

# Allocation of larval and adult resources to reproduction in a fruit-feeding butterfly

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## Summary

1. The source of nutritional resources allocated to reproduction strongly influences reproductive and foraging strategies. While we are beginning to understand the role of adult and larval resources for nectivorous Lepidoptera, essentially nothing is known for the large number of species that feed on fruit as adults.
2. We used stable isotopes to examine allocation of larval- and adult-derived resources to egg production in a tropical frugivorous butterfly, *Bicyclus anynana* (Butler, 1879), under both *ad libitum* and semistarvation conditions. The butterfly's larval and adult host plants differ from each other in both carbon and nitrogen isotopic ratios.
3. Adult fruit feeding is required for the onset of oviposition.
4. At peak, adult fruit feeding contributed 55% of the carbon found in eggs under both feeding conditions. This is similar to values for several nectivorous Lepidoptera with similar ovarian dynamics and egg C/N.
5. Egg <sup>15</sup>N declined rapidly during the first week of oviposition, suggesting that the adult diet was not contributing nitrogen to egg production. Values rose during the following 2 weeks, consistent either with adult contribution or with isotopic fractionation.
6. Our results are consistent with the hypothesis that fruit serves as a carbon source for egg production in a similar manner as nectar. However, more work is needed to elucidate fully fruit's role as a nitrogen source.

*Key-words:* Carbon turnover, egg production, nitrogen turnover, nutritional resources, stable isotopes

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## Introduction

The timing and source of nutritional resources allocated to reproduction have critical consequences for reproductive strategies and hence individual fitness. Allocated resources may be derived from stored reserves or current feeding (e.g. Sibly & Calow 1984; Wheeler 1996; Boggs 1997a). Organisms are therefore often categorized as 'capital breeders' vs 'income breeders' (e.g. Tammaru & Haukioja 1996). Nonetheless, organisms rarely are pure capital or income breeders, but rather draw nutrients to varying degrees from reserves and current feeding (e.g. Boggs 1997a; O'Brien, Boggs & Fogel 2004). Different resource types (carbohydrates, lipids, amino acids, etc.) may also be differentially drawn from reserves and current feeding (e.g. Boggs & Ross 1993; Boggs 1997b). This is particularly true for species whose diet changes with age – which includes most holometabolous insects. Given the need for resource congruence (the use of nutrient types in a

specified ratio; Bazzaz 1996), storage, foraging and reproduction are linked strategies for such insects. Understanding the relative reproductive importance of stored (usually larval-derived) and current (usually adult-derived) resources, and how that importance changes through time, is thus a critical element to understanding the functional basis of reproductive patterns.

Lepidoptera are holometabolous insects that exhibit a range of reproductive and foraging strategies, and hence are useful for exploring the impact of diverse feeding habits on allocation strategies. Almost all species have herbivorous larvae, with the known exception of a few partly carnivorous lycaenids (e.g. Elmes *et al.* 2001). As adults, many species are strict nectivores, while others supplement their nectar diet with substrates ranging from pollen to mud, dung or carrion (e.g. Gilbert 1972; Boggs 1987; Boggs & Jackson 1991; DeVries, Murray & Lande 1997; Beck, Mühlenberg & Fiedler 1999). The nutritional composition of nectar is fairly well understood (e.g. Pacini, Nepi & Vespini 2003), and we are making progress in understanding its reproductive role in butterflies (e.g. Boggs 1997b; O'Brien, Fogel & Boggs 2002; O'Brien, Boggs & Fogel 2003). In

general, nectars used by Lepidoptera are carbohydrate-rich, containing small amounts of lipids, amino acids and other compounds (see Boggs 1987 for a review). Nectar-derived carbon in particular can be used to make non-essential amino acids (O'Brien *et al.* 2002), and determines reproductive output in some species (Boggs & Ross 1993). Additionally, amino acid-containing nectars seem to be preferred over carbohydrate-only food sources by some species and sexes (e.g. Erhardt & Rusterholz 1998; Rusterholz & Erhardt 2000; Mevi-Schutz, Goverde & Erhardt 2003). Nonetheless, essential amino acids stored from larval feeding are likely ultimately limiting to reproduction.

Rotting fruit is also a major adult food source for a large number of Lepidoptera, particularly in the tropics (e.g. DeVries *et al.* 1997; DeVries & Walla 2001). Researchers have informally speculated that fruit could be a richer and more diverse source of nitrogenous compounds than is nectar; however, a recent paper suggests that amino acid concentrations in fruit are similar to those in nectar (Omura & Honda 2003). However, rotting fruit may provide yeast to fruit-feeding Lepidoptera, which is an excellent source of protein to insect frugivores (Good & Tatar 2001).

Although the impact of fruit-feeding on butterfly life histories has not been studied, a rich body of literature on frugivory in birds provides some insights on the potential nutritional quality of fruit for insects (reviewed in Levey & del Rio 2001). Fruit is similar to nectar in being fairly carbohydrate-rich and nitrogen-poor (Bosque & Pacheco 2000). Up to 25% of the nitrogen in fruit pulp can be non-proteinaceous, meaning it has little nutritional value (Levey, Bissell & O'Keefe 2000). The protein found in fruit is unbalanced in its amino acid content (Izhaki 1998), further decreasing its nutritional value to frugivorous animals. Birds maintained exclusively on fruit diets are in negative protein balance, and lose mass (Baierlein 1996). If rotting fruit is primarily a carbon source, then we would expect allocation and life-history patterns similar to those of nectivorous Lepidoptera. However, if rotting fruit provides significant amino acid resources to fruit feeding Lepidoptera, their life history may more closely resemble those Lepidoptera that supplement their diet with amino acid resources.

The best-studied example of amino acid supplementation in nectar-feeding Lepidoptera is that of pollen-feeding *Heliconius* butterflies. Pollen is a nitrogen-rich food source used in addition to nectar, which completely alters the life history in comparison with related butterflies that are solely nectivores: oogenesis is continuous throughout life, rather than stopping prior to adult emergence and life span is dramatically increased (Gilbert 1972; Dunlap-Pianka, Boggs & Gilbert 1977). These butterflies supplement larval-derived essential amino acids with essential amino acids from pollen feeding to some extent (O'Brien *et al.* 2003).

Here we use the tropical, fruit-feeding butterfly *Bicyclus anynana* (Butler, 1879) to explore allocation

of resources from larval herbivory and adult frugivory to egg production. We employ stable isotopes to trace dietary carbon and nitrogen, and describe allocation kinetics using a turnover model (O'Brien, Schrag & del Rio 2000). Using *ad libitum* fed and semistarved females, we ask two questions: is fruit an important source of carbon or nitrogen resources used in egg production and what is the relative importance of fruit in egg production in this species as compared with the importance of nectar in other lepidopteran species?

## Materials and methods

### STUDY ORGANISM AND EXPERIMENTAL POPULATION

*Bicyclus anynana* (Satyriinae) is a tropical butterfly distributed from southern Africa to Ethiopia, which feeds on a variety of fallen and decaying fruit, including that from *Ficus* trees (Larsen 1991; Brakefield 1997). A laboratory stock population of *B. anynana* was established at Leiden University in 1988 from over 80 gravid females collected at a single locality in Malawi. Several hundred adults are reared in each generation, maintaining high levels of heterozygosity (Saccheri & Bruford 1993). Butterflies from this stock population were used for this study.

### EXPERIMENTAL DESIGN

As in earlier studies (O'Brien *et al.* 2000, 2002, 2003, 2004), we used natural variation in the  $^{13}\text{C}$  content of plants to trace the dietary sources of egg carbon. The  $^{13}\text{C}$  content is expressed as the ratio ( $R$ ) of sample  $^{13}\text{C} : ^{12}\text{C}$  relative to a limestone carbon standard (PDB):  $\delta^{13}\text{C} = [(R_{\text{sample}}/R_{\text{standard}}) - 1] \times 1000$ . A more positive number indicates an increased abundance of the heavy isotope, and is referred to as 'enriched'. C3 plants typically have  $\delta^{13}\text{C}$  values of *c.*  $-28\text{‰}$ , whereas C4 plants have  $\delta^{13}\text{C}$  values of *c.*  $-14\text{‰}$  (O'Leary 1988). By raising butterflies on isotopically contrasting larval and adult diets, we could easily identify the dietary source of carbon in the eggs. Likewise, plants may also differ in  $^{15}\text{N}$  content, depending on the sources and pathways used in nitrogen assimilation. The ratio of  $^{15}\text{N} : ^{14}\text{N}$  is again expressed relative to the standard, atmospheric nitrogen, using the same formula as for carbon and yielding  $\delta^{15}\text{N}$ .

Female larvae were reared on their normal laboratory C4 host plants (maize – *Zea mays*;  $\delta^{13}\text{C} = -14.37\text{‰}$ ), whereas adults were fed on ripe (i.e. 'yellow'), moist banana (*Musa paradisiaca*), a C3 plant ( $\delta^{13}\text{C} = -25.33\text{‰}$ ). Butterflies were reared and maintained at 27 °C in a climate cell with high humidity and a photoperiod of L12 : D12. These conditions are similar to those at which the butterflies develop and reproduce during the favourable wet season in the field (Brakefield 1997). All females used in the experiments were kept separate from males on the day of eclosion, and were mated to virgin males the following day. The mating pairs were transferred

individually to 1-l plastic containers. After mating, males were removed from the containers. The females were supplied with a fresh maize cutting for oviposition, which was replaced daily. Likewise, eggs were collected daily, counted and frozen at  $-20^{\circ}\text{C}$  for later analysis.

We performed two separate experiments using different generations of the same *B. anynana* stock population. In *experiment 1*, all butterflies had access to moist bananas for adult feeding from the day of adult eclosion onwards. Egg-laying began the day after mating (defining oviposition day = 1). Thus females had access to bananas for 2 days (eclosion and mating day) before eggs were laid. All eggs were collected daily throughout the females' adult life to analyse carbon isotope turnover. Females not reaching 'normal' longevity and fecundity (cf. Fischer, Brakefield & Zwaan 2003) were excluded from the analyses. In *experiment 2*, males and females had no access to bananas before and during mating as well as on the following 2 days, but only to water. On the fifth day of life and thereafter, females were provided with bananas. Four of 11 females used for stable isotope analyses laid eggs on their sixth day of adult life (defining oviposition day = 1); seven females laid their first eggs on their seventh day of adult life, or oviposition day = 2. Thus some of the females had access to bananas for 1 day and some for 2 days before starting oviposition. This experiment was stopped on the female's ninth day of adult life. Throughout all experiments, bananas were replaced every other day to minimize potentially confounding effects of microbial activity.

#### EGG ELEMENTAL AND ISOTOPIC ANALYSIS

Egg  $^{13}\text{C}$  and  $^{15}\text{N}$  content for individual females' eggs was analysed either in *c.* 4-day intervals (*experiment 1*) or daily (*experiment 2*), using batches of four or five eggs (equivalent to *c.* 0.30–0.35 mg dry weight) per female and day measured. Eggs were oven dried prior to analysis at  $60^{\circ}\text{C}$ , and then placed into  $3.5 \times 5 \text{ mm}^2$  tin capsules (Costech Analytical Supply, Valencia, CA, USA) for analysis. Samples from *experiment 1* were analysed at the Carnegie Institution of Washington, Geophysical Laboratory (in the laboratory of Dr Marilyn Fogel). Samples from *experiment 2* were analysed at the Alaska Stable Isotope Facility. In both laboratories, egg  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  were measured using continuous flow isotope ratio mass spectrometry. A CE Instruments Elemental Analyser (Milan, Italy) was used to combust and separate sample gases, which were introduced into a Finnigan Delta XL Plus isotope ratio mass spectrometer in a continuous stream of helium, via the Conflo II interface (Thermo Finnigan, Bremen, Germany). *C/N*-values were obtained from the Elemental Analyser for both experiments;  $\% \text{C}$  and  $\% \text{N}$  were determined for *experiment 2* only.

#### STATISTICS

Throughout, means are given  $\pm 1$  SE. The daily trend in mean egg number was evaluated with linear regres-

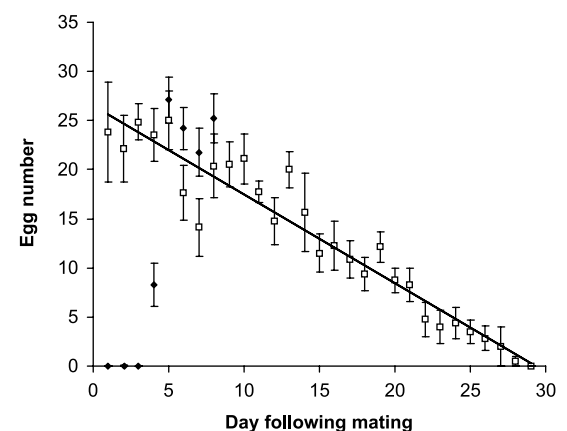
sion. Differences in fecundity between fed and starved females were evaluated with *t*-tests. Differences in egg  $\delta^{13}\text{C}$  per day were evaluated with ANOVA or ANCOVA, and residuals were evaluated for normality using the Shapiro–Wilks test. Non-linear fitting was performed using least-squares minimization (JMP version 3.2; SAS Institute, Cary, NC).

## Results

### FECUNDITY AND LONGEVITY

All eight females used in *experiment 1* were healthy and fecund as evidenced by their longevity ( $27.6 \pm 0.9$  days, min. 23, max. 30) and their realized lifetime fecundity ( $372.8 \pm 16.7$  eggs, min. 314, max. 452) (cf. Brakefield *et al.* 2001; Fischer *et al.* 2003). All eight initiated oviposition the day after mating. Mean egg number per day decreased linearly with female age ( $t_{1,28} = 17.93$ ,  $r = -0.96$ ,  $P < 0.0001$ ; Fig. 1).

Out of the 21 females used in *experiment 2*, only one female laid a single, unviable egg during the period of starvation. Thirteen (out of 21) females initiated oviposition the day after a banana was provided (including 4 of the 11 females used for isotope measurements), and all females had started to oviposit by the following day. On days 5–8 following mating, the previously starved females laid only slightly higher egg numbers compared with the egg numbers laid over the same time period by females being fed continuously in *experiment 1* ( $t_{1,27} = 2.08$ ,  $P = 0.047$ ; Fig. 1). Consequently, the total fecundity of starved females up to day 8 was significantly less than that of the females from *experiment 1* ( $106.6 \pm 5.0$  vs  $171.5 \pm 12.8$ ;  $t_{1,27} = -5.26$ ,  $P < 0.0001$ ).



**Fig. 1.** Mean daily egg number ( $\pm 1$  SE) for two groups of *Bicyclus anynana* females, one of which had access to banana for adult feeding from the eclosion day onwards (open squares;  $n = 8$  females), while the other was starved before and during mating as well as the following 2 days (i.e. 4 days in total; filled diamonds;  $n = 21$  females). For the latter group, the experiment was stopped on day eight after mating. The regression line shows the decline in daily fecundity for the group with access to banana throughout adult life.

**Table 1.**  $\delta^{13}\text{C}$  of insects and diets

Sample type	$\delta^{13}\text{C}$ (‰)	$\delta^{15}\text{N}$ (‰)	C/N
Larval host plant (maize) ( $n = 3$ )	$-14.37 \pm 0.06$	$1.60 \pm 1.00$	$13.60 \pm 0.85$
Prepupae ( $n = 3$ )	$-15.97 \pm 0.73$	$0.52 \pm 0.76$	$5.53 \pm 0.43$
Newly emerged females ( $n = 3$ )	$-14.88 \pm 0.95$	$0.61 \pm 1.64$	$4.20 \pm 0.28$
Adult diet (banana) ( $n = 3$ )	$-25.33 \pm 0.33$	$3.87 \pm 0.58$	$44.89 \pm 12.09$

#### EGG AND DIET C/N COMPOSITION

Maize C/N was  $13.60 \pm 0.85$  ( $n = 3$ ), whereas banana C/N was  $44.89 \pm 12.09$  ( $n = 3$ ; Table 1). In *experiment 1*, egg C/N was  $7.14 \pm 0.06$ . In *experiment 2*, egg C/N was  $7.08 \pm 0.04$ ; egg %C was  $51.53 \pm 0.19$ , and %N was  $8.53 \pm 0.19$ . This %N corresponds to a protein composition of 48.6%, using  $5.7 \text{ g protein g}^{-1} \text{ N}$  (O'Brien *et al.* 2004). C/N varied significantly among individual butterflies in both experiments (*experiment 1*,  $\min = 6.67$ ,  $\max = 7.49$ , ANOVA  $F_{7,42} = 3.71$ ,  $P = 0.0033$ ; *experiment 2*,  $\min = 6.65$ ,  $\max = 7.43$ , ANOVA  $F_{10,34} = 4.27$ ,  $P = 0.0009$ ). In neither experiment was there a pattern in C/N with oviposition day.

#### EGG $\delta^{13}\text{C}$ AND CARBON TURNOVER

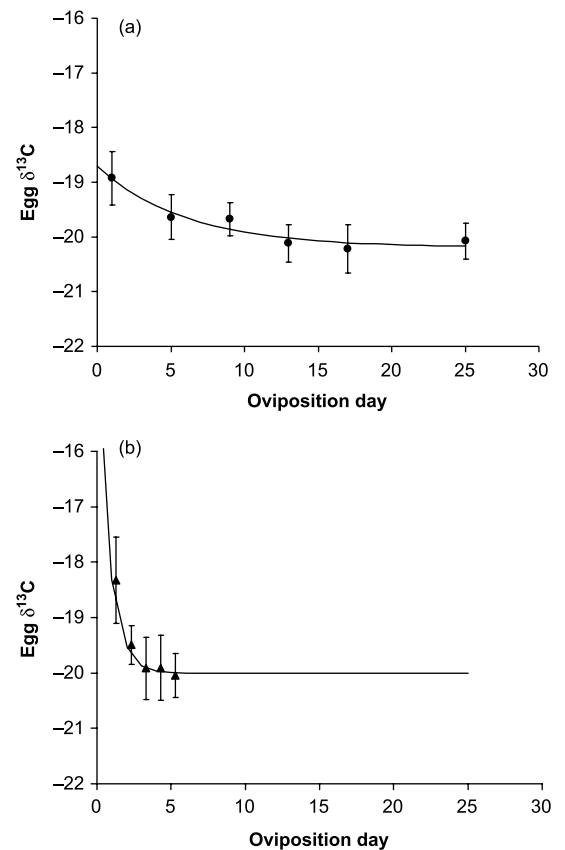
The larval host plant (maize) had a  $\delta^{13}\text{C}$  value of  $-14.37 \pm 0.03$ ‰ ( $n = 3$ ), while the adult diet (banana)  $\delta^{13}\text{C}$  was  $-25.33 \pm 0.14$ ‰ ( $n = 3$ ) (Table 1). Thus, the diets were clearly isotopically distinct. Samples of prepupae ( $-15.97 \pm 0.42$ ‰,  $n = 3$ ) and newly emerged females ( $-14.88 \pm 0.55$ ‰,  $n = 3$ ; prior to first feeding) were similar to the value of the larval diet (Table 1).

#### Experiment 1

Egg  $\delta^{13}\text{C}$  values were intermediate between the larval and the adult diets (Tables 1 and 2; Fig. 2). The first eggs laid were  $-19.04 \pm 0.17$ ‰ ( $n = 8$  females), and thus already reflected a significant carbon contribution from the adult diet. Eggs shifted slightly but highly significantly further toward the adult diet across female lifetime (ANOVA;  $F_{5,42} = 12.0$ ,  $P < 0.0001$ ; Fig. 2). This shift was well described by a turnover model similar to that applied to nectar-feeding Lepidoptera (O'Brien *et al.* 2000):

**Table 2.** Model parameter estimates from *experiment 1*, *experiment 2* and both sets of data combined. Also given are model sum of squared errors (SSE) and df

Model parameters	Experiment 1	Experiment 2	All data
$r$ ( $\text{days}^{-1}$ )	$0.17 \pm 0.06$	$1.26 \pm 0.43$	$1.07 \pm 0.33$
$\delta^{13}\text{C}_i$ (‰)	$-18.71 \pm 0.21$	$-14.03 \pm 2.79$	$-16.32 \pm 1.31$
$\delta^{13}\text{C}_f$ (‰)	$-20.19 \pm 0.14$	$-20.01 \pm 0.13$	$-19.98 \pm 0.07$
SSE	7.04	10.70	20.51
df	45	42	90

**Fig. 2.** Egg  $\delta^{13}\text{C}$  over time for *Bicyclus anynana* females being (a) fed with banana *ad libitum* (mean of eight females  $\pm 1$  SE) or (b) initially starved ( $n = 11$ ). The lines indicate the line of best fit of the following model:  $\text{egg } \delta^{13}\text{C} = e^{-rt}(\delta^{13}\text{C}_i - \delta^{13}\text{C}_f) + \delta^{13}\text{C}_f$ , where  $t$  = time in days,  $r$  = the fractional turnover rate, and  $\delta^{13}\text{C}_i$  and  $\delta^{13}\text{C}_f$  = the initial and final egg  $\delta^{13}\text{C}$ , respectively. The shift toward the adult diet is slight but highly significant, and occurs more rapidly in *experiment 2* (starved females). Egg isotope values are intermediate between the larval ( $-14.37$ ‰) and adult ( $-25.33$ ‰) diets.

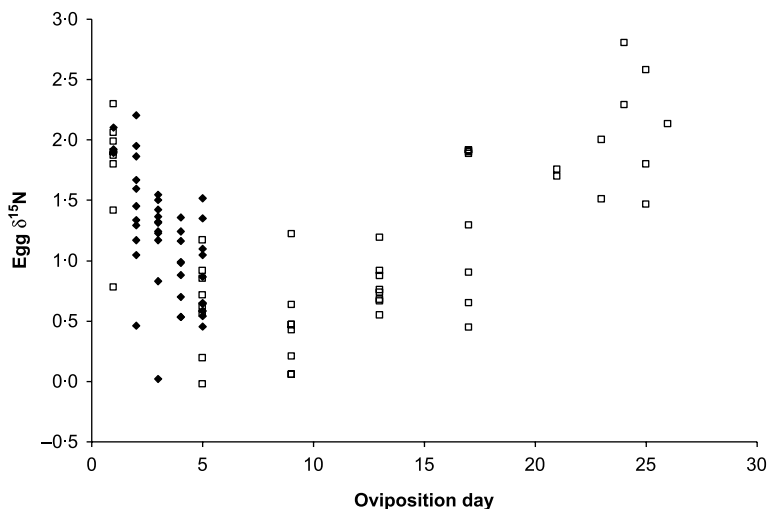
$$\text{egg } \delta^{13}\text{C} = e^{-rt}(\delta^{13}\text{C}_i - \delta^{13}\text{C}_f) + \delta^{13}\text{C}_f \quad \text{eqn 1}$$

where  $r$  = the fractional turnover rate,  $t$  = time in days,  $\delta^{13}\text{C}_i$  = initial egg  $\delta^{13}\text{C}$  (estimated value at oviposition day = 0) and  $\delta^{13}\text{C}_f$  = final egg  $\delta^{13}\text{C}$ . This model provided a close fit to the data; parameters are presented in Table 2.

The value at which egg  $\delta^{13}\text{C}$  stabilized ( $\delta^{13}\text{C}_f$ ) was  $-20.19$ ‰; this value reflects the maximal use of adult dietary carbon in oocyte provisioning. Using a mixing model (Eqn 2), we calculate that the proportion of *B. anynana* egg carbon deriving from the adult diet ( $p_{\text{adult}}$ ) at peak is 53%:

$$\text{Egg } \delta^{13}\text{C} = p_{\text{adult}}(\delta^{13}\text{C}_{\text{adult diet}}) + (1 - p_{\text{adult}})(\delta^{13}\text{C}_{\text{larval diet}}) \quad \text{eqn 2}$$

However, shifts in isotope ratio as a result of chemical reactions (fractionations) are common in metabolism and may be associated with use of dietary carbon in eggs. In other lepidopteran species, fractionation of



**Fig. 3.** Egg  $\delta^{15}\text{N}$  over time for *Bicyclus anynana* females being fed with banana *ad libitum* (experiment 1; open squares,  $n = 8$ ) or being initially starved (experiment 2; filled diamonds;  $n = 11$ ). These data represent the effect of oviposition day with maternal effects held constant. Because females varied significantly in egg  $\delta^{15}\text{N}$ , data were standardized to a per female mean of  $1.2\text{‰}$  to visualize the time component more clearly.

larval dietary carbon was negligible ( $0\text{--}1\text{‰}$ ); however, fractionation of adult dietary carbon was more substantial and variable (ranging from extremes of  $-1.9$  to  $+3.7\text{‰}$ ; O'Brien *et al.* 2000, 2004). If we assume a  $-2\text{‰}$  and a  $+2\text{‰}$  fractionation, respectively, our estimate of *B. anynana* egg carbon that derives from the adult fruit diet varies from 45% to 65%. Thus, while our estimate of 53% carries uncertainty, it is clear that the butterflies are deriving a large fraction of their egg carbon from banana.

#### Experiment 2

After 1 day's exposure to banana, females deprived of adult diet for 4 days (causing a delay in oviposition) laid eggs that were slightly more larval in isotopic content than the first eggs laid in experiment 1 ( $-18.32 \pm 0.39\text{‰}$ ,  $n = 4$  females). Those females that laid their first eggs after 2 days' exposure to banana in fact had eggs which had already shifted toward banana by more than  $1\text{‰}$  ( $-19.49 \pm 0.08\text{‰}$ ,  $n = 7$ ). From the initiation

of oviposition until the end of these analyses, there was a small but significant decrease in egg  $\delta^{13}\text{C}$  over time ( $F_{3,39} = 5.4$ ,  $P = 0.0034$ ; Fig. 2). As in experiment 1, egg  $\delta^{13}\text{C}$  equilibrated at  $c. -20\text{‰}$ . We applied the model (equation 1) to these data to estimate  $\delta^{13}\text{C}_i$ ,  $\delta^{13}\text{C}_f$ , and the fractional turnover rate ( $r$ ) (Table 2; Fig. 2). Turnover in the starved females was more rapid (1 vs 6 days for 50% turnover), and the estimated starting  $\delta^{13}\text{C}$  reflected a greater proportion of larval diet. These differences are reflected in a significantly different fit of the model from experiment 1 and experiment 2 ( $F_{3,87} = 4.52$ ;  $P = 0.0054$ ; Motulsky & Ransas 1987).

#### EGG $\delta^{15}\text{N}$ AND NITROGEN TURNOVER

Larval host plant and adult diet differed naturally in  $\delta^{15}\text{N}$ , although not as strikingly as in  $\delta^{13}\text{C}$ . Maize had a  $\delta^{15}\text{N}$  value of  $1.60 \pm 1.00\text{‰}$  ( $n = 3$ ), while the banana had one of  $3.87 \pm 0.58\text{‰}$  ( $n = 3$ ) (Table 1). Prepupae ( $0.52 \pm 0.76\text{‰}$ ,  $n = 3$ ) and newly emerged females ( $0.61 \pm 1.64\text{‰}$ ) were isotopically light relative to both diets, but more similar to the larval maize diet (Table 1).

#### Experiment 1

During the first week of oviposition, *B. anynana* eggs showed a rapid and highly significant decline in  $\delta^{15}\text{N}$ , of about  $-1.5\text{‰}$ , from a starting value of  $1.8 \pm 0.9\text{‰}$  (Fig. 3, Table 3). This pattern reversed for the second and third week of oviposition, with a highly significant but slower increase in egg  $\delta^{15}\text{N}$  to a higher value ( $2.6 \pm 0.4\text{‰}$ ) (Fig. 3; Table 3). Egg  $\delta^{15}\text{N}$  also varied significantly among individual females (Table 3). Because this individual variation partially obscures the pattern with oviposition day when plotted, data from each female were standardized to the mean per female value of  $1.2\text{‰}$  for visualization. These standardized data are presented against oviposition day in Fig. 3.

#### Experiment 2

Egg  $\delta^{15}\text{N}$  followed a very similar pattern with oviposition day in females that had been starved for 4 days (Fig. 3), dropping by  $c. -1.5\text{‰}$ . Both the slope and the

**Table 3.** Effect of oviposition day and butterfly identity on egg  $\delta^{15}\text{N}$  (tested with ANCOVA). Data are separated by experiment and two time periods: oviposition day 0–6 and oviposition day 7–27

Oviposition day	Slope	Effect	SS	d.f.	<i>F</i>	<i>P</i>
Experiment 1 (days 0–6)	$-0.28\text{‰ day}^{-1}$	Oviposition day	5.18	1	46.91	0.0002
		Butterfly	12.28	7	15.90	0.0008
		Error	0.77	7		
Experiment 2 (days 0–6)	$-0.27\text{‰ day}^{-1}$	Oviposition day	4.81	1	26.46	<0.0001
		Butterfly	13.88	10	7.63	<0.0001
		Error	6.00	33		
Experiment 1 (days 7–27)	$+0.11\text{‰ day}^{-1}$	Oviposition day	12.14	1	57.38	<0.0001
		Butterfly	13.61	7	9.19	<0.0001
		Error	4.86	23		

significance of the trend were similar to *experiment 1*, days 1–6 (Table 3). Standardized data from *experiment 2* are also presented in Fig. 3.

## Discussion

Our results reveal that adult feeding is essential for reproduction in *B. anynana*: females will not oviposit without adult dietary input. This finding suggests that adult diet here is even more important than in many nectivorous butterflies, which do lay eggs if fed only water. In nectivorous species from diverse taxonomic groups studied to date, fecundity increases two- to seven-fold for females with access to sugar as opposed to only water (e.g. Pieridae, Stern & Smith 1960; Nymphalidae, Murphy, Launer & Ehrlich 1983; Lycaenidae, Hill & Pierce 1989; Satyridae, Karlsson & Wickman 1990). The most extreme case to our knowledge is that of the lycaenid *Lycaena hippothoe*, which increased fecundity seven-fold given sugar as opposed to only water (Fischer & Fiedler 2001). Whether the pattern observed here is specific to *B. anynana*, typical of fruit-feeders or due to some other cause remains to be tested.

Likewise, the stable isotope analyses indicate that the adult diet is an important source of egg nutrients in *B. anynana*: approximately half the carbon came from banana. The carbon that banana provides is incorporated into developing eggs quite rapidly, as the first eggs laid already reflect a nearly even ratio of larval and adult carbon sources. Eggs laid subsequently show an additional shift toward adult dietary carbon, although the magnitude of the shift is slight. The shift in egg carbon from more larval to more adult sources is well described by a simple turnover model, similar to those applied to nectar-feeding moths and butterflies (O'Brien *et al.* 2000, 2003). The model reflects a physiological hypothesis: that egg carbon sources come to reflect dietary carbon to the extent that is possible, and that further adult dietary input is constrained by specific nutrient requirements. The tendency for the eggs in both experiments to plateau at identical isotopic values is consistent with this hypothesis.

The initially starved females began laying eggs within 1–2 days after adult food was provided, again indicating that adult food is quickly metabolized and incorporated into eggs. Nevertheless, the estimated starting  $\delta^{13}\text{C}$  reflected slightly more larval carbon input than the first eggs laid by continuously fed butterflies, and the eggs shifted more rapidly to the maximum contribution of adult dietary carbon. The isotopic change of eggs laid by previously starved butterflies is more than seven times more rapid than that of continuously feeding butterflies. This more rapid turnover probably reflects the depletion of larval stores in starved females due to metabolism or general maintenance. With smaller larval reserves and a similar rate of intake, turnover would be expected to occur more rapidly.

Both the percentage use of adult carbon in eggs and the C : N ratios for *B. anynana* eggs were within the

range of those documented for nectivorous butterflies by O'Brien *et al.* (2004). The earlier series of nectivorous butterflies examined included species that differed in the percentage of eggs mature at adult emergence, the amount of nectar eaten as an adult, and the C : N ratio of eggs. *B. anynana* ecloses with no eggs mature (K. Fischer, unpublished data); when compared with such nectivorous species with similar C : N ratios, the maximal percentage of adult carbon used in egg production is the same as for the pierid *Colias eurytheme* and similar to the nymphalid *Heliconius charitonia* (O'Brien *et al.* 2004) and the sphingid *Amphion floridensis* (O'Brien *et al.* 2000). These data suggest that fruit may play an equivalent role to nectar in egg production, with respect to carbon utilization.

The larval and the adult diets of *B. anynana* contrasted in  $\delta^{15}\text{N}$ , with the adult banana diet being over 2‰ more enriched in  $\delta^{15}\text{N}$  than the larval maize diet. Egg  $\delta^{15}\text{N}$  started at a value similar to the larval diet, and declined rapidly over the first week of oviposition. These data suggest the adult diet was not providing significant nitrogen for egg manufacture, at least not at the onset of oviposition. Furthermore, it suggests that egg manufacture selectively uses the lighter isotope of nitrogen, to an increasing extent over the first week of oviposition. In a study on nectar-feeding moths, moths synthesized non-essential amino acids from adult dietary carbon, using endogenous sources of amine groups (O'Brien *et al.* 2002). The reaction transferring amine nitrogen between amino acids has been shown to prefer  $^{14}\text{N}$ , causing a shift in isotope ratio of –8‰ (Macko *et al.* 1986, 1987). Thus, the initial drop in egg  $\delta^{15}\text{N}$  may reflect increasing levels of non-essential amino acid synthesis from adult-derived carbon sources, using endogenous nitrogen from the larval diet. Although the adult diet in this study did contain nitrogen, it was very nitrogen poor (with a C/N of 45 : 1). Thus, the fact that egg nitrogen resembled larval diet more than the adult diet supports the idea that the adult diet is primarily a source of carbon rather than nitrogen. Banana %N was only about 1–2% (dry weight), thus, they would have to consume a great deal to make up for their nitrogen losses to oviposition (estimated to total, on average, 2.4 mg N).

Interestingly, the trend in egg  $\delta^{15}\text{N}$  shifted direction after about 1 week of oviposition. Egg  $\delta^{15}\text{N}$  increased slowly but consistently, to a value slightly higher than initial egg  $\delta^{15}\text{N}$ . This reflects an isotopic enrichment of the source of egg nitrogen, which could happen via at least two means. First, the preferential use of  $^{14}\text{N}$  in egg manufacture could cause the nitrogen source to become enriched in  $^{15}\text{N}$ . This shift will ultimately be reflected in the isotope signature of the eggs. Secondly, the accumulation of nitrogen from the adult banana diet might start to increase the  $\delta^{15}\text{N}$  of the source pool. These factors will drive the  $\delta^{15}\text{N}$  of the eggs in the same direction, so our data do not allow us to evaluate the relative merits of these two hypotheses. However, egg nitrogen follows a similar pattern in butterflies

maintained on sucrose alone as adults (D. M. O'Brien, unpublished data), and thus does not require the input of banana nitrogen. Thus, the data do not clearly indicate that the adult diet provides nitrogen for egg manufacture, but neither do they rule it out in the later weeks of oviposition.

In summary, our study shows that reproduction in *B. anynana* depends on input from the adult fruit diet, without which no eggs are laid. Overall, egg composition is similar to that of nectivorous Lepidoptera, as is the extent to which the adult diet provides carbon for egg manufacture (O'Brien *et al.* 2000, 2004). However, the shift in egg carbon from larval towards an adult signature is less pronounced, because the first eggs laid already contained a large contribution of carbon from the adult diet. The only species with a comparably slight shift towards the adult diet and a high contribution of adult dietary nutrients to first-laid eggs is *Heliconius charitonia*, a nymphalid butterfly that feeds on nitrogen-rich pollen as well as nectar (O'Brien *et al.* 2004). In the experiment cited above, *H. charitonia* were provided sugar water only; but still drew rapidly and heavily on adult dietary input for egg manufacture. This suggests that the degree of dependence on adult income and the rapidity with which it is incorporated may be associated with the *expected* quality of the adult diet. If so, fruit may typically provide a richer resource for reproduction than is suggested by the data here, perhaps through the growth of yeast or other micro-organisms. Alternatively, the similarity might arise from a possible higher predictability of encountering adult food throughout much of the year in tropical regions. These issues deserve further investigation.

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### References

Baierlein, F. (1996) Fruit-eating in birds and its nutritional consequences. *Comparative Biochemistry and Physiology A* **113**, 215–224.

Bazzaz, F. (1996) *Plants in Changing Environments: Linking Physiological, Population and Community Ecology*. Cambridge University Press, Cambridge.

Beck, J., Mühlberg, E. & Fiedler, K. (1999) Mud-puddling behavior in tropical butterflies: in search of proteins or minerals. *Oecologia* **119**, 140–148.

Boggs, C.L. (1987) Ecology of nectar and pollen feeding in Lepidoptera. *Nutritional Ecology of Insects, Mites and Spiders and Related Invertebrates* (eds F. Slansky Jr & J.G. Rodriguez), pp. 369–391. John Wiley & Sons, New York.

Boggs, C.L. (1997a) Reproductive allocation from reserves and income in butterfly species with differing adult diets. *Ecology* **78**, 81–191.

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

Boggs, C.L. & Jackson, L.A. (1991) Mud puddling by butterflies is not a simple matter. *Ecological Entomology* **16**, 123–127.

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

Bosque, C. & Pacheco, M.A. (2000) Dietary nitrogen as a limiting nutrient in frugivorous birds. *Revista Chilena de Historia Natural* **73**, 441–450.

Brakefield, P.M. (1997) Phenotypic plasticity and fluctuating asymmetry as responses to environmental stress in the butterfly *Bicyclus anynana*. *Environmental Stress: Adaptation and Evolution* (eds R.R. Bijlsma & V. Loeschke), pp. 65–78. Birkhäuser, Basel.

Brakefield, P.M., El Filali, E., Van der Laan, R., Breuker, C.J., Saccheri, I.J. & Zwaan, B.J. (2001) Effective population size, reproductive success and sperm competition in the butterfly *Bicyclus anynana* in captivity. *Journal of Evolutionary Biology* **14**, 148–156.

DeVries, P.J. & Walla, T.R. (2001) Species diversity and community structure in neotropical fruit-feeding butterflies. *Biological Journal of the Linnean Society* **74**, 1–15.

DeVries, P.J., Murray, D. & Lande, R. (1997) Species diversity in vertical, horizontal, and temporal dimensions of a fruit-feeding butterfly community in an Ecuadorian rainforest. *Biological Journal of the Linnean Society* **62**, 343–364.

Dunlap-Pianka, H.L., Boggs, C.L. & Gilbert, L.E. (1977) Ovarian dynamics in heliconiine butterflies: programmed senescence versus eternal youth. *Science* **197**, 487–490.

Elmes, G.W., Thomas, J.A., Munguira, M.L. & Fiedler, K. (2001) Larvae of lycaenid butterflies that parasitize ant colonies provide exceptions to normal insect growth rules. *Biological Journal of the Linnean Society* **73**, 259–278.

Erhardt, A. & Rusterholz, H.P. (1998) Do peacock butterflies (*Inachis io* L.) detect and prefer nectar amino acids and other nitrogenous compounds? *Oecologia* **117**, 536–542.

Fischer, K. & Fiedler, K. (2001) Effects of adult feeding and temperature regime on fecundity and longevity in the butterfly *Lycaena hippothoe*. *Journal of the Lepidopterists Society* **54**, 91–95.

Fischer, K., Brakefield, P.M. & Zwaan, B.J. (2003) Plasticity in butterfly egg size: why larger offspring at lower temperatures? *Ecology* **84**, 3138–3147.

Gilbert, L.E. (1972) Pollen feeding and reproductive biology of *Heliconius* butterflies. *Proceedings of the National Academy of Sciences of the USA* **69**, 1403–1407.

Good, T.P. & Tatar, M. (2001) Age-specific mortality and reproduction respond to adult dietary restriction in *Drosophila melanogaster*. *Journal of Insect Physiology* **47**, 1467–1473.

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

Izhaki, I. (1998) Essential amino acid composition of fleshy fruits vs. maintenance requirements of passerine birds. *Journal of Chemical Ecology* **24**, 1333–1345.

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

Larsen, T.B. (1991) *The Butterflies of Kenya*. Oxford University Press, Oxford.

Levey, D.J. & del Rio, C.M. (2001) It takes guts (and more) to eat fruit: lessons from avian nutritional ecology. *Auk* **118**, 819–831.

- Levey, D.J., Bissell, H.A. & O'Keefe, S.F. (2000) Conversion of nitrogen to protein and amino acids in wild fruits. *Journal of Chemical Ecology* **26**, 1749–1763.
- Macko, S.A., Estep, M.L.F., Engel, M.H. & Hare, P.E. (1986) Kinetic fractionation of stable nitrogen isotopes during amino-acid transamination. *Geochimica et Cosmochimica Acta* **50**, 2143–2146.
- Macko, S.A., Fogel, M.L., Hare, P.E. & Hoering, T.C. (1987) Isotopic fractionation of nitrogen and carbon in the synthesis of amino acids by microorganisms. *Chemical Geology* **65**, 79–92.
- Mevi-Schutz, J., Goverde, M. & Erhardt, A. (2003) Effects of fertilization and elevated CO<sub>2</sub> on larval food and butterfly nectar amino acid preference in *Coenonympha pamphilus* L. *Behavioral Ecology and Sociobiology* **54**, 36–43.
- Motulsky, H.J. & Ransas, L.A. (1987) Fitting curves to data using non-linear regression: a practical and nonmathematical review. *FASEB Journal* **1**, 365–374.
- Murphy, D.D., Launer, A.E. & Ehrlich, P.R. (1983) The role of adult feeding in egg production and population dynamics of the checkerspot butterfly *Euphydryas editha*. *Oecologia* **56**, 257–263.
- O'Brien, D.M., Schrag, D.P. & del Rio, C.M. (2000) Allocation to reproduction in a hawkmoth: a quantitative analysis using stable carbon isotopes. *Ecology* **81**, 2822–2831.
- O'Brien, D.M., Fogel, M.L. & Boggs, C.L. (2002) Renewable and non-renewable resources: amino acid turnover and allocation to reproduction in Lepidoptera. *Proceedings of the National Academy of Sciences of the USA* **99**, 4413–4418.
- O'Brien, D.M., Boggs, C.L. & Fogel, M.L. (2003) Pollen feeding in the butterfly *Heliconius charitonia*. Isotopic evidence for essential amino acid transfer from pollen to eggs. *Proceedings of the Royal Society of London B* **270**, 2631–2636.
- O'Brien, D.M., Boggs, C.L. & Fogel, M.L. (2004) Making eggs from nectar: connections between butterfly life history and the importance of nectar carbon in reproduction. *Oikos* **105**, 279–291.
- O'Leary, M.H. (1988) Carbon isotopes in photosynthesis. *Bioscience* **38**, 328–336.
- Omura, H. & Honda, K. (2003) Feeding responses of adult butterflies, *Nymphalis xanthomelas*, *Kaniska canace* and *Vanessa indica*, to components in tree sap and rotting fruits: synergistic effects of ethanol and acetic acid on sugar responsiveness. *Journal of Insect Physiology* **49**, 1031–1038.
- Pacini, E., Nepi, M. & Vespini, J.L. (2003) Nectar biodiversity: a short review. *Plant Systematics and Evolution* **238**, 7–21.
- Rusterholz, H.P. & Erhardt, A. (2000) Can nectar properties explain sex-specific flower preferences in the Adonis blue butterfly *Lysandra bellargus*? *Ecological Entomology* **25**, 81–90.
- Saccheri, I.J. & Bruford, M.W. (1993) DNA fingerprinting in a butterfly, *Bicyclus anynana* (Satyridae). *Journal of Heredity* **84**, 195–200.
- Sibly, R.M. & Calow, P. (1984) Direct and absorption costing in the evolution of life cycles. *Journal of Theoretical Biology* **111**, 463–473.
- Stern, V.M. & Smith, R.F. (1960) Factors affecting egg production and oviposition in populations of *Colias philodice eurytheme* Boisduval (Lepidoptera: Pieridae). *Hilgardia* **29**, 411–454.
- Tammaru, T. & Haukioja, E. (1996) Capital breeders and income breeders among Lepidoptera – consequences to population dynamics. *Oikos* **77**, 561–564.
- Wheeler, D. (1996) The role of nourishment in oogenesis. *Annual Reviews of Entomology* **41**, 407–431.

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