Renewable and nonrenewable resources: Amino acid turnover and allocation to reproduction in Lepidoptera

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The allocation of nutritional resources to reproduction in animals is a complex process of great evolutionary significance. We use compound-specific stable isotope analysis of carbon (GC/combustion/isotope ratio MS) to investigate the dietary sources of egg amino acids in a nectar-feeding hawkmoth. Previous work suggests that the nutrients used in egg manufacture fall into two classes: those that are increasingly synthesized from adult dietary sugar over a female's lifetime (renewable resources), and those that remain exclusively larval in origin (nonrenewable resources). We predict that nonessential and essential amino acids correspond to these nutrient classes and test this prediction by analyzing egg amino acids from females fed isotopically distinct diets as larvae and as adults. The results demonstrate that essential egg amino acids originate entirely from the larval diet. In contrast, nonessential egg amino acids were increasingly synthesized from adult dietary sugars, following a turnover pattern across a female's lifetime. This study demonstrates that female Lepidoptera can synthesize a large fraction of egg amino acids from nectar sugars, using endogenous sources of nitrogen. However, essential amino acids derive only from the larval diet, placing an upper limit on the use of adult dietary resources to enhance reproductive success.

Classic models of life history assume a fundamental tradeoff between reproduction and determinants of survival, mediated by a single resource currency (e.g., energy) (1). In reality, allocation is more complex. Requirements for specific nutrients, such as amino acids or vitamins, can constrain reproduction when energetic resources are not limited (2, 3). Furthermore, different nutrients may follow different patterns of use and turnover (4). Researchers have increasingly argued that an understanding of the physiological processes underlying nutrient allocation would help clarify predictions about life history tradeoffs (2–8). In this study, we use stable isotopes to characterize the turnover and allocation of a suite of nutrients to reproduction in the Lepidoptera, focusing on specific amino acids, their dietary sources, and their role in potentially constraining fecundity.

The allocation dynamics of amino acids in Lepidoptera are of special interest because insect eggs consist primarily of protein (9), whereas the sugar-rich nectar diet of most butterflies and moths provides females with only trace amounts of amino acids (10). Therefore, the size of protein reserves stored from the larval stage is important in determining adult reproductive output (11–13). Because amino acids are likely to be important resources in egg manufacture, their allocation processes are particularly relevant to understanding nutritional constraints on reproduction.

We investigated allocation in a hawkmoth (*Amphion floridensis*), which feeds on grape leaves as a larva and nectar as an adult. Females emerge with mostly unprovisioned oocytes and lay eggs daily over their 3- to 4-wk adult lifespan (14). Females lay eggs singly, starting at about 50/day and decreasing after the first week (average total = 469 eggs) (14). Previous work has shown that females incorporate an increasing fraction of carbon from dietary sugar into eggs over time, to a stable plateau reflecting carbon input from both larval and adult diets (14). O'Brien *et al.* modeled this allocation pattern by positing two classes of egg nutrient: one that turns over with the carbon in the adult diet (renewable resources), and one that derives exclusively from the larval diet (nonrenewable resources). Each nutrient class contributes a fixed proportion of total carbon to the egg. Their model predicts that distinct classes of compounds can be identified with respect to their allocation dynamics: one that derives solely from the larval diet, and the other for which the dietary source changes from larval to adult over time. The above study found no evidence for the use of nectar amino acids in eggs (14); therefore, we focus here on dietary sugar only.

We predict that essential amino acids will derive entirely from the larval diet, because they have carbon skeletons that cannot be synthesized by animals. The origin of nonessential amino acids may be more complex. Their carbon skeletons can be synthesized from the sugars in nectar; however, their production also requires amine groups. These must be obtained from nitrogen-bearing compounds, probably amino acids, acquired from the larval diet. Whether moths store both essential and nonessential amino acids from the larval diet for use in eggs or synthesize nonessential amino acids from the carbon in dietary nectar is likely to depend on the availability of useable amine groups. These different physiological scenarios affect the degree to which females can rely on their adult diet for reproductive resources.

Methods

Experimental Design. We used natural variation in the ^{13}C content of plants as a tool for tracing the dietary sources of egg amino acid carbon. C_3 plants are ^{13}C-depleted relative to C_4 plants (15). By raising moths on isotopically contrasting larval and adult diets, we can easily identify the dietary sources of carbon in specific egg amino acids.

Females were reared as larvae on their normal C_3 host plants (*Vitis*, $\delta^{13}C^{\S} = -30.11‰$) (14) and maintained as described elsewhere (14). Adult females were hand fed daily on sucrose solution (30%) from either C_3 beet sugar ($\delta^{13}C = -24.76‰$) or C_4 cane sugar ($\delta^{13}C = -11.25‰$). Eggs were collected daily, dried, and stored. Previous analyses showed that the bulk carbon isotopic composition of eggs from these females stabilized after day 10 of oviposition, and that isotopic variation among individuals fed each diet was minimal (14). Eggs were selected from

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§$\delta X = [(R_{sample} - R_{std})/R_{std} \times 1{,}000)]$; X is the heavy isotope of N or C, and R = the ratio of heavy-to-light isotopes. [Standards: PeeDee belemnite (C) and atmospheric N_2 (N)].

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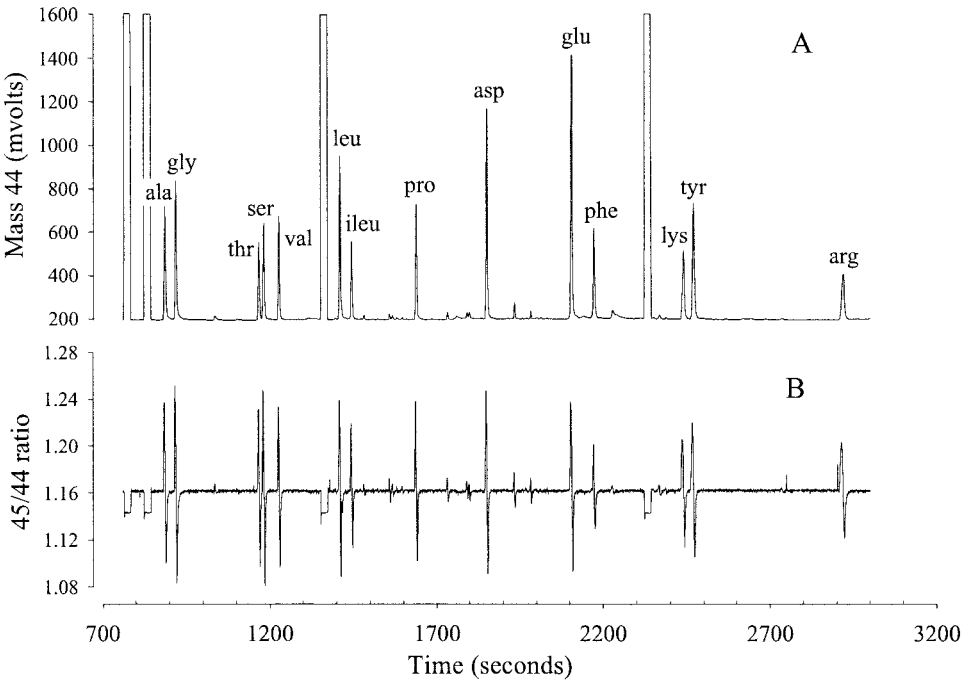

Fig. 1. Sample egg chromatography trace. (*A*) Voltage of the mass 44 ($^{12}CO_2$) ion collector over time. Large square peaks are internal gas standards. Voltages are also monitored at mass 45 ($^{13}CO_2$) and mass 46 (to correct for the presence of ^{18}O). (*B*) Ratio of 45 to 44 voltages over time. Lighter amino acids move through the column slightly more slowly, causing offset retention times.

two adult females for amino acid-specific isotope ratio analysis, fed on beet (C_3) and cane (C_4) sugar solution, respectively, and closely matched in age-specific fecundity. We also analyzed eggs from a female that was not fed as an adult ("unfed") and that laid eggs for the first 2 days of adult life only.

Amino Acid Composition. The amino acid composition of eggs from a 2-day-old ("young") and a 12-day-old ("old") female was analyzed by using postcolumn ninhydrin amino acid analysis (16).

Calculating the Proportion of Adult and Larval Carbon in Egg Amino Acids. The carbon isotope ratio of an egg amino acid is determined by the isotopic composition of its carbon sources, weighted by their proportional contributions (17), and potentially offset by isotopic shifts, or "fractionations" (18):

$$\delta^{13}C_{\text{egg aa}} = p\,(\delta^{13}C_{\text{adult carbon source}} + \Delta_a) + (1-p)\,(\delta^{13}C_{\text{larval carbon source}} + \Delta_l). \quad [1]$$

The parameter of greatest interest here is "p," the proportion of the amino acid's carbon deriving from the adult diet. The terms Δ_a and Δ_l are fractionation effects associated with amino acid synthesis or import from adult and larval diets. Fractionations are characteristic of particular biological processes and are independent of source isotope ratio (18–21). We can assume that Δ_a is the same for C_3- and C_4-fed females kept under controlled conditions, because the sugars have identical biochemical fates. We can then remove the isotopic fractionations by solving Eq. **1** for C_3 and C_4 adult diets simultaneously:

$$p = \frac{(\delta^{13}C_{\text{C4 egg aa}} - \delta^{13}C_{\text{C3 egg aa}})}{(\delta^{13}C_{\text{C4 adult diet}} - \delta^{13}C_{\text{C3 adult diet}})}. \quad [2]$$

Compound-Specific $\delta^{13}C$ Analysis of Amino Acids. Eggs laid on days 2, 6, 8, and 12 of adult life were analyzed from both C_3- and C_4-fed females. We measured the isotopic composition of egg amino acids individually using compound-specific stable isotope analysis (GC/combustion/stable isotope MS) (21, 22). Briefly, eggs were hydrolyzed to amino acids and derivatized to *N*-trifluoroacetic acid isopropyl esters as described elsewhere (21, 22). derivatized samples were injected into a Varian 3400 gas chromatograph for separation (using an HP-1 column), converted to CO_2 gas via a combustion interface, and analyzed with a Finnigan Delta Plus XL (Finnigan-MAT, San Jose, CA) isotope ratio mass spectrometer.

We could resolve 13 amino acids using these methods, six of which are considered nonessential for most insects (23)—alanine (Ala), glycine (Gly), serine (Ser), proline (Pro), aspartate (Asp), and glutamate (Glu)—and six of which are considered essential: threonine (Thr), valine (Val) leucine (Leu), isoleucine (Ile), phenylalanine (Phe), and lysine (Lys) (Fig. 1). The thirteenth, tyrosine (Tyr), can be synthesized from phenylalanine; however, we classify it here as essential or "nonrenewable," because animals cannot synthesize its aromatic ring *de novo*. Asparagine and glutamine are converted to aspartate and glutamate, respectively, during acid hydrolysis; therefore, these amino acids are indistinguishable.

Samples were run in triplicate and were derivatized and analyzed in several batches. Data from standards revealed that 8 of the 13 amino acids showed significant but fairly small batch effects in $\delta^{13}C$ (among-batch SDs varied 0.6–1.3‰ among the affected amino acids). However, C_3 and C_4 samples from the same day of oviposition were always run in the same batch to be comparable.

Calculating Amino Acid $\delta^{13}C$. Measurements of $\delta^{13}C$ for a given amino acid include carbon from both the amino acid and derivatization reagents, which can be heavily fractionated in amino acid-specific ways (22). Amino acid standards with known $\delta^{13}C$ were derivatized and analyzed along with each batch of samples. The results reflected the effect of carbon added by the isopropyl and *N*-trifluoroacetyl groups and provided the information required to calculate the $\delta^{13}C$ of amino acid carbon

Table 1. Amino acid composition of moth eggs laid on days 2 and 12 of adult life ("young" and "old")

	Moth eggs			
	Young		Old	
Amino acid	mol %	wt %	mol %	wt %
Glycine	15.6	8.5	15.7	8.6
Alanine	11.4	7.8	11.7	8.0
Glutamine/glutamate	11.0	13.5	11.2	13.8
Asparagine/aspartate	8.2	8.9	8.2	9.0
Leucine	7.0	7.6	7.0	7.6
Valine	6.1	5.8	6.0	5.7
Proline	5.8	5.4	5.9	5.6
Serine	5.6	4.6	5.8	4.8
Tyrosine	5.5	8.6	4.5	7.1
Lysine	4.5	5.6	4.2	5.3
Threonine	4.2	4.1	4.3	4.2
Arginine	3.9	5.8	3.7	5.7
Isoleucine	3.8	4.1	3.8	4.1
Phenylalanine	2.8	4.0	2.8	3.9
Histidine	2.3	3.0	2.6	3.5
Methionine	1.6	2.0	1.9	2.4
Cysteine*	0.6	0.6	0.7	0.7

*Values are minima.

skeletons in the samples (22). Final $\delta^{13}C_{aa_{sample}}$ values were assigned by using Eq. **3**:

$$\delta^{13}C_{aa_{sample}} = \frac{(\delta^{13}C_{aa_{dsa}} - \delta^{13}C_{aa_{dst}} + \delta^{13}C_{aa_{standard}} \cdot p_{std})}{p_{std}} \quad [3]$$

The labels dsa and dst refer to the derivatized sample and standard, respectively, and p_{std} = the proportion of carbon in the derivative from the amino acid. Because both $\delta^{13}C_{aa_{dsa}}$ and $\delta^{13}C_{aa_{dst}}$ are means of at least three runs, the SE of $\delta^{13}C_{aa_{sample}}$ was calculated as:

$$SE\ \delta^{13}C_{aa_{sample}} = 1/p_{std} \cdot \sqrt{(SD^2_{dsa}/n_{dsa} + SD^2_{dst}/n_{dst})}, \quad [4]$$

where n_{dsa} and n_{dst} = number of runs of the derivatized sample and standard, respectively. This expression accounts for the propagation of variance in mean measurements of the sample and standard through Eq. **3**, thus indicating total measurement error. Over all samples, these standard errors averaged 0.80‰. Average standard errors varied systematically among amino acids, ranging from 0.32‰ for leucine (least variable) to 1.34‰ for serine (most variable).

Statistical Analyses. All statistical analyses were performed in JMP IN Ver. 3.2.1 (student version, SAS Institute, Cary, NC). Values of $\delta^{13}C$ were analyzed by using factorial ANOVA. These analyses were performed on corrected data from individual run replicates, so that run variation factored into the total error of the model. Because calculations of p required that C_3 $\delta^{13}C$ be compared with C_4 $\delta^{13}C$ (Eq. **2**), we used mean $\delta^{13}C$ values for each sample (thus, $n = 1$ for p on each day of oviposition). Turnover parameters were estimated in JMP by using nonlinear fitting via least-squares estimation. The fit of parameter estimates between data sets was evaluated by using the test of Ratkowsky (24). This test compares the sums of squares of two data sets when a model is fit to them separately vs. when they are pooled, generating an F ratio.

Results

Egg Composition. Amino acid composition varied little to not at all between eggs laid early and late in life (Table 1). Glycine, alanine, and glutamine/glutamic acid were the most prevalent amino acids in eggs (16, 11, and 11 mol%, respectively; Table 1).

Amino Acid $\delta^{13}C$. The isotopic composition of amino acids in the host plant consumed by the larvae, eggs laid by an unfed moth, and eggs laid by C_3- and C_4-fed females are presented in Table 2. We tested whether adult diet (C_3 vs. C_4 sugar) and age (day 2, 6, 8, or 12) affected egg amino acid $\delta^{13}C$, analyzing essential and nonessential amino acids separately (Table 3). Among nonessential amino acids, diet had a large, highly significant effect on amino acid $\delta^{13}C$ (Table 3), indicating substantial incorporation of carbon from the adult diet. The extent to which adult dietary carbon was incorporated varied among different amino acids and over time (indicated by a highly significant day effect and interactions between diet × day and diet × amino acid; Table 3).

Adult diet also appeared to have a significant effect on $\delta^{13}C$ of essential amino acids; however, the effect was smaller than measurement error (0.80‰ on average) and in the wrong direction [least-squared means from the ANOVA model were −27.53‰ vs. −27.89‰ for C_3 (more negative) vs. C_4 (more positive) diets, respectively]. The interaction between diet and amino acid was because of C_3 vs. C_4 differences in Phe and Tyr, which were both within measurement error and in the wrong direction. We thus conclude that adult dietary carbon was not incorporated into any of the essential amino acids.

Table 2. Amino acid $\delta^{13}C$ (‰) of larval hostplant, eggs of unfed moths, and eggs laid on days 2, 6, 8, and 12 by moths fed C_3 and C_4 adult diets

Amino		Unfed	C_3-fed moth eggs				C_4-fed moth eggs			
acid	Host plant	moth eggs	Day 2	Day 6	Day 8	Day 14	Day 2	Day 6	Day 8	Day 12
Ala	−27.20	−27.47	−23.71	−20.05	−21.02	−19.75	−13.83	−5.96	−8.37	−7.48
Gly	−7.93	−8.54	−11.46	−13.23	−13.74	−9.57	−9.88	−6.58	−6.74	−2.19
Thr	−18.42	−14.13	−14.90	−15.08	−16.67	−16.61	−16.21	−15.51	−17.56	−17.40
Ser	−21.49	−13.70	−14.68	−11.09	−11.99	−11.74	−7.30	−3.60	−4.75	−3.47
Val	−32.24	−31.70	−31.07	−31.05	−32.31	−30.63	−31.35	−32.57	−32.53	−31.40
Leu	—	—	−37.02	−35.95	—	−35.23	−36.00	−34.49	—	−34.83
Ileu	−27.50	−28.16	−30.57	−26.57	−25.64	−27.18	−30.38	−26.71	−25.17	−27.67
Pro	−24.62	−24.49	−26.39	−22.75	−21.39	−19.21	−19.95	−12.69	−11.55	−8.33
Asp	−27.16	−24.09	−24.55	−19.23	−20.01	−19.82	−19.38	−14.27	−14.95	−11.45
Glu	−29.34	−27.33	−25.57	−21.19	−24.39	−19.05	−17.16	−9.92	−12.86	−8.07
Phe	−34.56	−29.66	−29.51	−31.66	−32.02	−30.24	−30.75	−33.16	−32.78	−30.74
Lys	—	−25.18	−26.84	−24.86	−27.37	−24.17	−25.42	−24.88	−27.35	−24.93
Tyr	−29.21	−27.55	−26.39	−25.83	−27.77	−27.18	−27.64	−27.49	−28.99	−27.82

Table 3. Effects of diet, day, and amino acid identity on egg amino acid $\delta^{13}C$, tested by ANOVA

	Essential				Nonessential			
Effect	SS	df	*F*	*P*	SS	df	*F*	*P*
Diet	5.76	1	4.28	0.0404	2,901.18	1	1,027.28	<0.0001
Day	10.60	3	2.62	0.0530	731.08	3	86.29	<0.0001
Amino acid	4,584.39	6	566.89	<0.0001	2,523.23	5	178.69	<0.0001
Diet × day	1.19	3	0.30	0.8290	54.75	3	6.46	0.0004
Diet × amino acid	21.39	6	2.65	0.0182	237.94	5	16.85	<0.0001
Day × amino acid	205.09	18	8.45	<0.0001	183.72	15	4.34	<0.0001
Error	198.13	147			369.96	131		

SS, sum of squares.

Both essential and nonessential amino acids varied widely in $\delta^{13}C$ (Table 3; amino acid effect), reflecting variation in amino acid $\delta^{13}C$ in the host plant (discussed in detail below). The significant day × amino acid term in both essential and nonessential amino acid ANOVA models reflects analytical variation among batches of analyses. Samples from different days of oviposition were not evenly distributed among batches of analyses; therefore, the variable "day" is confounded with a slight batch effect. Although that batch effect cannot account for the huge effect of "day" on nonessential amino acids, it probably does account for the marginally nonsignificant day effect observed in the essential amino acids. Because the batch effects were present only in 8 of the 13 amino acids, they are most obvious in the day × amino acid interaction term.

Proportion of Amino Acid Carbon Deriving from Adult Diet. Eggs reach a constant proportion of adult dietary carbon by day 12 (14). These eggs should show the greatest isotopic differences between amino acids that are and are not synthesized from the adult diet. The proportion of adult dietary carbon in all amino acids on day 12 is presented in Fig. 2. Error bars indicate measurement error, as described in *Methods*. All essential amino acids are within measurement error of zero (most are within one SD, and all are within two SD), indicating that they derive exclusively from the larval diet. In contrast, the carbon in nonessential amino acids derives primarily from the adult diet, from 50 to 60% of the carbon in Gly, Ser, and Asp to 80–100% of the carbon in Ala, Pro, and Glu.

Carbon Turnover in Nonessential Amino Acids. In all of the nonessential amino acids, the proportion of amino acid carbon deriving from the adult diet (p) increases over time, as described by the turnover model $p = p_{max}\ (1 - e^{-r \cdot day})$ (Fig. 3). Because females were not fed until day 1 of oviposition, $p = 0$ on day 0. The parameter p_{max} corresponds to the proportion of adult dietary carbon at which each amino acid equilibrates or plateaus, and r represents the fractional turnover rate. p_{max} and r varied among amino acids (Fig. 3) and were not correlated with each other (correlation coefficient = −0.322, $P = 0.469$). Comparisons of model fit between pairs of amino acids (24) reveal that turnover in Ala and Glu is identical, whereas turnover parameters for Pro, Gly, and Ser are all different from each other and from Ala and Glu (Fig. 3; Table 4). Only Asp fit the model relatively poorly and thus cannot be distinguished from Pro, Gly, or Ser in its turnover characteristics. Because multiple comparisons are required to test these differences, we also present significance values that are adjusted by using a sequential Bonferroni correction. By that conservative test, turnover of Ala and Glu still differs from that of Gly and Ser, but turnover of Pro and Asp is indistinguishable from that of any other amino acid (except Pro vs. Ser) (Table 4).

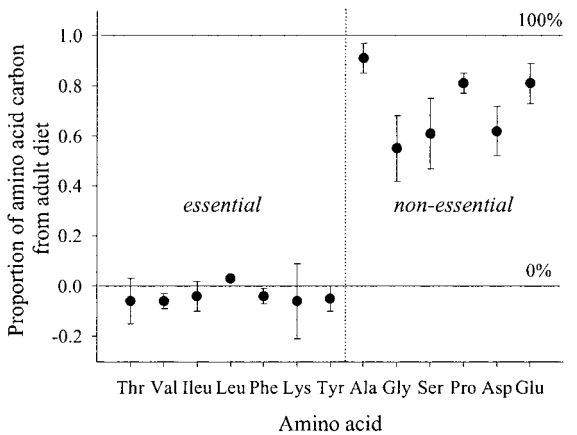

Fig. 2. The proportion of amino acid carbon deriving from adult diet "p" in moth eggs laid on day 12. Each data point is calculated as: ($\delta^{13}C$ C_4 egg aa − $\delta^{13}C$ C_3 egg aa)/($\delta^{13}C$ C_4 diet − $\delta^{13}C$ C_3 diet) (Eq. **2**). Error bars show the combined standard errors in $\delta^{13}C$, corrected for these calculations.

Transfer of Amino Acids from Host Plant to Eggs. Values of $\delta^{13}C$ range nearly 30‰ among amino acids in the larval host plant. Amino acid $\delta^{13}C$ in eggs laid by the unfed moth track this range of variation fairly closely (slope = 0.97; $R^2 = 0.88$; triangles in Fig. 4). Essential amino acids in the C_3- and C_4-fed moths follow an identical pattern, with no effect of either day or feeding treatment (Analysis of Covariance, slope = 0.99; Fig. 4). Because nonessential amino acids in the eggs of C_3- and C_4-fed females contain carbon other than that derived from the larval diet, we do not include them in this analysis. Egg amino acids tended to fall on or above the line $y = x$, indicating either no fractionation (Val, Ile, Ala, Pro, Gly) or a positive fractionation of 1–4‰ (Thr, Phe, Glu, Asp, Tyr; Ser is an exception).

Discussion

The processes by which essential and nonessential amino acids are allocated into egg proteins differ strikingly. Nearly half of the amino acids in egg protein are essential (Table 1) and derive exclusively from the larval diet. The requirement for these amino acids in eggs (they contribute 35% of the total egg carbon) restricts a female's ability to use surplus adult diet to increase reproduction. We can thus infer that the availability of essential amino acids for use in egg manufacture poses a significant constraint on a female's potential fecundity.

In contrast, the nonessential amino acids used in egg manufacture increasingly derive from the adult diet over time, as moths synthesize amino acid carbon skeletons from adult dietary sugars. Amino acid synthesis requires a source of endogenous nitrogen, which is most likely supplied by transamination from existing amino acids (25). This scenario suggests that moths are adept at conserving amine nitrogen, possibly by reusing amine

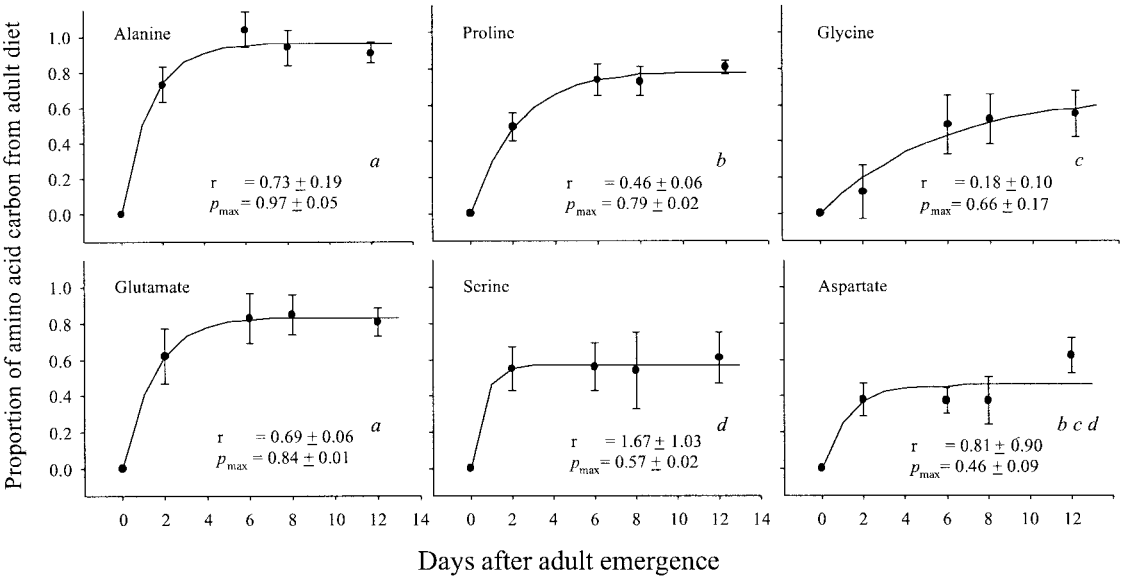

Fig. 3. The change in the proportion of amino acid carbon (p) in nonessential amino acids deriving from the adult diet over time. Error bars represent experimental error. Lines of best fit were generated with the model $p = p_{max}(1 - e^{-r \cdot day})$, and estimates of p_{max} and r are presented for each amino acid. Amino acids that share italicized lowercase letters are not statistically different in their turnover parameters.

groups from structural proteins [e.g., flight muscle (26)] as they are broken down in the course of routine protein turnover (27).

The pattern of isotopic change in the nonessential egg amino acids suggests a physiologically straightforward turnover model. The model requires that each egg amino acid be drawn from a pool, probably corresponding to the free amino acid in the hemolymph, which turns over isotopically as newly synthesized amino acids are added to it. The balance between sources of amino acids deriving from the larval diet and newly synthesized amino acids will determine the maximal percent of turnover observed in the eggs ("p_{max}"), whereas the rate of synthesis relative to the size of the pool will determine the turnover rate ("r"). Thus, these parameters provide a window into the sources and utilization rates of different amino acids. Among the nonessential amino acids, source (p_{max}) and turnover rate (r) varied widely and independently of each other.

Table 4. Comparisons of turnover models between pairs of amino acids

Contrasts	F_{ratio}	P value	$P > 0.05$?
Glu vs. Ser	56.90	0.001	y*
Glu vs. Gly	44.96	0.002	y*
Ala vs. Gly	43.82	0.002	y*
Ala vs. Ser	35.75	0.003	y*
Pro vs. Ser	24.50	0.006	y*
Pro vs. Gly	22.31	0.007	y
Ala vs. Asp	17.16	0.011	y
Ala vs. Pro	14.35	0.015	y
Gly vs. Ser	12.88	0.018	y
Glu vs. Pro	11.52	0.022	y
Asp vs. Glu	11.38	0.022	y
Asp vs. Pro	6.59	0.054	n
Ala vs. Glu	5.35	0.074	n
Asp vs. Ser	1.61	0.307	n
Asp vs. Gly	0.48	0.649	n

y, yes; n, no.

*The comparison is still significant when α is modified by using the adjusted Bonferroni method.

Turnover is determined by the rate at which amino acids and their precursors are used and replaced from adult dietary sugar. For example, alanine is synthesized from pyruvate, which is generated through sugar catabolism. Because sugar provides much of the flight energy in these females (28), alanine should turn over very rapidly, and it does. Similarly, glutamate exhibits fairly complete turnover with the adult diet. Glutamate serves as a central currency in amino acid metabolism, donating amino groups for transamination reactions and thus cycling rapidly with its carbon precursor, α-ketoglutarate. Because α-ketoglutarate is a Krebs cycle intermediate, it is also likely to have a strongly sugar-derived isotopic signal.

In contrast, aspartate exhibited relatively low turnover with the adult diet even though its precursor, oxaloacetate, is also a

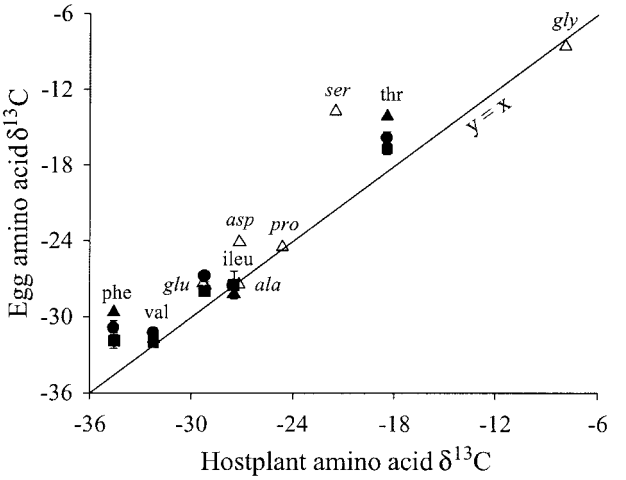

Fig. 4. The relationship between the $\delta^{13}C$ of amino acids in larval host plant and in eggs from three adult females. Filled symbols indicate essential amino acids; open symbols indicate nonessential amino acids (labels are italicized). Data from all amino acids are presented for eggs from the unfed moth (triangles). Only data from essential amino acids are presented from the two fed moths (squares and circles for C_3 and C_4 moths, respectively; points are means across days 2, 6, 8, and 12).

Krebs cycle intermediate. Aspartate is very abundant in the "methionine-rich storage protein" (29), a hexamerin expressed primarily in adult female Lepidoptera (30) that provides essential amino acids for egg provisioning (31, 32). Breakdown of this protein store should contribute aspartate with a larval isotopic signature throughout a female's lifetime, thus contributing to its low turnover with the adult diet. The breakdown and turnover of structural proteins formed during metamorphosis, such as flight muscle, could also yield amino acids that are larval in origin.

Amino acids in the larval host plant span a 30‰ range of variation in $\delta^{13}C$, which is generated through amino acid biosynthesis in plants (33). Larval-derived amino acids in eggs tracked the $\delta^{13}C$ of host plant amino acids closely. An offset between amino acid $\delta^{13}C$ in the host plant and that in the egg indicates isotopic fractionation generated by physiological processes in the moth; these tended to be fairly small (averaging 1–3‰ among essential amino acids). These modest fractionations are interesting given the massive morphological, physiological, and biochemical changes that the moths undergo during larval growth, metamorphosis, and reproduction. They suggest that the larval-derived amino acids used in eggs are relatively inactive metabolically, a view that accords with the possibility that storage proteins serve as a reservoir for these compounds (31, 32).

Nitrogen-containing compounds, amino acids in particular, have long been considered to be a limiting resource for reproduction in nectar-feeding Lepidoptera (12, 34). In this study we demonstrate, to our knowledge for the first time, that adult Lepidoptera can extensively synthesize nonessential egg amino acids from dietary sugar, accessing endogenous sources of amine nitrogen. The primary constraint on egg manufacture thus appears to be the availability of essential amino acids rather than the availability of nitrogen per se. Interestingly, essential amino acids are impossible for animals to synthesize primarily because of their complex carbon (or carbon and sulfur) structures rather than their nitrogen groups. This raises the unexpected possibility that carbon biochemistry limits the life histories of nectar-feeding Lepidoptera as much as (or even more than) nitrogen biochemistry. This point illustrates how an understanding of the physiology and biochemistry of particular nutrients can greatly aid our understanding of resource allocation, and subsequently, of the relationships between ecology and life history.

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