



Effects of weight loading on flight performance and survival of palatable Neotropical *Anartia fatima* butterflies

ROBERT B. SRYGLEY* AND JOEL G. KINGSOLVER

Department of Zoology, Box 351800, University of Washington, Seattle, Washington 98195–1800, U.S.A.

Received 19 February 1999; accepted for publication 10 December 1999

Previous studies show that the position of centre of body mass (cm_{body}) and the ratio of flight muscle to total body mass (flight muscle ratio, FMR) are good predictors of flight speed and manoeuvrability in butterflies. However, cm_{body} , FMR, and related morphometric traits are strongly correlated phenotypically, making it difficult to identify the causal determinants of flight performance. By experimentally gluing weights that amounted to ~15% body weight to a palatable Neotropical butterfly species (*Anartia fatima*), we tested the effects of altering FMR and repositioning cm_{body} on two measures of flight performance: flight speed and the ability to evade capture. We then tested their effects on survival in a natural setting. Flight performance studies detected no significant differences in airspeed or evasive flight ability among unweighted controls, weighted-loaded butterflies (WL), and those with cm_{body} positioned further posterior (CM). In two mark-release-recapture experiments, survival of treatment groups did not differ, but males survived longer than females. In one experiment, WL and CM butterflies were recaptured more frequently than controls, whereas the probability of recapture for females was higher than that for males in the second experiment. When significant, results for recapture were consistent with a causal relationship between FMR and flight speed. Presumably, a decrease in flight speed was due to a reduction in muscle mass-specific power output in the weighted butterflies. However, the results did not support a relationship between manoeuvrability and cm_{body} .

© 2000 The Linnean Society of London

ADDITIONAL KEYWORDS:—Lepidoptera – Nymphalidae – flight – dispersal – mortality – survival – predation.

CONTENTS

Introduction	708
Predictions	709
Material and methods	710
Study organism and study site	710
Experimental treatments	711
Flight performance	712
MRR experiments	713

* Corresponding author. Present address: Department of Zoology, South Parks Road, University of Oxford, Oxford OX1 3PS; E-mail bob.srygley@zoo.ox.ac.uk

Mass loss	714
Bird abundance on the MRR site	715
Results	715
Flight performance	715
MRR studies	717
Behaviours	719
Bird abundance and observations of predation on the MRR site	720
Discussion	720
Morphometric determinants of flight performance	720
Survival and resampling	721
Acknowledgements	723
References	723

INTRODUCTION

The adaptive significance of morphological change is one of the central tenets of evolution by natural selection. However, morphological features are frequently highly intercorrelated (Olson & Miller, 1958; Arnold, 1983; Crespi, 1990), making the relations between form and function difficult to establish.

Biomechanical analyses of hovering insects have identified a number of key morphological features that affect flight aerodynamics and energetics (Ellington, 1984a–c), but the relative importance of these features in forward flight and manoeuvrability is not well understood. Because aerial predators and prey are presumably selected to maximize flight performance, differences in palatability to avian insectivores that have evolved within butterfly taxa have been used to evaluate the evolution of flight biomechanics and test predictions from biomechanical theory (see table 1 in Srygley, 1994). Butterflies are an excellent taxonomic group for examining insect flight because some species fly fast to escape predators whereas others are protected by defensive chemicals and fly slowly (Wallace, 1865; Chai & Srygley, 1990; Srygley & Chai, 1990a).

Recently, two morphological features have been correlated with the ability of butterflies to escape from predatory attack. Mass allocation to the thorax, or flight muscle ratio (FMR), is a measure of the body mass-specific power available for flight. Marden and Chai (1991) proposed that FMR was related to linear acceleration and the ability to accelerate vertically against gravity to escape from predator attacks. Srygley and Dudley (1993) correlated mass allocation to the thorax with flight speed, arguing that a larger thorax was associated with greater muscle cross-sectional area, and hence, greater contractile force (Srygley & Chai, 1990b). Kingsolver and Srygley (2000) experimentally decreased FMR by adding inert weights to temperate *Colias* and *Pontia* butterflies; they showed that decreased FMR significantly reduced the probability of flight in the field, but had no consistent effects on dispersal or survival rates.

The position of centre of body mass (cm_{body}) is also associated with the ability to escape predator attacks (Srygley & Dudley, 1993). Relative to the long, thin abdomens of distasteful species, palatable butterflies possess short, squat bodies that position cm_{body} nearer to the wingbase. For any given body mass, their smaller abdominal mass also positions cm_{body} nearer to the wingbase. Ellington (1984b), Chai and Srygley (1990), and Srygley and Dudley (1993) proposed that the position of cm_{body} relative to the wing base was directly related to the insect's manoeuvrability. This hypothesis

was supported by the extremely high correlation among butterfly species between cm_{body} and the moment of rotational inertia (RI), a measure of the response of a butterfly's body to the torque applied by the wings (Srygley, 1994). Relative to distasteful species, palatable butterflies had lower RI. Theoretically, a lower RI permits faster radial acceleration and greater manoeuvrability for any given aerodynamic force.

Hence, these two traits, FMR and cm_{body} , both have theoretical and empirical relevance to escape from predation. However, they are also highly correlated with one another (Srygley, 1994). As a result, their independent effects on flight speed and manoeuvrability remain unclear.

One means of resolving the independent effects of FMR and cm_{body} is to alter these features experimentally by the addition of inert weights. To this end, we experimentally altered FMR and cm_{body} and measured the resulting flight performance of palatable butterfly species *Anartia fatima* in a Neotropical setting.

PREDICTIONS

The addition of an inert weight reduces FMR, resulting in a decrease in body mass-specific power available for flight. The predicted result is a decrease in flight speed (Srygley & Dudley, 1993). A decrease in FMR would also result in a decrease in linear acceleration (Marden & Chai, 1991) and manoeuvrability (Hedenström, 1992).

The addition of the same inert weight posterior to cm_{body} will result in an increase in RI, resulting in a decrease in manoeuvrability above and beyond that due to the decrease in FMR (Srygley & Dudley, 1993).

In the presence of appropriate predators, changes in flight speed and manoeuvrability caused by manipulation of FMR and cm_{body} will influence the butterflies' abilities to escape from predatory attack. Hence, we measured the survival of *A. fatima* butterflies in a natural setting after experimental manipulation of FMR and cm_{body} . A decrease in flight speed and manoeuvrability resulting from the addition of weights should reduce their survival in the field. Above and beyond the decrease in survival due to the addition of weights, repositioning cm_{body} further posterior should reduce the butterflies' manoeuvrability and survival.

Behavioural changes that are indirectly related to flight performance may also arise from weight-loading (Witter & Cuthill, 1993; Witter *et al.*, 1994, and references therein). For example, weighted butterflies may spend more time nectar feeding to account for the increased energetic expenditure of carrying the extra load. Weighted butterflies may also require higher body temperatures, and thus bask more, in order to generate the additional force required for take-off. Weight-loading and repositioning cm_{body} may also increase the frequency of collisions and the resulting damage during flight or landing (Witter & Cuthill, 1993). In a concurrent study, Srygley (unpublished data) measured the effects of weight-loading on dispersal. If they fly more slowly than unweighted butterflies, then weighted butterflies are predicted to disperse more slowly.

These four potential changes in the behaviours of weighted butterflies may also influence the estimates of survival. If weighted butterflies spend more time nectar feeding or basking, then they may be exposed more to predation relative to

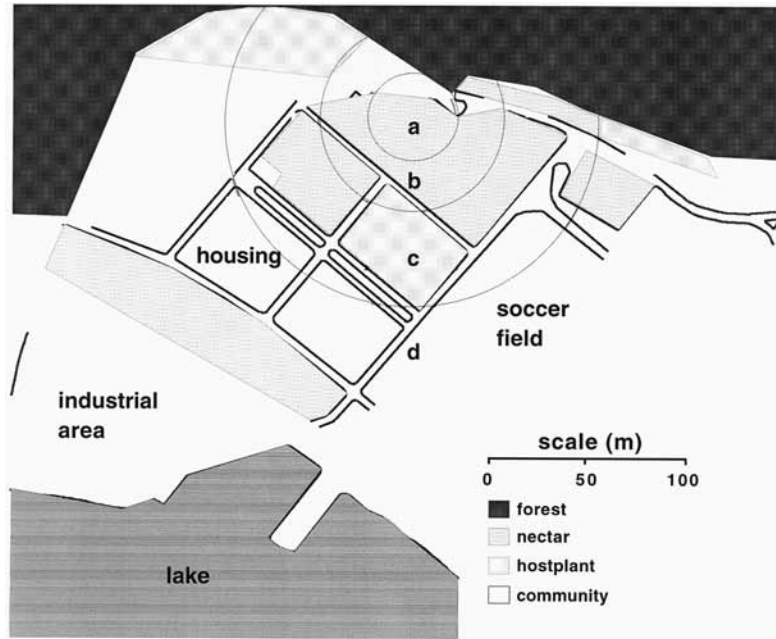


Figure 1. Map of the study site in Gamboa, Panama, and general characteristics of the habitats. Habitats abundant with nectar and hostplants were bounded by habitats of poor quality for the butterflies, including forest, community housing, grass parks, and the lake.

unweighted butterflies. In addition, because the survival estimates depend both on mortality and dispersal, differences in dispersal among treatment groups may affect estimates of survival.

MATERIAL AND METHODS

Study organism and study site

Anartia fatima Godart (Nymphalidae, Lepidoptera) is found exclusively in heavily disturbed areas of lowland Neotropical rainforest where immatures feed on *Blechnum* sp. (Acanthaceae) and adults feed on nectar. The species is palatable to avian insectivores. Rufous-tailed jacamars (*Galbula ruficauda*) attacked *A. fatima* in the field (Chai, 1986) and ate all of the butterflies made available during presentation experiments (Chai, 1990). Flycatchers and kingbirds (Tyrannidae) also attacked and consumed *A. fatima* in the field (R.B.S., pers. observ.). Estimates of the number of insectivorous and omnivorous birds on the study site during the survival experiments are given below.

The study site where butterflies were captured for measures of flight performance and released for mark-release-recapture studies was an abandoned settlement on the edge of Gamboa, Panama (Fig. 1). It was artificially divided by asphalt roadways, and was overgrown with a mixture of old pasture and young secondary forest stands. An abundance of *A. fatima* butterflies inhabited this area, feeding on adult nectar

TABLE 1. Effects of experimental treatments on male *Anartia fatima* from the 1996 rodeo. Butterfly morphology prior to (A) and following (B) treatment

(A)	n	mass	cm_{body} (mm)	RI (mg mm ²)	FMR (%)	ρ (mg/cm ²)
WL	15	87 ± 16	2.14 ± 0.27	1759 ± 301	44 ± 4	6.60 ± 1.13
CM	20	92 ± 14	2.17 ± 0.49	1803 ± 664	46 ± 5	6.85 ± 1.01
Controls	17	98 ± 15	2.21 ± 0.46	2152 ± 661	46 ± 3	6.98 ± 1.08
All butterflies*	52	92 ± 15	2.17 ± 0.42	1904 ± 598	45 ± 4	6.82 ± 1.06
(B)						
WL		100 ± 18	2.05 ± 0.23	1790 ± 304	39 ± 4	7.65 ± 1.31
CM		106 ± 16	3.01 ± 0.43	2587 ± 811	39 ± 4	7.93 ± 1.16

*Includes controls and experimental butterflies prior to treatments.

cm_{body} , position of centre of body mass relative to the wingbase; FMR, thoracic mass divided by body mass; ρ , wing loading; RI, moment of rotational inertia; WL, group in which weights were added near to cm_{body} ; CM, group of butterflies for which weights were added posterior to cm_{body} . Values are means ± SD.

plants (*Lantana horrida*), milkweeds, and composites) in the old pasture and laying eggs on its hostplant along the shaded pasture edge. The site was bounded by areas where resources for the butterflies were rare: old secondary forest on a steep hillside to the north and east; and Lake Gatún (forming a part of the Panama Canal), and asphalted industry or community areas to the south and west. The roadways within the study area served to divide the area into 12 sectors that were subsequently simplified into four zones for analysis of dispersal (see Fig. 1).

Experimental treatments

For flight performance experiments and mark-release-recapture (MRR) experiments, the methods for experimentally weighting butterflies were identical. During the morning hours (08:00–12:00), adult butterflies were hand-netted on the study site, and handled to ensure that damage and desiccation were minimized. Only those in reasonably young condition (e.g. freshly emerged or intermediate-fresh with little loss of wing scales) and occasionally those with intermediate wing-wear were used in the experiments. Butterflies were kept in humid Ziploc bags for up to 3 h in the field, and then placed in refrigeration or on ice in the nearby laboratory for up to 3 h until measurements and manipulations were performed. Each butterfly was sexed and weighed on a Sartorius balance (accuracy ± 0.5 mg). It was then held by the wings on a mounting board using a pair of spring-loaded hairpins fixed to the board, and the body length (L_{body}) was measured to the nearest 0.5 mm.

We aimed to reposition cm_{body} further posterior by approximately 2 standard deviations from the mean for *A. fatima*. This would bring cm_{body} and RI to an extreme of the natural variation in the population of emerging butterflies. Using Duro contact cement, we glued 15% of the body mass of each individual (rounded to the nearest milligram) in the form of a pre-cut tin-alloy solder weight (Alphametals: 0.050 American Wire Gauge, 60% tin 40% lead with rosin flux core) to a position on the abdomen corresponding to 26% of L_{body} from the wing base. This position for the additional mass was 10% further posterior than the mean cm_{body} for *A. fatima* at 16% of L_{body} from the wing base. This experimental group is termed CM. To estimate the magnitude of the manipulation on cm_{body} , FMR, and RI, rodeo butterflies were compared before and after the manipulation (Table 1). The position of cm_{body} for

CM males shifted 2 SD posterior to that of the same males prior to manipulation (all butterflies prior to manipulation were used to obtain the best estimate for SD), FMR decreased 1.5 SD, and RI increased 1.31 SD.

Weight-loaded butterflies (WL) were treated identically, except that the weight was added at cm_{body} which was estimated from previous measurements to be 16% of L_{body} from the wing base. The position of cm_{body} for WL males was 0.21 SD anterior to that for the same males prior to manipulation, FMR decreased 1.5 SD and RI increased 0.05 SD (Table 1). Unweighted controls were treated in the same manner, except that contact cement only was placed on the abdomen at 26% of L_{body} .

For flight speed and MRR experiments, comparisons among control butterflies and those with weights were conducted. Combined CM and WL butterflies are referred to below as weighted (CM + WL) butterflies.

Flight performance

Artificial predation experiments

The ability to evade predators was measured using artificial predation experiments (rodeos). At least 10 individuals for each of the three treatment groups were flown sequentially. During 1995, each butterfly was flown once with one of two catchers selected at random. During 1996, only male *A. fatima* were used for measures of flight performance. Unless it was lost or unable to fly, each butterfly was flown twice, once with each designated catcher. A butterfly was set on the ground within a 2 m radius circle. The designated catcher began in the circle and pursued the butterfly when it flew beyond the circle perimeter. A second person timed the pursuit, measured the distance, and recorded the number of swings required to capture the butterfly. Flight velocity (m/s) was estimated as distance divided by duration. Ambient temperature, weather conditions, wind speed and direction were measured following the pursuit. To minimize the effect of wind speed and direction on flight performance, experiments were carried out on generally still days only. Following a rodeo, butterflies were frozen, mass and winglength (L_{wing}) were measured. Wing loading was estimated as body mass/ L_{wing}^2 . The position of cm_{body} was measured by cutting the body to divide it into the head, thorax, and three abdominal sections. Masses and lengths of each section were measured. Each segment was assumed to be homogeneous in mass along its length, and the wing base was assumed to be in the middle of the thorax. The point at which the mass anterior balanced the mass posterior was then calculated, and positioned along the body axis relative to the wing base. The moment of rotational inertia (RI) was calculated as the sum of the segment masses multiplied by the squared distance of the segment from the wing base. FMR was estimated as thoracic mass divided by body mass. This estimate is near to the true FMR, because approximately 95% of the wet thoracic mass is muscle (Marden & Chai 1991).

Airspeed

During September 1995 and October 1996, flight speed was measured during release experiments over Lake Gatún. At least 10 individuals for each treatment group were manipulated the previous day, and fed a 10% honey-water solution on

the morning of the release. Butterfly airspeeds were measured in the mornings between 08.00 and noon in light to moderate winds (0.2–3.8 m/s). Individual butterflies were released over the lake, and followed with a 15 foot Boston Whaler powered with a 30 hp outboard motor until an even pace was maintained approximately 1 m aft or parallel to the starboard side for 10 s. Sampled butterflies adopted a definite flight direction within a few minutes of release, and remained within 1–2 m of the water's surface. A reliable estimate of airspeed was obtained by measuring the speed of the boat using a hand-held unidirectional anemometer (Kurz series 490). The anemometer probe was held laterally from the prow of the boat so that the probe tip was 1–1.5 m distant from the boat perpendicular to the insect's body axis and at the same height as the flying butterfly. Both the butterfly and anemometer were kept out of the flow field around the boat's hull. Airspeed, i.e. flight speed independent of wind, was thus measured directly and no assumptions concerning ambient wind were necessary. Between the lake centre and edge, a maximum of three measurements of airspeed were taken before capture (see also Srygley & Dudley, 1993; Dudley & Srygley, 1994).

MRR experiments

Release protocol

Two mark-release-recapture experiments (MRR) were performed in 1996: the first between 18 September and 14 October, and the second between 30 September and 14 October. Releases occurred during five of the first eight days of the first experiment (18–25 September 1996), and the first three days of the second experiment (30 September–2 October 1996).

Following the manipulations, butterflies were uniquely numbered on the ventral surface of both hindwings using a silver paint marker. When the paint had dried, the butterflies were placed in glassine envelopes, reweighed, returned to a nylon net bag, and transported to the field. The butterflies were released near a *Lantana* shrub at the centre of zone 'a' (see Fig. 1) between 15:00 and 17:00 h. During the release, the butterflies' probosci were hand-extended into a 20% sucrose solution upon which they were permitted to feed and then fly away of their own volition. For the first experiment (September 1996), 17 of 295 butterflies were not released (eight control, four WL, and five CM) because they were damaged. For the second experiment (October 1996), two butterflies (both WL) out of 179 were not released.

Resampling protocol

In order to measure survival in the field, butterflies were resighted or recaptured (resampled). A preliminary study in 1994 indicated that the majority of *Anartia* disappear from the population in 14 days, although a few may live for as long as 22 days. Resampling generally occurred in the morning between 08:30 and 12:30. Between one and four people resampled the population each day for a total of 121 person-hours during 19–30 September 1996 and 99 person-hours during 1–14 October 1996. Each person was designated a sector or sectors of the study site that they sampled for 1 h and then rotated with the others in order to avoid overlap.

When resampled, each butterfly's unique number, resampling site, activity when first observed (flying, feeding, basking with wings open, resting with wings closed,

courting, copulating, or searching for hostplant), wing-wear condition (fresh, intermediate-fresh, intermediate, intermediate-worn, worn, or very worn), and when possible, treatment were noted. Wings from marked dead individuals were collected to investigate causes of mortality, and confirmed predation by spiders, birds, and other animals were also noted. Although we checked whether butterflies had lost weights so that they could be re-weighted, none of the butterflies were re-weighted in either MRR experiment.

Statistical analyses

To estimate resampling and survival probabilities, a maximum likelihood analysis of the MRR data was performed using the SURPH program (survivorship probability hazard analysis, Skalski *et al.*, 1993). Following the methodology of Kingsolver (1995, 1996, 1999), the full model (analogous to the standard Cormack–Jolly–Seber model), including effects of sex, treatment, time period and their interactions on both resampling and survival probabilities was first estimated. The estimated dispersion parameter for the full model (McCullagh & Nelder, 1989) was 2.53 for the September 1996 study and 4.43 for the October 1996, indicating a reasonable fit of the model to these data. The best model for the resampling probabilities was then found by successively eliminating the variables time, treatment, sex and their interactions from the model and examining the Akaike Information Criteria (AIC). Using the best model for resampling probabilities, survival probabilities were then analysed, varying time, treatment, and sex, and the best model with the lowest AIC was selected. For the final time period, only the product of resampling probabilities and survival probabilities can be estimated; again the AIC was used to examine possible sex or treatment effects on this product. The best overall model for the data is selected as that yielding the lowest AIC.

It was predicted that (1) a decrease in survival would result from weight-loading (CM+WL) versus unweighted controls, and (2) altering the position of cm_{body} (i.e. CM butterflies) would result in a decrease in survival relative to WL butterflies (i.e. those that were weight-loaded at the position of cm_{body}). Hence, using the best resampling and best survival models as a starting point, we also varied parameters to compare relative resampling and survival of (1) unweighted versus weighted (CM+WL) butterflies and (2) control and WL butterflies combined versus CM. From the best model, resampling and survival probabilities were calculated with their standard errors for each time period, sex, and treatment group. Selection coefficients were also generated.

Mass loss

To investigate whether weighted butterflies reduced their food consumption to mitigate the effect of the weight on their ability to fly, an additional experiment to measure mass loss in the field was performed during 9–14 October 1996. Field-caught butterflies had either cm_{body} repositioned as described above ($n=29$ butterflies) or were treated as unweighted controls ($n=28$). They were released in the morning and then recaptured after at least 2 h, reweighed in the laboratory, and re-released soon thereafter. For butterflies recaptured more than once, an average was used for the field mass. The differences in mass between treatments and recaptures were compared among sexes, release date, and treatments.

Bird abundance on the MRR site

W.D. Robinson, an ornithologist and compiler of Panamanian bird songs (W. D. Robinson and G. Angher unpublished data), measured species richness and the abundance of birds on the study site using unlimited radius point counts (Blondel *et al.*, 1981). This method has been successfully applied to censusing Neotropical bird communities (Blake, 1992; Lynch, 1995). During the first week of October 1996, W.D. Robinson visited three points on the study site on each of three mornings. During an 8 min census, he recorded all birds that were seen or heard, and their direction and estimated distance from his position (estimated either visually or by the volume of the song). Censuses were conducted within 2 h after dawn in calm weather when birds are generally at peak song activity. The observation points were separated by 200 m to maximize the independence of the samples. To further minimize the chance of counting the same bird from neighbouring points, only detections within 75 m of a point were used in the calculations, which provided a buffer zone of 50 m between points. The open, secondary forest habitat ensured that songs and call notes for all species relevant to the present study were heard at 75 m. On each date, a bird species' abundance was estimated by summing the number of detections across all three points. Diet for each bird species was assigned by its primary food source (Karr *et al.*, 1990; Robinson *et al.*, 2000).

RESULTS

*Flight performance**Artificial predation experiments*

It was predicted that weighted butterflies (CM+WL) would have slower flight speeds, and therefore would be easier to capture than unweighted controls. Moreover, repositioning cm_{body} should reduce manoeuvrability, making CM butterflies more easy to capture than WL butterflies.

During 1995, butterflies released on 4 October did not differ significantly from those released on 9 October in velocity ($P=0.48$), log duration ($P=0.77$), or log distance ($P=0.90$). For this reason, data from the two dates were pooled. The weather was sunny to mostly cloudy on 4 October and overcast on 9 October, but winds were similar, averaging 1.3 ± 0.7 m/s (mean \pm SD) on 4 October and 1.3 ± 0.4 m/s on 9 October.

Treatments ($n=17$ WL, 18 CM, and 18 controls) did not differ significantly in velocity ($P=0.14$), log duration ($P=0.35$), or log distance ($P=0.49$). Sexes ($n=46$ males, 7 females) were not significantly different for these same three measures ($P>0.15$). Using one author's (R.B.S.) number of missed attempts to capture the butterflies, rank order of the treatment groups did not differ ($n=5$ WL, 4 CM, and 5 controls; Kruskal–Wallis $H=1.33$, $P=0.52$).

During 1996, there was no significant difference in the ability of the treatment groups to evade capture, as measured by the log distance and log duration of flights prior to capture (repeated ANOVA: $n=7$ controls, 8 WL, 5 CM; two replicates; duration $P=0.232$, distance $P=0.081$). The flight velocity of control butterflies showed a tendency to be greater than that for weighted (CM+WL) butterflies,

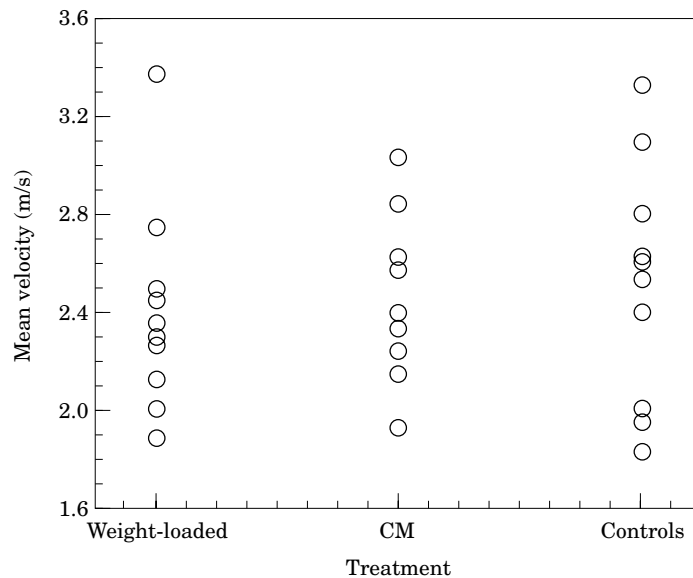


Figure 2. Airspeeds measured over Lake Gatún for each treatment group. Each point represents one individual. CM, group of butterflies for which weights were added posterior to cm_{body} .

although this was marginally significant ($n=8$ controls, 7 weighted (CM+WL); replicates=2; $P=0.051$). The number of attempts required by either chaser to capture each butterfly did not differ among treatments ($n=8$ WL, 10 CM, and 13 controls: Kruskal–Wallis $H=1.84$, $P=0.40$; $n=10$ WL, 9 CM, and 14 controls: Kruskal–Wallis $H=0.55$, $P=0.76$).

Airspeed

Over the lake, no significant difference in airspeed was found among treatment groups ($n=13$ WL, 2.5 ± 0.4 m/s, mean \pm SD; $n=9$ CM, 2.5 ± 0.4 m/s; $n=10$ controls, 2.5 ± 0.5 m/s; $P=0.96$; Fig. 2). Sexes were also not significantly different ($n=27$ males, 2.5 ± 0.4 ; $n=5$ females, 2.6 ± 0.2). Ambient temperature and light, measured when the butterflies were released, did not differ among treatment ($P=0.71$ and $P=0.22$, respectively). For all butterflies, airspeed was not related to ambient temperature ($n=20$, $r=0.16$, $P=0.51$) or light ($n=21$, Spearman's rank correlation $r_s = -0.11$, $P=0.61$).

For those butterflies that were used in artificial predation (rodeo) and lake airspeed experiments, the relationship between airspeed over the lake and velocity in the rodeo did not differ among treatment groups (ANCOVA: test for slopes, $P=0.52$; test for means, $P=0.91$). Pooling the data, airspeed over the lake V_a was a significant predictor of rodeo velocity V_r ($V_r = -1.6 + 1.5 V_a$, $n=21$, $P=0.005$). Hence, the data over land and water were consistent qualitatively. Quantitatively, for airspeeds or velocities less than 3 m/s, rodeo velocity was less than lake airspeeds; and for airspeeds or velocities greater than 3 m/s, rodeo velocity was greater than airspeed over the lake.

Interestingly, the mean airspeed of *A. fatima* butterflies intercepted while crossing the canal naturally was 3.7 m/s ($n=5$ males, 3.1 ± 0.3 m/s, mean \pm SD; $n=3$ females,

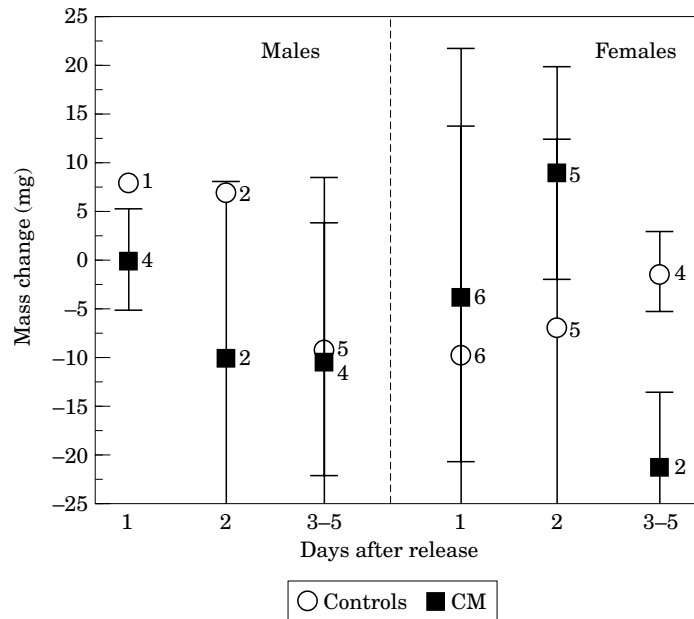


Figure 3. Change in mass when recaptured relative to the mass prior to release for control and experimental butterflies. Sample size is given beside each point. CM, group of butterflies for which weights were added posterior to cm_{body} .

4.2 ± 0.6 m/s; R.B. Srygley and R. Dudley unpublished data). These results suggest that handling affected motivation in all treatment groups. This reduction in flight speed when releasing butterflies over the lake relative to those crossing naturally has been found previously (Srygley & Dudley, 1993; Dudley & Srygley, 1994).

MRR studies

Mass loss

There was no evidence that CM butterflies reduced food consumption to mitigate the effect of the weight on their ability to fly. Mass loss was not dependent on sex ($t=0.05$, $P=0.96$) nor release date ($t=-0.12$, $P=0.90$). Among the unweighted-control and CM treatments, mass loss did not differ significantly ($n=16$ for each treatment, $t=-0.97$, $P=0.34$). On average, mass loss for control butterflies was greater than that for CM butterflies (mean loss \pm SE: 7.5 ± 5.0 mg and 1.9 ± 2.9 mg, respectively). Between 3–5 days (Fig. 3), average mass loss for CM males was 10 mg, which if from the abdomen, would negate the effect of the added weight (13 mg, on average). However, control butterflies lost a similar amount of mass, and thus the differential effect of the added weight on the CM males would remain. In the same period, few females were recaptured. However, from the available data, the average mass loss for CM females was 21 mg and the weight added was also 21 mg. There is no evidence that control females were losing less mass, and so the differential effect of the added weight on the CM females would remain.

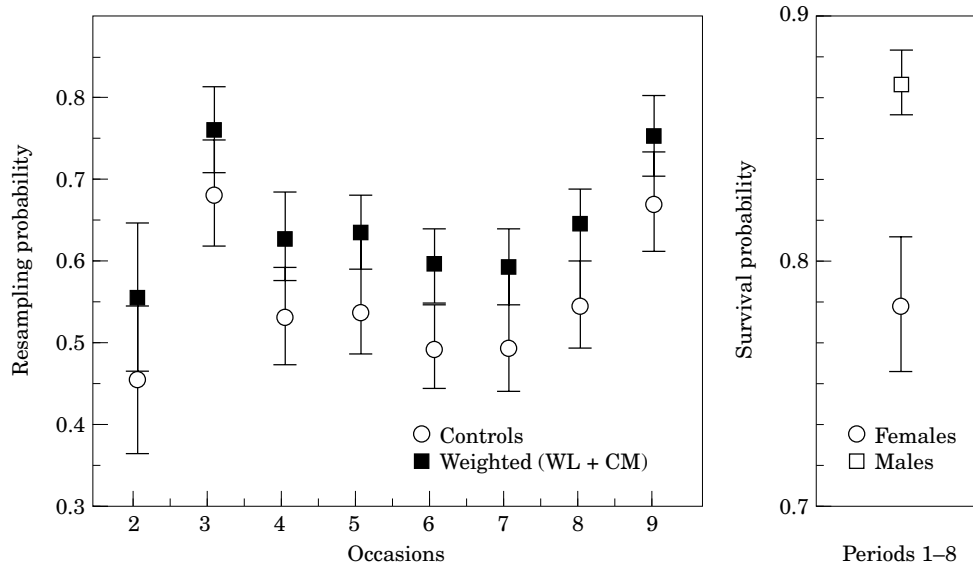


Figure 4. Resampling and survival probabilities from the mark-release-recapture experiment conducted in September 1996. Values are means \pm SE. For those insects known to be alive, control butterflies were less likely to be recaptured than weighted ones (CM + WL combined). Males and females differed in survival probabilities. CM, group of butterflies for which weights were added posterior to cm_{body} .

September 1996

Resampling probabilities differed among time periods and treatments. Combining weighted treatments (CM + WL) and comparing them to unweighted control butterflies yielded the best model for resampling (Fig. 4). In all time periods, weighted (CM + WL) butterflies were more likely to be resampled than controls given that they were known to be alive. Survival probabilities differed among the sexes, but not among time periods or treatments. Survival probabilities in both sexes were high (mean \pm SE: 0.78 ± 0.03 for females; 0.87 ± 0.01 for males). The best model included effects of sex and treatment on the product of resampling and survival in the final period (log likelihood = -936.71 , AIC = 1907.42). Overall, females had a lower product of survival and resampling than males. However, there was an interaction between treatment and sex, such that female controls had the lowest product and male controls had the highest product (Fig. 4). Testing directly for the effect of treatment, inclusion of all three treatments into the best model for resampling was not significantly different from distinguishing only weighted (CM + WL) and control butterflies ($df=1$, log linear statistic = 0.47 , $P=0.49$). Placing all three treatments into the best model for survival was not significantly better than including only sex ($df=4$, log linear statistic = 7.51 , $P=0.11$).

October 1996

Resampling probabilities differed among the sexes and time periods, but not among treatments. For those butterflies known to be alive, females were more likely

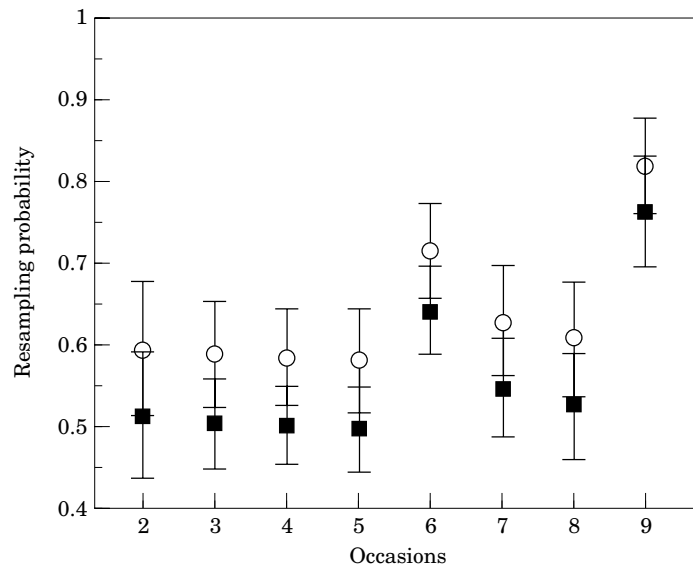


Figure 5. Resampling probabilities for males (■) and females (○) during the mark-release-recapture experiment conducted in October 1996. Values are means \pm SE.

to be resampled than males (Fig. 5). Survival probabilities differed among the sexes, but not among time periods or treatments. Survival probabilities for males (0.878 ± 0.014) were higher than that for females (0.803 ± 0.028) for any given period. The best model (log likelihood = -712.40 , AIC = 1448.81) was homogeneous for resampling by survival in the final period (product of survival and resampling = 0.613 ± 0.068 for all treatments and sexes). Recombinations of the treatments did not improve this model. Directly testing this model against those with treatments included in the hypothesis for resampling or survival did not significantly improve the model (resampling: $df=4$, log linear statistic = 2.29 , $P=0.683$; survival: $df=4$, log linear statistic = 1.78 , $P=0.775$).

Behaviours

Approximately 55% of the butterflies were resighted rather than recaptured. The MRR experiment in September 1996 had a greater proportion of resightings than that in October 1996 (61% *vs* 52%, log likelihood ratio = 15.22 , $P=0.0005$). Resightings were most likely to occur during nectar-feeding when butterflies were relatively still and held their wings closed. For both experiments combined, sexes and treatments did not differ in the proportion of resightings *vs* recaptures (likelihood ratio = 0.244 , $P=0.885$; likelihood ratio = 3.72 , $P=0.446$, respectively). Moreover, butterflies of different sexes and different treatments did not differ significantly in the frequencies of resting, basking, feeding, flight, and copulation (for sexes, log likelihood ratio = 8.19 , $P=0.085$; for treatments, log likelihood ratio = 11.84 , $P=0.159$).

Bird abundance and observations of predation on the MRR site

Combining the three point counts for each census date, insectivore abundance averaged 46 birds (range 42–50), and insectivore species abundance averaged 30 per census (range 29–31). Omnivore abundance averaged 22 birds (21–25) and 15 species (14–17). Other guilds recorded were nectarivores and frugivores or granivores. Insectivores amounted to 49% of the birds sampled (54% of the species) and omnivores made up 31% of the total (28% of the species).

During September 1996, direct observations of predation were relatively few. Two butterflies (one WL and one CM) may have fallen prey to birds, and one control butterfly was captured in a spider's web but later escaped. During October 1996, bird predation at the release point was high. Fourteen butterflies (four controls, six WL, and four CM) that were consumed were identified. Other butterflies fell prey to birds but could not be identified because the unique numbers on the wings were missing or damaged beyond recognition.

DISCUSSION

Morphometric determinants of flight performance

The associations among FMR, cm_{body} and flight performance that are observed among species in Neotropical butterfly communities (summarized in Srygley, 1994, table 1) were not supported by the experimental manipulations of morphological features within a single palatable species *Anartia fatima* reported here. Addition of weights representing 15% body mass to *A. fatima* did not have a consistent effect on flight performance, as measured by flight speed and the ability to evade capture, nor was there a detectable effect on survival. The only effects that the experimental addition of weights had were an increase in the probability of resampling butterflies that were known to be alive (i.e. recapture probability) and a reduction in dispersal rate relative to controls (Srygley, unpublished data).

Flight speeds of other experimentally loaded animals are consistently lower than those of unweighted ones (e.g. kestrels, Videler *et al.*, 1988; pigeons *Columba livia*, Gessaman & Nagy, 1988; Harris hawks *Parabuteo unicinctus*, Pennycuick *et al.*, 1989; long-eared bats *Plecotus auritus*, Hughes & Rayner, 1991; and honeybees *Apis mellifera*, Seeley, 1986). Without kinematic data for *A. fatima*, it is not possible to determine how they were able to accommodate the additional mass for most attributes of their performance. Recent work on bumblebees *Bombus lucernus* may lend insight into the ability of insects to generate the additional lift required to offset the added mass (Cooper, 1993). Hovering bumblebees that were loaded with honey-water (22% body mass, on average) increased their wingbeat frequency slightly. Foraging bumblebees were more heavily loaded (67% body mass, on average), and the additional lift to offset this increase in body weight came principally from an increase in the lift coefficient. As a result, changes in the angle of attack for the wing are presumed to have accommodated the majority of the additional mass in loaded bees. In slow climbing flight, qualitatively similar results were obtained: frequency increased by approximately 15%, and stroke amplitude increased by 16%, but an increase in the lift coefficient accounted for the majority of the additional required lift.

Butterflies may vary wingbeat frequency more than bumblebees in order to accommodate additional weight. For example, western white butterflies *Pontia occidentalis* (Pierinae) that had wing areas experimentally reduced by 15–20% increased wingbeat frequency by approximately 40% during hovering (Kingsolver, 1999). Therefore, the relative importance of changes in wingbeat frequency, which is much slower in butterflies (approximately 10–20 Hz) than bumblebees (approximately 200 Hz), stroke amplitude, and lift coefficient have yet to be investigated.

The large wing areas and low wing loading characteristic of butterflies may permit them to augment force production (Kingsolver, 1999). For example, butterflies and moths were capable of taking-off with FMR experimentally decreased to 14% by the addition of weights (Marden, 1987). This value is less than the FMR required for take-off in other insect orders (16–18%) with higher wing loadings (beetles, flies, bees, dragonflies, hawkmoths, Marden, 1987). Marden (1987) suggested that this difference may be due to the clap-and-fling mechanism for lift production that butterflies and moths share with damselflies (damselflies also had a relatively low FMR of 12% required for take-off). Note that the experimental FMR in the present study was 35%, well above the lower limit for take-off. Kingsolver and Srygley (2000) experimentally decreased FMR by 10–17% by adding inert weights to temperate *Colias* and *Pontia* butterflies, and showed that decreased FMR significantly reduced the probability of flight in the field.

Moreover, repositioning cm_{body} further posterior had no effect on manoeuvrability or survival. The manipulation positioned cm_{body} two standard deviations from the mean for recently emerged *A. fatima* (Table 1). For the CM males, the average non-dimensional cm_{body} was repositioned from 11.9% L_{body} (SD = 2.1%) when unloaded to 16.5% L_{body} (SD = 2.1%) when loaded. However, the objective was not to mimic the cm_{body} posterior to distasteful butterfly species, which may be as far as 35% L_{body} posterior to the wing base (Srygley, 1994). This more conservative experimental approach minimized disruption of the flight velocity that might have resulted from tilting the body angle and concomitantly the stroke plane angle backwards to an unnatural extent if cm_{body} was repositioned much further back. However, it limits extrapolation of the results to the effect of cm_{body} on the manoeuvrability of distasteful species.

No detectable difference in nectar feeding or flight behaviour was found between weighted and control individuals, suggesting that weighted butterflies did not require more food to fuel flight. In contrast, metabolic rate increased with loading for hovering bumblebees (Cooper, 1993) and pigeons (Gessaman & Nagy, 1988). Furthermore, weighted *A. fatima* butterflies were apparently no less capable of competing for nectar than controls.

Survival and resampling

Males survived longer than females in both MRR experiments. Between the two experiments, estimates for the survival probabilities did not differ significantly (males: 0.87 and 0.88; females: 0.78 and 0.80, for September and October respectively). Male western white *Pontia occidentalis* butterflies survived longer than females (Kingsolver, 1995, 1996, 1999) and this pattern is repeated in several *Colias* species (Coliadinae, Watt *et al.*, 1977). In palatable papilionid and pierid butterflies, Ohsaki

(1995) found that females were attacked by predators more frequently than males, as measured by the frequency of beak marks on the wings.

Including treatments in the survival probability model did not improve it significantly. However, estimates of survival probabilities differed significantly among the treatments during September 1996 within females (mean \pm SE, controls: 0.855 ± 0.043 ; WL: 0.688 ± 0.056 ; CM: 0.794 ± 0.040), but not within males (controls: 0.881 ± 0.022 ; WL: 0.888 ± 0.021 ; CM: 0.845 ± 0.027). Because survival probabilities were similar in males of all groups, addition of a weight to the thorax apparently did not interfere with the flexion of the thorax involved in the movement of the wings (Chapman, 1982). Kingsolver (1999) found no significant effects of experimental reductions in wing area (and hence, increases in wing loading without changing FMR) on either recapture or survival probabilities in *Pontia occidentalis* in eastern Washington. Similarly, Kingsolver and Srygley (2000) found no consistent effects of experimental reductions in FMR on dispersal rates or on recapture or survival probabilities in *Pontia occidentalis* or *Colias philodice* butterflies in eastern Washington.

Females have a larger body size and higher wing loading than males, which may, in part, explain the difference in survival between the sexes. Females may operate at nearer to maximum power output as a result of their heavier abdominal loads (Berrigan, 1991). Wallace (1865) hypothesized that this reproductive load led to greater selection on females relative to males, and hence, may in part explain sex-limited mimicry (also see Srygley & Chai 1990b; Ohsaki, 1995).

Control butterflies were resampled (resighted or recaptured) less often than the other treatments in the September MRR experiment. Perhaps control butterflies were more likely to flee when approached, and for this reason, they were resampled less often. Similarly, control butterflies may have been more dispersive. However, no difference in dispersal distance was found among the treatment groups in September (Srygley, unpublished data). Field observations suggested that individuals dispersed for a few days and then settled into areas that appeared much like home ranges. Hence, one would expect that resampling probabilities would increase during the first few days if they varied with the dispersive nature of the butterflies. In support of this hypothesis, resampling probabilities for all treatment groups tended to increase over time. Males tended to be resampled less often than females in the October MRR experiment. In this experiment, a significant difference in dispersal rates was found among the treatment groups (Srygley, unpublished data). However in this case, there was no tendency for resampling probabilities to increase over time.

Both mortality and dispersal contribute to estimates of survival. Hence, differential dispersal among treatment groups would bias estimates of survival. In a concurrent study, Srygley (unpublished data) found a difference in dispersal among weighted and unweighted treatment groups in one experimental release. During September 1996, when butterflies, particularly females, tended to differ in survival among treatments, there was no detectable difference in dispersal rates. However during October 1996, when treatment groups did not differ in survival, control butterflies dispersed significantly faster than weighted ones. Survival estimates for control butterflies are underestimated if these butterflies were more likely to disperse from the study site.

The survival analysis for October 1996 is particularly sensitive to dispersal because of the small sample sizes. If the survival of control butterflies was in fact greater

than that of weighted individuals, the difference in dispersal rates would only need to approximate the standard error of the survival estimates to make the differences due to mortality undetectable. During October 1996, if the difference in dispersal from the study site for control butterflies exceeded that for weighted ones by 1.4% for males and 2.8% for females, then differences among treatment groups would be undetectable. At the onset of the experiment, this difference was less than a single individual for each sex (0.56 males and 0.53 females), and declined with the number of surviving butterflies.

The study site was surrounded by habitats that were much less favourable for the survival of the butterflies, and this may have served to minimize the difference in dispersal out of the study site. Upon reaching the less favourable habitat, the butterflies migrating outward may have turned back towards the study site. As evidence that butterflies did not disperse regularly out of the study site, few marked butterflies were captured in the flower-rich habitat near to the lake shore (Fig. 1, zone d), even though a large fraction of the butterflies used in these experiments were captured in this highly suitable habitat.

The low dispersal rate of *A. fatima* may also minimize the effect of dispersal on the error in the survivorship estimates. In general, dispersal distances were much lower in September than October (Srygley unpublished data), which may have been due to overcast weather during the former month.

Reduced dispersal of WL and CM butterflies was consistent with the greater recapture probabilities of these groups relative to controls and the marginally significant change in flight velocity that we observed in one of the rodeos. *Anartia* butterflies occupy disturbed habitats in populations that are dense relative to many other tropical butterflies. Their maximum lifespan of approximately 22 days, high probability of resampling (45–85%, Figs 3 & 4), as well as the ease of resampling without direct interference (e.g. resightings rather than recaptures) make *Anartia* butterflies particularly amenable to future experimental analyses of flight performance and survival in the Neotropics.

ACKNOWLEDGEMENTS

We thank R. Aizprua, C. Batista, N. Castillo, and N. Zangen for assistance in the field, and the people of Gamboa for their patience with netters in their gardens. Measures of bird diversity on the study site would not have been possible without D. Robinson's expertise and interest. We also thank C. Ellington for discussion and A. Cooper for comments on the manuscript. The Autoridad Nacional del Ambiente (ANAM) granted permission to conduct the research. This project was funded by the National Geographic Society (to R.B.S.) and NSF grant IBN 9498150.

REFERENCES

- Arnold SJ. 1983.** Morphology, performance, and fitness. *American Zoologist* **23**: 347–361.
Berrigan D. 1991. Lift production in the flesh fly, *Neobellieria* (= *Sarcophaga*) *bullata* Parker. *Functional Ecology* **5**: 448–456.
Blake JG. 1992. Temporal variation in point counts of birds in a lowland wet forest in Costa Rica. *Condor* **94**: 265–275.

- Blondel J.** 1981. Point counts with unlimited distance. *Studies in Avian Biology* **6**: 414–420.
- Chai P.** 1986. Field observations and feeding experiments on the responses of rufous-tailed jacamars (*Galbula ruficauda*) to free-flying butterflies in a tropical rainforest. *Biological Journal of the Linnean Society* **29**: 161–189.
- Chai P.** 1990. Butterfly traits and bird responses: relationships between visual characteristics of rainforest butterflies and responses of a specialized insectivorous bird. In: Wicksten M, ed. *Adaptive Coloration in Invertebrates: Proceedings of Symposium Sponsored by American Society of Zoologists*. Galveston, Texas: Seagrant College Program, Texas A & M University, 31–60.
- Chai P, Srygley RB.** 1990. Predation and the flight, morphology, and temperature of Neotropical rainforest butterflies. *American Naturalist* **135**: 748–765.
- Chapman RF.** 1982. *The Insects: Structure and Function*. Cambridge, Massachusetts: Harvard University Press.
- Cooper AJ.** 1993. Limitations of bumblebee flight performance. Ph.D. dissertation, Cambridge University.
- Crespi BJ.** 1990. Measuring the effect of natural selection on phenotypic interaction systems. *American Naturalist* **135**: 32–47.
- Dudley R, Srygley RB.** 1994. Flight physiology of Neotropical butterflies: allometry of airspeeds during natural free flight. *Journal of Experimental Biology* **191**: 125–139.
- Ellington CP.** 1984a. The aerodynamics of hovering insect flight. II. Morphological parameters. *Philosophical Transactions of the Royal Society London B* **305**: 17–40.
- Ellington CP.** 1984b. The aerodynamics of hovering insect flight. III. Kinematics. *Philosophical Transactions of the Royal Society London B* **305**: 41–78.
- Ellington CP.** 1984c. The aerodynamics of hovering insect flight. V. A vortex theory. *Philosophical Transactions of the Royal Society London B* **305**: 115–144.
- Gessaman JA, Nagy KA.** 1988. Transmitter loads affect the flight speed and metabolism of homing pigeons. *Condor* **90**: 662–668.
- Hedenström A.** 1992. Flight performance in relation to fuel loads in birds. *Journal of Theoretical Biology* **158**: 535–537.
- Hughes PM, Rayner JMV.** 1991. Addition of artificial loads to long-eared bats *Plecotus auritus*: Handicapping flight performance. *Journal of Experimental Biology* **161**: 285–298.
- Karr JR, Robinson SK, Bierregaard RO Jr, Blake JG.** 1990. Birds of four neotropical forests. In: Gentry AH, ed. *Four Neotropical Rainforests*. New Haven, Connecticut: Yale University Press, 237–269.
- Kingsolver JG.** 1995. Viability selection on seasonally polyphenic traits: wing melanin pattern in western white butterflies. *Evolution* **49**: 932–941.
- Kingsolver JG.** 1996. Experimental manipulation of wing pigment pattern and survival in western white butterflies. *American Naturalist* **147**: 296–306.
- Kingsolver JG.** 1999. Experimental analyses of wing size, flight and survival in the western white butterfly. *Evolution* **53**: 1479–1490.
- Kingsolver JG, Smith SG.** 1995. Estimating selection on quantitative traits using capture-recapture data. *Evolution* **49**: 384–388.
- Kingsolver JG, Srygley RB.** 2000. Experimental analyses of body size, flight and survival in pierid butterflies. *Evolutionary Ecology Research* **2**: 593–612.
- Lynch JF.** 1995. Effects of point count duration, time-of-day, and aural stimuli on detectability of migratory and resident bird species in Quintana Roo, Mexico. In: Ralph CJ, Sauer, JR, Droege S, eds. *Monitoring Bird Populations by Point Counts*. USDA Forest Service General Technical Report PSW-GTR-149, 1–6.
- Marden JH.** 1987. Maximum lift production during takeoff in flying animals. *Journal of Experimental Biology* **130**: 235–258.
- Marden JH, Chai P.** 1991. Aerial predation and butterfly design: how palatability, mimicry, and the need for evasive flight constrain mass allocation. *American Naturalist* **137**: 15–36.
- McCullagh P, Nelder JA.** 1989. *Generalized Linear Models*. London: Chapman and Hall.
- Ohsaki N.** 1995. Preferential predation of female butterflies and the evolution of Batesian mimicry. *Nature (London)* **378**: 173–175.
- Olson EC, Miller RL.** 1958. *Morphological Integration*. Chicago: University of Chicago Press.
- Pennycuik CJ, Fuller MR, McAllister L.** 1989. Climbing performance of Harris' hawks (*Parabuteo unicinctus*) with added load: Implication for muscle mechanics and for radiotracking. *Journal of Experimental Biology* **142**: 17–29.

- Robinson WD, Brawn JD, Robinson SK. 2000.** Forest bird community structure in central Panama: Influence of spatial scale and biogeography. *Ecological Monographs* **70**: 209–235.
- Seeley TS. 1986.** Social foraging by honeybees: How colonies allocate foragers among patches of flowers. *Behavioral Ecology and Sociobiology* **19**: 343–354.
- Skalski JR, Hoffman A, Smith SG. 1993.** Testing the significance of individual- and cohort-level covariates in animal survival studies. In: Lebreton JD, North PM, eds. *Marked Individuals in the Study of Bird Populations*. Basel, Switzerland: Birkhauser, 9–28.
- Srygley RB, 1994.** Locomotor mimicry in butterflies? The associations of positions of centres of mass among groups of mimetic, unprofitable prey. *Philosophical Transactions of the Royal Society London B* **343**: 145–155.
- Srygley RB, Chai P. 1990a.** Predation and the elevation of thoracic temperature in brightly colored Neotropical butterflies. *American Naturalist* **135**: 766–787.
- Srygley RB, Chai P. 1990b.** Flight morphology of Neotropical butterflies: Palatability and distribution of mass to the thorax and abdomen. *Oecologia* **84**: 491–499.
- Srygley RB, Dudley R. 1993.** Correlations of the position of center of body mass with butterfly escape tactics. *Journal of Experimental Biology* **174**: 155–166.
- Srygley RB, Kingsolver JG. 1998.** Red-wing blackbird reproductive behaviour and the palatability, flight performance, and morphology of temperate pierid butterflies (*Colias*, *Pieris*, and *Pontia*). *Biological Journal of the Linnean Society* **64**: 41–55.
- Videler JJ, Vossebelt G, Gnodde M, Groenewegen A. 1988.** Indoor flight experiments with trained kestrels. I. Flight strategies in still air with and without added weight. *Journal of Experimental Biology* **134**: 173–183.
- Wallace AR. 1865.** On the phenomena of variation and geographical distribution as illustrated by the Papilionidae of the Malayan region. *Transactions of the Linnean Society London* **25**: 1–71.
- Watt WB, Chew FS, Snyder LRG, Watt AG, Rothschild DE. 1977.** Population structure of pierid butterflies. I. Numbers and movements of some montane *Colias* species. *Oecologia* **27**: 1–22.
- Witter MS, Cuthill IC. 1993.** The ecological cost of avian fat storage. *Philosophical Transactions of the Royal Society London B* **340**: 73–90.
- Witter MS, Cuthill IC, Bonser RHC. 1994.** Experimental investigations of mass-dependent predation risk in the European starling, *Sturnus vulgaris*. *Animal Behaviour* **48**: 201–222.