

REPRODUCTIVE ALLOCATION FROM RESERVES AND INCOME IN BUTTERFLY SPECIES WITH DIFFERING ADULT DIETS

CAROL L. BOGGS

Center for Conservation Biology, Department of Biological Sciences, Stanford University, Stanford, California 94305 USA, and Rocky Mountain Biological Laboratory, Crested Butte, Colorado 81224 USA

Abstract. Allocation of stored and incoming nutrients to reproduction determines an organism's age-specific fecundity curve. In holometabolous insects, differences among species in the shape of the curve are correlated with differences in the potential importance of adult food to reproduction. I examined allocation patterns underlying this association. Specific changes throughout life in body mass and reproductive effort were predicted to result from use of stored vs. incoming nutrients for reproduction and other metabolic needs at each age. Data for three nymphalid butterfly species, *Euphydryas editha*, *Speyeria mormonia*, and *Heliconius charitonius*, were compared with the predictions. These three species differ in adult diet and fraction of oocytes mature at adult emergence (hence, potential for adult nutrients to be used to make eggs), with *E. editha* showing the least potential for use of adult nutrients in egg production and *H. charitonius* showing the greatest potential. For all three species, body mass declined with age, although nonlinearly for *E. editha*. This indicated that metabolic expenditures were greater than intake at all ages, and that a constant fraction of stored nutrients was allocated to reproduction and other metabolic uses at each age for *E. editha*. Reproductive effort also declined with age for all three species. The specific patterns seen suggested that incoming nutrients may be stored, to some extent, early in life and then used late in life by both *S. mormonia* and *H. charitonius*. The similarity between *S. mormonia* and *H. charitonius* is rather surprising, given the qualitative differences in adult diet and suggests either that qualitative age-specific allocation patterns for incoming vs. stored nutrients may be independent of adult diet quality, or that the observed patterns are constrained by phylogenetic relatedness of these two species.

Key words: *age-specific body mass; age-specific fecundity; nutrient reserves; Nymphalidae; ovarian dynamics; reproductive allocation; reproductive effort.*

INTRODUCTION

Nutrients used in egg production by animals can be derived from juvenile feeding, adult female feeding, or, in some cases, nuptial gifts received by the female from the male at mating (e.g., Thornhill and Alcock 1983, Boggs 1990, 1995). The quality and quantity of nutrient resources available from each life stage vary among species. Differences in resource availability may affect the shape of each species' age-specific fecundity curve (Boggs 1986). Among holometabolous insects, in particular, the higher the quality of the adult diet and/or the potential importance to reproduction of adult female feeding, the longer the time that age-specific fecundity stays high and constant before its eventual decline (Fig. 1). The relative availability of nutrients from the juvenile and adult resource pools most likely drives this cross-species correlation between feeding habits and timing of reproduction.

The quantity of either adult- or larval-derived nutrients available for reproduction may vary with age. The quantity of nutrients available from juvenile feed-

ing is fixed by pupation and cannot increase with age. The quantity of nutrients available at any age from adult feeding may vary, depending on relative rates of feeding vs. use. If nutrients from adult feeding exceed expenditures at a particular age, adult-derived nutrients may be stored, joining juvenile-derived nutrients. In such cases, adult- and juvenile-derived, stored nutrients should be treated identically when feeding minus expenditure is no longer a positive value. Because of the differences in timing of feeding and expenditure of nutrients between juveniles and adults, nutrient types that are scarce or non-existent in adult food should limit reproduction, causing it to decline to zero as juvenile stores are depleted.

Three scenarios could describe the quantitative relationship between juvenile-derived and adult-derived nutrients when a given nutrient type is available from both stages. First, juvenile-derived nutrients initially may be completely used for reproduction, and then replaced with adult-derived nutrients. This would allow the new adult time to mature and to locate adult food sources, potentially reducing the risk of early mortality during foraging. Second, juvenile-derived nutrients may be used slowly and steadily through life, with

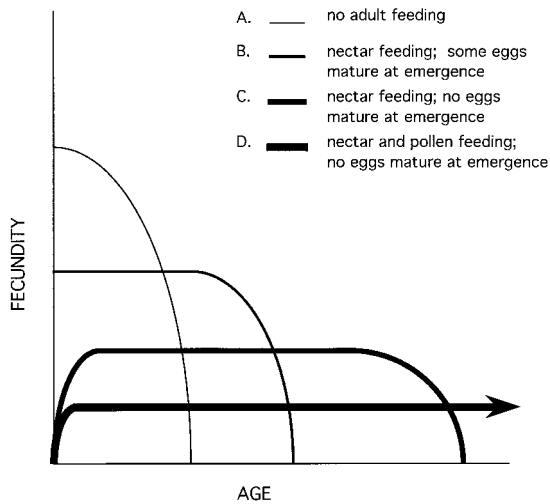


FIG. 1. Changes in age-specific fecundity among lepidopteran species as a function of the potential importance of adult nutrients to reproduction, measured by quality of the adult diet and proportion of eggs mature at adult emergence (which can, therefore, only be made from larval nutrients). Adapted from data in Boggs (1986).

nutrients derived from both juvenile and adult feeding used at the same time. This would reduce foraging requirements consistently through life, and some juvenile reserves would remain to be used in case of late-life shortages of adult food. Finally, juvenile-derived nutrients could be saved, and used only at the end of adult life. Such a strategy would also guard against possible late-life shortages of adult food.

Use of stored nutrients can be regarded as "capital expenditure," whereas use of nutrients as they are acquired can be regarded as "income expenditure," in terminology paralleling that of Sibly and Calow (1984, 1986). Here, I examine the expenditure strategy through time in holometabolous insects, and the implications for the female reproductive pattern. In particular, I explore the effects of adult diet quality and opportunity to use adult nutrients in reproduction on reproductive allocation dynamics (see Fig. 1). Diet quality is defined as the degree to which all needed nutrient types are present. That is, a low-quality diet might contain only carbohydrates, whereas a high-quality diet might contain diverse carbohydrates, proteins, and lipids. Opportunity to use adult nutrients in reproduction is measured by the percentage of eggs fully mature at adult emergence; the fewer mature eggs, the greater the opportunity for use of adult nutrients. I examine differences among species in age-specific changes in body mass and reproductive effort to address the following questions. Do organisms allocate current nutrients independently of the expected nutrient intake? If not, do organisms from species with high-quality expected nutrient intake hoard their stored reserves, or spend them freely in expectation of future intake? Do organisms with low-quality expected nu-

trient intake spend a fluctuating or a constant proportion or amount of nutrient stored reserves at each age, as they use juvenile-derived nutrients not available in the adult diet?

Answers to these questions are important to understanding the interaction between diet and allocation patterns. Various workers have examined the physiology of resource allocation in particular ecological contexts (see Boggs 1992 for a review). The question usually addressed in such work is the relative priority of survival, growth, and reproduction for allocation of scarce nutrients, which may yield trade-offs among life history parameters (e.g., Bazzaz et al. 1987, Gatto et al. 1989, Kooijman et al. 1989, Gurney et al. 1990). This present work addresses resource allocation at a finer scale, examining the relative priority of nutrient use from different life stages for one type of expenditure, reproduction. Such information will contribute to general physiological resource allocation models combining life history and foraging strategies.

EXPECTED AGE-SPECIFIC CHANGES IN BODY MASS AND REPRODUCTIVE EFFORT

Adult body mass and reproductive effort, here defined as egg mass per total body mass, should show age-specific patterns that depend on the use of stored vs. incoming nutrients for egg production. Stored nutrients include those from juvenile feeding plus any previous surplus from adult feeding. In what follows, I present the expected age-specific changes for body mass and reproductive effort, under different scenarios for use of stored and incoming nutrients for reproduction. These expectations can then be compared to experimental observations for species with differing adult food quality and percentage of eggs mature at adult emergence. This allows correlations of nutrient use with the quality, and relative possible reproductive importance, of adult food. For all the expectations outlined, the ratio of nonreproductive to reproductive costs is assumed to be constant with age. Justification for this assumption is presented in the *Discussion*.

Changes in body mass with age

The slope and curvature of changes in body mass with age reflect the use of stored reserves vs. intake through time. There are three major groups of predictions.

1) If nutrient expenditures are greater than intake, a negative slope for body mass vs. age should be seen (Fig. 2A). In this case, both stored and incoming nutrients are expended throughout life. This is likely to happen if adult feeding is minimal.

2) If nutrient expenditures equal intake, a slope of zero for body mass vs. age should be seen (Fig. 2B). This occurs if all incoming nutrients are spent, or if incoming nutrients replace stored nutrients exactly as stored nutrients are used.

3) If nutrient expenditures are less than intake, a

positive slope for body mass vs. age should be seen (Fig. 2C). This results from storage of incoming nutrients.

Further, the relationship between expenditure and intake may change with age, to either of the following:

4) If the ratio of expenditure to intake changes (while still remaining within one of the three classes outlined, e.g., expenditure > intake), curvature in the relationship between body mass and age should be seen. One possible cause of changes in the expenditure : intake ratio is the use of all incoming nutrients plus a constant fraction of stored nutrients at each age (Fig. 2D).

5) If expenditure minus intake of nutrients shifts among negative, positive, and zero, then hybrid curves based on classes 1–4 should be seen. For example, expenditures may equal intake for most of life, but with stored nutrients used in increasing amounts with incoming nutrients at the end of life (expenditures > intake, and the ratio of expenditure : intake continuously changing) (Fig. 2E).

Changes in reproductive effort with age

Age-specific changes in reproductive effort can also provide insight into allocation of stored vs. incoming nutrients to reproduction. The slope of the relationship between reproductive effort and age depends on age-specific changes in reproductive mass and body mass. There are again three major groups of predictions.

1) Predictions if expenditure equals intake:

a) If reproductive mass and body mass remain constant with age, then a zero slope for reproductive effort vs. age will be seen. In this case, only incoming nutrients are spent on reproduction.

b) If reproductive mass declines while body mass is constant with age, then a negative slope for reproductive effort vs. age will be seen. This is produced by decreasing intake or increasing nonreproductive expenditures with age. Again, only incoming nutrients are spent on reproduction.

c) If reproductive mass increases while body mass is constant with age, then a positive slope for reproductive effort vs. age will be seen. This is produced by increasing intake or decreasing nonreproductive expenditures with age. Only incoming nutrients are spent on reproduction.

2) Predictions if expenditures are greater than intake:

a) If the allometric relationship between reproductive and body masses is 1.0, then a zero slope for reproductive effort vs. age will be seen (Fig. 3A). In this case, both incoming and stored nutrients may be spent on reproduction.

b) If the allometric relationship between reproductive and body masses is >1.0, then a negative slope for reproductive effort vs. age will be seen (Fig. 3B). This allometric relationship reflects an increase with body mass in the ratio of reproductive expenditures to

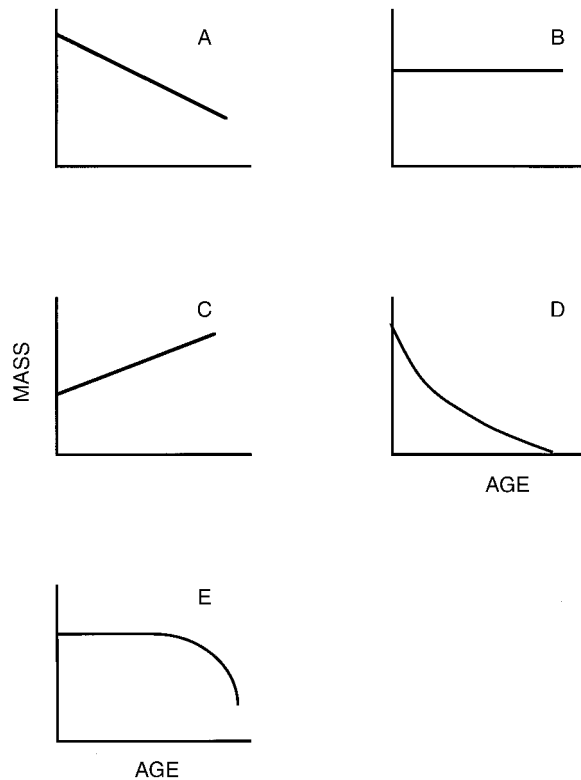


FIG. 2. Expected changes in lepidopteran body mass with age. (A) Expenditures exceed intake; (B) expenditures equal intake; (C) expenditures are less than intake; (D) expenditures equal intake early in life, then expenditures become increasingly greater than intake; (E) expenditures exceed intake, and a constant fraction of stored nutrients is used at each age.

other metabolic expenditures. This pattern could result, for example, if a constant fraction of nutrients were used for other metabolic expenses, while a constant amount of nutrients were used for reproduction.

c) If the allometric relationship between reproductive mass and body mass is <1.0, then a positive slope for reproductive effort vs. age will be seen (Fig. 3C). This allometric relationship reflects a decrease with body mass in the ratio of reproductive expenditures to other metabolic expenditures. This pattern could result, for example, if a constant fraction of available mass were used for reproduction, while a constant amount was used for other metabolic expenses.

3) Predictions if expenditures are less than intake:

a) If the allometric relationship between reproductive and body masses is 1.0, then a zero slope for reproductive effort vs. age will be seen (Fig. 3A). In this case, both incoming and stored nutrients may be spent on reproduction.

b) If the allometric relationship between reproductive and body masses is >1.0, then a negative slope for reproductive effort vs. age will be seen (Fig. 3B). This allometric relationship reflects an increase with body mass in the ratio of reproductive expenditures to

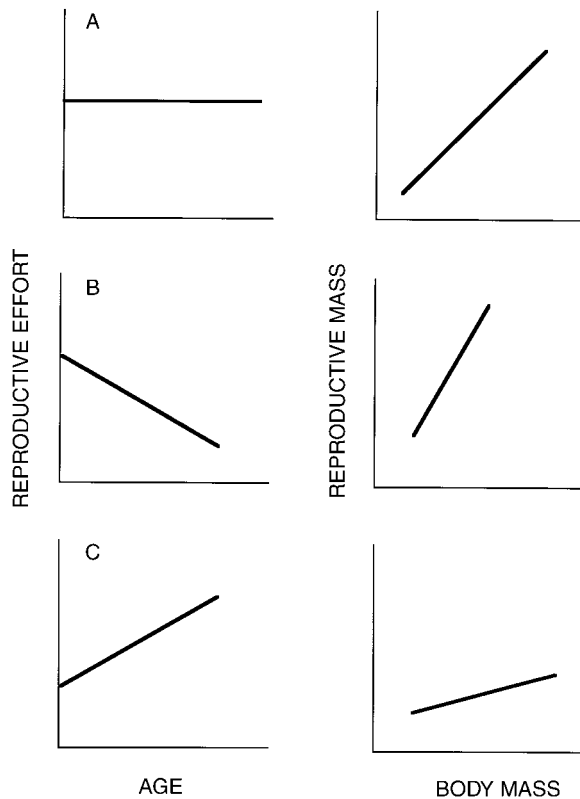


FIG. 3. Expected changes in reproductive effort with age, and the corresponding allometric relation between reproductive and body mass. (A) Expenditure \neq intake, and reproductive effort is unchanged with age. (B) Expenditure \neq intake, and reproductive effort declines with age. (C) Expenditure \neq intake, and reproductive effort increases with age.

other metabolic expenditures. The pattern could result if other metabolic expenses were constant, while use of nutrients in reproduction increased with body mass.

c) If the allometric relationship between reproductive mass and body mass is <1.0 , then a positive slope for reproductive effort vs. age will be seen (Fig. 3C). This allometric relationship reflects a decrease with body mass in the ratio of reproductive to other metabolic expenditures. The pattern could result if other metabolic expenditures were to increase with body mass and, hence, age, while reproductive expenditures remained constant.

As for body mass, the relationship between nutrient intake and expenditure may change with age, producing hybrid age-specific patterns. However, curvature in the age-specific patterns may be produced by either:

4) changes in the ratio of expenditure to intake as outlined for body mass; or

5) different rates of change in reproductive and body mass.

CHOICE OF STUDY ANIMALS

To explore patterns of allocation to reproduction from stored vs. incoming nutrients, I used three but-

terfly species (of family Nymphalidae) that differ in quality of the adult diet and in potential importance of adult nutrients to egg production.

The first, *Euphydryas editha*, fits the pattern described in Fig. 1B: the adult diet is low quality, a percentage of the eggs is made using only larval-derived resources, and egg production is limited. Adults feed on nectar, which primarily contains carbohydrates, along with a small amount of amino acids and lipids (reviewed by Boggs 1987). At mating, females receive from males nutrients that are used in egg production (Boggs 1995, 1997). However, females mate 1.4 times, on average, over their life-span (Labine 1964), so availability of male nutrients is limited. A mean of 17.8% (and up to 40%) of the eggs are already mature at adult emergence in the Jasper Ridge (San Mateo County, California) population of *E. editha* (Labine 1968); adult nutrients cannot be used to make those eggs. Nectar availability is important to production of later eggs (Murphy et al. 1983). Females emerge with a fixed number of oocytes in the ovaries. Egg production begins at a high level, remains there for ≈ 1 wk, and then declines (Labine 1968).

The second species, *Speyeria mormonia*, fits the pattern described in Fig. 1C: the adult diet is low quality, but no eggs are mature at adult emergence, although total egg production is limited. Adults feed on nectar, and older females may occasionally feed at mud, dung, or carrion (Boggs and Jackson 1991, Sculley and Boggs 1996). Females mate an average of 1.03 times over their life-span (Boggs 1986), so male-derived nutrient input is limited. No eggs are mature at adult emergence, so adult nutrients could be used in all eggs (Boggs 1986). Adult nutrients are important in maintaining fecundity. If females are semistarved, fecundity declines in direct proportion to the decrease in adult food (Boggs and Ross 1993). Females emerge with a fixed number of oocytes in the ovaries. Egg production initially increases, levels off, and then declines after ≈ 2 wk, depending on temperature (Boggs 1986) and probably other factors.

The third species, *Heliconius charitonius*, fits the pattern shown in Fig. 1D: the adult diet is high quality, containing diverse nutrient types that are of maximum potential importance in egg production, since no eggs are mature at adult emergence, and egg production does not decline as the female ages. Adults feed on both pollen and nectar, deriving carbohydrates, amino acids, and other compounds (Gilbert 1972). Pollen deprivation results in a marked decrease in fecundity. Females mate an average of 1.03 times over their lifetimes (Boggs 1979, 1990), so input of male-derived nutrients is limited. No eggs are mature at adult emergence, so adult nutrients could be used in all eggs. Oogenesis continues throughout adult life, as long as pollen is available to the female (Dunlap-Pianka et al. 1977). Egg production initially increases, then levels off and stabilizes.

METHODS

Female maintenance

Euphydryas editha.—Late last-instar larvae were collected from a population at Kirby Canyon, Santa Clara County, California. Larvae were fed *Plantago erecta* (Plantaginaceae) derived from seed collected at Kirby Canyon and at Jasper Ridge Preserve. *P. erecta* is a usual host plant for this population.

Dates of eclosion and mating were recorded for all females: 14 females mated on the 1st d of adult life, four mated on the 2nd d and three on the 3rd d. Females were caged with several males for mating, and the pair was removed when mating was begun, usually within 5 min.

Mated females were maintained in 0.3 m diameter \times 0.5 m tall cages, placed inside a greenhouse. Each cage had a plywood floor, net screen sides, and a top made from an inverted metal bowl to provide shade. Cages contained one pot of *P. erecta* for oviposition and three pots of flowering *Layia platyglossa* (Compositae) for nectar. *L. platyglossa* nectar is utilized in the field, and plants were grown from seed obtained locally. Females were placed on nectar and oviposition plants at least twice a day to guarantee that the plants were found by the females. However, the plants were constantly available, and females were often observed spontaneously ovipositing or nectaring.

Speyeria mormonia.—Females were collected as freshly eclosed and mated adults that had not yet oviposited. They came from a field population immediately south of the Rocky Mountain Biological Laboratory, Gothic, Gunnison County, Colorado. Females were maintained in cages as described by Boggs and Ross (1993). All cages contained one or two *Viola nuttallii* (Violaceae) leaves (a native larval host plant) in a dram vial (3.697 mL) filled with water and plugged with paper towel.

Females were fed ad libitum on a 25% honey : water solution in the morning and late afternoon. The honey : water solution is a good substitute for the normal adult diet in this species, yielding maximum survival and fecundity (Boggs and Ross 1993; C. L. Boggs, unpublished data).

Heliconius charitonius.—Females came from a free-flying greenhouse population at Stanford University. The population originated from San José, Costa Rica, and had been maintained in captivity on native host plants as a freely interbreeding population of \approx 50 individuals, with overlapping generations for \approx 40 generations.

Pupae and three males were put in 1.0 \times 0.5 \times 1.3 m net cages within a greenhouse. Females mated at, or soon after, eclosion. Dates of female eclosion and mating were noted. All extra males were removed from the cage after mating began.

Each cage contained one female, her mate, one potted *Passiflora caerulea* or *P. biflora* (Passifloraceae)



PLATE 1. Top: *Heliconius charitonius* feeding from *Psiguria umbrosa*. Bottom: *Speyeria mormonia* in grassland habitat in Colorado.

for oviposition, and one potted *Psiguria umbrosa* (Cucurbitaceae), a native source of nectar and pollen. Each cage contained at least one *P. umbrosa* flower per butterfly, providing sufficient nectar and pollen (V. K. Iyengar and C. L. Boggs, unpublished data). However, supplemental 25% honey : water solution, which was changed twice a week, was also placed in each cage.

Ovarian status at adult emergence in E. editha

It was possible that *E. editha* from different populations could differ in the number and maturation state of oocytes in virgin females, given that time to mating and first oviposition varies among populations (M.

Singer, *personal communication*). Kirby Canyon females had not previously been examined. Data were needed to provide information on the number of eggs that a female could possibly lay, for comparison with the number of eggs laid under experimental conditions. Therefore, 13 virgin females were weighed to the nearest 0.1 mg and dissected, and the numbers of mature and immature oocytes in the ovaries were counted.

*Age-specific changes in body mass
and reproductive effort*

All females were weighed to the nearest 0.1 mg daily at sunset, or, for *S. mormonia*, 1 h after the late afternoon feeding. All three species are diurnal, and females are quiescent by sunset. Eggs were collected at sunset, counted, and weighed as a group to the nearest 0.1 mg.

Seventeen *H. charitonius* were studied under ambient light: dark cycles, with maximum and minimum daily greenhouse temperatures of $31.0 \pm 3.8^\circ\text{C}$ (mean ± 1 SD) and $16.4 \pm 1.4^\circ\text{C}$, respectively. (Sixteen of these individuals were used for the body mass study; nine butterflies were killed by greenhouse spiders and did not live a full physiological life-span.) Cages for the 24 *S. mormonia* used were placed in an environmental chamber at $25^\circ\text{--}29^\circ\text{C}$ and a 12:12 L:D cycle. Twenty-one *E. editha* were studied under ambient light: dark cycles during the adult flight season, and temperatures fluctuating in parallel with those in the field. Representative mean daily maximum and minimum temperatures were $34.0 \pm 3.3^\circ\text{C}$ and $12.6 \pm 2.0^\circ\text{C}$, respectively ($n = 35$).

Data analysis

Nonlinear regressions were calculated for body mass against age, using both linear and quadratic terms to examine slope and curvature. Day 1 was the day before eggs were first laid. Daily body mass values used in the regression were age effects from an ANOVA for unbalanced design, using age and butterfly as category variables and body mass as the dependent variable.

To examine changes in reproductive effort through time, egg mass laid on a given day was divided by body mass on the previous evening. The first day of egg-laying was considered as day 1. Reproductive effort was regressed against age and age², again to detect both linear and quadratic changes in reproductive effort with age. As before, reproductive effort values used in the regression were age effects from an ANOVA for unbalanced design, using age and butterfly as category variables and body mass as the dependent variable.

For regressions of both body mass and reproductive effort against age, *t* values for coefficients for age and/or age² that were significant at $P < 0.001$ were further examined for significance. A jackknife statistic was used, first dropping data for butterflies living the shortest and longest number of days, then for butterflies

living the next longest and shortest number of days, etc. (Sokal and Rohlf 1981).

One alternative method of data analysis would have been a repeated-measures ANOVA. Unequal life-spans of individual butterflies, however, led to unequal numbers of repeated measures. This could have been overcome by assuming an underlying physiological process that is uniform among individuals, but varies only in whether it occurs at a fast or slow rate. With this assumption, data points could be interpolated evenly through the data sets for shorter lived individuals, giving a uniform life-span and allowing a repeated-measures test. I rejected this approach on two grounds: first, I have no basis for making the assumption, and second, such interpolation may amplify random deviations from linearity or other underlying pattern. Further, the statistical tests actually used are, if anything, conservative.

ANOVAs and regression analyses were done using SYSTAT (Wilkinson 1990).

RESULTS

Euphydryas ovarian status at emergence

Dissections of 13 newly eclosed female *E. editha* showed that $17.4 \pm 4.7\%$ of the oocytes, or 113.9 ± 32.7 eggs, were mature (all values $\bar{X} \pm 1$ SD). This corresponds to the mean number of eggs contained in the first 2.6 ± 1.6 egg batches, or laid during the first 1.7 ± 1.3 d of egg-laying in the greenhouse. Neither the number nor percentage of mature eggs was significantly correlated with female initial mass. However, the total number of oocytes in the ovaries increased with increasing female mass (number of oocytes = $150.6 + 2.0(\text{mass})$; $r = 0.67$; $F_{1,11} = 8.93$; $P = 0.01$; mass of dissected females = 261.0 ± 31.4 mg; 660 ± 91 oocytes per female).

Reliability of fecundity data

There was no significant difference for *E. editha* between mean number of eggs laid and mean potential fecundity (the number of oocytes at adult emergence), based on an ANCOVA with initial female mass as a covariate to control for differences due to female size (all values $\bar{X} \pm 1$ SD: 307 ± 209 eggs laid; initial body mass, 189.7 ± 40.1 mg; eggs laid vs. oocytes at emergence, $F_{1,31} = 2.56$, NS). This indicates that experimental females laid all their eggs, rather than resorbing some.

S. mormonia females laid significantly fewer eggs than the number of oocytes at emergence, with initial female body mass as a covariate (451 ± 63 oocytes at emergence (Boggs and Ross 1993); 262 ± 170 eggs laid; $F_{1,31} = 18.7$, $P < 0.001$). Eliminating females that laid < 300 eggs reduced the sample size from 24 to nine females but resulted in a subset of females whose egg output no longer differed significantly from the number of oocytes in the ovaries at adult emergence

TABLE 1. Regression of body mass on age for three nymphalid butterfly species, with body mass calculated as age effects from an ANOVA for unbalanced design with butterfly and age as category variables, and body mass as the dependent variable.

Variable	Coefficient	<i>t</i>	<i>P</i>	<i>t</i> _s (jackknife)	df	<i>P</i>
A) <i>Euphydryas editha</i> (multiple <i>r</i> = 0.98; regression: $F_{2,15} = 207.5$, <i>P</i> < 0.001)						
Constant	168.0	34.27	<0.001			
Age	-12.5	-10.55	<0.001	-5.41	9	<0.001
Age ²	0.4	6.14	<0.001	4.54	9	<0.01
B) <i>Speyeria mormonia</i> , all females (multiple <i>r</i> = 0.95; regression: $F_{1,19} = 185.63$, <i>P</i> < 0.001)						
Constant	170.0	48.80	<0.001			
Age	-3.8	-13.62	<0.001	-5.21	11	<0.001
C) <i>S. mormonia</i> , only females laying >300 eggs (multiple <i>r</i> = 0.95; regression: $F_{1,19} = 190.24$, <i>P</i> < 0.001)						
Constant	176.5	51.93	<0.001			
Age	-3.7	-13.79	<0.001	-5.07	3	<0.02
D) <i>Heliconius charitonius</i> , all females (multiple <i>r</i> = 0.98; regression: $F_{2,30} = 503.69$, <i>P</i> < 0.001)						
Constant	135.4	91.77	<0.001			
Age	-0.8	-4.03	<0.001	-2.34	7	<0.10
Age ²	-0.02	-3.73	0.001	-2.24	7	<0.10
E) <i>H. charitonius</i> , only females killed by greenhouse spiders (multiple <i>r</i> = 0.96; regression: $F_{1,31} = 364.20$, <i>P</i> < 0.001)						
Constant	136.4	105.31	<0.001			
Age	-1.3	-19.08	<0.001	-3.52	3	<0.05

(443 ± 107 eggs laid; $F_{1,16} = 2.4$, NS). Consequently, both the complete and reduced *S. mormonia* data sets were examined separately for changes in body mass and reproductive effort with age.

The number of oocytes at emergence is not fixed for *H. charitonius* females (Dunlap-Pianka et al. 1977). Hence, no estimate of potential egg numbers is possible.

Changes in body mass with age

Body mass declined with age for all species (Table 1, Fig. 4). The age² regression coefficient was significantly positive for *E. editha*, significantly negative for the full *H. charitonius* data set, but insignificant for *S. mormonia* and *H. charitonius* females killed by greenhouse spiders (Table 1). Results for *S. mormonia* were the same for both the complete data set and only those females laying >300 eggs. Data for all species are consistent with the hypothesis that nutrient expenditures are greater than nutrient intake throughout life. For *E. editha*, however, either expenditures equal intake late in life, or a constant fraction of stored nutrients is used throughout life, producing the significant quadratic regression coefficient.

Changes in reproductive effort with age

Daily reproductive effort declined with age (but not age²) for *E. editha* (Table 2A, Fig. 5). For both *S. mormonia* and *H. charitonius*, daily reproductive effort declined with age²; age was insignificant (Table 2, Fig. 5). Observed changes in reproductive effort with age did not differ between female *S. mormonia* laying >300 eggs and the whole *S. mormonia* data set, or between

female *H. charitonius* killed by greenhouse spiders and the whole *H. charitonius* data set (Table 2). Since the earlier analysis showed that nutrient expenditures exceed nutrient intake for all three species, the data are consistent with the prediction of a decrease in the ratio of reproductive to other metabolic expenditure with decreasing body mass. The nonlinearity for *S. mormonia* and *H. charitonius* suggests that, as the organisms age, changes in body mass are associated with disproportionately larger increases or decreases in reproduction.

DISCUSSION

Euphydryas ovarian status at emergence

The ovarian status of Kirby Canyon *E. editha* at adult emergence was essentially identical to that of females studied by Labine (1968) from the same subspecies. Labine (1968) found that 17.8% of the oocytes were mature in females from the Jasper Ridge population, as compared with 17.4% of the oocytes in this study. This similarity is not surprising, as the two populations are most likely members of the same metapopulation (Harrison et al. 1988). Further, the correlation of initial female body mass with total number of oocytes in the ovaries, but not with number of oocytes that are initially mature, suggests that the numbers of oocytes and of mature oocytes are unlinked.

Allocation patterns among species

E. editha: low quality and potential importance of adult food.—Of the three species examined, *E. editha* should have the lowest dependence on adult feeding

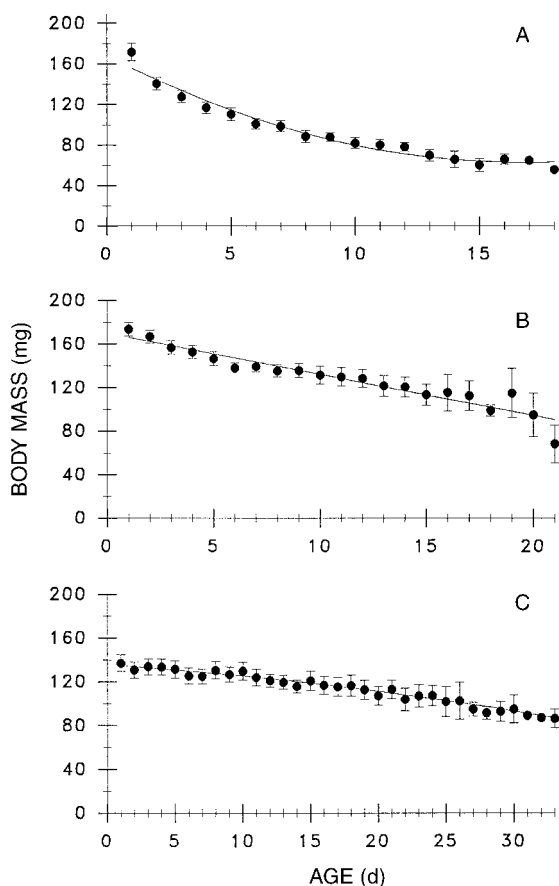


FIG. 4. Body mass as a function of age. Body mass data are the age effects means from an unbalanced-design ANOVA, with age and butterfly as category variables. Error bars represent standard errors of the age effect mean. The line is the regression of body mass on age from Table 1. (A) *E. editha*, no. butterflies at each age: 21, days 1–3; 20, days 4–5; 19, day 6; 18, day 7; 17, day 8; 15, day 9; 12, day 10; 11, day 11; 9, day 12; 7, day 13; 5, days 14–15; 4, day 16; 2, day 17; 1, day 18. (B) *S. mormonia*: 19, day 1; 24, days 2–5; 23, day 6; 22, day 7; 20, day 8; 18, day 9; 17, day 10; 16, day 11; 14, day 12; 12, day 13; 11, day 14; 8, day 15; 6, day 16; 3, days 17–18; 2, days 19–21. (C) *H. charitonius*: 16, days 1–5; 15, days 6–7; 14, days 8–13; 13, day 14; 11, days 15–19; 7, days 20–21; 6, days 22–24; 4, days 25–26; 2, days 27–33.

for reproduction, due to the number of oocytes already yolked at adult emergence and the scarcity in the adult nectar diet of some nutrient types, such as lipids and nitrogenous compounds. Combined results from examination of age-specific body mass and reproductive effort patterns suggest that (1) nutrients derived from adult feeding are not stored, and (2) a constant fraction of stored nutrients is allocated to reproduction and other functions at each age.

Spending a constant fraction of reserves is consistent with results of studies on allocation to individual eggs in species with similar adult feeding habits. Telfer and Rutberg (1960), Campbell (1962), and others have suggested that egg mass and/or oviposition rates decline

with age due to declining availability of reproductive reserves for oogenesis, and, hence, slower uptake rates of those reserves. This would happen if uptake depended on the concentration of available compounds, which declines as reserves decline. Wiklund and Karlsson (1984) provide supporting evidence that use of a constant proportion of reserves results in egg mass decline in the butterfly *Lasiommata megera*.

The lack of effect of age² on reproductive effort suggests that the importance of adult-derived nutrients to egg production remains relatively constant throughout life. Combined with earlier work on *E. editha*, showing that fecundity of older females was depressed if adult nutrients were not available (Murphy al. 1983), these data predict that stored nutrients should be used at a faster rate under conditions of low availability of adult nutrients.

S. mormonia: low quality, but high potential importance of adult food.—*S. mormonia* should have an intermediate dependence on adult feeding for reproduction, given the adult diet of nectar and the lack of mature eggs at adult emergence. As for *E. editha*, combined results for the whole *S. mormonia* data set from age-specific body mass and reproductive effort patterns suggest that (1) metabolic expenditures are greater than adult nutrient intake, and (2) as body mass decreases, allocation to reproduction decreases more rapidly than allocation to other metabolic expenses. However, the linear relationship between body mass and age indicates that a constant amount, rather than a constant fraction, of stored nutrients is spent at each age interval. Further, stored nutrients may be increasingly important in reproduction as the butterflies age, since the data indicate that a given change in body mass results in lower reproductive output for older individuals. One possible interpretation of these data is that some adult-derived nutrients are stored early in adult life (although not enough to produce a positive net intake–expenditure balance), and then these stored nutrients are increasingly used in reproduction as the butterfly ages. That is, storage of adult-derived nutrients would convert an age-specific body mass curve based on a constant proportion of stored nutrients spent at each age to a curve that looked like a constant amount of stored nutrients was spent at each age, tending to linearize the relationship. This hypothesis is supported by the observation that, for some individuals, restriction of adult food availability during the first 5 d of adult life leads to the same decreases in fecundity as restriction during the entire life-span (Boggs and Ross 1993; C. L. Boggs, unpublished data).

S. mormonia was the only species fed honey : water rather than nectar from a natural adult host. Although neither female fecundity nor intake differed among individuals fed 25% honey : water vs. nectar (C. L. Boggs, unpublished data), honey : water could differ in its relative composition from nectar and mud, dung, or carrion, with nutrients available in different ratios. Any

TABLE 2. Regression daily reproductive effort on age, with body mass calculated as age effects from an ANOVA for unbalanced design with butterfly and age as category variables, and daily reproductive effort as the dependent variable.

Variable	Coefficient	t	P	t_s (jackknife)	df	P
A) <i>Euphydryas editha</i> (multiple $r = 0.94$; regression: $F_{1,15} = 104.02$, $P < 0.001$)						
Constant	0.093	11.75	<0.001			
Age	-0.008	-10.20	<0.001	-4.22	9	<0.01
B) <i>Speyeria mormonia</i> , all females (multiple $r = 0.84$; regression: $F_{1,19} = 45.67$, $P < 0.001$)						
Constant	0.036	14.87	<0.001			
Age ²	-0.00008	-6.76	<0.001	-2.32	11	<0.05
C) <i>S. mormonia</i> , only females laying >300 eggs (multiple $r = 0.72$; regression: $F_{1,19} = 20.26$, $P < 0.001$)						
Constant	0.049	15.00	<0.001			
Age ²	-0.00007	-4.50	<0.001	-2.66	3	<0.10
D) <i>Heliconius charitonius</i> , all females (multiple $r = 0.73$; regression: $F_{1,31} = 35.16$, $P < 0.001$)						
Constant	0.017	13.00	<0.001			
Age ²	-0.00001	-5.93	<0.001	-2.66	7	<0.10
E) <i>H. charitonius</i> , only females killed by greenhouse spiders (multiple $r = 0.84$; regression: $F_{1,30} = 69.81$, $P < 0.001$)						
Constant	0.016	10.00	<0.001			
Age ²	-0.00003	-8.36	<0.001	-5.96	3	<0.01

differences in composition could affect allocation patterns, altering storage and use patterns of adult-derived nutrients.

H. charitonius: *high quality and potential importance of adult food*.—Of the species studied here, *H. charitonius* should have the highest dependence on adult resources, given an adult diet composed of pollen and nectar, and the opportunity for all adult-derived nutrients to be used in making all eggs. However, there were no qualitative, but only quantitative, differences between the allocation patterns for *H. charitonius* and the full *S. mormonia* data set. This was unexpected, and suggests that qualitative allocation patterns from stored vs. incoming nutrients may be somewhat independent of adult diet quality. That is, changes in adult diet quality may be associated only with changes in rates of stored nutrient usage, whereas the basic age-specific pattern of nutrient storage and expenditure may be dependent on other factors. Alternatively, phylogenetic constraint may play a role. *Heliconius* and *Speyeria* are members of the tribe Heliconiini within the subfamily Heliconiinae, but *Euphydryas* is a member of the tribe Melitaeini within the subfamily Nymphalinae (Harvey 1991). Thus, it is possible that the similarity between *H. charitonius* and *S. mormonia* is due to historical factors. This hypothesis remains to be tested.

Effects of nonreproductive expenditures

As noted earlier, allocation of resources to nonreproductive traits, such as respiration in support of basal metabolism or flight, is assumed here to have no effect on the interpretation of changes in body mass with age. This will be true if the ratio of nonreproductive to reproductive costs is constant with age. This assumption has not been verified, but is reasonable. Flight

should become less expensive as body mass decreases with age, but the degree to which wings fray with age will also affect wing loading and, hence, flight efficiency. The necessity to fly to find oviposition sites and food decreases towards the end of life for *E. editha* and *S. mormonia*, as both fecundity and adult feeding decrease (Labine 1968, Boggs 1986, Boggs and Ross 1993).

Should the assumption not hold, for example, if respiratory costs were to remain constant or increase while reproductive allocation declined, expectations associated with different age-specific patterns of body mass and reproductive effort would need to be revised. There are two possible approaches to resolving this issue. First, construction of age-specific energy budgets separating reproductive energy expenditures from all other expenditures would directly test the assumption. Second, a more detailed analysis of allocation of stores and incoming food to reproduction using radiotracers would provide a direct and strong test of the interpretation of patterns observed here. The second approach has been taken for two of the three species studied here (Boggs 1997).

Conclusions

Among species, increasing potential importance of adult food to egg production is associated with a shift from initial use of reserves (most likely from larval feeding) in egg production towards greater maintenance of stored nutrients (either larval- or adult-derived) until the end of life. Although expenditures are greater than intake throughout life for all three species, *H. charitonius* (and probably to a lesser extent *S. mormonia*), with high potential use of nutrients in egg production, appears to maintain more nutrient stores for a

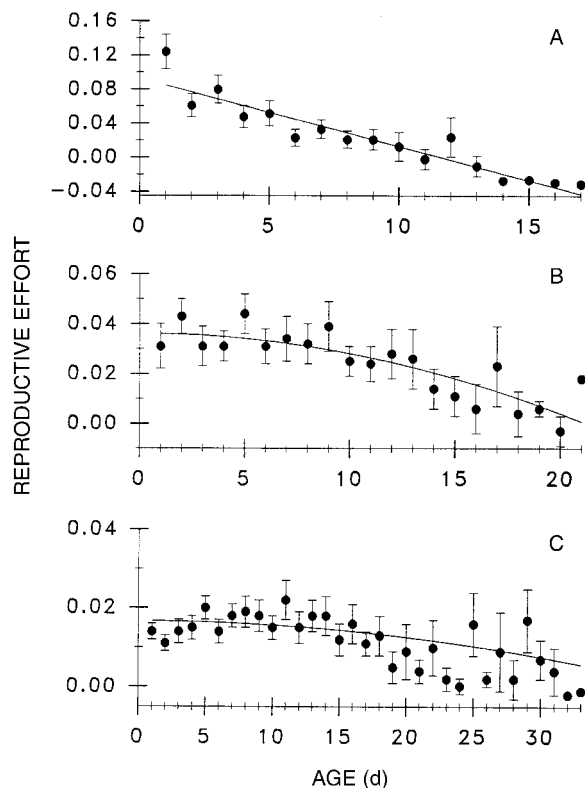


FIG. 5. Daily reproductive effort with age. Reproductive effort data are the age effects means from an unbalanced-design ANOVA, with age and butterfly as category variables. Error bars represent standard errors of the age effect mean. The line is the regression of reproductive effort on age (Table 2). Negative values for reproductive effort late in life are due to use of age effect means; actual values for each butterfly were, of course, positive. (A) *E. editha*, no. butterflies at each age: 21, days 1–2; 20, days 3–5; 18, day 6; 17, day 7; 15, day 8; 13, day 9; 11, day 10; 9, day 11; 7, day 12; 5, days 13–15; 3, day 16; 1, day 17. (B) *S. mormonia*: 24, days 1–4; 23, day 5; 22, day 6; 21, day 7; 19, day 8; 17, day 9; 16, day 10; 14, day 11; 13, day 12; 11, day 13; 8, day 14; 6, day 15; 4, day 16; 3, day 17; 2, days 18–20; 1, day 21. (C) *H. charitonus*: 17, days 1–4; 16, days 5–6; 15, days 7–12; 14, day 13; 13, day 14; 12, days 15–18; 10, day 19; 8, day 20; 7, day 21–23; 6, day 24; 5, day 25; 4, day 26; 3, days 27–32; 2, day 33.

longer period than does *E. editha*, with low potential use.

Various dipterans have morphs that require a blood meal for ovarian development (anautogeny) or that can mature eggs without a blood meal (autogeny). These morphs occupy the ends of the range of potential importance of adult feeding to egg production studied here. In both forms of the screw-worm fly, *Chrysomya bezziana*, the fat body (=stored nutrients) decreases with age; the decrease is slower in anautogenous morphs with access to proteinaceous food (Spradberry and Sands 1981). This pattern is similar to that detected for butterflies here, with stored nutrients maintained to a greater extent as adult food becomes more important to egg maturation.

This association among potential use of adult nutrients in egg production, use of stored vs. incoming nutrients in egg production, and age-specific fecundity suggests that feasible age-specific fecundity patterns in holometabolous organisms may be limited by allocation patterns of stored vs. incoming resources. The questions remain: are stored, larval-derived nutrients maintained in storage, or replaced by nutrients taken in by adults? Are all nutrient types, such as carbohydrates and proteins, treated equivalently, or are types absent in adult feeding treated differently from those that are present? Answers to these questions are needed in order to construct resource allocation models examining the constraints on age-specific fecundity imposed by a holometabolous lifestyle.

ACKNOWLEDGMENTS

I thank G. Daily, G. Henzie, C. Meister, J. Richmond, C. Ross, K. Strickler, J. Switzer, and S. Weiss for technical assistance; L. Moses for statistical advice; A. Brody, J. H. Cushman, B. Karlsson, A. Launer, K. Oberhauser, W. Watt, and two anonymous reviewers for comments on the manuscript; and the U.S. Fish and Wildlife Service for the necessary permits. The work was funded by a Whitehall Foundation grant, and site access and further support came from Waste Management Incorporated.

LITERATURE CITED

- Bazzaz, F. A., N. R. Chiarello, P. D. Coley, and L. F. Pitelka. 1987. Allocating resources to reproduction and defense. *BioScience* **37**:58–67.
- Boggs, C. L. 1979. Resource allocation and reproductive strategies in several heliconiine butterflies. Dissertation. University of Texas, Austin, Texas, USA.
- . 1986. Reproductive strategies of female butterflies: variation in and constraints on fecundity. *Ecological Entomology* **11**:7–15.
- . 1987. Ecology of nectar and pollen feeding in Lepidoptera. Pages 369–391 in F. Slansky, Jr. and J. G. Rodriguez, editors. *Nutritional ecology of insects, mites, and spiders, and related invertebrates*. John Wiley, New York, New York, USA.
- . 1990. A general model of the role of male-donated nutrients in female insects' reproduction. *American Naturalist* **136**:598–617.
- . 1992. Resource allocation: exploring connections between foraging and life history. *Functional Ecology* **6**:508–518.
- . 1995. Male nuptial gifts: phenotypic consequences and evolutionary implications. Pages 215–242 in S. R. Leather and J. Hardie, editors. *Insect reproduction*. CRC Press, New York, New York, USA.
- . 1997. Dynamics of reproductive allocation from juvenile and adult feeding: radiotracer studies. *Ecology* **78**:192–202.
- Boggs, C. L., and L. A. Jackson. 1991. Mud puddling by butterflies is not a simple matter. *Ecological Entomology* **16**:123–127.
- Boggs, C. L., and C. L. Ross. 1993. The effect of adult food limitation on life history traits in *Speyeria mormonia* (Lepidoptera: Nymphalidae). *Ecology* **74**:433–441.
- Campbell, I. M. 1962. Reproductive capacity in the genus *Choristoneura* Led. (Lepidoptera: Tortricidae). I. Quantitative inheritance and genes as controllers of rates. *Canadian Journal of Genetics and Cytology* **4**:272–288.
- Dunlap-Pianka, H. L., C. L. Boggs, and L. E. Gilbert. 1977.

- Ovarian dynamics in heliconiine butterflies: programmed senescence versus eternal youth. *Science* **197**:487–490.
- Gatto, M., C. Matessi, and L. B. Slobodkin. 1989. Physiological profiles and demographic rates in relation to food quantity and predictability: an optimization approach. *Evolutionary Ecology* **3**:1–30.
- Gilbert, L. E. 1972. Pollen feeding and reproductive biology of *Heliconius* butterflies. Proceedings of the National Academy of Sciences (USA) **69**:1403–1407.
- Gurney, W. S. C., E. McCauley, R. M. Nisbet, and W. W. Murdoch. 1990. The physiological ecology of *Daphnia*: a dynamic model of growth and reproduction. *Ecology* **71**:716–732.
- Harrison, S., D. D. Murphy, and P. R. Ehrlich. 1988. Distribution of the bay checkerspot butterfly, *Euphydryas editha bayensis*: evidence for a metapopulation model. *American Naturalist* **132**:360–382.
- Harvey, D. 1991. Appendix B. Higher classification of the Nymphalidae. Pages 255–268 in H. F. Nijhout, editor. The development and evolution of butterfly wing patterns. Smithsonian Institution Press, Washington, D. C., USA.
- Kooijman, S. A. L. M., N. van der Hoeven, and D. C. van der Werf. 1989. Population consequences of a physiological model for individuals. *Functional Ecology* **3**:325–336.
- Labine, P. A. 1964. Population biology of the butterfly *Euphydryas editha*. I. Barriers to multiple inseminations. *Evolution* **18**:335–336.
- . 1968. The population biology of the butterfly *Euphydryas editha*. VIII. Oviposition and its relation to the pattern of oviposition in other butterflies. *Evolution* **22**:799–805.
- Murphy, D. D., A. E. Launer, and P. R. Ehrlich. 1983. The role of adult feeding in egg production and population dynamics of the checkerspot butterfly *Euphydryas editha*. *Oecologia* **56**:257–263.
- Sculley, C. E., and C. L. Boggs. 1996. Mating systems and sexual division of foraging effort affect puddling behavior by butterflies. *Ecological Entomology* **21**:193–197.
- Sibly, R. M., and P. Calow. 1984. Direct and absorption costing in the evolution of life cycles. *Journal of Theoretical Biology* **111**:463–473.
- Sibly, R. M., and P. Calow. 1986. Physiological ecology of animals. Blackwell Scientific Publications, Oxford, UK.
- Sokal, R. R., and F. J. Rohlf. 1981. Biometry. The principles and practice of statistics in biological research. W. H. Freeman, San Francisco, California, USA.
- Spradberry, J. P., and D. P. A. Sands. 1981. Larval fat body and its relationship to protein storage and ovarian development in adults of the screw-worm fly *Chrysomya bezziana*. *Entomologia experimentalis et applicata* **30**:116–122.
- Telfer, W. H., and L. D. Rutberg. 1960. The effects of blood protein depletion on the growth of the oocytes in the cecropia moth. *Biological Bulletin* **18**:352–366.
- Thornhill, R., and J. Alcock. 1983. The evolution of insect mating systems. Harvard University Press, Cambridge, Massachusetts, USA.
- Wiklund, C., and B. Karlsson. 1984. Egg size variation in satyrid butterflies: adaptive vs. historical, “Bauplan”, and mechanistic explanations. *Oikos* **43**:391–400.
- Wilkinson, L. 1990. SYSTAT. Evanston, Illinois, USA.