

## ALLOCATION TO REPRODUCTION IN A HAWKMOTH: A QUANTITATIVE ANALYSIS USING STABLE CARBON ISOTOPES

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**Abstract.** There is great interest in the importance of nectar nutrients to fecundity in the Lepidoptera, but nutrient allocation has been difficult to measure quantitatively. Here we trace the allocation of nectar nutrients in the hawkmoth *Amphion floridensis* using naturally occurring variation in plant stable carbon isotopes and thereby derive a descriptive model of carbon flow into eggs. Because <sup>13</sup>C content (expressed as  $\delta^{13}\text{C}$ , the <sup>13</sup>C:<sup>12</sup>C ratio relative to a standard) depends on photosynthetic mode, moths were fed sucrose solution made with either either C<sub>3</sub> or C<sub>4</sub> sugar (beet or cane), both of which were distinct from larval host plant. In addition, two of four experimental diets contained an amino acid supplement distinct in  $\delta^{13}\text{C}$  from either sugar or larval host plant. Females were hand fed daily from experimental diets, and their eggs were collected and analyzed for  $\delta^{13}\text{C}$ . Egg  $\delta^{13}\text{C}$  increased rapidly from a value resembling larval  $\delta^{13}\text{C}$ , and followed an asymptotic pattern of carbon incorporation. The presence of amino acids in the diet had no effect on either fecundity or egg  $\delta^{13}\text{C}$ . Because egg  $\delta^{13}\text{C}$  equilibrated at a value lower than  $\delta^{13}\text{C}$  diet, we invoke an allocation model in which carbon is contributed to eggs by two separate pools. One pool of carbon comes into isotopic equilibrium with adult diet, whereas the other does not, contributing carbon with an exclusively larval signature across a female's lifetime. Carbon fractional turnover rate and the relative contribution of the two pools were estimated by fitting the model to the data with nonlinear regression. The resulting model fitted the data well and indicated that 50–60% of egg carbon is derived from adult nectar sugars after the “mixing pool” has come into equilibrium. Thus, this study demonstrates that adult nectar sugars provide an important source of egg carbon and explores how properties of nutrient mixing and turnover can generate patterns of reproductive allocation.

**Key words:** allocation; carbon turnover; Lepidoptera; nectar feeding; reproduction; Sphingidae; stable isotopes.

### INTRODUCTION

Reproductive resource allocation is a fundamental aspect of life history with profound ecological and evolutionary consequences. Allocation decisions in the Lepidoptera are particularly interesting because larval and adult diets are nutritionally distinct, and because species vary widely in the importance of adult feeding to fecundity (Dunlap-Pianka et al. 1977, Hebert 1983, Boggs 1997a, Miller 1997). In addition, interest in Lepidoptera as pollinators as well as concern for threatened populations has focused attention on the factors limiting their survivorship and fecundity (Buchman and Nabhan 1996). Understanding the fate of nectar nutrients provides a mechanistic basis for understanding the relative importance of adult nutrition to different components of fitness.

Numerous studies have demonstrated that adult nec-

tar feeding enhances fecundity in butterflies and moths (e.g., Murphy et al. 1983, Hill 1989, Hill and Pierce 1989, Ziegler 1991, Boggs and Ross 1993). However, this association does not necessarily indicate a direct allocation of nectar nutrients into eggs. Nectar could be used to provide water (Norris 1936, Miller 1988) or energy for mating, egg manufacture, and oviposition. In these scenarios, nectar feeding will enhance fecundity even if eggs are provisioned from larval stores alone. To disentangle the direct allocation of specific nutrients from the general effects of nutrition on fecundity, nutrients from different dietary sources must be distinct and amenable to tracing.

Mechanistic studies of nutrient allocation have been hampered by the lack of quantitative methodology for nutrient labeling. In general, radiotracers have been used to follow the fate of nutrients fed to or injected into organisms. This method allows qualitative documentation of nutrient flow into eggs, for example, male-donated nutrients (e.g. Gilbert 1972, Boggs 1981a) or nutrients from larval and adult diets (Boggs and Gilbert 1979, Boggs 1997b). Radiotracers fed or injected into individuals, however, are introduced as a single pulse

Manuscript received 23 November 1998; revised 6 September 1999; accepted 9 September 1999.

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into a dynamic system of nutrient flow. Without knowing the resultant specific activity of the nutrient pool and its turnover dynamics, the amount of radiolabel in eggs is difficult to interpret quantitatively.

Naturally occurring variation in stable carbon isotopes provides a potential solution to the difficulties inherent in nutrient labeling. The ratio of  $^{13}\text{C}$  to  $^{12}\text{C}$  in plant tissues varies with photosynthetic mode (O'Leary 1988, Farquhar et al. 1989) such that  $\text{C}_3$  plants are strikingly depleted in  $^{13}\text{C}$  relative to  $\text{C}_4$  plants. Many studies have made use of this difference to infer diet in extant populations of animals (e.g. Boutton et al. 1980, Ambrose and DeNiro 1986, Fleming et al. 1993, Ostrom et al. 1997) and in paleo-remains (e.g., Vogel and Van der Merwe 1977, Koch et al. 1994), as well as to assess the physiological fates of different nutritional components of diet (Tieszen and Fagre 1993).  $\text{C}_3$  and  $\text{C}_4$  diets have been used in the laboratory to observe the kinetics of tissue carbon turnover (Tieszen et al. 1983, Hobson and Clark 1992, Ostrom et al. 1997), and of reproductive investment in birds (Hobson 1995) and dairy cows (Boutton et al. 1988, Metges et al. 1990). The success with which stable isotopes have been applied to problems of nutrient tracing in ecosystems and within organisms makes them a good candidate for documenting resource allocation patterns in the Lepidoptera.

In this study we use stable carbon isotopes to trace the allocation of nutrients derived from larval vs. adult feeding into eggs by the diurnal nectarivorous hawkmoth, *Amphion floridensis*. The host plant of *A. floridensis* caterpillars is  $\text{C}_3$  (*Vitis* species), whereas the adults are fed sucrose solution in the laboratory. Sucrose is the predominant sugar in hawkmoth nectars (Baker and Baker 1983), and is commercially available as either beet sugar or cane sugar ( $\text{C}_3$  and  $\text{C}_4$  plants, respectively). We trace the allocation of these dietary sugars into eggs by analyzing egg  $^{13}\text{C}$  content across a female's lifetime. In addition, we use an isotopically distinct amino acid supplement to address whether nectar amino acids are an important source of egg nutrient, given their typical abundance in plant nectars. We describe the observed carbon kinetics of eggs in *A. floridensis* with a model that parameterizes the timing and amount of incorporation of adult diet, as well as the number of resource pools contributing carbon and their dietary source. In so doing we present a more complete model for reproductive allocation in Lepidoptera than has formerly been possible.

## METHODS

### *Moth trapping and rearing*

Adult *Amphion floridensis* were trapped in Princeton, New Jersey during the summers of 1995 and 1997. Traps were baited with a fermented banana/beer/sugar mixture and hung at forest edges providing both host plant and natural flowers for nectar foraging (Platt

1969). Trapped females were housed in  $0.6 \times 0.9 \times 1.2$ -m flight cages and provided 30% (by mass) sugar solution for food and potted grape plants (*Vitis vinifera*) for oviposition. Eggs were removed from host plants daily. Larvae were reared in 14 cm diameter plastic dishes on freshly collected leaves of host plant (Family Vitaceae), primarily wild grape (*Vitis novae-angliae*) but also including fox grape (*Vitis labrusca*), European ampelopsis (*Ampelopsis brevipedunculata*), and Virginia creeper (*Parthenocissus quinquefolia*). Adults, eggs, and larvae were kept at  $27^\circ\text{C}$  on a 16L:8D photoperiod. Humidity was maintained at 70–80%.

Prepupae were removed from dishes and allowed to burrow into darkened boxes of moist peat moss. *Amphion floridensis* overwinters as pupae; therefore, after one month of pupation at  $27^\circ\text{C}$  pupae were stored at  $4^\circ\text{C}$  for 6–13 mo. Experimental adults emerged 10–14 days after being returned to  $27^\circ\text{C}$  and a 16L:8D photoperiod.

### *1996 and 1998 experiments*

Experiments took place in fall of 1996 and spring of 1998. In 1996, moths were kept in a greenhouse on 16L:8D photoperiod and with a mean daytime temperature of  $\sim 27^\circ\text{C}$ . Females emerged after a full year of diapause, were reluctant to mate, and did not begin to lay eggs until the second or third day after eclosion. Poor mating and oviposition success restricted 1996 sample size to four females ( $\times 10$  egg samples per moth [mean] = 40 egg samples total). In 1998, moths were kept with the same photoperiod but with higher daytime temperatures,  $\sim 32^\circ\text{C}$ . In 1998, females experienced a shorter diapause (5 mo), mated on the day of eclosion, and usually began to lay eggs the following day. Higher mating and oviposition success (100%) in 1998 allowed greater sample sizes ( $N = 16$  females  $\times 6.3$  egg samples per moth [mean] = 100 egg samples total). Due to these differences between the two years, data were analyzed separately.

### *Experimental protocol*

Freshly eclosed experimental females were housed separately in 61 cm square nylon mesh cages with 1–3 males and a potted grape plant for oviposition. Females were hand-fed daily to satiation from 0.6 ml centrifuge vials containing one of four experimental diets. Vials were weighed on a Mettler microbalance model MT5 (Mettler, Columbus, Ohio, USA) before and after feeding to quantify intake. Eggs were collected daily, counted, and frozen for later analysis. Females laid eggs for  $18 \pm 1$  d (mean  $\pm$  SE), and were fed for the duration of their natural lifespan or until they were too feeble either to lay eggs or to take food.

### *Diets*

Experimental females were assigned to one of four artificial nectar diets:  $\text{C}_3$  sugar,  $\text{C}_4$  sugar,  $\text{C}_3$  sugar with amino acids, or  $\text{C}_4$  sugar with amino acids. All diets

contained 30% sucrose (by mass) deriving either from beet ( $C_3$ ) or cane ( $C_4$ ) sugar. One diet of each sugar type also contained amino acids derived from hydrolyzed casein. Casein was purified from Mexican milk; because subtropical rangeland offers predominantly  $C_4$  plants for grazing cattle, milk casein was enriched in  $^{13}C$  relative to  $C_3$  plants. Amino acids were added to the  $C_3$  and  $C_4$  sugar diets to a final concentration of 0.266 g/L sucrose solution. This amino acid concentration is typical for moth-visited flower nectar ( $\sim 0.2$  g/L; Baker and Baker 1973). Solutions were aliquotted into 0.6 mL centrifuge vials for feeding and frozen until used.

The casein fraction was extracted from reconstituted milk through acid precipitation with HCl to pH 4.6, filtered through Whatman #1 filters (Whatman, Clifton, New Jersey, USA), and lyophilized. The powdered precipitate was washed in petroleum ether and refiltered four times, until remaining lipid residues were negligible. The casein was resuspended in sodium phosphate buffer (0.2 mol/L, pH 7) and incubated with the proteolytic enzymes trypsin, elastase, and carboxypeptidase B for 60 min at 37°C (Sigma Biochemical, St. Louis, Missouri, USA). This method yielded 95% hydrolysis or better, as determined using a Bradford assay for total protein before and after hydrolysis. To remove incompletely hydrolyzed protein fragments, the raw hydrolysate was filtered through Centriprep 30 centrifuge filters (Millipore, Bedford, Massachusetts, USA) in a Sorvall ultracentrifuge (Newtown, Connecticut, USA) at 3663 rpm (1500 g). Final amino acid concentrations were measured with a fluorescamine spectrofluorometric assay (Aminco Bowen Spectrofluorometer, Spectronic Unicam, Rochester, New York, USA). Amino acids were added to sugar solutions in a small amount of phosphate buffer (final concentration sodium phosphate = 0.007 mol/L).

#### Determination of isotope ratios

Egg  $^{13}C$  content was analyzed in batches of 10–15 eggs from each day. Eggs were oven dried to 2–3 mg dry mass at 60°C, placed in quartz tubes with 3 g of Cu:CuO (1:3) pellets, and flame sealed under vacuum. Samples were combusted at 900°C for 9 hr, until all organic carbon was completely oxidized to  $CO_2$ . Quartz tubes were opened into a vacuum distillation line in which water vapor was collected on a dry ice/ethanol trap and  $CO_2$  was collected on a liquid nitrogen trap. After remaining sample gas was pumped away (predominantly  $N_2$ ), sample  $CO_2$  was thawed, recondensed in a pyrex tube, and flame sealed. Collected  $CO_2$  was then transferred to an automated isotope ratio mass spectrometer (VG Optima, Micromass UK, Manchester, UK) for  $\delta^{13}C$  analysis. The procedure for analyzing  $\delta^{13}C$  of larval host plant and dietary components was identical. Sample preparation and mass spectrometry were performed in the Princeton Isotope Geochemistry Laboratory (Princeton, New Jersey, USA; 1996 sam-

ples) and the Harvard Laboratory for Geochemical Oceanography (Cambridge, Massachusetts, USA; 1998 samples).

The  $^{13}C$  content is expressed as the ratio ( $R$ ) of sample  $^{13}C:^{12}C$  relative to a standard, the Pee Dee Belemnite, according to the following notation:

$$\delta^{13}C = ((R_{\text{sample}}/R_{\text{standard}}) - 1) \times 1000. \quad (1)$$

$C_3$  plants typically have  $\delta^{13}C$  values  $\sim -28\text{‰}$ , whereas  $C_4$  plants have  $\delta^{13}C$  values  $\sim -14\text{‰}$  (O'Leary 1988). All plants are depleted in  $^{13}C$  relative to the standard (negative  $\delta^{13}C$ ); a less negative  $\delta^{13}C$  indicates a relative  $^{13}C$  enrichment, whereas a more negative number indicates relative  $^{13}C$  depletion. The standard deviation of carbon standards combusted, distilled, and analyzed together with samples was 0.028‰.

#### Egg protein composition and ovarian dynamics

The elemental composition of five batches of 10 eggs each was determined using a Fisons CHNS analyzer (Micromass UK, Manchester, UK). Egg protein content was calculated using a nitrogen to protein conversion factor of 5.7 g protein/g N. This conversion factor was calculated from the amino acid composition of *A. floridensis* eggs, measured as mole percentage (Beckman 6300 Amino Acid Analyzer, Fullerton, California, USA) and converted to mass percentage (D. M. O'Brien and C. L. Boggs, unpublished data). The amino acid composition is multiplied by the percentage N by mass of the constituent amino acids to determine protein percentage N, 0.175 g/g. Because N:protein ratios vary among tissues and species in plants (Milton and Dintzis 1981), it is preferable to calculate N to protein directly rather than rely on the standard protein conversion factor of 6.25 for animal tissue (Simonne et al. 1997). Percentage carbon in protein was estimated similarly and was 0.53 g C/g protein.

To characterize ovarian dynamics in *A. floridensis*, ovaries were dissected from 13 newly emerged females and the ratio of fully provisioned to partially- or non-provisioned oocytes was counted (as in Dunlap-Pianka et al. 1977).

#### Statistical analyses

All statistical analyses were performed in JMP version 3.1 (SAS Institute, Cary, North Carolina, USA). Means are presented  $\pm 1$  SE unless otherwise noted. The effects of year, sugar type, and amino acids on the duration of oviposition and total fecundity were tested with ANOVA. The effect of the amino acid supplement on egg  $\delta^{13}C$  was tested in the 1998 data set with ANOVA, including sugar type, day, and the interaction between sugar and amino acids as effects. The decline in meal size over time was tested with linear regression. Nonparametric Spearman rank tests are used to test the decline in egg laying over time, because the residuals do not meet the assumptions of linear regression. Non-linear curve fitting and parameter estimation was per-

FIG. 1. Ovarian status of a representative newly emerged *Amphion floridensis* female. The mean percentage of oocytes that were fully provisioned was 2.5% ( $N = 13$ ). In this ovary there are at least three fully provisioned oocytes. The photograph was taken at a magnification of 15–20 $\times$  through a dissecting scope.



formed in JMP using nonlinear least squares minimization.

## RESULTS

### Ovarian dynamics

The mean number of oocytes counted in freshly emerged females was  $466 \pm 26$ , with <3% mature on average ( $2.6\% \pm 0.5\%$ ). Most oocytes varied continuously from being partially provisioned to unprovisioned (Fig. 1). Counts of total oocytes in dissected females did not differ significantly from the total numbers of eggs laid by fed females ( $F_{1,33} = 0.5273$ ,  $P = 0.47$ ), suggesting that females emerge with a fixed number of oocytes, lay them all, and do not manufacture oocytes de novo across their adult lifetime.

### Egg protein composition

Elemental analysis revealed egg batches to contain  $9.88\% \pm 0.14\%$  nitrogen (g/g), and  $49.2\% \pm 0.05\%$  carbon (g/g). Therefore, the protein composition of *A. floridensis* eggs is  $9.88 \text{ g N/g egg} \times 5.7 \text{ g protein/g N} = 56\%$ . Because this study focuses on egg carbon composition, we are also interested in knowing what fraction of egg carbon derives from protein. This fraction can be calculated from the percentage C of the eggs, the percentage C of egg protein, and the percentage protein in the eggs. The percent of egg carbon deriving from protein is thus  $(0.57 \text{ g protein/g egg} \times 0.53 \text{ g C/g protein}) / 0.49 \text{ g C/g egg} = 0.61$  or 61%.

### Life history

Fed experimental females laid a mean of 469 eggs over the course of 16 days ( $N = 20$ ). Three unfed females laid many fewer eggs ( $90.0 \pm 14.0$ ,  $F_{1,23} = 45.32$ ,  $P < 0.0001$ ); therefore, nectar feeding significantly affects fecundity in this species. Year, sugar type ( $C_3$  vs.  $C_4$ ), and the addition of amino acids had no significant effects on either the duration of egg laying or total fecundity. The effect of amino acids on fecundity was marginally nonsignificant ( $428 \pm 31$  vs. 510

$\pm 31$  eggs [least squared means],  $P = 0.0527$ ), indicating a weak tendency for moths fed amino acids to lay more eggs than those fed sugar only.

Daily meal sizes were fairly constant until day five, and then decreased in both 1996 ( $R^2 = 0.30$ ,  $P < 0.0001$ ; Fig. 2) and 1998 ( $R^2 = 0.49$ ,  $P < 0.0001$ ; Fig. 2). Meal sizes decreased more rapidly in 1998, starting almost twice as high but decreasing to near zero in the same period of time. Egg laying rates also declined with time after day five both in 1996 ( $r_s = -0.7148$ ,  $P < 0.0001$ ; Fig. 2) and in 1998 ( $r_s = -0.6523$ ,  $P < 0.0001$ ; Fig. 2). The axes on the right-hand side of Fig. 2 express the data in mg carbon; in both years females took in several times more carbon as sucrose than they laid as eggs per day. The decrease in sample size with time is also plotted in Fig. 2. The apparent difference in survivorship between the years reflects different experimental procedure: In 1996 females continued to be fed until they were found dead, whereas in 1998 females were removed after they ceased to lay eggs. True differences in longevity, therefore, cannot be assessed between the two years.

### $\delta^{13}\text{C}$ of larval and adult dietary components

Samples of larval host plant (including *V. labrusca*, *V. novae-angliae*, and *A. brevipedunculata* from several collection sites) ranged in  $\delta^{13}\text{C}$  from  $-28.97\text{‰}$  to  $-31.11\text{‰}$  (Table 1). These values are within the range of  $\delta^{13}\text{C}$  for  $C_3$  plants, but fall near the extreme end of  $^{13}\text{C}$  depletion (O'Leary 1988). Both cane and beet sugars were readily distinguishable in  $\delta^{13}\text{C}$  from larval host plant (Table 1). Although larval host plant and beet both use  $C_3$  photosynthesis, their carbon signatures fall to either end of the range of  $\delta^{13}\text{C}$  values found in  $C_3$  plants (O'Leary 1988), and are thus quite distinct. The casein amino acid supplement was intermediate in carbon composition between the two sugars (Table 1).

### Initial egg $\delta^{13}\text{C}$

The  $\delta^{13}\text{C}$  of eggs laid by unfed females was  $-29.47\text{‰} \pm 0.16\text{‰}$  ( $N = 7$ ; Table 1). Eggs laid by

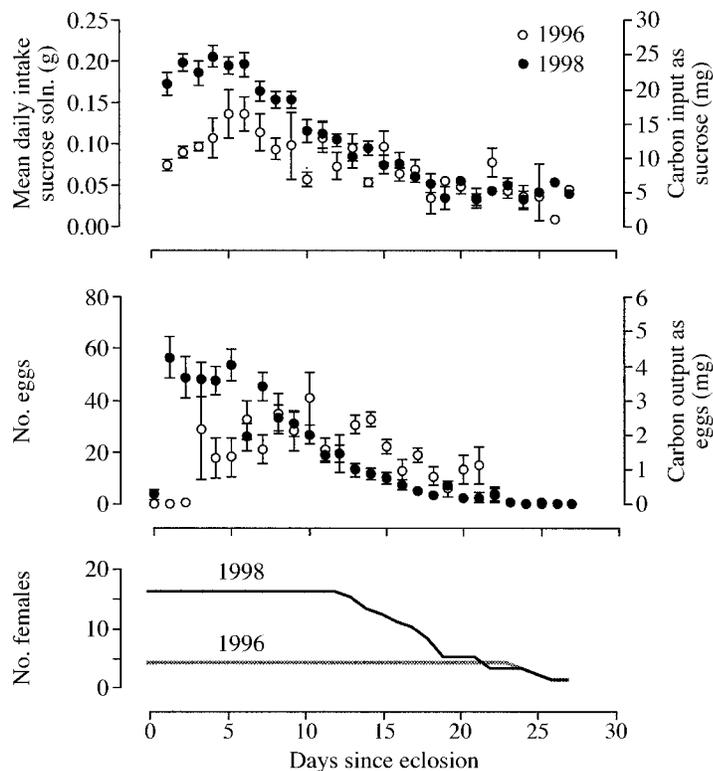


FIG. 2. Intake amounts, eggs laid, and sample sizes over time. Intake and egg-laying declined significantly after day 5 in both 1996 and 1998. Oviposition began later in 1996 (days 2–3) than in 1998 (days 0–1). Data are labeled on the right as carbon inputs and outputs; these divided by time correspond to intake rate and egg-laying rate in the model diagram (Fig. 4). Error bars represent  $\pm 1$  SE.

experimental moths prior to their first feeding were included in the analysis. The  $\delta^{13}\text{C}$  of unfed moth eggs provides an initial value for egg  $\delta^{13}\text{C}$ , referred to as  $\delta^{13}\text{C}_0$ . The similarity of this value to larval host plant (Table 1) indicates that there is little net carbon isotope fractionation (a shift in isotope ratio due to isotope discrimination) associated with manufacturing eggs from larval diet.

#### *Incorporation of dietary carbon from amino acids into eggs*

There was no difference in egg  $\delta^{13}\text{C}$  between moths fed diets with and without the amino acid supplement (tested with 1998 data;  $F_{1,100} = 0.01$ ,  $P = 0.9275$ ; Table 2, Fig. 3 [open vs. solid symbols]). A power test re-

vealed that our methods could detect a mean effect as small as 0.015‰, which would correspond to having only 0.34% of total egg carbon derive from dietary amino acids. This result thus indicates that nectar amino acids do not contribute significantly to egg manufacture.

#### *Incorporation of dietary carbon from sugar into eggs*

Eggs laid by fed moths show a smooth and rapid elevation of egg  $\delta^{13}\text{C}$  over time, indicating incorporation of adult dietary carbon (Fig. 3). The pattern of incorporation is very similar between the two years, following a negative exponential increase from  $\delta^{13}\text{C}_0$ . The relationship closely resembles that expected from turnover due to constant flow through a single, well-mixed chamber, with one important caveat. A single-chamber model predicts that the chamber should equil-

TABLE 1.  $\delta^{13}\text{C}$  values frequently referred to in the text: larval host plant, eggs laid by unfed females, and adult dietary constituents.

Sample	$\delta^{13}\text{C}$ (mean $\pm$ 1 SE)	N
Larval host plant ( $\text{C}_3$ )	$-30.11\text{‰} \pm 0.34\text{‰}$	14
Unfed moth eggs	$-29.47\text{‰} \pm 0.16\text{‰}$	7
Cane sugar ( $\text{C}_4$ )	$-11.26\text{‰}^\dagger$	1
Beet sugar ( $\text{C}_3$ )	$-24.76\text{‰}^\dagger$	1
Casein hydrolysate (amino acids)	$-18.85\text{‰}^\dagger$	1

$^\dagger$  All adult diets were made from single batches of sugar and amino acids, therefore  $N = 1$  for those samples and their SE is that associated with sample preparation and analysis ( $<0.001\text{‰}$ ).

TABLE 2. Analysis of variance table for 1998 egg  $\delta^{13}\text{C}$  data.

Effect	ss	df	F ratio	P
Amino acids	0.02	1	0.01	0.9275
Sugar	756.15	1	281.71	$<0.0001$
Day	802.53	21	14.24	$<0.0001$
Sugar $\times$ Amino acids	6.17	1	2.30	0.1336
Error	201.31	75		

Notes: Day is treated as a categorical variable because it does not covary linearly with egg  $\delta^{13}\text{C}$ . Amino acid content had no significant effect on the carbon isotopic composition of eggs.

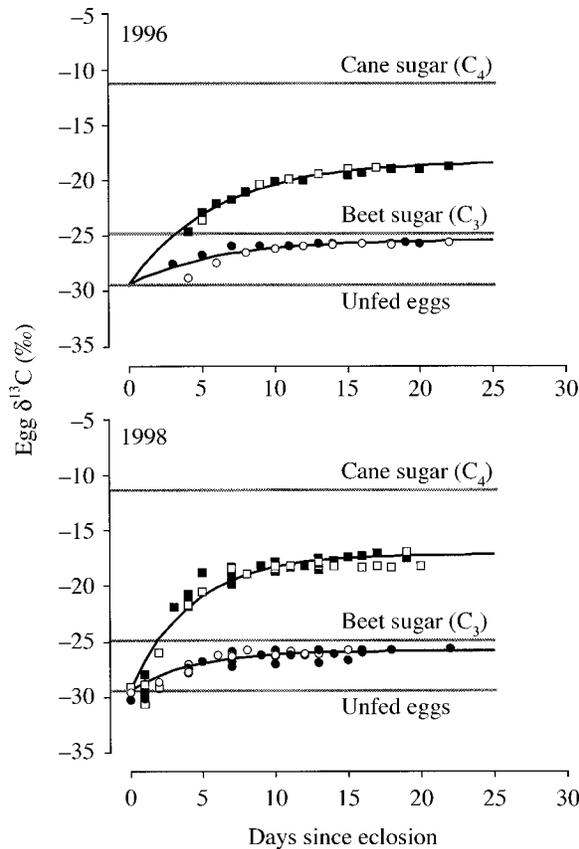


FIG. 3. The time pattern of egg  $\delta^{13}\text{C}$  for 1996 and 1998 females. Squares denote eggs laid by females fed  $\text{C}_4$  sugar diets; circles denote eggs laid by females fed  $\text{C}_3$  sugar diets. Filled symbols indicate that diets were supplemented with amino acids. Horizontal lines indicate the  $\delta^{13}\text{C}$  values of the nectar sugars ( $\delta^{13}\text{C}_{\text{diet}}$ ) and the baseline value for egg  $\delta^{13}\text{C}$  ( $\delta^{13}\text{C}_0$ ). Lines fit through the data were generated by the model proposed in Fig. 4 (Eq. 4), using nonlinear fitting to estimate parameters.

ibrate with the isotopic composition of the adult diet. Egg  $\delta^{13}\text{C}$ , in contrast, equilibrates at a value considerably lower in  $\delta^{13}\text{C}$  than adult diet.

To address this discrepancy between egg  $\delta^{13}\text{C}$  at equilibration and dietary  $\delta^{13}\text{C}$ , we propose a two-compartment model of carbon flow into eggs (Fig. 4). One carbon pool mixes with adult diet, accounting for the exponential equilibration dynamics observed. The other carbon pool does not mix with adult diet, accounting for the offset between egg  $\delta^{13}\text{C}$  at equilibration and dietary  $\delta^{13}\text{C}$ . The second pool contributes carbon with a constant  $\delta^{13}\text{C}$  determined only by larval diet; we assume that this pool is large enough not to be emptied entirely across the course of egg laying. The simple two compartment model, therefore, can be expressed as the following:

$$\delta^{13}\text{C}_{\text{egg}} = \alpha \times (\delta^{13}\text{C}_{\text{mixingpool}}) + (1 - \alpha) \times (\delta^{13}\text{C}_{\text{nonmixingpool}}) \quad (2)$$

where  $\alpha$  = the fraction of total egg carbon contributed by each compartment, or carbon pool.

The  $\delta^{13}\text{C}$  of the mixing pool is modeled as the following:

$$\delta^{13}\text{C}_{\text{mixingpool}} = \delta^{13}\text{C}_0 + [(\delta^{13}\text{C}_{\text{diet}} + f_a) - \delta^{13}\text{C}_0] \times (1 - e^{-r \times \text{Day}}) \quad (3)$$

where  $\delta^{13}\text{C}_0$  =  $\delta^{13}\text{C}$  of eggs laid by unfed females ( $\delta^{13}\text{C}_0$  represents the baseline or initial  $\delta^{13}\text{C}$  of eggs, and will also be substituted into Eq. 2 as an estimate of the value of nonmixing pool carbon);  $r$  = the fractional turnover rate, defined as the flow rate into the pool divided by its volume;  $f_a$  = the fractionation associated with manufacturing eggs from adult dietary carbon.

Inserting Eq. 3 into Eq. 2,

$$\delta^{13}\text{C}_{\text{egg}} = \alpha \times \{ \delta^{13}\text{C}_0 + [(\delta^{13}\text{C}_{\text{diet}} + f_a) - \delta^{13}\text{C}_0] \times (1 - e^{-r \times \text{Day}}) \} + (1 - \alpha) \times (\delta^{13}\text{C}_0). \quad (4)$$

The parameters  $\alpha$ ,  $f_a$ , and  $r$  were estimated separately for the 1996 and 1998 data by fitting the data to the above expression using least squares methods (Fig. 3; Table 3). Estimating the parameters separately for the two years provided a significantly better fit than pooling the data ( $F_{3,134} = 27.69$ ,  $P < 0.0001$ , using the significance test described in Motulsky and Ransnas 1987). The estimated parameter standard errors in Table 3 indicate that  $\alpha$  and  $r$  are known with relatively more confidence than  $f_a$ . Their differences are therefore likely to have a bigger effect on model fit than  $f_a$ , which may not actually differ between the years.

#### Contribution of adult diet to egg provisioning

The percentage contribution of adult dietary carbon to eggs can now be traced over time, solving the following expression for  $p$ :

$$\delta^{13}\text{C}_{\text{egg}} = p \times (\delta^{13}\text{C}_{\text{adultdiet}} + f_a) + (1 - p) \times (\delta^{13}\text{C}_{\text{larvaldiet}} + f_1). \quad (5)$$

Here  $p$  is the percentage contribution of adult diet to eggs,  $f_a$  is the fractionation associated with manufacturing eggs from adult diet, and  $f_1$  is the fractionation associated with manufacturing eggs from larval stores. Fractionation of adult diet ( $f_a$ ) was estimated using the two-compartment model for egg  $\delta^{13}\text{C}$  (Table 3), and  $\delta^{13}\text{C}_{\text{larval diet}} + f_1$  is estimated as  $\delta^{13}\text{C}_0$  (Table 1). Calculating  $p$  permits the change in egg composition over time to be expressed independently of dietary  $\delta^{13}\text{C}$  (Fig. 5). Note that  $p$  at equilibrium equals  $\alpha$  (Table 3, Eq. 4). Fig. 5 shows  $p$  plotted against time for both years; it emphasizes the similarity in incorporation pattern across all individuals and shows that nectar sugars come to provide over half of the carbon in eggs after several days of adult nectar feeding.

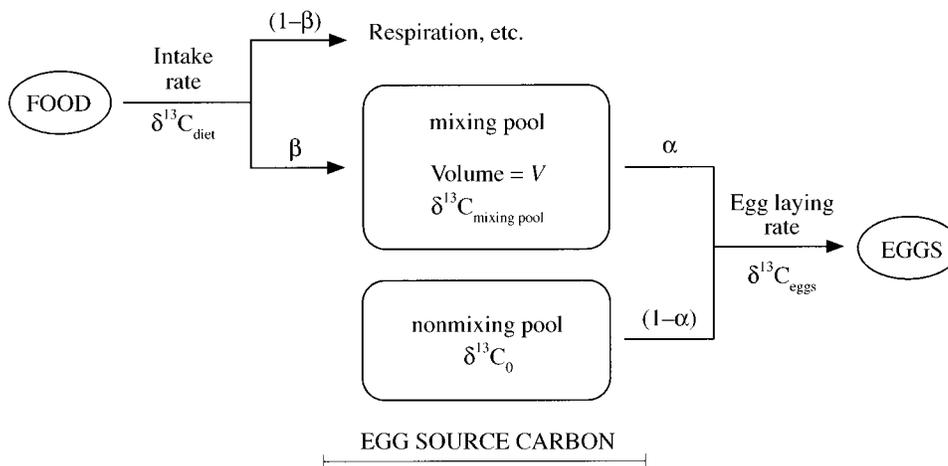


FIG. 4. Two-compartment carbon flow model suggested by patterns of egg  $\delta^{13}\text{C}$ . One carbon pool mixes with adult dietary carbon, whereas the other does not and retains its larval isotopic signature. The sizes of these two pools are unknown. Carbon flow in from food is split into a fraction available for egg provisioning ( $\beta$ ) and a fraction lost to respiration or other physiological fates ( $1 - \beta$ ). Carbon flows into the mixing pool with a rate of  $(\text{Intake rate}) \times \beta$  and mixes with a fractional turnover rate of  $[(\text{Intake rate}) \times \beta] / V$ . Carbon is lost from the mixing and nonmixing pools at the rate of  $(\text{Egg laying rate}) \times \alpha$  and  $(\text{Egg laying rate}) \times (1 - \alpha)$ , respectively. Egg  $\delta^{13}\text{C}$  is equal to the sum of the  $\delta^{13}\text{C}$  from each pool weighted by its proportional contribution to egg carbon.  $\delta^{13}\text{C}_0$  equals the  $\delta^{13}\text{C}$  of eggs laid by unfed moths; we use this value to represent both  $\delta^{13}\text{C}$  from the nonmixing pool and the initial  $\delta^{13}\text{C}$  of carbon from the mixing pool. Values for  $\delta^{13}\text{C}_0$  and  $\delta^{13}\text{C}_{\text{diet}}$  can be found in Table 1. Values for carbon flow rates in as nectar and out as eggs can be seen in Fig. 2; however, they are not included in the mathematical solution.

## DISCUSSION

### Importance of nectar nutrients

These results demonstrate that nectar sugars can be a significant source of egg nutrient in *A. floridensis*, here supplying 20–30% of egg carbon after only two days of egg laying and coming to supply a consistent 50–60% of egg carbon after  $\sim 1$  wk. This result conforms to the observation that *A. floridensis* females emerge with eggs primarily unprovisioned, a strategy that allows them to take advantage of nectar nutrients for egg manufacturing. It is also consistent with the 81% reduction in fecundity observed in unfed females. Although a relationship between fecundity and nectar feeding does not necessarily indicate the allocation of nectar nutrients into eggs, here nectar is not only required for maximal fecundity but also provides an important supply of egg nutrient.

Nectar amino acids, in contrast, do not contribute to egg provisioning. This result is not surprising in light of the nectar intake and oviposition rates observed in

this study: the amount of amino acids contained in the mean meal size was only 1% (a nonetheless detectable fraction) of the total egg protein laid in an average day of egg laying. The Lepidoptera have been predicted to capitalize upon nectar amino acids as a source of dietary protein (Murphy et al. 1983, Alm et al. 1990); however, most studies have found that amino acids in nectar do not increase fecundity, longevity, or foraging preference in nectarivorous butterflies (Murphy et al. 1983, Moore and Singer 1987, Hill 1989, Hill and Pierce 1989, Erhardt 1991, 1992, but see Alm et al. 1990). Because nectar amino acids are very dilute, the increased foraging time required by a butterfly or moth that relies on nectar for protein may outweigh the long-term benefits to overall fecundity.

### Dynamics of adult nutrient allocation

The incorporation dynamics of dietary carbon suggests two distinct classes of egg nutrient, defined by their turnover properties. Although we label these nu-

TABLE 3. Parameters estimated by the two-compartment model for egg  $\delta^{13}\text{C}$ .

Parameter	1996	1998
$r$ (fractional turnover rate of the mixing pool)	$0.168 \pm 0.008$	$0.235 \pm 0.016$
$\alpha$ (percentage of egg carbon from mixing pool)	$52.3 \pm 1.4$	$63.3 \pm 2.1$
$f_a$ (fractionation term)	$1.2 \pm 2.1$	$3.1 \pm 0.3$

Notes: Parameters are presented  $\pm 1$  estimated SE. Separate parameter estimation for 1996 and 1998 yields a better fit than pooling the data from the two years. Poor confidence in the estimate of  $f_a$  from the 1996 data suggests that  $f_a$  may not differ between the two years; however, estimated SE values are not appropriate for strict statistical inference.

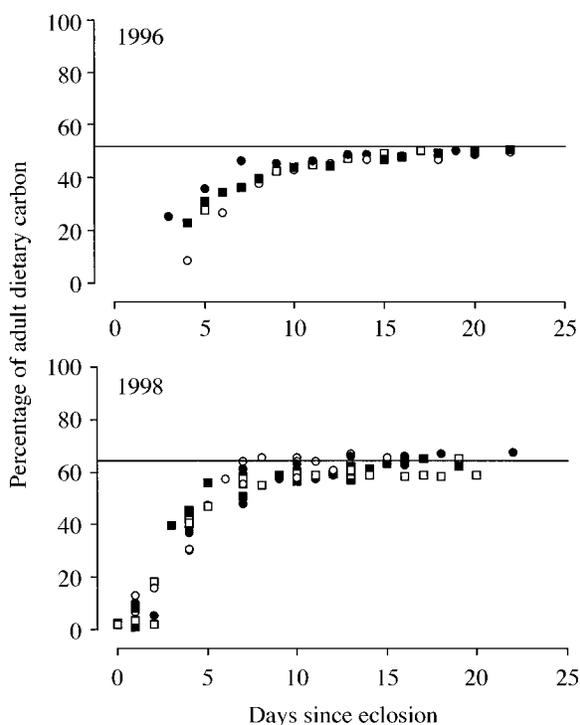


FIG. 5. The proportion of egg carbon deriving from nectar sugar, 1996 and 1998. Symbols are as in Fig. 3; there were no differences between diets in allocation. The percentage of adult dietary carbon in eggs ( $p$ ) was calculated using the following equation:  $\text{egg } \delta^{13}\text{C} = p \times (\delta^{13}\text{C diet} + f_a) + (1 - p) \times (\delta^{13}\text{C}_0)$ , using the values for  $f_a$  estimated by the carbon flow model (Fig. 4, Table 3). Incorporation of nectar carbon at equilibrium was  $>50\%$  in both years.

trient classes as pools, it is important to emphasize that they correspond neither to discrete anatomical structures nor to specific metabolic pathways. Rather, they are operationally defined: the mixing pool includes those sources of egg carbon which exchange with and are replaced by adult dietary carbon over time, whereas the nonmixing pool consists of those reserves which retain an exclusively larval carbon signature. Once the mixing pool has come into isotopic equilibrium with adult diet, the two pools correspond to larval vs. adult derived resources (as in Boggs 1997a, b). Initially, however, both pools have a larval carbon isotopic signature, as do the eggs.

Because *Amphion floridensis* does not use nectar amino acids in egg provisioning, one might predict that the protein fraction of eggs must derive entirely from larval stores (thus corresponding to the nonmixing pool). Several storage proteins have been described in the Lepidoptera, including a methionine-rich protein present primarily in females and likely involved in yolk protein synthesis (Kanost et al. 1990, Telfer and Kunkel 1991, Hauerland 1996). Were all of egg protein to derive from larval storage proteins, however, the percentage of carbon deriving from nectar feeding could

not be as high as it is (up to 63%). At least 24% of total egg carbon and 40% of the carbon in egg proteins has to be in protein derived from adult diet. We arrived at these figures by making the following conservative assumptions: if all nonprotein egg carbon (39%) is derived from the adult diet, then all of the egg carbon deriving from the larval diet must be in the form of protein ( $100\% - 63\% = 37\%$  of total egg carbon). The remaining 24% of the unaccounted carbon in eggs ( $100\% - 39\% - 37\% = 24\%$ ) must be derived from adult diet and must be in the form of protein. Because carbon in protein comprises 61% of the total, nearly 40% (i.e.,  $(24/61) \times 100\% = 39\%$ ) of egg protein carbon must be derived from adult feeding. This result requires that the carbon skeletons of a significant proportion of egg amino acids be synthesized from sucrose, with amino groups supplied from other proteins.

Despite the evidence that some amino acid synthesis occurs in egg provisioning, essential amino acids must be provided by the larval diet. A physiological interpretation of the nonmixing pool, therefore, is that it is comprised of those egg nutrients (chiefly essential amino acids) which cannot be manufactured from adult diet and which constitute a constant and significant proportion of egg nutrients across a female's lifetime. The amino acid composition of *Amphion floridensis* eggs indicated that 29% (g/g) of egg protein is carbon deriving from essential amino acids (D. M. O'Brien and C. L. Boggs, unpublished data). Eggs are  $\sim 57\%$  protein by mass; therefore, the percentage of egg weight made up of carbon from essential amino acids is 17%. Because eggs contain 49% total carbon by mass, we estimate that the percentage of egg carbon which derived from essential amino acids is  $17/49 \times 100 = 35\%$ . This value is high enough to be consistent with  $(1 - \alpha)$ , the estimated carbon contribution from the nonmixing pool (between one third and one half of total egg carbon).

The dynamics of the mixing pool follow a negative exponential pattern of turnover, with half of the larval-derived carbon replaced by nectar carbon within four days. This turnover is relatively rapid, given that oviposition can continue for  $\leq 3$  wk. The fractional turnover rate  $r$  (flow rate/pool size) was estimated as a constant, which requires either constant flow into a pool of fixed size, or a flow rate and pool size which decrease proportionately. Although the former scenario is implausible, the latter is less so: intake rates declined over time (Fig. 2), and female *A. floridensis* lose mass even when prevented from ovipositing (O'Brien 1999). Alternatively,  $r$  could vary across a female's lifetime. Were flow into the pool to decrease more rapidly than pool volume, for example,  $r$  would be a decreasing function of time. In this case, the apparent decelerating approach of egg  $\delta^{13}\text{C}$  to a stable asymptote could in part result from progressively slower carbon turnover. Because neither  $\beta$  nor  $V$  are known in this study (Fig. 4), we cannot evaluate the potential role played by

variation in  $r$ . An experiment in which turnover was systematically varied by restricting intake and/or varying activity levels (and therefore respiratory carbon loss) could clarify the potential role played by variation in  $r$ . However, for the purposes of this study the more simple assumption of a constant  $r$  is reasonable and well supported by the data.

#### Comparative implications

How applicable are these results to other species? Because this model is quite simple, it should also be very general. The values of the parameters  $\alpha$  (the fraction of egg carbon deriving from a source which mixes with diet) and  $r$  (the fractional turnover rate of that carbon pool), however, are likely to vary widely with life-history differences. Interspecific differences in the relative importance of larval vs. adult feeding should manifest themselves as differences in  $\alpha$ . Interspecific differences in feeding rates, mass change patterns, and the allocation of dietary nutrient to respiration vs. reproduction should manifest themselves as differences in  $r$ . Species that are similar in diet, lifespan, ovarian dynamics, and the importance of nectar to fecundity may be fairly similar in their patterns of allocation. *Amphion floridensis* resembles two classic models for studies of lepidopteran life history in these respects: the Nymphalid butterflies *Dryas julia* (Dunlap-Pianka et al. 1977, Boggs 1981b) and *Speyeria mormonia* (Boggs and Ross 1993, Boggs 1997a, b). Whether these similarities in life history translate into similar patterns of resource allocation can be addressed quantitatively, using the above proposed model as a framework for interspecific comparison.

#### ACKNOWLEDGMENTS

This manuscript was greatly improved by suggestions from Carol Boggs, Ben Bolker, William Bradshaw, Lila Fishman, Lenny Gannes, Tom Hahn, Hope Hollocher, Henry Horn, Lukas Keller, Paul Koch, Dan Rubenstein, Diane Wagner, and two anonymous reviewers. For laboratory help we thank Mark Abruzzese, Dan Bryant, and Ethan Goddard. For greenhouse help we thank Jerry Dick and Dave Wilson. This work was supported by a Sigma Xi Grant-in-Aid of Research to D. M. O'Brien, a National Science Foundation Dissertation Improvement Grant (IBN 95-20626) to D. M. O'Brien, and an National Science Foundation Grant (OCE-9733688) to D. P. Schrag.

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