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Experimental analyses of body size, flight and survival in pierid butterflies

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ABSTRACT

To explore the consequences of allocation of body mass for flight and fitness in butterflies, we experimentally increased body weight and measured the effects on flight activity and survival in the field for two co-occurring pierid species (*Pontia occidentalis* and *Colias philodice*) that differ in palatability, mass allocation and flight speed. We predicted that increasing body weight would reduce flight activity in both *Pontia* and *Colias*, but that reductions in survival would be greater in the more palatable *Colias* than in the less palatable *Pontia*. Behavioural observations during three mark–recapture experiments showed that weighted butterflies flew less frequently than control butterflies in both *Pontia* and *Colias*. Adding weights significantly reduced survival in one study with *Pontia*, but not in a second study. Contrary to our predictions, adding weights had no significant effects on survival in the more palatable *Colias*. In combination with previous experimental manipulations of wing area, these results support the hypothesis that flight muscle ratio – the ratio of thoracic flight muscle to total body mass – is an important determinant of flight frequency in these species. However, the results do not indicate that flight muscle ratio is important for predator escape by these butterflies at our study sites. Different aspects of body size and shape may affect different components of butterfly flight, and seasonal variation in predation at our sites may modulate any relationship between flight and survival.

Keywords: body size, butterflies, flight, flight muscle ratio, mark–recapture, survival.

INTRODUCTION

How do body size and shape affect locomotory performance? One common approach to this central question in evolutionary morphology has been to examine aspects of locomotion that enable escape from potential predators. Because predator avoidance has a direct impact on fitness, one would expect morphometric traits that influence maximal locomotory performance and predator escape to be under strong if intermittent selection. The relationships among locomotion, predator escape and body, limb and tail size and shape have been explored in fish, lizards and a variety of other animals (Feder and Lauder, 1986). However, because morphometric traits are typically strongly correlated phenotypically and functionally, identifying how specific traits determine maximal locomotion

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and predator escape is problematic. In addition, such traits may have other effects on performance and fitness beyond their effects on predator escape, making it difficult to determine how selection may result in evolution of such traits.

Recent studies with neotropical butterflies have addressed some of these issues by comparing the morphometric and locomotory characteristics of unpalatable and palatable butterfly lineages (Chai and Srygley, 1990; Srygley and Chai, 1990b; Marden and Chai, 1991; Srygley and Dudley, 1993; Srygley, 1994). Unpalatable butterflies possess noxious chemicals that render them distasteful, and thus are rarely attacked by avian predators, whereas palatable butterflies must instead rely on crypsis and evasive take-off and flight manoeuvres to escape predators. Comparative analyses have indicated that palatable butterflies have relatively larger thoracic and flight muscle masses, higher wing loading, higher wing-beat frequencies and faster forward flight speeds than related unpalatable butterflies; these associations have evolved independently in several different butterfly lineages. However, palatable and unpalatable butterflies also differ in a host of other morphological and behavioural characteristics (Chai and Srygley, 1990; Srygley and Chai, 1990b; Marden and Chai, 1991; Srygley and Dudley, 1993; Srygley, 1994). Wing loading (total body mass/wing surface area), flight muscle ratio (thoracic flight muscle mass/total body mass) and the position of the centre of mass have all been proposed as major morphological determinants of evasive flight and predator escape, but the causal relationships between body size and shape, flight speed and predator escape remain undetermined.

Experimental manipulations of phenotype provide one important tool for establishing the causal relationships between morphology, performance and fitness (Arnold, 1983; Jayne and Bennett, 1989; Sinervo *et al.*, 1992; Kingsolver, 1996). Such manipulations have been used to explore some aspects of how wing and body morphology affect flight and survival in fitness. For example, Marden (1987) added weights near the centre of mass to a variety of insects and determined their effects on maximal lift production during take-off. Similarly, adding weights reduced flight ability and mating success of male dragonflies (Marden, 1989). Marden interpreted these results in terms of the effects of flight muscle ratio; however, these manipulations simultaneously alter wing loading as well. In the Western White Butterfly, *Pontia occidentalis*, experimental reductions in wing area (and hence increases in wing loading) increased wing-beat frequency during hovering, but did not significantly alter flight activity or survival in the field (Kingsolver, 1999).

Here we use experimental manipulations of mass to explore the causal relationships between body size, flight and survival in pierid butterflies (Family Pieridae) that differ in palatability. Studies at our field sites in central Washington demonstrate that *Colias philodice* butterflies (subfamily Coliadinae) have significantly greater palatability to avian predators than *Pontia occidentalis* butterflies (subfamily Pierinae) (Srygley and Kingsolver, 1998). In addition, *Colias philodice* have a higher wing loading, a greater thoracic and flight muscle mass relative to body mass, and a greater capacity to escape potential predators than *Pontia occidentalis*. By adding weights at the centre of mass of the butterfly, we can determine experimentally whether decreasing flight muscle ratio decreases flight performance in *Pontia* or *Colias* butterflies in the field. If flight muscle ratio determines successful escape from predators in this system, we predict that these experimental manipulations will have a greater impact on survival in the palatable *Colias philodice* than in the less palatable *Pontia occidentalis*.

MATERIALS AND METHODS

Study system

Our studies involve two species of pierid butterflies that co-occur in many areas of western North America. *Pontia occidentalis* Reakirk (subfamily Pierinae), the Western White Butterfly, is a common butterfly throughout western North America, primarily at higher elevations and latitudes. It occurs primarily in open, meadow habitats, where its larval host plants are various herbaceous Crucifers. *Colias philodice* L. (subfamily Coliadinae), the Common Sulphur Butterfly, is a common butterfly in many open habitats throughout North America, where it feeds on a variety of weedy and agricultural leguminous larval host plants. In eastern Washington state (including our study site) and many other regions where *C. philodice* is abundant, this species co-occurs and often hybridizes with *Colias eurytheme* Boisduval, the Orange Sulphur; the resulting hybrids are diverse and difficult to classify. These two *Colias* species are very similar ecologically and morphologically. In the present paper, we use the name *Colias philodice* to refer collectively to *C. philodice* and their likely hybrids with *C. eurytheme* at our study site.

The field research on these species described here was conducted at the Corfu site in central Washington in the Columbia National Wildlife Refuge (Grant Co., Washington) (Kingsolver, 1995a,b). The site, approximately 10 ha in area, consists of dense stands of weedy host plants and nectar sources for these butterflies surrounded by regions of dry sage-brush scrub. The site supports dense adult populations (500–5000 adults) of *P. occidentalis* during April–August, and frequently supports sizeable populations (~500–2000) of *C. philodice* in July and August. There is a cattail marsh in the centre of the site in which Redwing Blackbirds breed each year.

The available evidence on palatability of pierid butterflies to avian predators suggests that there are important differences in palatability among different pierids. Neotropical studies have indicated that coliadines typically have higher palatabilities to avian predators than pierines (Chai, 1986, 1990). Paired-choice experiments using Canadian Jays in Colorado showed that *Pontia occidentalis* have similar (Ley and Watt, 1989) or somewhat lower (Kingsolver, 1987) palatabilities than the *Colias* species that occur in the same habitats. However, our feeding studies on the territories of Redwing Blackbirds nesting at the Corfu study site suggest that *Pontia* have significantly lower palatability than *Colias*, although *Pontia* are taken regularly during late May when birds are feeding young at the nest (Srygley and Kingsolver, 1998). Because of the higher palatability of *Colias*, we would expect that experimental manipulations that alter flight performance relevant to predator escape will have a greater impact on survival in *Colias* than in *Pontia*. In addition, *Colias* have greater flight muscle ratios and higher wing loading, and are more difficult to capture by human 'predators' than *Pontia* (Srygley and Kingsolver, 1998); these are typical of the morphometric and locomotory differences commonly seen between palatable and unpalatable groups (Chai and Srygley, 1990; Srygley and Chai, 1990b; Marden and Chai, 1991; Srygley and Dudley, 1993; Srygley, 1994).

Measuring and manipulating morphology

We experimentally increased total body mass in *Pontia occidentalis* and *Colias philodice* to evaluate the effects of flight muscle ratio and wing loading on flight performance and

survival. For each butterfly, the pubescent scales on the dorsum of the posterior metathorax were removed. The butterfly was then subjected to one of two experimental treatments: 'added-weight', in which a small piece of lead solder (see below) was glued on the dorsum of the posterior metathorax near the butterfly's centre of mass; and 'controls', on which a small drop of glue was placed on the dorsum of the posterior metathorax near the butterfly's centre of mass. The control treatment thus controlled for the effects of handling and of glue on the butterfly. The total mass of each butterfly before and after the manipulation was measured to ±1 mg on an electronic balance (Fisher XT); the length of the right forewing was also measured with a hand-rule.

The 'added-weight' manipulations increased total mass by an average of 16.6% (males) and 14.2% (females) of *P. occidentalis* in the 1994 study, 10.4% (males) and 11.3% (females) of *P. occidentalis* in the 1995 study, and 15% (males and females) of *C. philodice* in the 1996 study (see next section). These represent changes in mass of about 0.8–1.0 standard deviations in males and 0.5–0.8 standard deviations in females (standard deviations estimated from control individuals in each study). Thus, these manipulations represent a substantial increase in total mass (and hence decrease in flight muscle ratio and increase in wing loading), but well within the observed range of phenotypic variation in mass seen in these populations. Note that this manipulation both increases wing loading and decreases flight muscle ratio, and thus cannot distinguish between these two different hypotheses about the morphological determinants of flight and predator escape. However, we have previously used experimental reductions of wing area (and thus increases in wing loading) with *P. occidentalis* in this population. The combination of these two experiments can thus provide information that will allow us to detect the separate effects of wing loading and flight muscle ratio in this species (see Discussion).

In the first study with *P. occidentalis* in 1994, we used superglue (Duco) in these experiments; however, we found that 15 of 210 butterflies (7.1%) in the 'added-weight' treatment group lost their weights, in nearly all cases before the first recapture. This experimental failure provided an interesting additional control: we could ask whether 'added-weight' individuals that immediately lost their weights had similar recapture and survival probabilities to control individuals (see below). In subsequent experiments with *P. occidentalis* and with *C. philodice*, we used contact cement (Duro); in these studies, only a handful of 'added-weight' individuals lost their weights, and these individuals were excluded from the analyses.

Mark–recapture studies

We use mark–release–recapture (MRR) experiments on manipulated butterflies to estimate flight activity, initial dispersal rates, and survival and recapture probabilities of each species. Our MRR studies were conducted at the Corfu site using a multiple-release, multiple-recapture method on cohorts of adults; only butterflies with little or no initial wing wear before treatment (wing wear classes 1 or 2: see Kingsolver, 1995a,b) were used in these studies.

For each study, wild butterflies at the study site were captured with hand nets, placed in glassine envelopes, and placed in a cooler. Later the same day, the butterflies were taken to our field laboratory near Corfu (Othello, WA, USA) and, within each sex, were haphazardly assigned to the 'added-weight' or control treatment groups. An identification number was written with a permanent felt-tip marker (extra-fine Sharpie) on the medial region of both

ventral forewings of each butterfly. All butterflies were then released in the central region of the study site in the evening of capture. Capture, marking and release continued for 4–5 consecutive days during each experiment, until at least 400 individuals in the population had been marked and released (see below).

Intensive recapture efforts were made every day following the initial release, for 4–6 hours per day by each of 2–4 observers (8–20 person-hours per day). Recapturing was done by spending fixed amounts of time in each of 12 subsites (13 sub-sites for *Colias*), including areas of both high and low densities of host plants and nectaring plants. Upon recapture, the time and location of recapture and the wing wear were noted for each individual. Recapture efforts continued daily for 9–10 days; the experiment was terminated when the number of recaptured individuals on a sample day was less than 1–2% of the total number of individuals marked in the population.

Three separate MRR studies were conducted, two with *Pontia occidentalis* and one with *Colias philodice*:

- 8–16 June 1994 with *P. occidentalis* ($n = 210$ 'added-weight' and 213 control butterflies);
- 15–26 May 1995 with *P. occidentalis* ($n = 166$ 'added-weight' and 178 control butterflies); and
- 18–28 June 1996 with *C. philodice* ($n = 175$ 'added-weight' and 177 control butterflies).

Behavioural activities and dispersal rates

Behavioural observations of the individual upon first sighting were also made as part of the recapture studies. Behaviour was assigned to five categories: basking, flight, nectar-feeding, resting and startle (take-off and flight after apparent disturbance by the observer). Because we were interested in how weather conditions may affect the flight behaviour of 'added-weight' and control individuals in different ways, we compared differences in flight behaviour in morning (09:00–11:00 Pacific daylight time, PDT) versus afternoon (12:00–14:00 PDT) time periods during the June 1994 and May 1995 studies with *P. occidentalis*, and morning (07:30–10:00 PDT) versus midday (11:00–13:00 PDT) time periods during the June 1996 study with *C. philodice*. The effects of treatment and of interactions between time period and 'added-weight' and control treatment in influencing flight activity are of particular interest. To evaluate this, we used generalized linear models to analyse the probability that a recaptured individual was observed in flight upon first sighting, where presence or absence of flight was treated as a binomially distributed variable (McCullagh and Nelder, 1989). The effects of time period, date, sex, treatment and their interactions were estimated using Splus Statistical Software.

We used our data on movements among subsites following the initial release to determine whether the experimental treatments altered the rates of local dispersal within the study population. We classified our 12 subsites (13 for *Colias*) into four (ordered) distance categories (0–3) that index the distance of each subsite from the central subsites in which all butterflies were initially released, and defined four (ordered) time periods (morning and afternoon on each of 2 days) for the 2 days following the initial release of each butterfly. Using the recapture data, we then created a multi-way contingency table giving the number of recaptured individuals for each date as functions of initial release, sex, treatment group, distance and time period, and fit a surrogate Poisson log-linear model to these data (McCullagh and Nelder, 1989; Venables and Ripley, 1997). Analysis of deviance

was used to test whether treatment group had a significant effect on dispersal rates, as identified by significant interactions between treatment and distance or between treatment and time.

Estimating survival and recapture probabilities

Mark–recapture data can be used to estimate two key parameters: survival probabilities and recapture probabilities. More formally, let ϕ_{ik} be the conditional probability that an individual of class k survives to day $i+1$ given survival up to day i. Similarly, let P_{ik} be the conditional probability that an individual of class k is recaptured on day i, given that it is alive. [We note that although the term 'survival' is typically used, in general (and in the present case) one cannot distinguish between mortality and permanent emigration from the study site: but see Discussion.] Using generalizations or special cases of the Cormack-Jolly-Seber (CJS) model, one can use maximum likelihood methods to evaluate how survival or recapture probabilities vary among time periods and among phenotypic classes (e.g. between treatment groups or between sexes) (see Manly, 1985, for a discussion).

The mark–recapture analyses reported here were conducted using the SURPH software program (Skalski *et al.*, 1993). We used a logit (log-odds) link function to model both survival and recapture probabilities, one of a family of possible link functions that are appropriate for such data (Manly, 1976, 1977; Williams, 1990; Lebreton *et al.*, 1992). Our goal was to estimate survival and recapture probabilities for each treatment group, sex and time period, and to test the possible effects of treatment group on survival and recapture probabilities. All model parameters were estimated simultaneously by maximizing the likelihood function.

We follow Lebreton *et al.* (1992) in our modelling procedures. We first compute the likelihood L of the model where survival and recapture probabilities depend on time period, sex, treatment and their interactions; the estimates of survival and recapture probabilities from this model are identical to those from the standard CJS model, except that the CJS model does not constrain these probabilities to values ≤1.0. We then consider various special cases of this model, in which time, sex, treatment and/or interaction effects are omitted, first for modelling recapture probabilities and then for modelling survival probabilities. For each model, we compute the Akaike Information Criterion (AIC) = $-2\ \ln(L) + 2p$, where p is the number of parameters in the model. The AIC represents a compromise between the total likelihood of (support for) the model given the data and the number of parameters required to achieve that likelihood. We define the 'best' model as that with the lowest AIC (see Lebreton *et al.*, 1992, for a discussion of this approach). We use likelihood ratio tests to evaluate whether the 'added-weight' treatment contributes significantly to the likelihood. We also report the estimates of the recapture and survival probabilities for the best model for each study, as identified by the AIC.

A primary goal of these analyses was to estimate whether the 'added-weight' and control treatment groups differ in recapture or survival probabilities. However, as noted above, in the June 1994 study, 7% of the individuals in the 'added-weight' group lost their weights before first recapture, whereas the rest kept their weights throughout the study. In effect, this experimental failure created two sets of 'added-weight' individuals: those that kept their weights throughout the study ('weight-on') and those that lost their weights soon after release ('weight-off'). This provides a second 'control' in this experiment for the possible

effects of handling and glueing the butterflies. Thus, in our analyses of the 1994 study, we consider three groups – 'weight-on', 'weight-off' and control – and model whether recapture and survival probabilities of the 'weight-off' group are similar to those of the control group. Some 'added-weight' individuals were never recaptured, so we could not assess whether they retained their weights; for the 1994 analyses, we randomly assigned 7% of these individuals to the 'weight-off' group, and the remainder to the 'weight-on' group. Subsequent analyses showed that excluding these individuals from the analysis did not affect our qualitative results or conclusions.

RESULTS

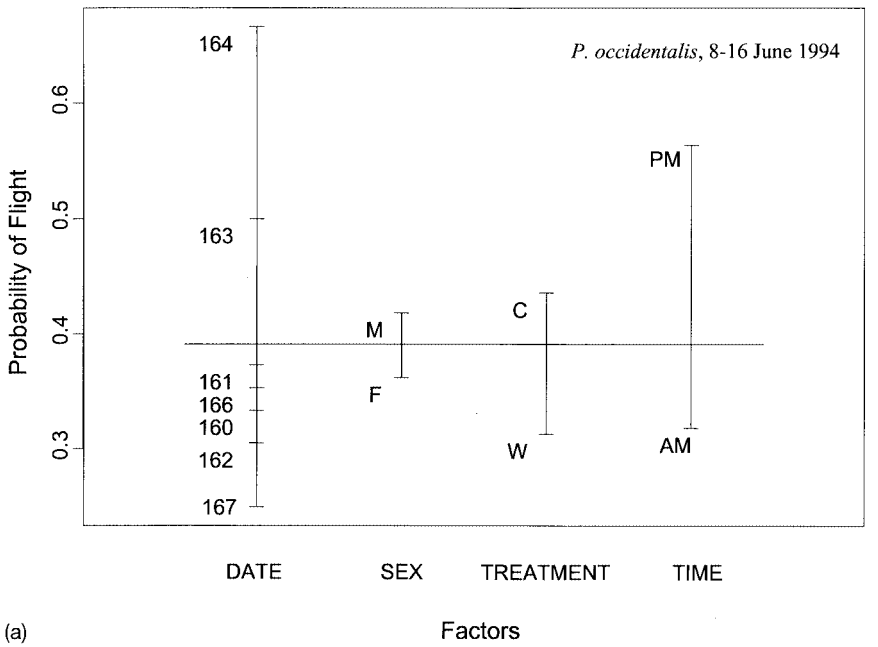
Flight performance: Flight activity and dispersal rates

Analyses of the probability of flight prior to recapture during the MRR studies suggested several consistent patterns across the three studies (Fig. 1a–c). First, there was considerable variation in flight probabilities between days within each study, probably associated with daily variation in weather conditions. Second, flight probabilities were consistently greater for males than females, and greater in afternoon than morning time periods, although the magnitude of these effects varied among studies. Third, flight probabilities were greater for control butterflies than for 'added-weight' butterflies in each study. Generalized linear modelling (Table 1a–c) showed that the effects of the 'added-weight' treatment on flight probabilities were significant in the June 1994 (*P. occidentalis*) and the June 1996 (*C. philodice*) studies, but not in the May 1995 (*P. occidentalis*) study.

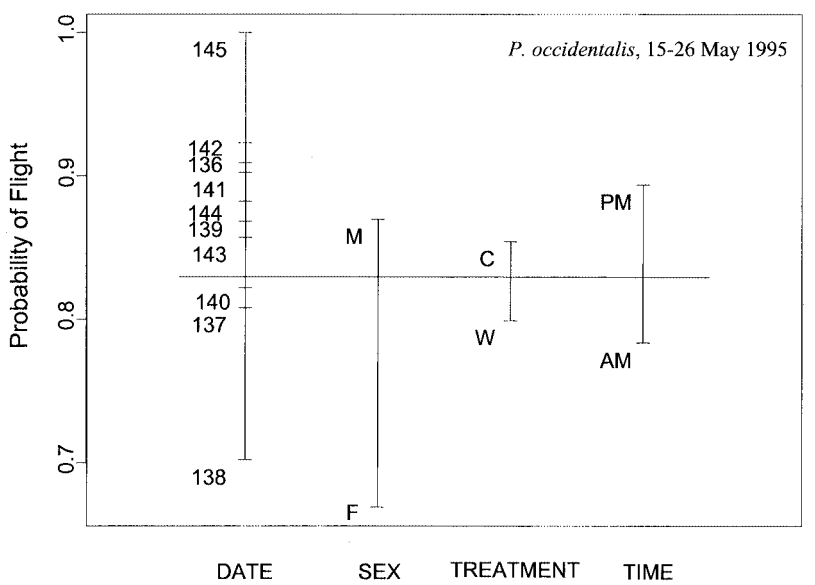
Analyses of deviance of the rate of dispersal from the initial subsite of release for each study are given in Table 2. Recall that our main interest is in possible effects of the 'added-weight' treatment (relative to the control group) on dispersal rates, as indicated by the interaction of experimental treatment with distance or with time period. In none of the three studies were these interactions significant, suggesting that the 'added-weight' and control treatment groups had similar initial dispersal rates in these studies.

Recapture and survival probabilities

The experimental manipulations of body mass provide a direct way of assessing the effects of flight muscle ratio and wing loading on recapture and survival. The values for the AIC, our criterion for identifying the best model for the MRR studies, are presented in Tables 3–5. For the June 1994 study with *P. occidentalis* (Table 3), the best model of recapture probabilities included effects of the 'weight-on' treatment and time period, no effect of sex, and no interaction between time period and treatment. (Interestingly, 'weight-off' and control individuals had similar recapture and survival probabilities in this study.) 'Weight-on' butterflies had consistently higher recapture probabilities than control butterflies throughout the 1994 study (Fig. 2), and omitting 'weight-on' effects from the recapture model marginally decreased the model's likelihood ($\chi^2 = 3.148$, d.f. = 1, $P = 0.07806$). Similarly, the best model of survival probabilities in this study included effects of the 'weight-on' treatment, no effects of time period or sex, and no interaction between time period and treatment. Survival probabilities (ϕ) were consistently lower for 'weight-on' butterflies ($\phi = 0.390 \pm 0.040$) than for control butterflies ($\phi = 0.557 \pm 0.032$) in this study, and omitting 'weight-on' effects from the survival model significantly decreased the model's

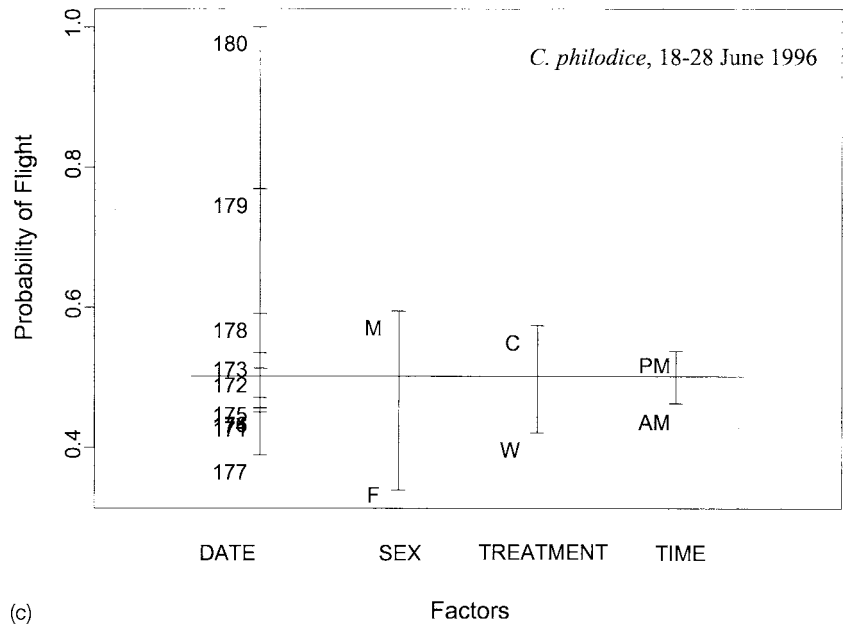


(a)



(b)

Fig. 1. Mean probabilities of flight for pierid butterflies as functions of date (Julian date), sex (M = males, F = females), treatment group (C = control, W = 'added-weight'), time of day (AM = morning, PM = afternoon) in the mark–recapture studies at Corfu, WA, 1994–96. (a) 8–16 June 1994 study with *Pontia occidentalis*; (b) 15–26 May 1995 study with *Pontia occidentalis*; (c) 18–28 June 1996 study with *Colias philodice*.



(c)

likelihood ($\chi^2 = 10.079$, d.f. = 1, $P = 0.00150$). Thus, the 'weight-on' treatment consistently increased recapture and decreased survival of *P. occidentalis* butterflies in the May 1994 study.

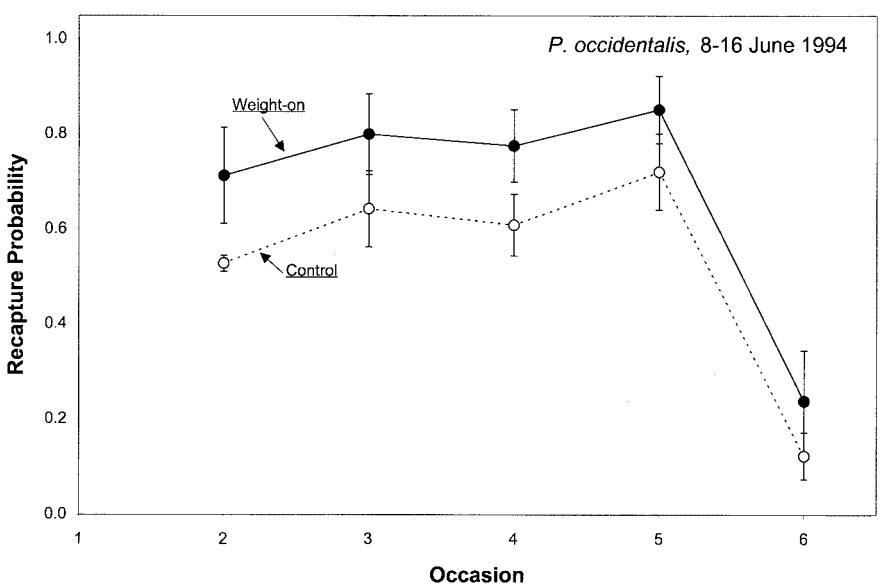


Fig. 2. Conditional probabilities of recapture (given alive) estimated for control (open circles, dashed line) and 'weight-on' (solid circles, solid line) *Pontia occidentalis* butterflies at Corfu, WA, in the 8–16 June 1994 study. The estimates are for the model yielding the lowest AIC value for the data (see Table 3). Error bars indicate standard errors. Occasions: 1 = 9 June, 2 = 10 June, 3 = 11 June, 4 = 12 June, 5 = 13 June, 6 = 14–15 June.

Table 1. Analysis of deviance tables for the probability of flight for 'added-weight' versus control treatments of pierid butterflies at Corfu, WA

	d.f.	Deviance	Residual d.f.	Residual deviance	$Pr(\chi)$
June 1994 study with *Pontia occidentalis*					
Null			316	424.3155	
Date	6	10.49838	310	413.8171	0.1051731
Sex	1	1.69512	309	412.1220	0.1929275
Treatment	1	5.60143	308	406.5206	0.0179458
Time	1	20.37518	307	386.1454	0.0000064
Sex × treatment	1	2.14369	306	384.0017	0.1431577
Sex × time	1	0.08630	305	383.9154	0.7689358
Treatment × time	1	1.08547	304	382.8299	0.2974770
Sex × treatment × time	1	1.43227	303	381.3977	0.2313939
May 1995 study with *Pontia occidentalis*					
Null			495	460.6363	
Date	9	26.06342	486	434.5729	0.0019947
Sex	1	17.96286	485	416.6100	0.0000225
Treatment	1	2.44749	484	414.1625	0.1177132
Time	1	4.56915	483	409.5934	0.0325527
Sex × treatment	1	0.80412	482	408.7893	0.3698635
Sex × time	1	0.32201	481	408.4673	0.5704032
Treatment × time	1	0.06301	480	408.4043	0.8018029
Sex × treatment × time	1	0.02315	479	408.3811	0.8790759
June 1996 study with *Colias philodice*					
Null			306	425.5891	
Date	9	12.56143	297	413.0277	0.1834754
Sex	1	17.55711	296	395.4706	0.0000279
Treatment	1	5.11035	295	390.3602	0.0237835
Time	1	4.24584	294	386.1144	0.0393465
Sex × treatment	1	0.07396	293	386.0404	0.7856592
Sex × time	1	0.02149	292	386.0189	0.8834513
Treatment × time	1	0.00025	291	386.0187	0.9872855
Sex × treatment × time	1	0.21322	290	385.8054	0.6442535

The results for the May 1995 study with *P. occidentalis* were quite different (Fig. 3; Table 4). For this study, the best model of recapture probabilities included effects of the 'added-weight' treatment, of time period and interactions between time and treatment, but no effect of sex. 'Added-weight' butterflies had higher recapture probabilities than control butterflies in some time periods, but lower recapture probabilities in other time periods (Fig. 3); averaged over the entire study, the treatments did not differ substantially in recapture probabilities. Omitting 'added-weight' effects from this recapture model significantly decreased the model's likelihood in this study ($\chi^2 = 29.035$, d.f. = 7, $P = 0.000143$). The best model of survival probabilities in this study included effects of sex and time period, no effect of treatment, and no interactions between time period and sex. The

Table 2. Analysis of deviance tables for the probability of dispersal for 'added-weight' versus control treatments of pierid butterflies at Corfu, WA

	d.f.	Deviance	Residual d.f.	Residual deviance	$Pr(\chi)$
June 1994 study with *Pontia occidentalis*					
Null			191	527.7560	
Date	3	24.3980	188	503.3580	0.0000206
Sex	1	0.0046	187	503.3534	0.9458776
Treatment	1	11.1605	186	492.1929	0.0008356
Distance	2	210.0944	184	282.0984	0.0000000
Period	3	107.4388	181	174.6597	0.0000000
Treatment × distance	2	0.8161	179	173.8436	0.6649498
Treatment × period	3	1.6419	176	172.2017	0.6499318
Distance × period	6	7.6131	170	164.5886	0.2678412
Treatment × distance × period	6	3.5008	164	161.0878	0.7438588
May 1995 study with *Pontia occidentalis*					
Null			383	951.4685	
Date	5	144.6466	378	806.8220	0.0000000
Sex	1	86.5285	377	720.2934	0.0000000
Treatment	1	1.3095	376	718.9840	0.2524917
Distance	3	259.0635	373	459.9205	0.0000000
Period	3	50.7917	370	409.1288	0.0000000
Treatment × distance	3	3.7018	367	405.4269	0.2955110
Treatment × period	3	1.4998	364	403.9271	0.6823149
Distance × period	9	56.8944	355	347.0327	0.0000000
Treatment × distance × period	9	8.3389	346	338.6938	0.5003865
June 1996 study with *Colias philodice*					
Null			319	760.7623	
Date	4	3.5197	315	757.242	0.4748839
Sex	1	27.4203	314	729.8223	0.0000002
Treatment	1	0.0133	313	729.8089	0.9080722
Distance	3	301.8192	310	427.9897	0.0000000
Period	3	74.2008	307	353.7889	0.0000000
Treatment × distance	3	3.5869	304	350.2020	0.3096639
Treatment × period	3	1.8027	301	348.3993	0.6143542
Distance × period	9	52.2870	292	296.1123	0.0000000
Treatment × distance × period	9	14.0353	283	282.0770	0.1210748

survival probabilities of 'added-weight' butterflies ($\phi = 0.788 \pm 0.029$ for females, $\phi = 0.634 \pm 0.047$ for males) were similar to those for control butterflies ($\phi = 0.743 \pm 0.031$ for females, $\phi = 0.598 \pm 0.056$ for males), and adding 'added-weight' effects to the survival model did not significantly increase the model's likelihood in this study ($\chi^2 = 16.011$, d.f. = 14, $P = 0.30336$). Thus, in contrast to the 1994 study, for the 1995 study the 'added-weight' treatment did not consistently increase recapture or decrease survival probabilities in *P. occidentalis*.

Table 3. The number of parameters and AIC values for recapture and survival probability models including (+) or excluding (0) effects of treatment (see text), 'weight-on', sex, time, first-order interactions, and the product of recapture and survival for the final time period (final Pϕ), for *Pontia occidentalis* at Corfu, WA, in June 1994

Treatment	Weight	Sex	Time	Treatment × time	Weight × time	Sex × time	Final Pϕ	No. of parameters	AIC
Recapture (P)									
+	—	+	+	+	—	+	+	66	1097.76
+	—	+	+	0	0	0	+	46	1075.3
+	—	+	0	0	0	0	+	42	1085.62
0	0	0	+	0	0	0	+	41	1073.79
0	0	+	+	0	0	+	+	46	1080.97
+	—	0	+	+	—	0	+	51	1074.77
0	+	0	+	0	0	0	+	46	1072.96
0	0	+	+	0	0	0	+	42	1074.93
+	—	0	+	0	0	0	+	43	1072.58
0	**+**	**0**	**+**	**0**	**0**	**0**	**+**	**42**	**1071.88***
0	0	+	0	0	0	0	+	38	1084.08
+	—	0	0	0	0	0	+	39	1082.92
0	+	0	0	0	0	0	+	38	1081.31
0	0	0	0	0	0	0	+	37	1083.22

Survival (ϕ)										
	+	—	+	+	0	0	0	+	22	1053.97
	0	—	+	+	0	0	+	+	22	1060.09
	+	—	0	+	+	—	0	+	27	1061.19
	0	+	0	+	0	+	0	+	22	1053.92
	0	0	+	+	0	0	0	+	18	1059.25
	+	—	0	+	0	0	0	+	19	1050.42
	0	+	0	+	0	0	0	+	18	1048.42
	+	—	+	0	0	0	0	+	18	1053.68
	0	—	0	+	0	0	0	+	17	1058.15
	0	0	+	0	0	0	0	+	14	1057.55
	+	—	0	0	0	0	0	+	15	1049.96
	0	**+**	**0**	**0**	**0**	**0**	**0**	**+**	**14**	**1048.17***
	0	0	0	0	0	0	0	+	13	1056.25
Best model										
P→	**0**	**+**	**0**	**+**	**0**	**0**	**0**	**+**	**14**	**1048.17**
ϕ→	**0**	**+**	**0**	**0**	**0**	**0**	**0**			

Note: The model indicated in **bold** yielded the lowest AIC value (*).

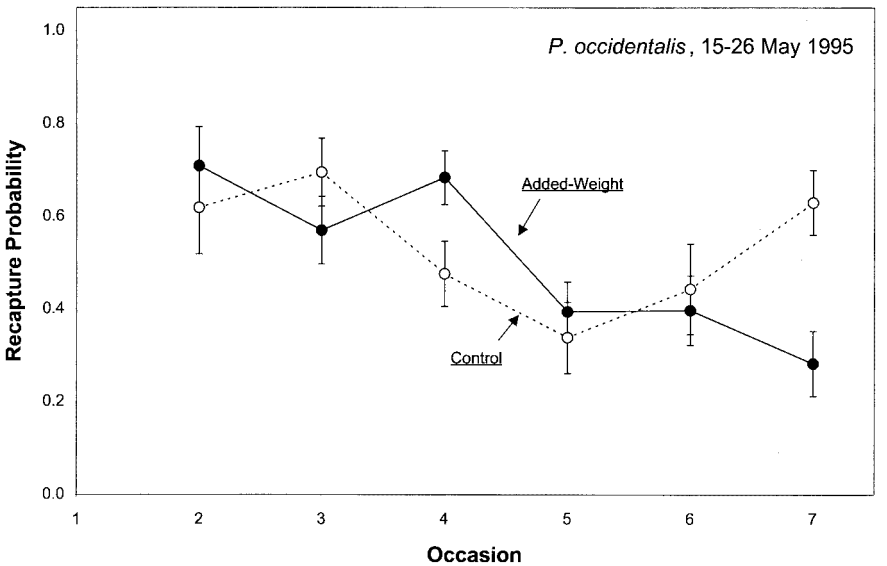


Fig. 3. Conditional probabilities of recapture (given alive) estimated for control (open circles, dashed line) and 'added-weight' (solid circles, solid line) *Pontia occidentalis* butterflies at Corfu, WA, in the 15–26 May 1995 study. The estimates are for the model with the lowest AIC value for the data that included treatment effects on both survival and recapture (see Table 4). Error bars indicate standard errors. Occasions: 1 = 16 May, 2 = 17 May, 3 = 18 May, 4 = 19 May, 5 = 20 May, 6 = 21–22 May, 7 = 23–25 May.

For the June 1996 study with *Colias philodice*, the best model of recapture probabilities included effects of time period, but no effects of the 'added-weight' treatment or sex (Fig. 4; Table 5). Recapture probabilities for 'added-weight' and control butterflies were similar throughout the study period (Fig. 4), and adding 'added-weight' effects to this recapture model did not significantly increase the model's likelihood in this study ($\chi^2 = 0.038$, d.f. = 1, $P = 0.845$). The best model of survival probabilities in this study did not include effects of treatment, sex or time period. The survival probabilities of 'added-weight' butterflies ($\phi = 0.657 \pm 0.028$) and control butterflies ($\phi = 0.654 \pm 0.026$) were very similar in this study, and adding 'added-weight' effects to the survival model did not significantly increase the model's likelihood ($\chi^2 = 0.038$, d.f. = 1, $P = 0.845$). Thus, for the 1996 study with *Colias*, the 'added-weight' treatment had no detectable effects on either recapture or survival probabilities.

DISCUSSION

Body size and flight in butterflies

Most aspects of body size are positively correlated phenotypically in butterflies (Chai and Srygley, 1990; Srygley and Chai, 1990a,b), including in *Pontia* and *Colias* (Srygley and Kingsolver, 1998). These positive correlations limit the range of natural phenotypic variation in flight muscle ratio, wing loading and other morphological features relevant to

Table 4. The number of parameters and AIC values for recapture and survival probability models including (+) or excluding (0) effects of 'added-weight' (weight), sex, time, first-order interactions, and the product of recapture and survival for the final time period (final Pϕ), for *Pontia occidentalis* at Corfu, WA, in May 1995

Weight	Sex	Time	Weight × time	Sex × time	Final Pϕ	No. of parameters	AIC
Recapture (P)							
+	+	+	+	+	+	60	1471.58
+	+	+	0	0	+	42	1468.33
+	+	0	0	0	+	36	1475.82
0	0	+	0	0	+	39	1465.10
0	+	+	0	+	+	46	1465.04
+	**0**	**+**	**+**	**0**	**+**	**46**	**1463.66***
0	+	+	0	0	+	40	1464.96
+	0	+	0	0	+	40	1467.00
0	+	0	0	0	+	34	1472.82
+	0	0	0	0	+	34	1474.36
0	0	0	0	0	+	33	1472.58
Survival (ϕ)							
+	+	+	0	0	+	33	1454.65
0	+	+	0	+	+	32	1449.58
+	0	+	+	0	+	32	1472.06
0	+	+	0	0	+	26	1452.62
+	0	+	0	0	+	26	1465.71
+	+	0	0	0	+	22	1446.76
0	0	+	0	0	+	25	1465.67
0	**+**	**0**	**0**	**0**	**+**	**20**	**1444.21***
+	0	0	0	0	+	20	1457.01
0	0	0	0	0	+	19	1456.20
Best model							
P→ **+**	**0**	**+**	**+**	**0**	**0**	**17**	**1438.58**
ϕ→ **0**	**+**	**0**	**0**	**0**			

Note: The model indicated in **bold** yielded the lowest AIC value (*).

flight performance, and reduce our ability to identify the morphological determinants of flight and survival. Here we have used experimental increases in body weight to evaluate more directly how changes in body mass may affect aspects of flight and survival. Previous experimental manipulations of wing area with *Pontia occidentalis* at our study site indicated that increased wing loading did not detectably alter flight, recapture or survival probabilities (Kingsolver, 1999). As a result, we can interpret the effects of our manipulations of weight in the current study in terms of effects of flight muscle ratio (Marden, 1987, 1989; Marden and Chai, 1991).

Our observations of flight activity during the MRR studies indicate that experimentally increasing body mass decreased the probability of flight in each of the three studies, with a statistically significant effect detected in two of the three studies (Fig. 1; Table 1). The

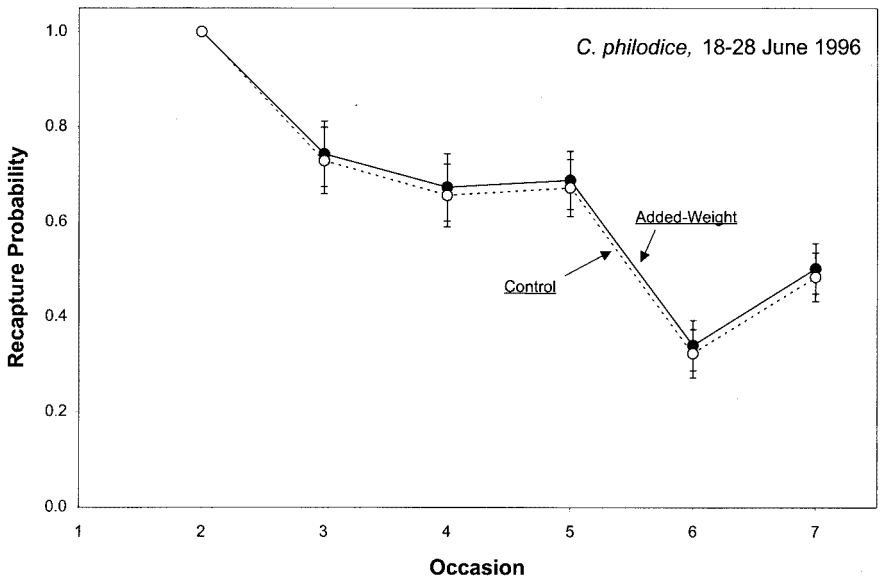


Fig. 4. Conditional probabilities of recapture (given alive) estimated for control (open circles, dashed line) and 'added-weight' (solid circles, solid line) *Colias philodice* butterflies at Corfu, WA, in the 18–28 June 1996 study. The estimates are for the model with the lowest AIC value for the data that included treatment effects on both survival and recapture (see Table 5). Error bars indicate standard errors. Occasions: 1 = 19 June, 2 = 20 June, 3 = 21 June, 4 = 22 June, 5 = 23 June, 6 = 24 June, 7 = 25–27 June.

smaller effect (in both magnitude and statistical significance) during the 1995 study with *P. occidentalis* is associated with the smaller experimental alteration of body mass in this study (10–11% increase) compared with the 1994 (14–16% increase) and 1996 (15% increase) experiments (see Methods). These results suggest that decreased flight muscle ratio reduces the frequency or extent of flight activity in the field in both *Pontia* and *Colias*.

Our results are consistent with previous experimental studies with other insects, indicating that flight muscle ratio affects take-off angle, maximal lift production and mating success (Marden, 1987, 1989). However, the effects of flight muscle ratio on flight speed and manoeuvrability remain unclear. For example, in ruby-throated hummingbirds, reduced body mass did not alter maximum forward flight speeds in a wind tunnel (Chai and Dudley, 1999).

Flight muscle ratio could affect flight activity in our studies in a variety of ways. For example, decreasing flight muscle ratio could increase the energetic costs of flight, leading to less flight and more time spent nectaring to replenish energy stores. However, our analyses of the probability of nectaring did not detect significant differences between treatment groups in nectaring probability in any of the three studies; indeed, nectaring probability was higher for the control than for the 'weight-on' group in the May 1994 study. Flight muscle ratio could also affect forward flight speed (Srygley and Dudley, 1993), the minimum temperature threshold for flight (Srygley and Chai, 1990a,b) and other features that would have an impact on flight probabilities as measured here.

Table 5. The number of parameters and AIC values for recapture and survival probability models including (+) or excluding (0) effects of 'added-weight' (weight), sex, time, first-order interactions, and the product of recapture and survival for the final time period (final Pϕ), for *Colias philodice* at Corfu, WA, in June 1996

Weight	Sex	Time	Weight × time	Sex × time	Final Pϕ	No. of parameters	AIC
Recapture (P)							
+	+	+	+	+	+	60	1441.16
+	+	+	0	0	+	42	1416.48
+	+	0	0	0	+	36	1458.20
0	**0**	**+**	**0**	**0**	**+**	**39**	**1411.59***
0	+	+	0	+	+	46	1420.14
+	0	+	+	0	+	46	1421.17
0	+	+	0	0	+	40	1413.43
+	0	+	0	0	+	40	1412.56
0	+	0	0	0	+	34	1454.49
+	0	0	0	0	+	34	1454.47
0	0	0	0	0	+	33	1452.51
Survival (ϕ)							
+	+	+	0	0	+	21	1395.62
0	+	+	0	+	+	25	1396.07
+	0	+	+	0	+	25	1399.45
0	+	+	0	0	+	19	1392.54
+	0	+	0	0	+	19	1392.78
+	+	0	0	0	+	15	1392.78
0	0	+	0	0	+	18	1390.84
0	+	0	0	0	+	13	1389.53
+	0	0	0	0	+	13	1389.74
0	**0**	**0**	**0**	**0**	**+**	**12**	**1387.78***
Best model							
P→ **0**	**0**	**+**	**0**	**0**	**+**	**12**	**1387.78**
ϕ→ **0**	**0**	**0**	**0**	**0**			

Note: The model indicated in **bold** yielded the lowest AIC value (*).

In contrast to the above results, our analyses of dispersal detected no significant treatment effects on the rate of initial dispersal in any of the three studies (Table 2), suggesting that the 'added-weight' manipulations did not alter rates of movement between subsites within the study area. This result also suggests that any differences in survival estimated in the MRR analyses are probably not the result of differences in dispersal rate between the treatment groups (see next section).

Probabilities of recapture during the MRR studies also reflect aspects of flight. In the studies with *P. occidentalis* in 1994 and 1995, the best models for the data included the 'added-weight' manipulations on recapture probabilities (Tables 3 and 4), but the effect in the two studies was quite different. In June 1994, the 'added-weight' butterflies had

consistently higher recapture probabilities than control butterflies (Fig. 3). In contrast, in the May 1995 study, the 'added-weight' butterflies had higher recapture than controls in some time periods but lower recapture in others, with no consistent effect of the treatment overall (Fig. 4). The reasons for these differences between studies with *P. occidentalis* are unclear. For *Colias philodice*, the 'added-weight' manipulations had no detectable impact on recapture probabilities (Fig. 4; Table 5). In summary, our manipulations of body weight did not consistently alter recapture probabilities in these studies.

Body size, survival and palatability

The above results suggest that increasing body mass and decreasing flight muscle ratio can reduce flight activity in the field in pierid butterflies. Do these effects on flight translate into effects on survival? Our results for *P. occidentalis* show that experimentally increasing body weight decreased the conditional survival probabilities of 'weight-on' butterflies by 30% each time period relative to controls in the 1994 study, but there were no detectable effects in the 1995 study. Whether the greater effect on survival in the 1994 study is associated with the greater experimental alteration of body mass in 1994 (14–16% increase) than 1995 (10–11% increase) is unclear. However, increasing body weight also had no detectable effect on survival in the 1996 study with *Colias philodice*, in which a 15% increase in body mass was recorded. Recall that we predicted that decreasing flight muscle ratio would have a greater impact on survival in *Colias* than in *Pontia* because of the greater palatability of *Colias* (Srygley and Kingsolver, 1998); clearly, our results do not support this prediction.

In summary, the results of our experimental studies suggest that flight muscle ratio has important effects on flight in these species, but these effects do not translate into consistent effects on survival at our study site; nor are effects on survival greater in more palatable species, as would be expected if flight muscle ratio determined predator escape in this system. Three issues are relevant to interpreting these results. First, increased wing loading and decreased flight muscle ratio may increase the energetic cost of locomotion as a result of increased wing-beat frequencies, and may thereby affect some other aspect of fitness such as mating success or realized fecundity. Our experiments do not rule out this possibility, although our analyses do not reveal significant increases in nectaring activities in response to added weights, as might be expected based on energetic costs. Second, the abundance and types of potential predators may change seasonally. In particular, Red-winged Blackbirds were actively feeding young on nests in the cattails at the centre of our study site during the studies on *Pontia* in mid-May and early June, but the young had fledged by the study of *Colias* in late June. In contrast, the salticid spider *Phidippus claris*, which we have seen successfully attacking *Pontia occidentalis* on flowers, were abundant during both the May and June studies. The relative and absolute impacts of these predators on *Pontia* and *Colias* at our study site are unknown. Unfortunately, *Colias* do not typically reach population levels sufficient for us to do MRR studies until late June at our study site.

Third, other aspects of body shape may be more important in determining aspects of flight relevant to predator escape and survival. For example, the position of the centre of mass (relative to the centre of lift) may affect turning rates and manoeuvrability in palatable butterflies (Srygley and Chai, 1990a; Srygley and Dudley, 1993). However, recent studies with the neotropical butterfly *Anartia fatima* suggest that manipulations of the position of

the centre of mass do not significantly alter survival in the presence of bird predators (Srygley and Kingsolver, in press). Although many features of wing and body size and shape may alter important aspects of flight in butterflies, the specific morphometric traits that determine successful escape from predators in the field remain unclear.

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