P. (2002). Does flight morphology relate to flight performance? An experimental test with the butterfly *Pararge aegeria. Funct. Ecol.* **16**, 484-491.

Berwaerts, K., Aerts, P. and Van Dyck, H. (2006). On the sex-specific mechanisms of butterfly flight: flight performance relative to flight morphology, wing kinematics, and sex in *Pararge aegeria. Biol. J. Linn. Soc. Lond.* **89**, 675-687.

Boggs, C. L. (1981). Nutritional and life-history determinants of resource allocation in holometabolous insects. *Am. Nat.* **117**, 692-709.

Brower, L. P. (1984). Chemical defence in butterflies. In *The Biology of Butterflies* (ed. R. I. Vane-Wright and P. R. Ackery), pp. 109-134. London: Academic Press.

Brown, J. J. and Chippendale, G. M. (1974). Migration of the monarch butterfly *Danaus plexippus*: energy sources. *J. Insect physiol.* **20**, 1117-1130.

Burger, J. and Gochfeld, M. (2001). Smooth-billed ani (*Crotophaga ani*) predation on butterflies in Mato Grosso, Brazil: risk decreases with increased group size. *Behav. Ecol. Sociobiol.* **49**, 482-492.

Chai, P. and Srygley, R. B. (1990). Predation and the flight, morphology, and temperature of neotropical rain-forest butterflies. *Am. Nat.* **135**, 398-411.

Dempster, J. P. (1984). The natural enemies of butterflies. In *The Biology of Butterflies* (ed. R. I. Vane-Wright and P. R. Ackery), pp. 97-104. New York: Academic Press.

Ide, J.-Y. (2006). Sexual and seasonal differences in the frequency of beak marks on the wings of two *Lethe* butterflies. *Ecol. Res.* **21**, 453-459.

James, R. S. and Johnston, I. A. (1998). Influence of spawning on swimming performance and muscle contractile properties in the short-horn sculpin. *J. Fish Biol.* **53**, 485-501.

Kingsolver, J. G. and Srygley, R. B. (2000). Experimental analyses of body size, flight and survival in pierid butterflies. *Evol. Ecol. Res.* **2**, 593-612.

Knopp, M. C. N. and Krenn, H. W. (2003). Efficiency of fruit juice feeding in *Morpho Peleides* (Nymphalidae, Lepidoptera). *J. Insect Behav.* **16**, 67-77.

Kullberg, C., Fransson, T. and Jakobsson, S. (1996). Impaired predator evasion in fat Blackcaps (*Sylvia atricapilla*). *Proc. R. Soc. Lond. B Biol. Sci.* **263**, 1671-1675.

Kullberg, C., Jakobsson, S. and Fransson, T. (2000). High migratory fuel loads impair predator evasion in sedge warblers. *Auk* **117**, 1034-1038.

Kullberg, C., Houston, D. C. and Metcalfe, N. B. (2002a). Impaired flight ability – a cost of reproduction in female blue tits. *Behav. Ecol.* **13**, 575-579.

Kullberg, C., Metcalfe, N. B. and Houston, D. C. (2002b). Impaired flight ability during incubation in the pied flycatcher. *J. Avian Biol.* **33**, 179-183.

Lee, S. J., Witter, M. S., Cuthill, I. C. and Goldsmith, A. R. (1996). Reduction in escape performance as a cost of reproduction in gravid starlings, *Sturnus vulgaris. Proc. R. Soc. Lond. B Biol. Sci.* **263**, 619-624.

Marden, J. H. (1987). Maximum lift production during takeoff in flying animals. *J. Exp. Biol.* **130**, 235-258.

Marden, J. H. (2000). Variability in the size, composition, and function of insect flight muscles. *Annu. Rev. Physiol.* **62**, 157-178.

Marden, J. H. and 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.

Miles, D. B., Sinervo, B. and Frankino, W. A. (2000). Reproductive burden, locomotor performance, and the cost of reproduction in free ranging lizards. *Evolution* **54**, 1386-1395.

Morse, D. H. (1975). Ecological aspects of adaptive radiation in birds. *Biol. Rev.* **50**, 167-214.

Norberg, U. M. (1995). How a long tail and changes in mass and wing shape affect the cost for flight in animals. *Funct. Ecol.* **9**, 48-54.

Pinheiro, C. E. G. (1996). Palatability and escaping ability in Neotropical butterflies: tests with wild kingbirds (*Tyrannus melancholicus*, Tyrannidae). *Biol. J. Linn. Soc. Lond.* **59**, 351-365.

Pullin, A. S. (1987). Adult feeding time, lipid accumulation, and overwintering in *Aglais urticae* and *Inachis io* (Lepidoptera: Nymphalidae). *J. Zool. Lond.* **211**, 631-641.

Scherer, C. and Kolb, G. (1987). The influence of colour stimuli on visually controlled behaviour in *Aglais urticae* L. and *Pararge aegeria* L. (Lepidoptera). *J. Comp. Physiol. A* **161**, 891-898.

Seigel, R. A., Huggins, M. M. and Ford, N. B. (1987). Reduction in locomotor ability as a cost of reproduction in gravid snakes. *Oecologia* **73**, 481-485.

Shaffer, L. R. and Formanowicz, D. R., Jr (1996). A cost of viviparity and parental care in scorpions: reduced sprint speed and behavioural compensation. *Anim. Behav.* **51**, 1017-1024.

Srygley, R. B. (1994). Locomotor mimicry in butterflies? The associations of positions of centres of mass among groups of mimetic, unprofitable prey. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* **343**,145-155.

Srygley, R. B. and Chai, P. (1990). Flight morphology of Neotropical butterflies: palatability and distribution of mass to the thorax and abdomen. *Oecologia* **84**, 491-499.

Srygley, R. B. and Dudley, R. (1993). Correlations of the position of centre of body mass with butterfly escape tactics. *J. Exp. Biol.* **174**, 155-166.

Srygley, R. B. and Kingsolver, J. G. (2000). Effects of weight loading on flight performance and survival of palatable Neotropical *Anartia fatima* butterflies. *Biol. J. Linn. Soc. Lond.* **70**, 707-725.

Stutt, A. and Willmer, P. (1998). Territorial defence in speckled wood butterflies: do the hottest males always win? *Anim. Behav.* **55**, 1341-1347.

Swaddle, J. P. and Witter, M. S. (1997). The effect of moult on the flight performance, bodymass, and behaviour of European starlings (*Sturnus vulgaris*): an experimental approach. *Can. J. Zool.* **75**, 1135-1146.

Swaddle, J. P., Williams, E. V. and Rayner, J. M. V. (1999). The effect of simulated flight feather moult on escape take-off performance in starlings. *J. Avian Biol.* **30**, 351-358.

Tsuji, J. S., Kingsolver, J. G. and Watt, W. B. (1986). Thermal physiological ecology of *Colias* butterflies in flight. *Oecologia* **69**, 161-170.

Vallin, A., Jakobsson, S., Lind, J. and Wiklund, C. (2006). Crypsis versus intimidation – anti-predation defence in three closely related butterflies. *Behav. Ecol. Sociobiol.* **59**, 455-459.

Van Dyck, H. and Matthysen, E. (1998). Thermoregulatory differences between phenotypes in the speckled wood butterfly: hot perchers and cold patrollers? *Oecologia* **114**, 326-334.

Wickman, P.-O. (1992). Sexual selection and butterfly design – a comparative study. *Evolution* **46**, 1525-1536.