

DYNAMICS OF REPRODUCTIVE ALLOCATION FROM JUVENILE AND ADULT FEEDING: RADIOTRACER STUDIES

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Abstract. Nutrients used in reproduction may come from adult feeding or reserves stored from the juvenile stage. The dynamics of allocation from these sources are predicted to differ among nutrient types, depending on the relative availability of each nutrient type from adult and juvenile feeding. Using radiotracer techniques, I examined reproductive allocation of glucose and amino acids from adult and juvenile sources in two nymphalid butterflies, *Euphydryas editha* and *Speyeria mormonia*. The species used were intermediate in expected importance of adult nutrients to egg production, with abundant carbohydrates but few nitrogenous compounds available from the adult diet. As predicted, for compounds abundantly available in the adult diet, incoming nutrients were used in preference to stored nutrients. For compounds present in low amounts in the adult diet, juvenile reserves were used throughout adult life, although adult sources were used if available. Nutrients received by the female from the male at mating, although expected to be treated as stored reserves, were immediately used in egg production. Thus, restriction of adult or juvenile feeding may cause different nutrient types (e.g., carbohydrates, nitrogenous compounds) to become limiting to reproduction. These results are consistent with earlier allocation studies examining age-specific changes in body mass and reproductive effort, and the effects on fecundity of quantitative adult food reduction. The work has implications for understanding the evolution of nutrient types donated by males to females, the effects of a holometabolous lifestyle on age-specific fecundity, and the effects of using stored reserves vs. income in reproduction. The present results allow further predictions concerning effects of food supply perturbation on fecundity and, hence, population dynamics, and suggest ways in which species and individuals will differ in sensitivity to those perturbations.

Key words: age-specific fecundity; *Euphydryas editha*; holometabolous insects; limiting factors; male nutrient donations; Nymphalidae; reproductive allocation; *Speyeria mormonia*.

INTRODUCTION

An organism's reproduction is fueled by nutrients that may be gathered during both its juvenile and adult stages. Nutrients spent on reproduction but derived from juvenile feeding must be stored until adulthood. These nutrients are "capital reserves" (in terms like those of Sibly and Calow 1984, 1986). Nutrients from adult feeding may be either stored or immediately allocated to reproduction. In the latter case, adult feeding leads to "income expenditure" (cf. Sibly and Calow 1984, 1986). A combination of the quality and quantity of juvenile nutrient reserves carried to adulthood, the quality, quantity, and predictability of the adult food supply, and the age-specific pattern of use or storage of nutrients from juvenile and adult sources should shape age-specific reproductive patterns (Fig. 1) (Pianka 1976, Boggs 1986, 1992, 1997, Boggs and Ross 1993).

Both population dynamics and individual fitness are partially determined by the dynamics of reproductive allocation of nutrients from different sources. The

amount of nutrients allocated affects the number and success of offspring, which, in turn, may affect population size and stability over time. Dynamics of allocation will also affect the organisms' available reserves in the event of food scarcity in the environment. In turn, available reserves affect the resiliency of fecundity and population size in the face of environmental variation.

Reproductive allocation from juvenile stored reserves and adult feeding may vary within or among species. For example, depletion of juvenile stored reserves may occur rapidly, or reserves may be used at a low constant rate, in effect providing an annuity for the organism. Nutrients from adult feeding may be used immediately, or could be stored as a buffer against later fluctuations in adult food availability.

Variation within or among species in allocation patterns could be a function of two factors: (1) expected or actual adult diet quality or quantity and (2) timing of reproductive allocation relative to timing of adult food intake. For example, if some nutrient types, such as nitrogenous compounds, are missing from the adult diet, or a long period of behavioral maturation is needed before the onset of adult feeding, juvenile reserves may

be relatively important in reproduction. Further, timing of oogenesis relative to the onset of adult feeding may set constraints on reproductive allocation from juvenile and adult sources for females.

Lepidopteran species provide an opportunity to examine the effects of adult diet and timing of oogenesis on reproductive allocation of diverse nutrient types. Among species, there is a correlation between the age-specific fecundity curve and the completeness of the adult diet, combined with percentage of oocytes completely mature at adult eclosion, which can only be formed from larval reserves. As the possible importance of adult food increases, age-specific fecundity plateaus for a longer time before declining (see Boggs 1997: Fig. 1) (Boggs 1986). This suggests that allocation patterns may vary among species, as well as among nutrient types found to differing degrees in the adult and larval diets.

Previous work shows that allocation patterns do differ among species. By examining age-specific changes in body mass and reproductive effort, I was able to determine the relative expenditure on egg production of reserves (from larval or adult sources) vs. income through adult life (Boggs 1997). Using three nymphalid species with differing possibilities of using adult nutrients for reproduction, I found that, as the possibility of using adult-derived nutrients increased, the pattern of reserve use changed from one where a constant fraction of available reserves was allocated to reproduction to one where reserves were hoarded until late in life.

With this background, I turn to an examination of differences in allocation of different nutrient types. I used radiotracer techniques to examine allocation patterns for two different nutrient types, glucose and amino acids, from larval and adult stages in two nymphalid butterfly species. The two species chosen span the intermediate range of potential importance of adult nutrients to reproduction, as both feed on nectar. They differ, however, in percentage of oocytes mature at adult eclosion, and in allocation patterns inferred from changes in body mass and reproductive effort with age. For these two species, I ask: if the adult diet is rich in carbohydrates but poor in nitrogenous compounds, are larval stores of carbohydrates and nitrogenous compounds allocated differently? The answer will be important to accurate modelling of the allocation processes connecting life-stage specific foraging habits with reproductive or life history patterns.

GENERAL PREDICTIONS: AGE-SPECIFIC CHANGES IN SOURCE OF NUTRIENTS USED IN EGG PRODUCTION

Juvenile and adult diets are qualitatively different in many organisms, especially in holometabolous insects. Specific nutrient types may be available only from the juvenile diet, only from the adult diet, or from both. Such availability will affect reproductive allocation patterns, with the following specific predictions:

1) Nutrient types available only (or primarily) from

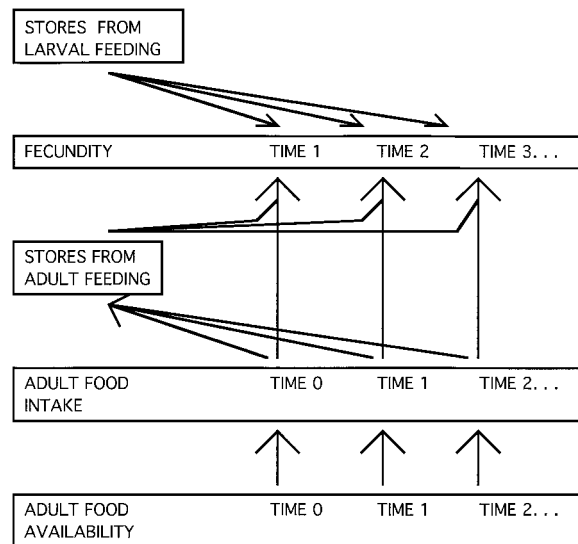


FIG. 1. Nutrients from larval and adult sources affect age-specific fecundity.

the juvenile diet must be drawn from reserves throughout adult life (see Boggs 1997):

a) If reproduction is maintained at a constant level with age, a constant amount of juvenile reserves should be used at each age class.

b) If chemical diffusion processes affect allocation to reproduction, a constant fraction of juvenile reserves should be used at each age class, resulting in decreasing absolute allocation with age.

2) Nutrient types found in both the adult and juvenile diets may be treated in one of two ways.

a) If food is available and is eaten from the beginning of adult life, adult-derived nutrients should have primacy in allocation to reproduction. Juvenile reserves should be kept in storage, or slowly and consistently used to supplement adult feeding throughout life. This prediction arises because of the added cost of storing and then remobilizing food eaten in the adult stage, compared to immediately allocating that food to reproductive use. Such a strategy could also be favored in habitats with a variable food supply, since juvenile reserves would remain to be called on in cases of adult food shortage.

b) If there is a delay in the onset of adult foraging, reserves may be exhausted immediately, followed by use of nutrients from adult sources. Such a strategy could be favored in stable adult food environments, if predation pressure and other costs of foraging are non-negligible.

3) Nutrient types available only (or primarily) from the adult diet should be spent as they accrue, with any surplus nutrients stored. This prediction arises from an expectation that it is cheaper to spend nutrients immediately, rather than to store and remobilize them.

TABLE 1. Regression against age of disintegrations per minute (dpm) per egg from labelled glucose fed to larval females of two butterfly species. Dpm/egg is calculated as age effects from an ANOVA for unbalanced design, with butterfly and age as category variables and dpm/egg as the dependent variable.

Variable	Coefficient	<i>t</i>	<i>P</i>	<i>t_s</i> (jackknife)	df	<i>P</i>
A) <i>Euphydryas editha</i> , ¹⁴ C (multiple <i>r</i> = 0.84; regression: <i>F</i> _{1,9} = 22.4, <i>P</i> = 0.001)						
Constant	256.7	10.89	<0.001			
Age	-16.5	-4.74	0.001	-2.06	4	<0.15
B) <i>Speyeria mormonia</i> , ¹⁴ C (multiple <i>r</i> = 0.80; regression: <i>F</i> _{2,27} = 54.88, <i>P</i> < 0.001)						
Constant	130.6	18.88	<0.001			
Age	-8.9	-8.80	<0.001	-20.68	2	<0.01
Age ²	0.2	7.13	<0.001	23.85	2	<0.01
C) <i>S. mormonia</i> , ³ H (multiple <i>r</i> = 0.96; regression: <i>F</i> _{2,17} = 95.90, <i>P</i> < 0.001)						
Constant	126.2	15.63	<0.001			
Age	-13.6	-7.70	<0.001	-4.92	2	<0.05
Age ²	0.4	4.74	<0.001	5.49	2	<0.05

STUDY ANIMALS

I used two nymphalid butterfly species, chosen for two reasons. First, they both have adult diets rich in carbohydrates but poor in nitrogenous compounds, allowing contrasts between usage of these two nutrient types within the same species. Second, they span the intermediate range of probable importance of adult nutrients to egg production. In both species, the adult diet is incomplete, but not all eggs are mature at adult emergence, so adult nutrients can be used to manufacture eggs. However, the species differ in the proportion of immature eggs at emergence.

Larvae of *Euphydryas editha* feed on a variety of hostplants, but primarily *Plantago erecta* (Plantaginaceae) in the population studied (Ehrlich 1965, Singer 1972; C. L. Boggs, *personal observation*). Adults feed on nectar (Murphy et al. 1983), which is primarily carbohydrate, but can contain some amino acids and lipids

(Baker and Baker 1975, 1983, Boggs 1987). As in other Lepidoptera, males pass a spermatophore to females at mating; it is absorbed by the female and used in egg production (Boggs 1995). Such nutrients can be classed as adult "feeding," but may arrive in stored form for the female. The total number of oocytes is fixed at adult emergence, and is positively correlated with body mass (Boggs 1997). At adult female emergence, ≈18% of these oocytes are already yolked and mature; this number corresponds to the number of eggs laid during the first 1.5 d of egg-laying (Labine 1968, Boggs 1997). Adult nutrients are important for achieving full potential fecundity (Murphy et al. 1983). Fecundity is initially high for a short time, and then declines with age (Labine 1968).

Females of *Speyeria mormonia* feed on *Viola* (Violaceae) as larvae and on nectar as adults (Boggs 1986). Older adult females may feed at mud, dung, or carrion

TABLE 2. Regression against age of disintegrations per minute (dpm) per egg from labelled glucose fed to adult females, with dpm/egg calculated as age effects from an ANOVA for unbalanced design, with butterfly and age as category variables and dpm/egg as the dependent variable.

Variable	Coefficient	<i>t</i>	<i>P</i>	<i>t_s</i> (jackknife)	df	<i>P</i>
A) <i>Euphydryas editha</i> , ³ H (multiple <i>r</i> = 0.90; regression: <i>F</i> _{2,10} = 21.06; <i>P</i> < 0.001)						
Constant	-33.3	-0.45	NS			
Age	144.3	5.89	<0.001	1.82	6	<0.15
Age ²	-8.7	-5.11	<0.001	-1.79	6	<0.15
B) <i>Speyeria mormonia</i> , ¹⁴ C (multiple <i>r</i> = 0.77; regression: <i>F</i> _{4,13} = 4.79, <i>P</i> = 0.014)						
Constant	2.6	0.04	NS			
Age	189.0	4.04	0.001	3.68	2	<0.10
Age ²	-35.1	-3.64	0.003	-3.19	2	<0.10
Age ³	2.5	3.30	0.006	2.85	2	<0.15
Age ⁴	-0.1	-3.04	0.01	2.62	2	<0.15
C) <i>S. mormonia</i> , ³ H (multiple <i>r</i> = 0.95; regression: <i>F</i> _{3,30} = 84.91, <i>P</i> < 0.001)						
Constant	504.4	4.12	<0.001			
Age	104.0	4.15	<0.001	2.00	4	<0.15
Age ²	-7.1	-6.00	<0.001	-8.06	4	<0.01
Age ⁴	0.003	5.59	<0.001	6.98	4	<0.01

TABLE 3. Regression against age of disintegrations per minute (dpm) per egg from labelled glucose fed to males, with dpm/egg calculated as age effects from an ANOVA for unbalanced design, with butterfly and age as category variables and dpm/egg as the dependent variable.

Variable	Coefficient	<i>t</i>	<i>P</i>	t_s (jackknife)	df	<i>P</i>
A) <i>Euphydryas editha</i> , ^{14}C (multiple $r = 0.68$; regression: $F_{1,14} = 12.38$, $P = 0.003$)						
Constant	1.7	5.01	<0.001			
Age	0.1	3.52	0.003	16.57	2	<0.01
B) <i>Speyeria mormonia</i> , ^{14}C (multiple $r = 0.59$; regression: $F_{1,17} = 9.25$, $P = 0.007$)						
Constant	1.6	6.64	<0.001			
Age	-0.1	-3.04	0.007	-1.54	1	<0.4
C) <i>S. mormonia</i> , ^3H (multiple $r = 0.41$; regression: $F_{1,15} = 3.07$, $P = 0.1$)						
Constant	6.0	3.70	0.002			
Age	-0.3	-1.75	0.1			

(Boggs and Jackson 1991, Sculley and Boggs 1996). Butterflies get sodium, and possibly nitrogenous compounds, from feeding at dung and mud (Arms et al. 1974, Adler and Pearson 1982, Pivnick and McNeil 1987). In many species, young males predominate at mud and dung, and contribute sodium to females at mating (Pivnick and McNeil 1987). However, in species whose females only mate once, such as *S. mormonia*, older females tend to be found feeding at mud and dung, presumably after having depleted nutrients received at mating (Sculley and Boggs 1996). As in *E. editha*, the total number of oocytes in the ovaries is fixed at adult emergence. However, the ovaries initially contain no fully yolkeggs, so adult nutrients can be used to make all eggs (Boggs 1986). Adult nutrients are critical in maintaining fecundity. If females are semistarved, fecundity decreases in direct proportion to the quantitative decrease in adult food availability. Under normal laboratory conditions, egg production initially increases to a plateau, and then declines after ≈ 2 wk, depending on environmental temperature (Boggs 1986).

SPECIFIC PREDICTIONS

Since there is no delay in initiation of feeding, then, based on general predictions, adult-derived carbohydrates should be used in reproduction, with larval- and male-derived carbohydrates kept in reserve or used slowly and consistently to supplement adult feeding. However, in comparison with *Speyeria mormonia*, *Euphydryas editha* should show a higher initial level of use of larval-derived carbohydrates in egg production, and a delay in use of adult-derived carbohydrates, since 18% of the eggs are already mature at female emergence.

Nitrogenous compounds are primarily available from larval and male sources. For both species, larval- and male-derived nitrogenous compounds should be used consistently in reproduction throughout life; adult-derived compounds should be used immediately if they are available.

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*. Resulting females were caged with several males for mating. Only females mating on the 1st or 2nd d of adult life were used in the study, as mating is believed to occur rapidly after female emergence in the field. Mated females were kept in 0.3 m diameter \times 0.5 m high cages that allowed behavioral thermoregulation (Boggs 1997). Each cage held one pot of *P. erecta* for oviposition, and three pots of flowering *Layia platyglossa* (Compositae), a native nectar source. Cages were placed inside a greenhouse with ambient daylength. Temperatures were relatively uncontrolled, fluctuating from 12° to 34°C in parallel with ambient temperature outside the greenhouse.

Speyeria mormonia.—Eggs were collected from wild-caught females from a population near Gothic, Gunnison County, Colorado. Resulting larvae were raised on potted *Viola soraria* in greenhouses at Stanford University. Females were mated with nonsiblings and were kept in 12 cm diameter \times 13.5 cm high cages as described in Boggs and Ross (1993). Each cage contained one or two violet leaves in a water-filled dram vial (3.697 mL) plugged with paper towel. Adult females were kept at 26°–29°C, on a 16:8 L:D (light:dark) cycle. Females were fed ad libitum on a 1:3 honey:water solution (volume:volume) in the morning and again in late afternoon. This diet yields survival and fecundity comparable to an ad libitum nectar diet (C. L. Boggs, unpublished data).

Radiotracer experiments

The age-specific use in egg production of nutrients from larval feeding, adult female feeding, and male spermatophores was determined using double-label radiotracer techniques, with ^{14}C and ^3H . The experiments were repeated with labelled glucose and labelled amino acid mixture (ICN), so that differences in allocation patterns between carbohydrate- and nitrogen-derived pools might be detected.

To label nutrient pools derived from larval feeding, 37 kBq of glucose or amino acids (contained in a 1.0 μL aqueous solution) was painted on a small leaf of

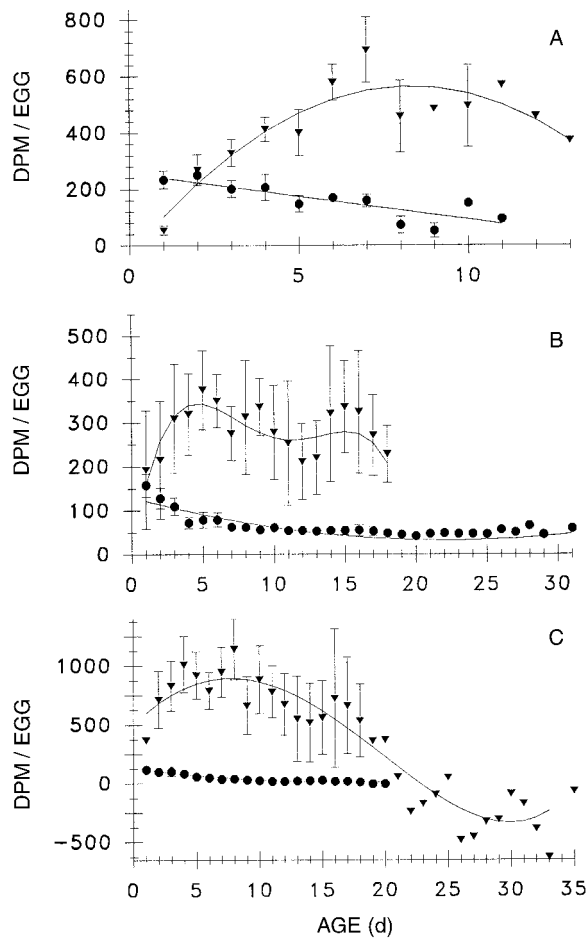


FIG. 2. Age-specific use in eggs of labelled glucose from larval and adult feeding by females. Radioactive disintegrations per minute (dpm) per egg are the age-effects means from an unbalanced-design ANOVA, with age and butterfly as category variables. Error bars represent standard errors of the age-effects means. Negative values are due to use of age-effects means. The lines are regressions of dpm per egg against age, from Tables 1 and 2. For the larval label, day 1 is the first day of oviposition; for the adult label, day 1 is the day after the first adult feeding. ●, larval; ▼, adult. (A) *Euphydryas editha*, ^{14}C glucose fed to larvae, ^3H glucose fed to adults. No. butterflies laying eggs at each age, larval label: day 1, 9; day 2, 6; day 3, 9; days 4–5, 6; days 6–7, 4; day 8, 2; day 9, 3; day 10, 1; day 11, 2; adult label: day 1, 13; day 2, 5; day 3, 11; day 4, 9; day 5, 8; day 6, 4; day 7, 5; day 8, 3; day 9–10, 2; day 11–13, 1. (B) *Speyeria mormonia*, ^{14}C glucose fed to both larvae and adults. No. butterflies laying eggs at each age: larval label: day 1–2, 7; day 3, 6; day 4, 5; day 5–9, 4; day 10, 3; day 11–17, 2; day 18–31, 1; adult label: day 1, 3; day 2, 4; day 3, 6; day 4–6, 7; day 7, 6; day 8, 4; day 9–10, 3; day 11, 2; day 12, 3; day 13–15, 4; day 16, 3; day 17–18, 2. (C) *S. mormonia*, ^3H glucose fed to both larvae and adults. No. butterflies laying eggs at each age, larval label: day 1, 6; day 2–5, 5; day 6, 3; day 7, 4; day 8, 2; day 9, 3; day 10–13, 2; day 14–15, 3; day 16–18, 2; day 19–20, 1; adult label: day 1, 2; day 2, 5; day 3–4, 8; day 5–6, 9; day 7–8, 8; day 9, 7; day 10–11, 9; day 12, 7; day 13–14, 4; day 15–16, 3; day 17–18, 2; day 19–35, 1.

the larval host plant. For *V. soraria*, the stem of the leaf was placed in a dram vial (3.697 mL) with water and was stoppered with a paper towel. For *P. erecta*, an entire small plant was used. After the solution had dried, a mid-last instar larva was placed on the leaf. The leaf was not replaced until it had been entirely eaten, generally 2–12 h later.

To label nutrient pools derived from adult feeding, females were fed 3.7 kBq of glucose or amino acids contained in a 5 or 10 μL water solution, with a few crystals of glucose to increase the sugar concentration and induce feeding. If the solution was not completely eaten, the amount remaining was measured, and the amount of label ingested was calculated. Adults were fed radiolabel in the late afternoon, every other day for *E. editha* and every third day for *S. mormonia*.

To label nutrient pools derived from male nutrient donations at mating, females were mated to males that had been fed radiolabelled compounds as last-instar larvae.

Each female contained two labelled pools, e.g., a ^{14}C larval pool and a ^3H adult female feeding pool, or a ^{14}C male-derived pool and a ^3H larval pool, etc. All possible combinations were explored with *S. mormonia*; that is, both ^3H - and ^{14}C -labelled amino acids and glucose were used to examine input to eggs from each possible source. This allowed verification that ^3H and ^{14}C behaved similarly. With this established, ^{14}C was used to trace larval and male donation sources; ^3H was used to trace adult female sources in *E. editha*, in order to minimize the number of individuals used.

Daily at sunset, each female's eggs were collected, counted, and weighed as a group to the nearest 0.01 mg. The group of eggs was then homogenized in 1 mL distilled water, added to 8.5 mL EcoLume, and counted three times, for 10 min each time, in a liquid scintillation counter. After subtracting background counts, mean counts per minute were corrected to disintegrations per minute (dpm) for each isotope, using a quench correction curve for double-label counting. Dpm for each female's daily egg output were then converted to daily dpm per egg. Although some of the resulting numbers are thus relatively small (cf. Fig. 3), they are derived from much larger counts per minute for a group generally totalling ≥ 30 eggs.

To examine changes with age in radiotracer incorporation into eggs, nonlinear regressions were calculated for dpm per egg against age, using linear, quadratic, and higher order terms to examine slope and curvature. Daily dpm per egg values used in the regression were age effects means from an ANOVA for unbalanced design, using age and butterfly as category variables and dpm per egg as the dependent variable. For regressions of dpm per egg against age, t values for regression coefficients that were significant at $P = 0.05$ or better were further examined for significance using a jackknife statistic, first dropping data for butterflies living the longest and shortest number of days,

then for butterflies living the next longest and shortest number of days, etc. (Sokal and Rohlf 1995). This analysis method was chosen because it accounts for effects due to individual butterflies; repeated-measures ANOVA was not possible because of unequal numbers of samples per butterfly (see also Boggs 1997). ANOVAs and regression analyses were done using SYSTAT (Wilkinson 1990).

Age 1 was not the actual first day of adult life, but rather was adjusted to synchronize individuals within a species and treatment by onset of egg-laying, or by first adult tracer feeding. That is, for larval and spermatophore data, age 1 was the first day of oviposition; for adult female feeding data, age 1 was the first day after the first adult feeding, regardless of whether or not oviposition had actually started.

The regression procedure was repeated for effects of mass per egg on dpm per egg for all data sets.

RESULTS

Age-specific incorporation of glucose-derived compounds

Incorporation of label from larval-, adult female-, and male-derived glucose into eggs showed a similar pattern for ^3H and ^{14}C in *Speyeria mormonia* (Tables 1, 2, and 3; Figs. 2 and 3). This means that the results were not specific to one radiolabel.

Dpm per egg from larval glucose declined with age in both *S. mormonia* and *Euphydryas editha*, as predicted (Table 1, Fig. 2). The decline was significantly linear for *E. editha*. However, for *S. mormonia*, dpm per egg declined until about day 5–7 of egg-laying, and then stabilized at a low level.

Incorporation into eggs of labelled glucose fed to adult female *S. mormonia* and *E. editha* initially increased with age, as expected (Table 2, Fig. 2). For *E. editha*, dpm per egg first increased with age, and then levelled off and declined. *S. mormonia* showed a much more complex pattern, with significant fluctuations in incorporation of label through time.

Dpm per egg from glucose fed to males increased significantly with age in *E. editha* (Table 3, Fig. 3A). Dpm per egg from males declined with age in *S. mormonia*, although the jackknife statistic suggests that the significance of the decline is questionable (Table 3, Fig. 3B).

Age-specific incorporation of amino acid-derived compounds

Incorporation patterns of label into eggs from larval-, adult female-, and male-derived amino acids generally showed a similar pattern for ^{14}C and ^3H in *S. mormonia* (Tables 4, 5, and 6; Figs. 4 and 5). As for glucose, the results are not specific to one radiolabel.

Incorporation of label from larval-derived amino acids into eggs decreased with age in *S. mormonia*, although the jackknife statistic has a relatively low sig-

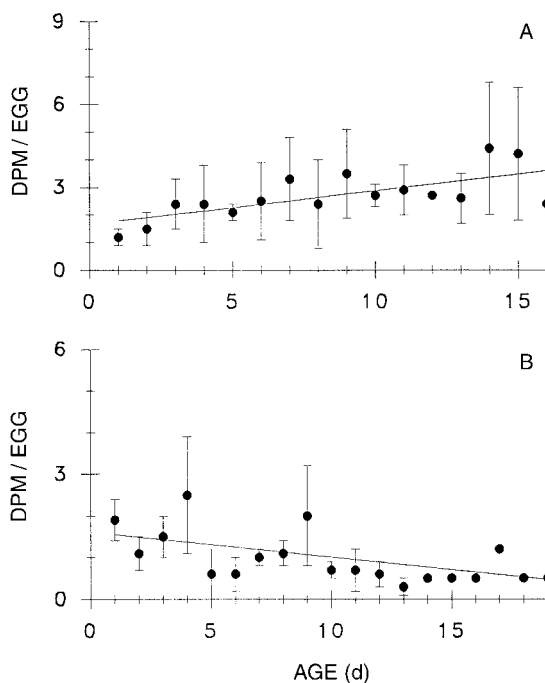


FIG. 3. Age-specific use in eggs of labelled glucose from male nutrient donations at mating. Statistics are as in the Fig. 2 legend. The lines are regressions of disintegrations per minute (dpm) per egg against age, from Table 3. Day 1 is the first day of oviposition. (A) *Euphydryas editha*, ^{14}C . No. butterflies laying eggs at each age: day 1, 7; day 2, 5; day 3, 6; day 4, 5; day 5–8, 4; day 9, 5; day 10–11, 2; day 12, 1; day 13–14, 3; day 15, 4; day 16, 1. (B) *Speyeria mormonia*, ^{14}C . No. butterflies laying eggs at each age: day 1–2, 5; day 3–4, 4; day 5, 3; day 6, 4; day 7–8, 3; day 9–11, 4; day 12–13, 3; day 14, 2; day 15–19, 1.

nificance level for ^3H (Table 4, Fig. 4B, C). In contrast, dpm per egg derived from larval feeding in *E. editha* initially increased with age, and then levelled off and declined (Table 4, Fig. 4A).

The pattern of incorporation of label from adult female feeding on amino acids showed parallels to the results for glucose. For *E. editha*, dpm per egg initially increased with age, then levelled off and declined (Table 5, Fig. 4A). *S. mormonia* showed a much more complex pattern, with an initial increase followed by significant fluctuations through time (Table 5, Fig. 4B, C).

The pattern of incorporation of amino acid-derived label from spermatophores differed between species. Initially, dpm per egg increased, stabilized, and then declined with age in *E. editha* (Table 6, Fig. 5A). For *S. mormonia* mated to a ^{14}C -labelled male, dpm per egg declined linearly with age, but the decline was not significant for females mated to ^3H -labelled males (Table 6, Fig. 5B).

Effects of egg mass on changes of dpm per egg with age

Egg mass can change with age in many butterflies, including the species studied here (Murphy et al. 1983,

TABLE 4. Regression against age of disintegrations per minute (dpm) from labelled amino acids fed to larval females, with dpm/egg calculated as age effects from an ANOVA for unbalanced design, with butterfly and age as category variables and dpm/egg as the dependent variable.

Variable	Coefficient	<i>t</i>	<i>P</i>	<i>t_s</i> (jackknife)	df	<i>P</i>
A) <i>Euphydryas editha</i> , ¹⁴ C (multiple <i>r</i> = 0.61; regression: $F_{2,11} = 3.28$, $P = 0.08$)						
Constant	423.4	15.44	<0.001			
Age	14.3	1.81	0.01	2.74	3	<0.1
Age ²	-1.0	-2.19	0.05	-2.15	3	<0.15
B) <i>Speyeria mormonia</i> , ¹⁴ C (multiple <i>r</i> = 0.82; regression: $F_{1,23} = 46.71$, $P < 0.001$)						
Constant	180.6	24.96	<0.001			
Age	-3.3	-6.84	<0.001	-3.27	2	<0.1
C) <i>S. mormonia</i> , ³ H (multiple <i>r</i> = 0.88; regression: $F_{1,16} = 57.92$, $P < 0.001$)						
Constant	154.3	17.04	<0.001			
Age	-6.4	-7.61	<0.001	-1.44	2	0.3

Boggs 1986). Changes in egg mass may equal changes in the amount of material per egg that could potentially be labelled. Thus, the observed changes in dpm per egg with age could simply be due to changes in egg mass, or material that could be labelled. If true, this would alter the interpretation of the changes in dpm per egg with age.

In all but two cases, mean daily mass per egg had no effect on dpm per egg. One exception was ³H amino acids fed to larval *S. mormonia*, for which dpm per egg decreased with increasing mass per egg ($t = -3.175$, $df = 16$, $P = 0.006$). Thus, larger eggs contained relatively less larval label. The second exception was ¹⁴C glucose fed to adult *S. mormonia*, for which dpm per egg increased with increasing mass per egg ($t = 2.32$, $df = 13$, $P = 0.04$). Thus, larger eggs contained relatively more adult label.

In all cases, including the two in which mass per egg had significant effects on dpm per egg, the relationship between dpm per egg and age remained unchanged by inclusion of mass per egg in the regression. Thus,

changes in dpm per egg with age cannot be due to changes in egg mass, with consequent changes in opportunity for incorporation of labelled compounds.

DISCUSSION

Fit of results to specific predictions: glucose

Both species showed an initial use of larval-derived glucose in egg production that declined with age while use of adult-derived glucose increased to a variable plateau. Use of male-derived glucose declined in *Speyeria mormonia* with age, but increased for *Euphydryas editha*.

These patterns are broadly consistent with the prediction: adult-derived glucose was expected to be used in preference to larval- or male-derived glucose. Thus, use of larval-derived glucose declined rapidly to a low level, coincident with the use of adult-derived glucose; however, male-derived glucose was not held in reserve as predicted.

Note that the pattern of use of larval-derived glucose

TABLE 5. Regression against age of disintegrations per minute (dpm) from labelled amino acids fed to adult females, with dpm/egg calculated as age effects from an ANOVA for unbalanced design, with butterfly and age as category variables and dpm/egg as the dependent variable.

Variable	Coefficient	<i>t</i>	<i>P</i>	<i>t_s</i> (jackknife)	df	<i>P</i>
A) <i>Euphydryas editha</i> , ³ H (multiple <i>r</i> = 0.96; regression: $F_{2,9} = 59.64$, $P < 0.001$)						
Constant	21.8	0.49	NS			
Age	106.9	7.29	<0.001	11.72	2	<0.01
Age ²	-5.2	-5.10	0.001	-8.59	2	<0.02
B) <i>Speyeria mormonia</i> , ¹⁴ C (multiple <i>r</i> = 0.92; regression: $F_{3,13} = 24.22$, $P < 0.001$)						
Constant	3.4	0.33	NS			
Age ²	2.8	3.12	0.008	7.40	2	<0.02
Age ³	-0.3	-2.72	0.02	-6.66	2	<0.05
Age ⁴	0.01	2.64	0.02	6.40	2	<0.05
C) <i>S. mormonia</i> , ³ H (multiple <i>r</i> = 0.91; regression: $F_{3,13} = 35.88$, $P < 0.001$)						
Constant	-391.7	-5.83	<0.001			
Age	142.6	9.22	<0.001	2.26	2	<0.2
Age ²	-7.1	-8.43	<0.001	-2.26	2	<0.2
Age ⁴	0.004	7.59	<0.001	4.26	2	<0.15

TABLE 6. Regression against age of disintegrations per minute (dpm) from labelled amino acids fed to males, with dpm/egg calculated as age effects from an ANOVA for unbalanced design, with butterfly and age as category variables and dpm/egg 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> , ¹⁴ C (multiple <i>r</i> = 0.81; regression: $F_{2,20} = 18.87$, <i>P</i> < 0.001)						
Constant	1.2	2.54	0.02			
Age	0.5	6.08	<0.001	2.86	2	<0.15
Age ²	-0.02	-6.12	<0.001	-2.12	2	<0.1
B) <i>Speyeria mormonia</i> , ¹⁴ C (multiple <i>r</i> = 0.74; regression: $F_{1,16} = 19.15$, <i>P</i> < 0.001)						
Constant	2.2	18.74	<0.001			
Age	-0.05	-4.38	<0.001	-4.99	4	<0.01
C) <i>S. mormonia</i> , ³ H (multiple <i>r</i> = 0.14; regression: $F_{1,18} = 0.37$, NS)						
Constant	5.7	4.62	<0.001			
Age	-0.06	-0.61	NS			

is consistent with two scenarios. One is that larval-derived glucose is held in reserve, except for that used in initial oocyte production during metamorphosis. The second is that larval-derived glucose is present only in small quantities in the adult female, and is exhausted immediately. Analysis of radioactivity remaining in dead females suggests that the first hypothesis is correct, as a mean 12 354 dpm ¹⁴C larval glucose remained at death in female *E. editha*, and 65 425 dpm ¹⁴C and 14 166 dpm ³H larval glucose remained, on average, in *S. mormonia* (C. L. Boggs, unpublished data).

S. mormonia and *E. editha* differed in the specific pattern of decline in use of larval-derived glucose, with *E. editha* showing a constant decline, whereas the decline levelled off in *S. mormonia*. This pattern could have had either of two causes. First, it could have been due to the shorter laboratory life-span of *E. editha*, so that a levelling off was not seen. Second, it could have resulted from the greater degree of maturity of eggs of *E. editha* at adult emergence, so that more eggs contained more larval-derived compounds before adult feeding started.

Age-specific use of male-derived glucose was inconsistent with the predicted results. Male-derived glucose was expected to be either held in reserve and used near the end of life, or used slowly to supplement adult feeding. Instead, use of male-derived glucose decreased with age in *S. mormonia* and increased in *E. editha*. These data suggest that male-derived nutrients were incorporated into developing oocytes as soon as possible (with the delay in *E. editha* being due to the number of eggs already mature), rather than being held in reserve. Thus, male-derived nutrients were used immediately, similar to adult-derived nutrients.

Fluctuations observed in the use of adult-derived glucose by *S. mormonia* were probably due to an interplay between the periodicity of tracer feeding and the dynamics of oogenesis. Such fluctuations probably also would have been seen in *E. editha*, if that species had a longer life-span.

Fit of results to specific predictions: amino acids

Use in egg production of both larval- and adult-derived amino acids first increased and then decreased with age in *E. editha*, whereas larval-derived amino acids decreased with age and adult-derived amino acids increased with age in *S. mormonia*. Use of male-derived amino acids increased, levelled out, and then decreased with age in *E. editha*, whereas *S. mormonia* showed a decrease with age for ¹⁴C and no significant trend for ³H.

Observed patterns differed somewhat from the prediction. Larval- or male-derived amino acids were expected to be used at a constant rate through the female's life, supplemented by immediate use of adult-derived amino acids whenever available. Use of adult-derived amino acids fit the prediction. The observed decline in use of larval-derived amino acids was unexpected. The decline occurred not only on a per egg basis, but also on a per milligram of egg basis, suggesting either that the pool from which larval reserves were drawn was being diluted by storage of adult nutrients, or that egg composition changed with age.

Integration with previous data

The radiotracer data were generally consistent with conclusions concerning these species' allocation patterns, based on changes in body mass and reproductive effort through time (Boggs 1997). Earlier data for *E. editha* suggested that a constant fraction of overall reserves was allocated to reproduction and other functions at each age class. Thus, the absolute contribution from incoming adult sources must have increased relative to that from larval sources throughout life; this was true for labelled glucose until near the end of life, when use of both adult and larval glucose declined. Earlier data for *S. mormonia* suggested either that a constant amount (not fraction) of reserves was spent at each age, or that some nutrients from adult feeding were being stored. Radiotracer data were consistent with the hypothesis that nutrients from adult feeding are being stored, because the decline in incorporation

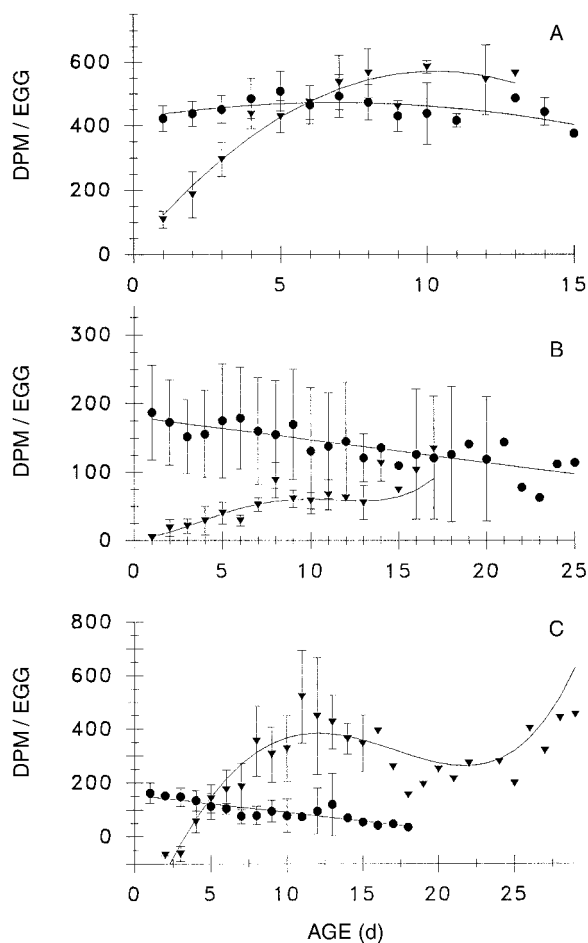


FIG. 4. Age-specific use in eggs of labelled amino acids from larval and adult feeding by females. Statistics are as in the Fig. 2 legend. The lines are regressions of disintegrations per minute (dpm) per egg against age, from Tables 4 and 5. For the larval label, day 1 is the first day of oviposition; for the adult label, day 1 is the day after the first adult feeding. ●, larval; ▼, adult. (A) *Euphydryas editha*, ^{14}C amino acids fed to larvae, ^3H amino acids fed to adults. No. butterflies laying eggs at each age, larval label: day 1, 9; day 2–3, 7; day 4–5, 5; day 6, 4; day 7, 6; day 8, 5; day 9–11, 3; day 13, 1; day 14, 2; day 15, 1; adult label: day 1, 6; day 2, 2; day 3, 7; day 4, 2; day 5, 5; day 6, 6; day 7, 3; day 8, 4; day 9–13, 2. (B) *Speyeria mormonia*, ^{14}C amino acids fed to both larvae and adults. No. butterflies laying eggs at each age, larval label: day 1–3, 7; day 4, 6; day 5–7, 5; day 8, 4; day 9, 5; day 10, 2; day 11–13, 3; day 14–15, 1; day 16–18, 2; day 19, 1; day 20, 2; day 21–25, 1; adult label: day 1, 2; day 2, 5; day 3, 6; day 4, 3; day 5–6, 5; day 7–10, 3; day 11, 2; day 12, 1; day 13–14, 2; day 15–17, 1. (C) *S. mormonia*, ^3H amino acids fed to both larvae and adults. No. butterflies laying eggs at each age, larval label: day 1–3, 6; day 4, 4; day 5, 3; day 6, 2; day 7–9, 3; day 10, 2; day 11, 1; day 12–13, 2; day 14–18, 1; adult label: day 2, 2; day 3, 3; day 4, 4; day 5, 6; day 6, 7; day 7, 4; day 8–9, 6; day 10, 7; day 11–13, 3; day 14, 4; day 15, 2; day 16–29, 1.

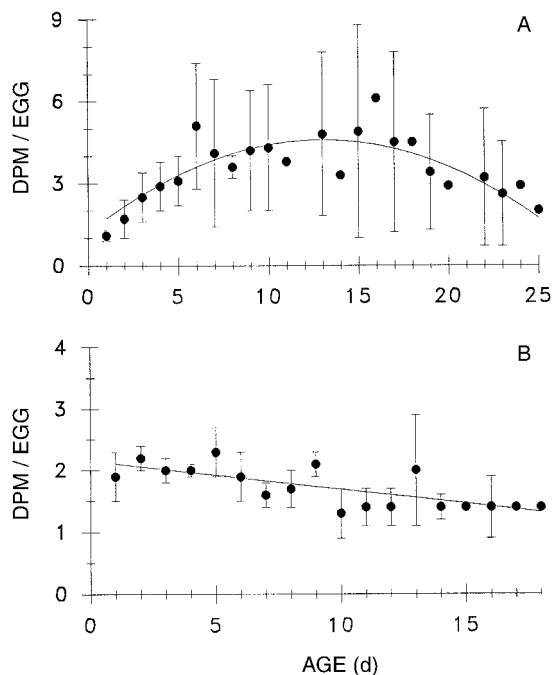


FIG. 5. Age-specific use in eggs of labelled amino acids from male nutrient donations at mating. Statistics are as in the Fig. 2 legend. The lines are regressions of disintegrations per minute (dpm) per egg against age, from Table 6. Day 1 is the first day of oviposition. (A) *Euphydryas editha*, ^{14}C . No. butterflies laying eggs at each age: day 1, 6; day 2–5, 5; day 6–8, 2; day 9–10, 3; day 11, 1; day 13, 3; day 14, 1; day 15, 2; day 16, 1; day 17, 2; day 18, 1; day 19, 2; day 20, 1; day 22–23, 2; day 24–25, 1. (B) *Speyeria mormonia*, ^{14}C . No. butterflies laying eggs at each age: day 1, 10; day 2, 9; day 3, 7; day 4–5, 8; day 6, 7; day 7, 4; day 8, 5; day 9, 4; day 10–11, 3; day 12–14, 4; day 15, 1; day 16, 2; day 17–18, 1.

of larval-derived label, independent of changes in egg mass, could be due to dilution of stored reserves by nonlabelled adult nutrients.

The present data examine allocation under conditions of ad libitum feeding. With restricted adult food, *S. mormonia* resorbs eggs and reduces fecundity to an extent equal to the reduction in adult food supply, while maintaining life-span (Boggs and Ross, 1993). The radiotracer data reported here suggest that the reduction is due to the unvarying importance of adult-derived glucose to egg production throughout the female's life (see also Boggs 1994).

Ecological and evolutionary implications

The age-specific patterns seen here bear on the role of nutrient reserves and income in producing observed age-specific reproductive patterns, strategies in the use of male-derived nutrients, the effects of a holometabolous lifestyle on life history strategies, and the likely resiliency of fecundity to different types of nutrient stress.

The two species studied here differ primarily in the opportunity for adult incoming nutrients to be used in

producing eggs laid early in a female's life. This difference had been suggested to underlie differences in age-specific fecundity (Boggs 1986). *S. mormonia* has a longer plateau of egg production than does *E. editha*, and the suggestion was that *S. mormonia* had a greater reliance on adult income for egg production. The data are consistent with this hypothesis, as use of larval glucose declined to a low baseline for *S. mormonia*, but not for *E. editha*; this could, however, be due to differences in life-span between the two species.

Females of both species rapidly incorporated both male-derived amino acids and glucose into eggs. *E. editha* females treated male-derived amino acids similarly to both larval- and adult-derived amino acids, whereas male-derived glucose was treated most similarly to adult female-derived glucose. *S. mormonia* treated both nutrient types from males most similarly to larval reserves. Very little is known about the precise form of nutrients donated by males and used by females in Lepidoptera, but the present data suggest that it should be different from compounds comprising stored larval reserves.

Rapid use of male nutrient donations is expected in species where females mate several times, since males should be selected to insure that "their" nutrients are used to form their own offspring (see Boggs 1995). However, female *S. mormonia* mate a mean 1.03 times ($n = 67$; Boggs 1986), whereas female *E. editha* mate a mean 1.4 times ($n = 23$), with most second matings apparently occurring immediately after the first, before the mating plug hardens (Labine 1964). Rapid use of male nutrients in this case could be the result of a phylogenetic constraint on the types of compounds included in male nutrient donations. Alternatively, selection pressures may be operating on the composition and ease of use of male nutrients. Such pressures might be associated either with ease of construction of male nutrient donations, or with improving the female's chance of reproductive success by providing easy-to-use nutrients early in the female's life, if foraging efficiency increases with female age.

Results presented here highlight the effects of a holometabolous lifestyle on age-specific fecundity and survival. Holometabolous insects have the unique opportunity to reallocate larval resources during metamorphosis. Among lepidopteran species, at least, the proportion of resources stored as reserves vs. that used to make body tissue is linked to expected reproductive output minus adult resource intake (Boggs 1981, May 1985, Karlsson 1994) and/or to life-span (with a resulting need for a durable body) (Karlsson and Wikman 1989). Species-specific availability of larval reserves for adult use is thus closely tied to adult life history and foraging traits. The radiotracer data on relative use of adult vs. larval nutrients in egg production throughout a female's life further show that different nutrient types are treated independently within a species. Thus, nutrient types, such as glucose, that are normally avail-

able in the adult diet of nectivores in a nectar-rich environment may limit reproduction if adult feeding is curtailed. Similarly, availability of larval food will limit reproduction for nutrient types that are scarce in the adult diet. The impact on fecundity of restricting access to, or altering the composition of, food in either the adult or larval stage thus depends on the relative importance of nutrients from that stage to fecundity.

Fecundity is one contributor to both individual fitness and population size in the next generation. The data reported here support the importance of mechanistic approaches to understanding life histories embodied by allocation models, such as the "y-model" of de Jong and van Noordwijk (1992). Further, fecundity is the "key factor" determining population size for some species (e.g., Hayes 1981, Nelemans et al. 1989, Southwood et al. 1989, Hassall and Dangerfield 1990). From either a management or an evolutionary perspective, the present data allow predictions as to the effects of perturbations in the food supply available to adults vs. larvae, and show that species will differ in sensitivity to those perturbations.

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