Fecundity, Lifespan and Egg Mass in Butterflies: Effects of Male-Derived Nutrients and Female Size

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Fecundity, lifespan and egg mass in butterflies: effects of male-derived nutrients and female size

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Summary

1. Effects of larval reserves and nutrients received as adults on fecundity and lifespan in female *Danaus plexippus* (the Monarch Butterfly) were measured to determine the relative importance of different sources of nutrients for reproduction and somatic maintenance.

2. Egg-laying lifespan was correlated with female size but not with the amount of male-derived nutrients or adult food concentration.

3. Lifetime fecundity was higher when females received a large first spermatophore, but was not affected by female size when lifespan was controlled or by adult food concentration.

4. At the end of their lives, females contained unlaid eggs and retained, on average, 88% of their initial mass. This proportion was unchanged in two years, although mean egg-laying lifespan varied from 22·5 to 28·7 days.

5. Egg mass decreased over the female lifespan, and was correlated with female size.

6. These results suggest that larval reserves are more important for somatic maintenance than adult income, but that the protein-rich nutrients received from males contribute to egg production. This supports theoretical predictions and empirical studies of other Lepidoptera showing that larval reserves are less likely to affect fecundity when the adult income can contribute substantially to egg production.

Key-words: Danaus plexippus, Lepidoptera, Monarch Butterflies, paternal investment, spermatophores

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Introduction

In many insects, materials for egg production are available from three sources: larval feeding, the adult diet and nutrients transferred by males during mating. This is represented by the following formula (Boggs 1990):

egg mass = mass from (larval reserves + adult diet + male-derived nutrients),

or

egg mass = mass from (larval reserves + adult income). eqn 1

It is useful to think of nutrients sequestered during the larval stage as 'capital', since they are irreplaceable after pupation, and those ingested as adults or received from males as 'income', since they are replaceable (Boggs 1997a). The relative importance of these materials should vary with (1) the timing of egg production, (2) the quality of the adult diet and (3) the quality and quantity of male donations (Boggs 1986; Wiklund & Kaitala 1995). Larval nutrients are expected to be most important when females eclose with most of their eggs mature. When females eclose without mature eggs, adult income can contribute significantly to egg production. Male-derived nutrients should be most important when females mate multiply and the adult diet lacks protein, and least important when females are monandrous and their diet contains protein-rich foods such as pollen.

Larval reserves and adult income must also be used for somatic maintenance and other metabolic expenditures, and the importance of non-reproductive expenditures should vary with adult lifespan. Since lifespan will also affect female fitness, there might be trade-offs in the usage of nutrients for reproduction or maintenance.

Two approaches were used to assess the importance of larval and adult resources on egg production and somatic maintenance in *Danaus plexippus* (L.) (Monarch Butterfly). First, variation in fecundity and lifespan with different amounts of material available from the three sources was measured. Female mass was used to indicate larval reserves, while income from the adult diet and males were experimentally manipulated. In the second approach, changes in female and egg mass were measured over the repro-

ductive period. Because mass at eclosion consists solely of larval reserves, mass lost during the adult stage reflects the amount of capital being spent on reproduction and maintenance (Boggs 1997a). Egg mass in excess of lost body mass must come from adult income, and equation 1 can be rearranged to calculate the minimum amount of income spent on eggs:

egg mass from adult income = egg mass – mass lost during adult stage. eqn 2

This amount is a minimum because it assumes that all mass lost during the adult stage is used in egg production, ignoring somatic maintenance.

These issues are relevant to many insects, but discussion in this paper emphasizes the Lepidoptera. Variation within the Lepidoptera in the timing of oogenesis, and the quality and quantity of adult income and adult longevity, make them ideal for studying how larval reserves and adult income are used. Females of some species eclose with most of their eggs mature, while others eclose with no mature eggs. Some adults ingest protein-rich pollen, while others do not eat at all. Both the number and size of the protein-rich spermatophores that females receive from males vary. Finally, some adults live for only a few days, while others live for several months. *Danaus plexippus* is a highly polyandrous, nectivarous species. Females eclose without mature eggs, so adult income, particularly male-derived nutrients, can contribute to egg production. They lay eggs over a relatively long (3–5 weeks) adult lifespan, so somatic maintenance is likely to be an important component of the overall resource budget.

Methods

Experimental *D. plexippus* were reared on fresh cuttings of *Asclepias syriaca* (Common Milkweed) under ambient photoperiod and temperature. Adults eclosed between 28 June and 10 July 1994. The day after eclosion they were individually marked and weighed to the nearest 0·01 mg, and their forewings measured to the nearest 0·1 mm. Female mass at eclosion ranged from 288 to 624 mg, reflecting a wide range of larval reserves. Butterflies were kept in glassine envelopes and fed a 20% honey solution *ad libitum* every other day until they were 4–5-days old. At this time, when females had begun to yolk oocytes and were likely to mate (Oberhauser & Hampton 1995), they were placed in outdoor flight cages (2 x 2 x 2 m^3) with males. Males ranged in age from 5 to 11 days, and were either virgins or had mated 1 day previously. These two male types transfer spermatophores of over 25 and ≈ 7 mg, respectively (Oberhauser 1988), and are designated large and small spermatophore donors.

The morning after mating, females were put into separate oviposition cages (0·7-m cubes) with potted host plants, *Asclepias curassavica*. At this time, they were assigned to one of five mating treatments. Treatments (followed by the sizes and order of spermatophores received) were matings to: (a) two virgin males (large, large), (b) a virgin followed by a non-virgin (large, small), (c) a non-virgin followed by a virgin (small, large), (d) two non-virgins (small, small) or (e) one non-virgin (small). Beginning on the third day of egg-laying, one or two males of the assigned type for the second mating were put into each female's cage in the afternoon. This was repeated until females remated. Any that had not remated within 7 days were removed from the experiment.

Females were removed from their cages and fed each morning. Every other day they were weighed before being fed. They were kept until they had laid no eggs for 7 days, could no longer fly, or died. Eggs laid by each female were counted daily. Ten eggs from each female, in batches of five, were weighed to the nearest 0·01 mg every day.

After they died or were removed from the experiment, females were frozen for later dissection to determine whether they contained oocytes and fat bodies. They were dissected several months after the end of the experiment, at which time the ovarioles had disintegrated enough to make exact determination of the number of oocytes that remained difficult. The mature oocytes in the most intact ovariole were counted, and this number multiplied by eight (the total number of ovarioles) to estimate the total number of oocytes. Most females contained at least one intact ovariole, so this method provided a reasonably accurate estimate of the number of mature oocytes remaining when egg-laying ceased. The state of fat bodies was categorized as none (no fat visible) or some (a little fat visible).

Initial sample sizes were 12 females in each of the double-mating treatments and six in treatment (e) (small). Nine females were removed from the experiment because they did not remate, laid no eggs, or laid eggs for fewer than 6 days. The final total sample size was 47 females, with 6–12 females per treatment.

A 1988 study has been described previously, assessing the effects of mating frequency on fecundity in *D. plexippus* (Oberhauser 1989). New analyses of data from this study are relevant to the present study. In 1988, females were used in a two-by-two factorial experiment, in which they were fed either a 15% or 30% honey solution daily and mated either once or *ad libitum* as often as once every 3 days. Eggs were counted daily, and females weighed every third day until they died. The summer of 1988 was exceptionally hot and dry in the upper midwestern USA, where this experiment was carried out.

Results

TIMING OF MATING AND EGG-LAYING

Females in 1994 mated for the first time at ages 4–11 days. Second matings occurred 3–7 days after the first, with the exception of one female that remated

before being removed from the flight cage the morning after her initial mating. The mean intermating interval for females that received a small spermatophore first was 4·33 days (N = 21, SE = 0·31), while that for females that received a large spermatophore first was 5·15 days (N = 20, SE = 0·18). These intervals are significantly different (T [unequal variances] = 2·33, P = 0·033).

Fecundity peaked during the first week of egg-laying, then gradually decreased (Fig. 1). There was a great deal of variation in daily fecundity, which is partly because of ambient conditions; females lay few eggs on cool or rainy days, and many on warm, sunny days.

Age at first mating affected fecundity on the first day of egg-laying. Females that mated at age 4–5 days, when they have no or few mature oocytes (Oberhauser & Hampton 1995), laid an average of 4·0 eggs the day after mating (SE = 2·54). Those that mated at age 6–8 days, when they have mature oocytes (Oberhauser & Hampton 1995), laid an average of 48·8 eggs the day after mating (SE = 8·96), while those that mated later laid 12·9 eggs (SE = 7·15). The number of eggs laid on day one by the group that mated at age 6–8 days is significantly greater than the other two groups (ANOVA $F_{2,44}$ = 8·55, P = 0·0007). However, by the second day of egg-laying there was no significant difference among these groups (ANOVA $F_{2,44}$ = 1·78, P = 0·18).

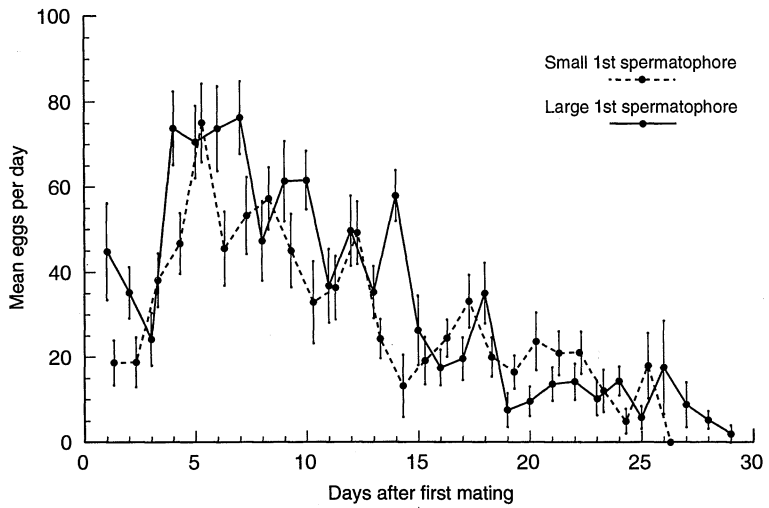

Fig. 1. Daily fecundity of females that received a large (solid line) or small (dashed line) spermatophore first in 1994. Bars represent one SE. Fecundity was high during the first 2 weeks of egg laying, then decreased until females died. When spermatophore nutrients were being broken down, and were still available to females, those that received large spermatophores first laid more eggs than those that received small spermatophores first; after this, there are no differences.

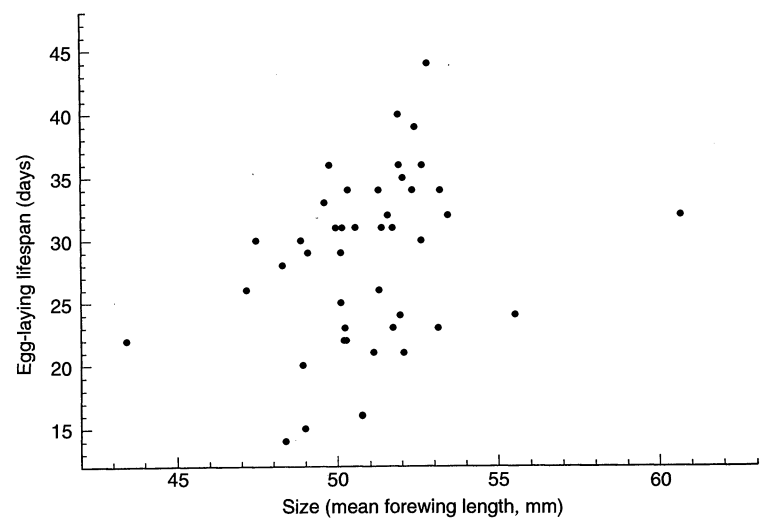

Fig. 2. Egg-laying lifespan (number of days from eclosion to the last day of egg laying) as a function of the mean length of female forewings. There is a significant positive correlation between female size and lifespan.

APPROACH 1: EFFECTS OF VARYING LARVAL RESERVES AND ADULT INCOME ON LIFESPAN AND FECUNDITY

Lifespan

'Egg-laying life', the time from eclosion to the last day of egg laying, is used instead of actual lifespan. This varied from 14 to 45 days (mean = 28·7). A stepwise multiple linear regression model was used to test effects of different factors on female lifespan; in this and other models, predictors were added when a partial F-test determined that their addition significantly (P<0·05) improved the model. Predictors included female morphometry (forewing asymmetry, mean forewing length and 'fatness'), the amount of male-derived nutrients received, and fecundity in the first 4 days of egg laying. Forewing asymmetry was calculated as the absolute value of the difference between the two forewing lengths. The mean of actual asymmetry (right–left), –0·042 (SE = 0·09), is not significantly different from 0 (t=–0·45, P=0·658). Residuals from the regression of forewing length on initial mass (r^2=0·70, P=0·000) indicate fatness; females with a positive residual are light for their winglength, while those with a negative residual are heavy. The total amount of spermatophore material was estimated using previously collected data on spermatophore masses (Oberhauser 1988). Early fecundity was included to determine if there was a trade-off between early reproductive effort and somatic maintenance.

Lifespan increased with increasing female size (lifespan = –30·1 + 1·15 × winglength, P=0·020, r^2=0·120; Fig. 2), but did not vary with other female attributes, the total amount of spermatophore material received, or early fecundity.

Lifespans previously reported for 1988 females were time from eclosion to death (Oberhauser 1989). These ranged from 15 to 53 days, while egg-laying life ranged from 11 to 32 days (mean = 22·5). This was significantly shorter in 1988 (the hot, dry year) than in 1994 (t = –3·94, df = 71, P = 0·0002). In 1988, egg-laying lifespan did not vary with feeding or mating treatments or with female size at eclosion. However, within the multiple-mating treatment

group, there was a significant correlation between the number of matings and egg-laying lifespan ($r^2 = 0{\cdot}432$, $P = 0{\cdot}022$).

Fecundity

Effects of several predictors on fecundity were tested using multiple regression analysis. Some of these were biologically relevant, while others were included to determine the importance of uncontrolled factors. Uncontrolled factors included female date of eclosion, the interval between first and second matings, and age at first mating. Because females eclosed over a 13-day interval, ambient conditions could have varied in a way that affected fecundity. However, the slope of the regression of fecundity *vs* date of eclosion is not significantly different from zero (Fig. 3), and there is no pattern when the residuals are plotted against time, so the date of emergence was not included in statistical models. Both the age at first mating and the interval between the first and second matings could have consistent effects if fecundity increases when females begin laying eggs or receive spermatophore nutrients earlier. These two factors were thus included in regression models. Other predictors included female morphometry, egg-laying lifespan, and the amount of spermatophore material received. Because daily fecundity is higher earlier in a female's life (Fig. 1), the size of the first spermatophore could be disproportionately important to a female's lifetime fecundity, so this was included as a predictor in addition to the total amount of material received. The female that remated the morning after her first mating was not included in models that included the size of the first spermatophore, because all of her eggs were laid after her second mating.

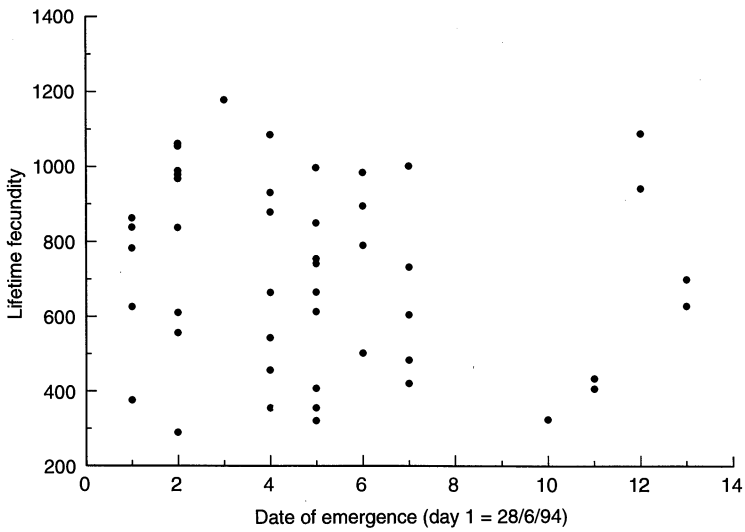

Fig. 3. Fecundity as a function of the date of female eclosion. There is no relationship between lifetime fecundity and the uncontrolled factor of the date of female emergence.

Average fecundity in 1994 was 715 eggs (Table 1, range 290–1179). There was a significant mating treatment effect on lifetime fecundity (ANOVA $F_{4,42} = 2{\cdot}98$, $P = 0{\cdot}030$). When all potential predictors are included in a multiple regression model, only lifespan and the size of the first spermatophore have significant effects (Table 2a). Females that lived longer laid more eggs, as did those that received a large first spermatophore. There was no effect of the timing of either mating, any morphometric characteristics, or the total amount of spermatophore material received. Fecundity did increase with increasing female size ($r^2 = 0{\cdot}109$, $P = 0{\cdot}015$), but controlling for lifespan made the effect of size insignificant in the regression analysis.

All females had oocytes remaining at the end of their egg-laying life. The number of mature oocytes in an intact ovariole ranged from 3 to 15, thus the total number of mature oocytes in all eight ovarioles ranged from approx. 24 to 120. This number did not vary with mating treatment, female size or lifespan. Females also had immature oocytes in the distal ends of the ovarioles. All females had either no or few fat bodies remaining in their abdomens.

Both mating treatment and lifespan affected fecundity in 1988 (Table 2b). Females in the multiple-mating treatment laid more eggs than singly-mated females (Oberhauser 1989). There were no effects of female mass, nor did females receiving the higher honey concentration lay more eggs. Females laid significantly fewer eggs in 1988 than they did in 1994 (1988 mean = 476, SE = 42·4; $t = -4{\cdot}07$, 71 df, $P = 0{\cdot}0001$). There was no difference in mass at eclosion in the two years ($t = -0{\cdot}58$, $P = 0{\cdot}562$).

Table 1. Treatment means for lifetime fecundity, reproductive effort (total egg mass/initial female mass) and income invested in eggs (total egg mass – mass lost during egg laying, in mg). Means followed by the same letter are not significantly different at the 0·05 level of confidence (Tukey LSD comparisons). Numbers in parentheses are 1 SE

Treatment	*N*	Mean fecundity	Reproductive effort	Income into eggs
a large, large	11	$711{\cdot}36^{ab}$ (80·97)	$0{\cdot}697^{ab}$ (0·0727)	$302{\cdot}9^{ab}$ (41·1)
b large, small	9	$936{\cdot}22^{a}$ (52·93)	$0{\cdot}899^{a}$ (0·0471)	$345{\cdot}4^{a}$ (26·5)
c small, large	12	$596{\cdot}38^{b}$ (73·40)	$0{\cdot}619^{b}$ (0·179)	$230{\cdot}4^{bc}$ (31·5)
d small, small	9	$700{\cdot}38^{ab}$ (73·51)	$0{\cdot}684^{ab}$ (0·0715)	$252{\cdot}7^{abc}$ (22·4)
e small	6	$664{\cdot}50^{ab}$ (85·70)	$0{\cdot}610^{ab}$ (0·0687)	$189{\cdot}6^{c}$ (34·3)
Total	47	714·77 (33·83)	0·702 (0·0301)	264·2 (14·8)

Table 2. Predictors of fecundity, female mass and egg mass

a Lifetime fecundity – 1994

Predictor	Coefficient (SE)	Student's t	P
Constant	281·2 (158·0)	1·78	0·082
Egg-laying lifespan	22·96 (4·23)	5·43	0·000
Small first spermatophore	–142·6 (55·36)	–2·58	0·013

$N = 47$, adj. $R^2 = 0{\cdot}446$. (no effect of age at first mating; intermating interval; female size, fatness or forewing asymmetry; or total amount of spermatophore material received).

b Lifetime fecundity – 1988

Predictor	Coefficient (SE)	Student's t	P
Constant	–163·8 (105·7)	–1·55	0·135
Egg-laying lifespan	25·51 (4·46)	5·72	0·000
Multiple mating treatment	123·0 (53·6)	2·29	0·031

$N = 26$, adj. $R^2 = 0{\cdot}605$. (no effect of female mass or feed treatment).

c Fecundity during first four days of egg-laying

Predictor	Coefficient (SE)	Student's t	P
Constant	1016 (347·8)	2·92	0·006
Small first spermatophore	–59·45 (28·24)	–2·11	0·043
Female size	–14·34 (6·84)	–2·10	0·044

$N = 35$, adj. $R^2 = 0{\cdot}202$. (no effect of age at first mating; or female fatness or forewing asymmetry).

d Proportion female mass remaining – 1994

Predictor	Coefficient (SE)	Student's t	P
Constant	1·48 (0·0602)	24·52	0·000
Age	–0·0155 (0·00107)	–14·37	0·000
Mass at emergence	–0·000698 (0·000125)	–5·57	0·000
Treatment (e) × age	–0·00435 (0·00149)	–2·91	0·0038
Treatment (a) × age	0·00841 (0·00231)	3·64	0·0003

$N = 455$, adj. $R^2 = 0{\cdot}4068$.

e Proportion female mass remaining – 1994

Predictor	Coefficient (SE)	Student's t	P
Constant	0·966 (0·0215)	44·9	0·000
Age	–0·0143 (0·00194)	–7·33	0·000
Age × multiple mating treatment	0·00926 (0·00181)	5·12	0·000
30% Feed Treatment	0·0948 (0·0208)	4·56	0·000

$N = 147$, adj. $R^2 = 0{\cdot}403$. (no effect of initial female mass).

f Egg mass

Predictor	Coefficient (SE)	Student's t	P
Constant	0·620 (0·221)	2·80	0·0052
Log TSM	–0·280 (0·024)	–11·67	0·000
Female size	0·036 (0·004)	8·44	0·000
Treatment (e)	0·0176 (0·005)	–3·53	0·000
Treatment (b)	–0·0161 (0·004)	–4·13	0·000

$N = 614$, adj. $R^2 = 0{\cdot}231$. (TSM = time, in days, since mating; no effect of interactions between treatments and TSM, or first spermatophore size).

Factors affecting fecundity during the first 4 days of egg laying in 1994 were studied to remove any variation in fecundity resulting from the second mating or lifespan. Only females that had not remated before this time were included. Lifespan was not included as a predictor, since it should not contribute to fecundity in the first 4 days. The other two factors that were correlated with lifetime fecundity, female size and the size of the first spermatophore, affected early fecundity. However, the effect of female size was negative (Table 2c). There was no effect of the age at first mating or other female attributes on early fecundity.

APPROACH 2: FEMALE MASS, EGG MASS AND REPRODUCTIVE EFFORT

Female mass decreased with age in both years. In 1994, the interaction between treatment (e) (small) and age had a negative effect on mass, while the interaction between treatment (a) (large, large) and age had a positive effect (Table 2d). This means that females in treatment (e) lost more mass than other females and those in treatment (a) lost less. There was a small, but significant, negative effect of initial female mass in 1994; larger females retained a smaller proportion of their initial mass. There were no effects of any of the treatments alone. In 1988, there was a significant effect of the interaction between mating treatment and age; females in the multiple-mating treatment lost mass more slowly (Table 2e). The interaction between feed treatment and age did not have a significant effect, thus the rate of mass loss did not vary with feeding treatment, but females fed the 30% honey solution consistently weighed more. There was no effect of initial female mass on mass loss in 1988. Analyses for both years only included masses while females were laying eggs. After this, those that were alive often gained mass because they could not fly or lay eggs, but were still being fed. One female weighed 946 mg, 1·78 times her initial mass, on her last weighing.

By the end of their egg-laying lives, females in both years weighed about 88% of their initial mass (1994 mean 87·7%, SE = 2·5%; 1988 mean 87·9%, SE = 3·2%). There were significant treatment effects on the proportion of a female's mass remaining in both years (Table 3). In 1994, treatment (a) females (large, large) retained a larger proportion of their mass than treatment (e) females (small). In 1988, females in both treatments fed the 30% honey solution retained a larger proportion of their initial mass than females in the single mating, 15% honey treatment.

Egg mass decreased over the period of egg laying (Fig. 4). The shape of the plot of egg mass on day of egg laying is concave, and using the log of time as a predictor significantly improved the regression. Larger females laid larger eggs, and females in treatments (b) (large, small) and (e) (small) laid smaller eggs than other females (Table 2f). The size of the first spermatophore did not affect egg mass. There

Table 3. Treatment means for the proportion of initial mass remaining at the end of egg-laying life. Means followed by the same letter are not different at the 0·05 level of confidence (Tukey's LSD comparison). Proportions could be (and sometimes were) greater than 1, so untransformed data were used in the ANOVA. Robustness of the normality assumption was tested by plotting residuals

1994 treatment	*N*	Proportion mass remaining (SE)	1988 treatment	*N*	Proportion mass remaining (SE)
(a) Large, large	10	$0{\cdot}999^{a}$ (0·0392)	15% honey, mate once	7	$0{\cdot}764^{b}$ (0·0423)
(b) Large, small	9	$0{\cdot}860^{ab}$ (0·0453)	30% honey, mate once	5	$0{\cdot}979^{a}$ (0·0872)
(c) Small, large	12	$0{\cdot}879^{ab}$ (0·0442)	15% honey, multiple mate	7	$0{\cdot}850^{ab}$ (0·0484)
(d) Small, small	8	$0{\cdot}868^{ab}$ (0·0629)	30% honey, multiple mate	6	$0{\cdot}968^{a}$ (0·0572)
(e) Small	6	$0{\cdot}779^{b}$ (0·0445)			
Total	45	0·877 (0·0252)		25	0·879 (0·0323)

were no significant interactions between treatments and the day of egg laying, so the rate of egg mass decrease did not vary among treatments.

The amount of adult income invested in eggs (equation 2) can be estimated by calculating the total egg mass produced by each female and then subtracting mass lost during her egg-laying life (her minimum investment from larval reserves). There was a significant treatment effect on income spent on eggs (Table 1), with females in treatment (a) (large, large) investing more than females in treatment (e) (small), and those in treatment (b) (large, small) investing more that those in treatments (c) and (e) (small, large and small).

Total lifetime reproductive effort by 1994 females, measured as the egg mass produced by each female divided by her initial mass, ranged from 0·302 to 1·14, with a mean of 0·702 (Table 1). This amount varied between mating treatments (ANOVA $F_{4,41} = 2{\cdot}90$, $P = 0{\cdot}033$). There was no effect of initial female size on total reproductive effort.

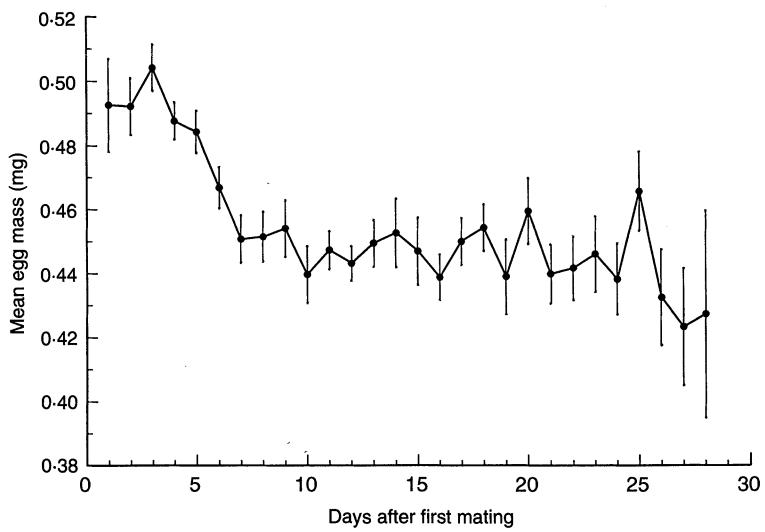

Fig. 4. Egg mass over the period of egg laying. Bars represent one SE.

Discussion

EFFECTS OF LARVAL RESERVES ON LIFESPAN AND FECUNDITY

The analyses of both fecundity and mass changes suggest that female *D. plexippus* use larval reserves for somatic maintenance, but not to increase egg production. The importance of larval reserves for somatic maintenance is indicated by results from both experimental approaches. First, there was a positive correlation between lifespan and size in 1994; females that eclosed with more reserves lived longer. There was no correlation between size and lifespan in 1988, but the extreme weather conditions that shortened lifespans could have counteracted beneficial effects of additional reserves. Second, females lost an average of 12% of their mass in both 1988 and 1994, despite laying eggs over a longer period in 1994; they seemed to lay eggs as long as they had the reserves to support oviposition activity. The small amount of fat left at the end of females' egg-laying lives is further evidence that reserves were used up. At this point, females did not always die, but they could not fly and would have died in the wild. The negative relationship between female mass at eclosion and the proportion of mass remaining (Table 2d) suggests that maintenance costs vary with size – larger females require a larger proportion of their reserves for maintenance.

Larval reserves did not affect fecundity. Although female size and fecundity were positively correlated in 1994, large females lived longer, and controlling for lifespan made the effect of size insignificant (Table 2a). In 1988, there was no relationship between female size and fecundity. However, larval reserves do affect female reproductive success, despite the lack of a direct connection between mass and fecundity. In captive *D. plexippus*, time to lay eggs that are produced, and not egg production itself, appears to limit realized fecundity; females run out of reserves needed for maintenance with many eggs unlaid.

Other studies of lepidopteran fecundity provide mixed support for the prediction that larval reserves

should affect fecundity when adult income is normally low, but not when adult income is high. The following studies of nectivarous, monandrous species (with low adult income) found correlations between female size and fecundity, supporting this prediction: Suzuki (1978) and Jones, Hart & Bull (1982), *Pieris rapae crucivora* (however, while Sugawara (1979) reports that this species is monandrous, Svärd & Wiklund (1989) found over two spermatophores per female in *P. r. rapae*); and Jones, Odendall & Ehrlich (1986), *Euphydryas editha* and *E. chalcedona*. This correlation was also found in the monandrous *Epirrita autumnata* (Haukioja & Neuvonen 1985), a moth in which adults do not feed. Studies of the monandrous *Pararge aegeria* have conflicting results; Wiklund & Persson (1983) found no correlation between female size and fecundity, while Karlsson & Wickman (1990) found size-dependent fecundity. Lederhouse (1981) also found a relationship between female size and fecundity in *Papilio polyxenes*. There is some polyandry in this species, but male *P. polyxenes* transfers relatively small spermatophores (Lederhouse 1981) that might not be an important source of nutrients for females.

On the other hand, some studies of monandrous Lepidoptera have not found a relationship between female size and fecundity: Wiklund (1984), *Lasiommata megera*; and Boggs (1986) and Boggs & Ross (1993), *Speyeria mormonia*. Boggs & Ross did find that female size affected the number of eggs that were unlaid at death in *Speyeria mormonia*, suggesting that larval reserves affect the number of eggs that are produced, as opposed to laid, in this species.

Larval reserves, as reflected by female size, should not be important determinants of fecundity when females receive substantial nutrient contributions from males. In addition to the current study, this prediction is supported by the following studies of polyandrous species: Svärd & Wiklund (1988), *D. plexippus;* Wiklund & Kaitala (1995), *Pieris napi*.

EFFECTS OF ADULT INCOME ON FECUNDITY

The large reproductive effort of female *D. plexippus*, measured as total egg mass divided by initial body mass (Table 1), indicates that much of the material used in eggs comes from adult income. Because the adult diet consists of nectar, a poor source of protein, ingested nutrients should be less important than the protein-rich spermatophores (Oberhauser 1992) received from males. Supporting this is the fact that a two-fold difference in honey-water concentration in 1988 had no effect on fecundity. It is possible that females fed the lower honey concentration simply imbibed more solution. However, females that received the higher concentration weighed more and thus must have ingested more calories (Table 2f).

Other researchers have found that the adult diet in nectar-feeding Lepidoptera affects fecundity, but in several cases lower fecundity occurred when females were given no carbohydrates at all (Norris 1935; *Pieris rapae*; David & Gardiner 1962; *P. brassicae*; Murphy, Launer & Ehrlich 1983; *Euphydryas editha*; Karlsson & Wickman 1990; *Pararge aegeria*). When Boggs & Ross (1993) gave *Speyeria mormonia* females either one-half or one-third the volume of honey-water consumed by females fed *ad libitum*, fecundity also decreased. Likewise, *Euploea core* (Hill 1989) and *Jalmenus evagoras* (Hill & Pierce 1989) females laid more eggs when they received 25% *vs* 1% sugar solutions. These results suggest that a threshold amount of carbohydrates from the adult diet is needed for females to realize maximum fecundity. My feeding regime provided this threshold, whereas others summarized above did not.

Male-derived income did affect fecundity in this study. Females that received a large first spermatophore in 1994 or were allowed to mate multiply throughout the egg-laying period in 1988 showed increased fecundity.

There is an increasing body of evidence that polyandrous insects use nutrients obtained during mating to increase fecundity (lepidopteran examples include Rutowski, Gilchrist & Terkanian 1987, *Colias eurytheme*; Watanabe 1988, *Papilio xuthus*; Oberhauser 1989 and current study, *Danaus plexippus;* Wiklund *et al.* 1993, *Pieris napi*; Tamhankar, Gothi & Rahalkar 1993, *Earis vittella*; Tamhankar 1995, *E. insulana;* Ward & Landolt 1995, *Trichoplusia ni*). In monandrous species, on the other hand, varying the amount of male-derived nutrients that females receive rarely affects fecundity (Greenfield 1982, *Plodia interpunctella*; Jones *et al.* 1986, *Euphydryas chalcedona* & *E. editha*; Svärd & Wiklund 1991, *Papilio machaon*). In fact, only one study of a monandrous lepidopteran has shown a relationship between the amount of male-derived nutrients and fecundity (Royer & McNeil 1993, *Ostrinia nubilalis*), and the authors suggest that this effect occurred because of sperm depletion in females mated to recently mated males, and not because of nutrient differences.

The above studies of monandrous species have been interpreted as evidence that male-derived nutrients are not important to fecundity. However, perhaps we should not expect this effect in monandrous species. A possible explanation for the differences between monandrous and polyandrous Lepidoptera is that it is possible to vary the amount of male-derived nutrients more when females mate multiply. Another explanation is that monandrous females have not evolved to utilize male-derived nutrients to make eggs. Once this occurs, there should be strong selection pressure for females to mate multiply, or forage (*sensu* Kaitala & Wiklund 1994) for additional nutrients.

Female *D. plexippus* appear to use male-derived nutrients relatively quickly after receipt. Multiply mating females in 1988 were allowed to mate at 3-day

intervals throughout their lives, while those in 1994 mated only twice early in egg laying. Fecundity differences between the 1988 mating treatments were apparent throughout the egg-laying period (Oberhauser 1989), whereas after about 10 days of egg laying there were no differences between females that received large and small first spermatophores in 1994 (Fig. 1). Spermatophores are completely broken down within 1–7 days after mating (Oberhauser 1992) and the nutrients they contain begin to be incorporated into eggs almost immediately (Boggs & Gilbert 1979; Wells, Wells & Rogers 1993). Wiklund *et al.* (1993) and Boggs (1997b) have found male-derived compounds in *Pieris napi,* and *Euphydryas editha* and *Speyeria mormonia* eggs, respectively, for over 2 weeks after mating, but in both cases the amount of these compounds in eggs decreased rapidly within the first week after mating. Rutowski *et al.* (1987) also found that the increase in egg production due to receipt of larger spermatophores by female *Colias eurytheme* lasted only during the time that the spermatophores were being broken down. These results suggest that most male-derived nutrients are used as they become available to female Lepidoptera, rather than being held in reserve. This strategy makes sense for polyandrous females, since they can obtain additional nutrients by remating.

EFFECTS OF ADULT INCOME ON LIFESPAN

Female *D. plexippus* do not appear to use adult income to increase their lifespan, at least within the income ranges provided in these experiments. Females that received the higher honey-water concentration in 1988 did not live longer, nor did increased income from males increase lifespans in either year. The only suggestion that females use income to increase lifespan is the fact that within the multiple-mating treatment in 1988, those that mated more times lived longer. However, there is no way to separate cause and effect in this case.

Two studies of monandrous Lepidoptera also showed no effect of spermatophore size on female lifespan (*Ostrinia nubilalis,* Royer & McNeil 1993; *Papilio machaon*, Svärd & Wiklund 1991). However, in three polyandrous species, females that received more male-derived nutrients lived longer (*Colias eurytheme*, Rutowski *et al.* 1987; *Pieris napi*, Wiklund *et al.* 1993; *Earis insulana*, Tamhankar 1995). Boggs (1990) also showed a correlation between the number of matings and female lifespan in *Heliconius cydno*, but butterflies in this study mated *ad libitum*, making it impossible to separate cause and effect. These studies of polyandrous species provided more variation in the number of spermatophores received than did my 1994 study, and it is possible that my treatments were not different enough to allow detection of such an effect. There were larger treatment differences in 1988, but the severe weather conditions could have negated any effects. The fact that Boggs & Gilbert (1979) found male-derived nutrients in female somatic tissue as well as eggs suggests that females could use these nutrients for maintenance, and my results cannot rule out a relationship between lifespan and male-derived nutrients under a different test regime.

In nectar-feeding Lepidoptera, completely withholding carbohydrates from the adult diet shortens lifespans (*Pieris rapae*, Norris 1935; *P. brassicae*, David & Gardiner 1962; *Euphydryas editha*, Murphy *et al.* 1983; *Pararge aegeria*, Karlsson & Wickman 1990), as does providing only a 1% sugar solution (*Euploea core*, Hill 1989; *Jalmenus evagoras*, Hill & Pierce 1989). However, when female *Speyeria mormonia* were fed one-half or one-third the volume of a 25% honey solution as that imbibed by females fed *ad libitum*, there was no effect of feeding treatment on lifespan, Boggs & Ross 1993). These studies, in combination with work on *D. plexippus*, indicate that nectar-feeding Lepidoptera need to obtain some sugar as adults to realize a full lifespan, but there is probably a threshold amount, over which no additional benefits are realized.

TIMING OF MATING

The lack of relationship between the age at first mating and lifetime fecundity indicates that the exact timing of the first mating might not be crucial to female *D. plexippus*. If they mate before they have mature eggs, they must carry the additional mass of a spermatophore. However, spermatophore nutrients are available earlier in egg production, and females avoid the potential risk of not finding a mate when they have mature eggs. Mating late would be risky if any egg-laying days are lost, especially when females have a finite chance of dying at any time in their lives. Some females apparently did mate before they had mature oocytes; those that mated before the age of 6 days laid fewer eggs on their first day of egg laying than those that mated at ages 6–8 days, probably owing to a lack of mature eggs (Oberhauser & Hampton 1995). Interestingly, females that mated after age 8 days also laid significantly fewer eggs on their first day of laying. It is possible that having mature eggs, but no sperm to fertilize them, has an inhibitory effect on oviposition behaviour, or that this late-mating subset of females matured eggs later.

Females that mated first to a virgin male remated later than those that mated first to a non-virgin male. This supports previous evidence that large spermatophores increase the female refractory period (Oberhauser 1989).

EGG MASS

Female *D. plexippus* lay smaller eggs as they age, and larger females lay larger eggs (Table 2f and Fig. 4).

These effects suggest that larval reserves affect egg mass; female size reflects the amount of larval reserves, and as females lose mass, they lay smaller eggs. The relationship between egg mass and the adult diet cannot be ascertained from this study, since female diet was not varied during the year that egg mass was measured. The effect of male-derived nutrients is unclear; there were significant negative effects of two mating treatments [treatments (b) (large, small) and (e) (small)], but these two treatments differed greatly in the amount of male-derived material received. An indication that male-derived nutrients are not important determinants of egg mass is the fact that egg mass dropped most rapidly over days 3–7 of egg laying (Fig. 4), when females were still breaking down spermatophores, and thus receiving these nutrients (Oberhauser 1992).

Egg mass decreases with time in many Lepidoptera (Jones *et al.* 1982; Murphy *et al.* 1983; Wiklund & Persson 1983; Karlsson & Wiklund 1984, 1985; Wiklund & Karlsson 1984; Boggs 1986; Svärd & Wiklund 1988). Wiklund and his collaborators (above references) have concluded that, in general, there appears to be a minimum size required for egg viability, and that variation over this size does not result in higher fitness. They suggest that females use a proportion of available reserves to produce eggs; as these reserves are depleted, females lay smaller eggs. As long as eggs are above the minimum size this does not result in less fit offspring (see especially Wiklund & Karlsson 1984). While I did not rear any of the eggs, my data support this resource depletion hypothesis.

There is no clear relationship between female and egg size in Lepidoptera studied to date. Jones *et al.* (1982) found a negative correlation in *Pieris rapae*, Boggs (1986) a positive correlation in *Speyeria mormonia*, Wiklund & Karlsson (1984) no correlation in 10 satyrid butterflies, and Svärd & Wiklund (1988) no correlation in *D. plexippus*. Wiklund, Karlsson & Forsberg (1987) argue that when fecundity is limited by the number of eggs females can actually lay and not by egg production, as seems to be the case in *D. plexippus*, there will be non-adaptive scaling of egg size to body size. However, when fecundity is limited by egg production, females should produce the smallest eggs possible, within limits posed by viability constraints.

Conclusion

Female *D. plexippus* appear to use increased amounts of male-derived income to increase egg production, and increased larval reserves to increase their egg-laying lifespan. However, realized fecundity is affected by both egg production and the time over which females lay eggs, so both capital and income are important to female fitness. In fact, females seem to run out of reserves for somatic maintenance before egg production terminates.

Even though increased male-derived income and larval reserves affect different components of female fitness, there are not separate nutrient pools, with larval reserves being used only for somatic maintenance and male-derived nutrients for egg production. Additional nutrients from males were not used to make larger eggs, but there was scaling between female size and egg size, suggesting that larval reserves are being put into eggs, or at least that larger females invest more nutrients in each egg. Another interesting relationship between female size and egg production was that larger females laid fewer eggs during the first 4 days of oviposition in 1994 (Table 2c). Large females also produced fewer eggs early in their lives in another study (Oberhauser & Hampton 1995). It is possible that there is a trade-off between reproduction and lifespan, and that females that can 'expect' to live relatively long lives do not produce as many eggs early. If this is the case, early reproduction could draw from reserves for somatic maintenance.

It should be noted that lifespan and fecundity, like other life-history traits, are affected by a great deal of environmental 'noise' (e.g. Price & Schluter 1991). This makes it difficult to establish a relationship between a single causal feature (e.g. body size or the quantity of adult income) and these traits, even under carefully controlled conditions. The lack of a statistically significant relationship in a single study is not final evidence that there is no relationship between the causal feature in question and the life-history trait.

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References

Boggs, C.L. (1986) Reproductive strategies of female butterflies: variation in and constraints on fecundity. *Ecological Entomology* **11,** 7–15.

Boggs, C.L. (1990) Effects of male-donated nutrients on female fitness in insects. *American Naturalist* **136,** 598–617.

Boggs, C.L. (1997a) Reproductive allocation from reserves and income in butterfly species with differing adult diets. *Ecology* **78,** 181–191.

Boggs, C.L. (1997b) Dynamics of reproductive allocation from juvenile and adult feeding: radiotracer studies. *Ecology* **78,** 192–202.

Boggs, C.L. & Gilbert, L.E. (1979) Male contribution to egg production in butterflies: evidence for transfer of nutrients at mating. *Science* **206,** 83–84.

Boggs, C.L. & Ross, C.L. (1993) The effect of adult food limitation on life history traits in *Speyeria mormonia* (Lepidoptera: Nymphalidae). *Ecology* **74,** 433–441.

David, W.A.L. & Gardiner, B.O.C. (1962) Oviposition and the hatching of the eggs of *Pieris brassicae* in a laboratory culture. *Bulletin of Entomological Research* **53,** 91–109.

Greenfield, M.D. (1982) The question of paternal investment in Lepidoptera: male-contributed proteins in *Plodia interpunctella*. *International Journal of Invertebrate Reproduction* **5,** 323–331.

Haukioja, E. & Neuvonen, S. (1985) The relationship between size and reproductive potential in male and female *Epirrita autumnata* (Lepidoptera, Geometridae). *Ecological Entomology* **10,** 267–270.

Hill, C.J. (1989) The effect of adult diet on the biology of butterflies. 2. The common crow butterfly, *Euploea core corinna*. *Oecologia* **81,** 258–266.

Hill, C.J. & Pierce, N.E. (1989) The effect of adult diet on the biology of butterflies. 1. The common imperial blue, *Jalmenus evagoras*. *Oecologia* **81,** 249–257.

Jones, R.E., Hart, J.R. & Bull, G.D. (1982) Temperature, size and egg production in the Cabbage Butterfly (*Pieris rapae* L.). *Australian Journal of Zoology* **30,** 223–232.

Jones, K.N., Odendall, F.J. & Ehrlich, P.R. (1986) Evidence against the spermatophore as paternal investment in Checkerspot Butterflies (Euphydryas: Nymphalidae). *American Midland Naturalist* **116,** 1–6.

Kaitala, A. & Wiklund, C. (1994) Polyandrous female butterflies forage for matings. *Behavioral Ecology and Sociobiology* **35,** 358–388.

Karlsson, B. & Wickman, P.-O. (1990) Increase in reproductive effort as explained by body size and resource allocation in the Speckled Wood butterfly, *Pararge aegeria* (L.). *Functional Ecology* **4,** 609–617.

Karlsson, B. & Wiklund, C. (1984) Egg weight variation and lack of correlation between egg weight and offspring fitness in the wall brown butterfly *Lasiommata megera*. *Oikos* **43,** 376–385.

Karlsson, B. & Wiklund, C. (1985) Egg weight variation in relation to egg mortality and starvation endurance of newly hatched larvae in some satyrid butterflies. *Ecological Entomology* **10,** 205–211.

Lederhouse, R.C. (1981) The effect of female mating frequency on egg fertility in the black swallowtail, *Papilio polyxenes asterius* (Papilionidae). *Journal of the Lepidopterists' Society* **34,** 266–277.

Murphy, D.D., Launer, A.E. & Ehrlich, P.R. (1983) The role of adult feeding in egg production and population dynamics of the checkerspot butterfly *Euphydryas editha*. *Oecologia* **56,** 257–263.

Norris, M.J. (1935) A feeding experiment on the adults of *Pieris rapae*. *Entomology* **68,** 125–127.

Oberhauser, K.S. (1988) Male monarch butterfly spermatophore mass and mating strategies. *Animal Behaviour* **36,** 1384–1388.

Oberhauser, K.S. (1989) Effects of spermatophores on male and female monarch butterfly reproductive success. *Behavioral Ecology and Sociobiology* **25,** 237–246.

Oberhauser, K.S. (1992) Rate of ejaculate breakdown and intermating intervals in monarch butterflies. *Behavioral Ecology and Sociobiology* **31,** 367–373.

Oberhauser, K.S. & Hampton, R. (1995) The relationship between mating and oogenesis in monarch butterflies (Lepidoptera: Danainae). *Journal of Insect Behavior* **8,** 701–714.

Price, T. & Schluter, D. (1991) On the low heritability of life-history traits. *Evolution* **45,** 853–861.

Royer, L. & McNeil, J.N. (1993) Male investment in the European corn borer, *Ostrinia nubilalis* (Lepidoptera: Pyralidae): impact on female longevity and reproductive performance. *Functional Ecology* **7,** 209–215.

Rutowski, R.L., Gilchrist, G.W. & Terkanian, B. (1987) Female butterflies mated with recently mated males show reduced reproductive output. *Behavioral Ecology and Sociobiology* **20,** 319–322.

Sugawara, P. (1979) Stretch reception in the bursa copulatrix of the butterfly, *Pieris rapae crucivora*, and its role in behavior. *Journal of Comparative Physiology A* **130,** 191–199.

Suzuki, Y. (1978) Adult longevity and reproductive potential of the small cabbage white, *Pieris rapae crucivora* Boisduval (Lepidoptera: Pieridae). *Applications in Entomological Zoology* **13,** 312–313.

Svärd, L. & Wiklund, C. (1988) Fecundity, egg weight, and longevity in relation to multiple matings in females of the monarch butterfly. *Behavioral Ecology and Sociobiology* **23,** 39–43.

Svärd, L. & Wiklund, C. (1989) Size and production rate of ejaculates in relation to monandry/polyandry in butterflies. *Behavioral Ecology and Sociobiology* **24,** 395–402.

Svärd, L. & Wiklund, C. (1991) The effects of ejaculate mass on female reproductive output in the European swallowtail butterfly, *Papilio machaon* (L.) (Lepidoptera: Papilionidae). *Journal of Insect Behavior* **4,** 33–42.

Tamhankar, A.J. (1995) Host influence on mating behavior and spermatophore reception correlated with reproductive output and longevity of female *Earis insulana* (Boisduval) (Lepidoptera: Noctuidae). *Journal of Insect Behavior* **8,** 499–512.

Tamhankar, A.J., Gothi, K.K. & Rahalkar, G.W. (1993) Host-induced augmented reproduction in spotted bollworm, *Earis vittella* (Fabricius) (Lepidoptera: Noctuidae). *Insect Science Applications* **14,** 371–375.

Ward, K.E. & Landolt, P.J. (1995) Influence of multiple matings on fecundity and longevity of female cabbage looper moths (Lepidoptera: Noctuidae). *Annals of the Entomological Society of America* **88,** 768–772.

Watanabe, M. (1988) Multiple matings increase the fecundity of the yellow swallowtail butterfly, *Papilio xuthus*, L., in summer generations. *Journal of Insect Behavior* **1,** 17–30.

Wells, H., Wells, P.H. & Rogers, S. (1993) Is multiple mating an adaptive feature of monarch butterfly winter aggregation? *Biology and Conservation of the Monarch butterfly*. (eds S. B. Malcolm & M. P. Zalucki), pp. 61–68. Natural History Museum of Los Angeles County, Los Angeles, CA.

Wiklund, C. (1984) Egg-laying patterns in butterflies in relation to their phenology and the visual apparency and abundance of their host plants. *Oecologia (Berlin)* **63,** 23–29.

Wiklund, C. & Kaitala, A. (1995) Sexual selection for large male size in a polyandrous butterfly: the effect of body size on male versus female reproductive success in *Pieris napi*. *Behavioral Ecology* **6,** 6–13.

Wiklund, C. & Karlsson, B. (1984) Egg size variation in satyrid butterflies: adaptive *vs* historical 'Bauplan', and mechanistic explanations. *Oikos* **43,** 391–400.

Wiklund, C. & Persson, A. (1983) Fecundity, and the relation of egg weight variation to offspring fitness in the speckled wood butterfly *Pararge aegeria*, or why don't female butterflies lay more eggs? *Oikos* **40,** 53–63.

Wiklund, C., Karlsson, B. & Forsberg, J. (1987) Adaptive versus constraint explanations for egg-to-body size relationships in two butterfly families. *American Naturalist* **130,** 828–838.

Wiklund, C., Kaitala, A., Lindfors, V. & Abenius, J. (1993) Polyandry and its effect on female reproduction in the green-veined butterfly (*Pieris napi* L.). *Behavioral Ecology and Sociobiology* **33,** 25–33.

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