

The Amino Acids Used in Reproduction by Butterflies: A Comparative Study of Dietary Sources Using Compound-Specific Stable Isotope Analysis

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ABSTRACT

It is a nutritional challenge for nectar-feeding insects to meet the amino acid requirements of oviposition. Here we investigate whether egg amino acids derive from larval diet or are synthesized from nectar sugar in four species of butterfly: *Colias eurytheme*, *Speyeria mormonia*, *Euphydryas chalcedona*, and *Heliconius charitonia*. These species exhibit a range of life history and differ in degree of shared phylogeny. We use ¹³C differences among plants to identify dietary sources of amino acid carbon, and we measure amino acid ¹³C using compound-specific stable isotope analysis. Egg essential amino acids derived solely from the larval diet, with no evidence for metabolic carbon remodeling. Carbon in nonessential amino acids from eggs derived primarily from nectar sugars, with consistent variation in amino acid turnover. There was no relationship between the nonessential amino acids of eggs and host plants, demonstrating extensive metabolic remodeling. Differences between species in carbon turnover were reflected at the molecular level, particularly by glutamate and aspartate. Essential amino acid ¹³C varied in a highly consistent pattern among larval host plants, reflecting a common isotopic "fingerprint" associated with plant biosynthesis. These data demonstrate conservative patterns of amino acid metabolism among Lepidoptera and the power of molecular stable isotope analyses for evaluating nutrient metabolism in situ.

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Introduction

A striking aspect of animal diversity is dietary: all animals are heterotrophic and consume diets as diverse and specialized as animals are themselves. Differences between the elemental composition of animals and their diets have provided a powerful framework for understanding aspects of animal ecology and life history (Elser et al. 2000; Sterner and Elser 2002). In particular, there has been growing appreciation for the importance of specific elements (primarily N and P) in constraining growth (Elser et al. 1996). Requirements for growth and reproduction include an additional level of complexity, in that many of the pathways required to synthesize essential compounds have been lost in animals. Thus, an understanding of nutritional biochemistry may help refine diet-based ecological predictions even further. For example, the capability for ascorbic acid synthesis varies among bird and mammal species (Birney et al. 1980; Martinez del Rio 1997), and requirements for specific sterols as hormone precursors vary among insects (Nation 2002). A more fundamental example is the inability of animals to synthesize many of the amino acids required in proteins.

Protein requirements are particularly complex because, of the 20 amino acids used to make proteins, nine cannot be synthesized by animals: methionine, tryptophan, threonine, valine, leucine, isoleucine, lysine, phenylalanine, and histidine (Reeds 2000). A tenth, arginine, is essential in many (e.g., fish: Akiyama et al. 1997; insects: Dadd 1973; Nation 2002) but not all (e.g., humans: Laidlaw and Kopple 1987) lineages. Two more amino acids require essential amino acids as precursors: cysteine and tyrosine are synthesized from methionine and phenylalanine, respectively (Berg et al. 2002). Thus, most animals are dependent on dietary sources for the majority of the amino acids found in their proteins (12 out of 20). The dietary requirement for these amino acids constrains the types of diets animals can rely on for growth and reproduction.

Many insects feed on plant phloem or nectars for part or all of their life. These diets are rich in sugars but generally poor or unbalanced in amino acids (Baker and Baker 1973, 1986; Baker 1977; Douglas 1993). These insects exhibit diverse and sometimes bizarre strategies for meeting their protein budgets, from supplementing diets with pollen, carrion, or dung (Gilbert

1972; Dunlap-Pianka et al. 1977; Alm et al. 1990; Erhardt and Rusterholz 1998; Hall and Willmott 2000; Mevi-Schutz and Erhardt 2002) to relying on symbionts for amino acids synthesis de novo (Febvay et al. 1999; Douglas et al. 2001; reviewed by Baumann et al. 2000; Moran and Baumann 2000). Not surprisingly, essential amino acids have been shown to be of primary importance to a number of these behaviors (e.g., Shigenobu et al. 2000; O'Brien et al. 2003). Amino acids from the larval diet may also be stored for later allocation into metamorphosis, reproduction, and adult survival. Larval storage proteins that are rich in essential amino acids have been identified in female Lepidoptera (Telfer and Kunkel 1991) and appear to serve as an amino acid reservoir for ovipositing adult females (Pan and Telfer 1996, 2001; Wheeler et al. 2000). Understanding the amino acid nutrition of these species can help shed light onto both their life histories and the evolutionary process of dietary specialization.

In a previous experimental study, we found that carbon isotope ratios ($^{13}\text{C}/^{12}\text{C}$) could be used to identify the dietary origin of amino acids used in egg manufacture by a nectar-feeding hawkmoth (*Amphion floridensis*). Essential amino acids used in eggs were identified as exclusively larval in origin, whereas egg nonessential amino acids were synthesized from nectar sucrose to a large but varying extent (O'Brien et al. 2002). These data suggested that females mobilize endogenous nitrogen with which to synthesize nonessential amino acids fairly easily. However, it is not known whether the patterns of amino acid use seen in one species are characteristic of amino acid utilization and synthesis in nectar-feeding species more generally. A subsequent comparative study demonstrated that nectar sugars provided a large component of egg carbon (from 39% to 81%) in four species of butterfly (O'Brien et al. 2004). We speculated that this variation in adult dietary input may have been determined by the proportion of essential amino acids in eggs. In most species, the amount of egg carbon not provided by sugar corresponded roughly to the percentage of egg carbon existing in the form of essential amino acids (O'Brien et al. 2004).

In this study, we investigate whether egg amino acids derive from larval or adult dietary sources in *Colias eurytheme*, *Speyeria mormonia*, *Euphydryas chalcedona*, and *Heliconius charitonia*, and we will examine the role of specific amino acids in determining differences in their carbon turnover. These four species exhibit a range of variation in life span and age-specific fecundity and differ in degree of shared phylogenetic history. Three of the four species are in the family Nymphalidae and include the two most extreme examples of life-history variation: *E. chalcedona*, a short-lived species that oviposits heavily immediately after adult emergence and mating, and *H. charitonia*, a longer-lived species that oviposits only after a week of adult feeding and lays relatively few eggs per day (O'Brien et al. 2004). The nymphalid *S. mormonia* and the pierid *C. eurytheme* are

intermediate in oviposition pattern and life span, yet the pierid is phylogenetically more distant from the three nymphalids.

This study takes advantage of naturally occurring differences among plants in $^{13}\text{C}/^{12}\text{C}$. All plants discriminate to some extent against ^{13}C during photosynthetic fixation of carbon. The extent to which plants discriminate depends primarily on photosynthetic mode (C_3 vs. C_4) and secondarily on water use efficiency and other physiological characteristics (Farquhar et al. 1989). These isotopic differences in fixed carbon are passed on to the macromolecules subsequently synthesized by the plant. Thus, the carbon isotope ratio of plant amino acids reflects both photosynthetic physiology and the subsequent isotopic discriminations of amino acid synthesis (Abelson and Hoering 1961; Macko et al. 1987; Fogel and Tuross 1999). By contrasting two adult sucrose diets, isotopically distinct from each other and from the larval host plant, we can attribute the carbon source of egg amino acids with high accuracy (O'Brien et al. 2002).

Material and Methods

Butterfly Rearing and Experimental Design

Experiments were carried out in the spring of 2000 at the Herrin Greenhouses at Stanford University, as described elsewhere (O'Brien et al. 2004). Briefly, all species were reared on their natural host plants in greenhouses maintained on a 16L : 8D photoperiod, with daytime and nighttime temperatures of 27°C and 15°C, respectively. Females were mated on emergence as adults and hand-fed twice daily from a 30% sucrose solution made from either cane or beet sugar. Free-living *Heliconius charitonia* feed on pollen in addition to nectar; however, experimental insects had access to only sugar as adults, allowing us to focus on the role of sugar per se in reproduction. Eggs were collected daily and dried at 50°C.

Cane and beet plants exhibit C_4 and C_3 photosynthesis, respectively, and thus have contrasting carbon isotopic signatures (Farquhar et al. 1989). These signatures are expressed as $\delta^{13}\text{C} = (R_{\text{sample}}/R_{\text{standard}} - 1) \times 1,000$, where R is the ratio of heavy to light isotopes, and the standard is PeeDee Belemnite. The cane sugar used in this study had an isotope ratio of $-11.1\text{‰} \pm 0.06\text{‰}$, whereas the beet sugar was $-24.8\text{‰} \pm 0.08\text{‰}$ (mean \pm SE, $N = 3$ replicate samples from each sugar source). The isotope ratios of the larval host plant for each species in the study are given in Table 1. All were C_3 plants and had $\delta^{13}\text{C}$ lower than that of beet sugar.

Previously published data on egg $\delta^{13}\text{C}$ from these females showed that the carbon from dietary sugar was incorporated into the eggs of all four species, in each case reaching a maximum level by a certain age of the ovipositing female (O'Brien et al. 2004). We selected eggs laid late in oviposition from four female butterflies of each species: two fed on beet sugar solution and two fed on cane sugar solution. In all cases, butterflies had

Table 1: Host plant species and host plant leaf tissue bulk $\delta^{13}\text{C}$ for each butterfly species in this study (mean \pm SD)

Butterfly	Host Plant	Host Bulk $\delta^{13}\text{C}$ (‰)	<i>N</i>
<i>Colias eurytheme</i>	<i>Vicia villosa</i>	$-25.85 \pm .56$	4
<i>Euphydryas chalcedona</i>	<i>Scrophularia californicus</i>	-28.25 ± 1.61	3
<i>Speyeria mormonia</i>	<i>Viola soraria</i>	-33.04 ± 1.94	10
<i>Heliconius charitonia</i>	<i>Passiflora caerulea</i>	$-31.86 \pm .92$	3

Note. *N* refers to the number of individual plants sampled for analysis. All plants were maintained in the Herrin Greenhouses at Stanford University during the spring of 2000.

reached the age at which incorporation of sugar carbon into eggs was at its maximal point.

Compound-Specific $\delta^{13}\text{C}$ Analysis of Amino Acids

We measured the isotopic composition of egg amino acids using compound-specific stable isotope analysis (via gas chromatography/combustion/isotope ratio mass spectrometry). These analyses were conducted at the Carnegie Institution of Washington Geophysical Laboratory. Eggs were hydrolyzed to amino acids and derivatized to N-trifluoroacetic acid isopropyl esters as described elsewhere (Silfer et al. 1991; O'Brien et al. 2002). Derivatized amino acids were separated, converted to CO_2 gas, and analyzed using a Varian 3400 gas chromatograph (with an HP-1 column) connected to a Finnigan Delta Plus XL isotope ratio mass spectrometer via a combustion interface.

We could reliably detect and resolve 13 amino acids using these methods, six of which are nonessential for insects (Nation 2002): alanine, glycine, serine, proline, aspartate, and glutamate. Asparagine and glutamine are converted to aspartate and glutamate, respectively, during acid hydrolysis; therefore, these amino acids are indistinguishable. Six detected amino acids are essential: threonine, valine, leucine, isoleucine, phenylalanine, and lysine. Tyrosine is nonessential in that it can be synthesized from phenylalanine; however, we classify it here as essential because animals cannot synthesize its aromatic ring de novo from sugar carbon (O'Brien et al. 2002). When possible, we also measured the essential amino acid arginine, although its peak was not always present.

Calculating Amino Acid $\delta^{13}\text{C}$

All samples were run in triplicate, and C_3 and C_4 samples from the same species were derivatized and run in the same batch to control for possible batch effects. The $\delta^{13}\text{C}$ of each peak includes carbon from both the amino acid of interest and the derivatization reagents, which can be heavily fractionated (Silfer et al. 1991). To correct for these unknown fractionations (shifts in isotope ratio), amino acid standards with known $\delta^{13}\text{C}$ were derivatized and analyzed along with each batch of samples. These standards were typically run before and after each triplicate run per sample and were used to correct for the carbon

added by the isopropyl and N-trifluoroacetyl groups during derivatization as follows:

$$\delta^{13}\text{Caa}_{\text{sample}} = \frac{\delta^{13}\text{Caa}_{\text{dsa}} - \delta^{13}\text{Caa}_{\text{dst}} + \delta^{13}\text{Caa}_{\text{standard}} \times p_{\text{std}}}{p_{\text{std}}} \quad (1)$$

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. The standard error of $\delta^{13}\text{Caa}_{\text{sample}}$ was calculated as

$$\text{SE } \delta^{13}\text{Caa}_{\text{sample}} = \frac{1}{p_{\text{std}}} \times \left(\frac{\text{SD}_{\text{dsa}}^2}{n_{\text{dsa}}} + \frac{\text{SD}_{\text{dst}}^2}{n_{\text{dst}}} \right) \wedge \frac{1}{2}, \quad (2)$$

where n_{dsa} and n_{dst} = number of runs of the sample and standard, respectively. This expression takes into account the propagation of variance in mean measurements of the sample and standard through Equation (3), thus indicating measurement error. Over all samples, these standard errors averaged 0.85‰. Average standard errors varied among amino acids, ranging from 0.54‰ for leucine to 1.61‰ for serine.

Calculating % C_{adult} : The Proportion of Adult Dietary 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 (Schwarcz 1991) and potentially offset by fractionation (Boutton et al. 1983):

$$\delta^{13}\text{C}_{\text{egg aa}} = \% \text{C}_{\text{adult}} (\delta^{13}\text{C}_{\text{adult carbon source}} + \Delta_a) + (1 - \% \text{C}_{\text{adult}}) (\delta^{13}\text{C}_{\text{larval carbon source}} + \Delta_l). \quad (3)$$

The parameter of greatest interest here is % C_{adult} , the proportion of the amino acid's carbon deriving from the adult diet (presented $\times 100\%$ as a true percentage in Fig. 5). The terms Δ_a and Δ_l are fractionation effects associated with amino acid synthesis or import from adult and larval diets, respectively. Fractionations are characteristic of particular biological processes

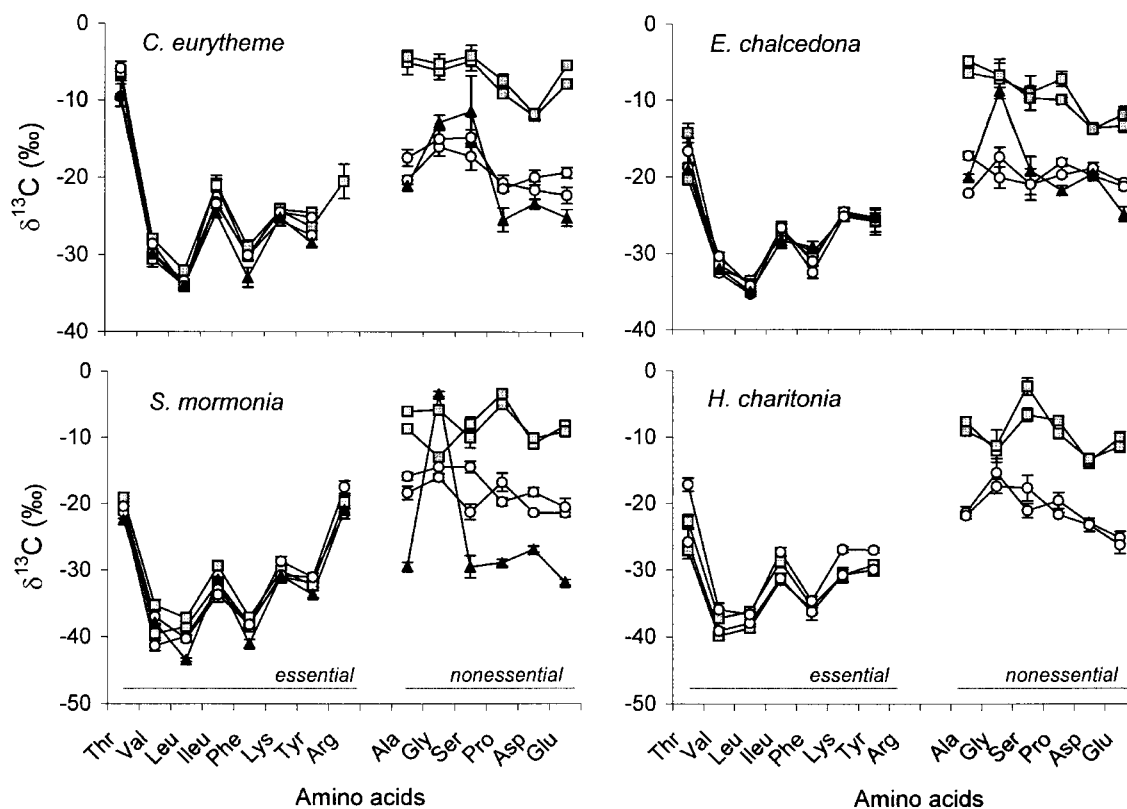


Figure 1. Egg and host plant amino acid $\delta^{13}\text{C}$ for each species. From left to right, the essential amino acids are given first, followed by the nonessential amino acids. With each category, amino acids are shown in chromatographic order. Because each sample was run in triplicate, data are given as \pm measurement error SE. Gray squares indicate eggs laid by females fed on cane (C_4) sugar solution, and open circles indicate eggs laid by females fed on beet (C_3) sugar. Solid triangles indicate host plant amino acid $\delta^{13}\text{C}$ in the species for which these data are available. *Thr* = threonine, *Val* = valine, *Leu* = leucine, *Ileu* = isoleucine, *Phe* = phenylalanine, *Lys* = lysine, *Tyr* = tyrosine, *Arg* = arginine, *Ala* = alanine, *Gly* = glycine, *Ser* = serine, *Pro* = proline, *Asp* = aspartate, and *Glu* = glutamate.

and are independent of source isotope ratio (e.g., Tieszen et al. 1983). We assume that Δ_a is the same for C_3 - and C_4 -fed females kept under controlled conditions. We can then remove the isotopic fractionations by solving Equation (3) for C_3 and C_4 adult diets simultaneously:

$$\%C_{\text{adult}} = \frac{\delta^{13}\text{C}_{\text{C}_4 \text{ egg aa}} - \delta^{13}\text{C}_{\text{C}_3 \text{ egg aa}}}{\delta^{13}\text{C}_{\text{C}_4 \text{ diet}} - \delta^{13}\text{C}_{\text{C}_3 \text{ diet}}}. \quad (4)$$

Statistical Analyses

All statistical analyses were performed in JMP IN version 3.2.1 (student version, SAS Institute, Duxbury). Values of $\delta^{13}\text{C}$ were analyzed using ANOVA, with egg $\delta^{13}\text{C}$ as the dependent variable and dietary sugar type, species, and amino acid as independent variables. Data from essential and nonessential amino acids were analyzed separately. Post hoc contrasts on amino acid or species-level differences used Tukey's HSD, and these effects

were reported as least squares means (means in which all other significant effects were held constant). Because calculations of $\%C_{\text{adult}}$ required that C_3 $\delta^{13}\text{C}$ be compared to C_4 $\delta^{13}\text{C}$ (Eq. [2]), we compared the average of two C_3 egg samples with the average of two C_4 egg samples for each species. Therefore, each species has only a single measurement of $\%C_{\text{adult}}$ for each amino acid, based on data from four samples. These measurements are reported \pm range.

Results

The four species showed similar patterns in egg amino acid $\delta^{13}\text{C}$ (Fig. 1). Because essential amino acids (EAAs) and non-essential amino acids (NEAAs) behave differently with respect to the larval and adult diets (Fig. 1), we analyze and present results for EAAs and NEAAs separately.

Essential Amino Acids

There was no effect of the dietary sugar type on EAA $\delta^{13}\text{C}$ (Table 2). However, there was a consistent pattern of EAA $\delta^{13}\text{C}$

Table 2: Effects of dietary sugar type, amino acid identity, and butterfly species on essential and nonessential amino acid $\delta^{13}\text{C}$

Effect	Essential Amino Acids				Nonessential Amino Acids			
	SS	df	F	P	SS	df	F	P
Sugar type	1.38	1	.28	.6010	2,879	1	561	<.0001
Amino acid	4,439	7	126	<.0001	250	5	9.75	<.0001
Species	1,207	3	80.1	<.0001	102	3	6.62	.0004
Error	512	102	441	86

Note. Essential amino acid $\delta^{13}\text{C}$ varies significantly between species and among amino acids but shows no effect of adult dietary sugar type, whereas nonessential amino acids also show a large, highly significant effect of dietary sugar type on $\delta^{13}\text{C}$.

variation that held across all species (Table 2; Figs. 1, 2). Post hoc contrasts showed three distinct isotopic groupings of EAAs: threonine and arginine were the heaviest EAAs ($\sim -16\%$ to -17%); isoleucine, lysine, and tyrosine were intermediate ($\sim -27\%$ to -28%); and valine, leucine, and phenylalanine were lightest ($\sim -33\%$ to -36%). The least squares means for each amino acid are presented in Figure 2; $N = 16$, except for lysine ($N = 15$) and arginine ($N = 3$).

These isotopic patterns in egg EAAs reflected $\delta^{13}\text{C}$ variation in the EAAs of the larval host plant. Figure 3 shows mean egg EAA $\delta^{13}\text{C}$ for each species (\pm SE) versus larval host plant EAA $\delta^{13}\text{C}$. In those species for which we had host plant data (*Colias eurytheme*, *Euphydryas chalcedona*, and *Speyeria mormonia*), there was a tight 1 : 1 relationship between mean egg EAA $\delta^{13}\text{C}$ and larval host plant EAA $\delta^{13}\text{C}$ (Fig. 3). This relationship held true for within-species data: *S. mormonia* ($y = 0.93x - 0.75$; $R^2 = 0.96$), *C. eurytheme* ($y = 1.03x + 2.54$; $R^2 = 0.98$), and *E. chalcedona* ($y = 1.06x + 1.84$; $R^2 = 0.96$).

Variation among species was also highly significant (Table 2), with *S. mormonia* and *Heliconius charitonia* EAAs the lightest (least squares means = -31.1% and -30.2% , respectively), *E. chalcedona* EAAs intermediate (-25.8%), and *C. eurytheme* EAAs the heaviest (-23.0%). This ranking followed the values of bulk $\delta^{13}\text{C}$ for the host plant of each species (Table 1).

Nonessential Amino Acids

The largest factor determining NEAA $\delta^{13}\text{C}$ was adult dietary sugar. The NEAA $\delta^{13}\text{C}$ of eggs laid by C_4 -fed females was significantly heavier than that of eggs laid by C_3 -fed females (on average, -8.6% vs. -19.5% ; Fig. 1; Table 2). NEAA $\delta^{13}\text{C}$ also varied significantly among amino acids, although the magnitude of that variation was diminished relative to that of the EAAs. This difference reflected the influx of adult dietary carbon with a fixed isotopic value to NEAAs. Average NEAA $\delta^{13}\text{C}$ ranged from -16.6 (aspartate) to -12.5 (glycine). There was no relationship between host plant NEAA $\delta^{13}\text{C}$ and egg NEAA $\delta^{13}\text{C}$ (Fig. 4).

Variation among species in NEAA $\delta^{13}\text{C}$ was also highly significant (Table 2), with *H. charitonia* and *E. chalcedona* NEAAs the lightest (least squares means = -15.3% and -14.7% , respectively), and *S. mormonia* and *C. eurytheme* EAAs the heaviest (-13.2% and -12.9% , respectively). These rankings did not follow the values of bulk host plant $\delta^{13}\text{C}$ but rather reflected both host plant $\delta^{13}\text{C}$ and the extent to which adult dietary carbon was incorporated into eggs.

$\%C_{\text{adult}}$ in Nonessential Amino Acids

The significant effect of adult diet on egg NEAA $\delta^{13}\text{C}$ indicates the use of sucrose carbon in amino acid synthesis. Because $\%C_{\text{adult}}$ was calculated from comparing average C_4 and C_3 values for each amino acid within each species (Eq. [4]; $N = 2$ per diet), error bars are \pm range. $\%C_{\text{adult}}$ varied among amino acids (Fig. 5), with alanine, glutamate, and proline consistently high in all species (80%–100% of all amino acid carbon deriving from adult dietary sucrose) and aspartate consistently low ($\sim 70\%$ or less in all species). Glycine and serine tended to be especially variable, which partly reflects lower analytic precision for these amino acids (across all samples, average analytical error for glycine and serine was 1.42% compared to 0.75% average error for all other amino acids). The extent of sucrose incorporation into aspartate and glutamate was particularly low in *E. chalcedona* (Fig. 5).

Discussion

These data demonstrate the dietary sources of amino acids used in reproduction by a selected sample of nectar-feeding Lepidoptera. The data reinforce the pattern observed previously from a single species of hawkmoth (O'Brien et al. 2002). The four butterflies in this study are only distantly related to hawkmoths and exhibit a range of life histories: from a shorter-lived species that oviposits heavily immediately after adult emergence (*Euphydryas chalcedona*) to a longer-lived species that oviposits only after a week of adult feeding and lays relatively few eggs per day (*Heliconius charitonia*; O'Brien et al. 2004). In all four

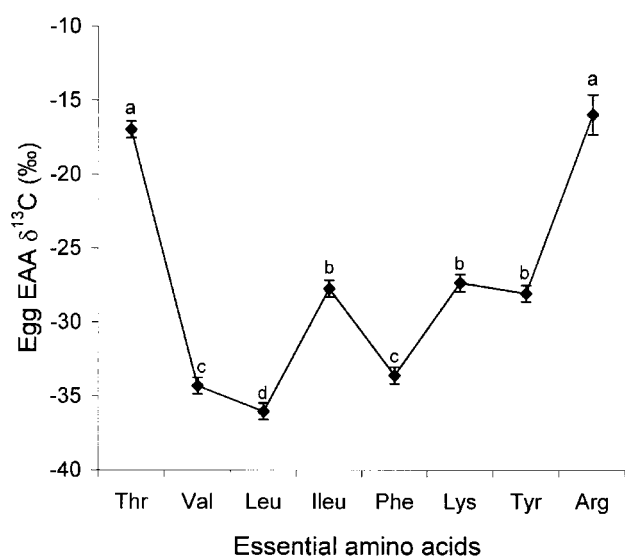


Figure 2. Pattern of $\delta^{13}\text{C}$ in egg essential amino acids (EAA). Shown are the least squares means \pm SE from the ANOVA model presented in Table 1; these represent amino acid means with species effects held constant. Sample sizes were $N = 16$ for each amino acid except lysine ($N = 15$) and arginine ($N = 3$). Amino acids are presented in chromatographic order, and lowercase letters indicate means significantly different at $P < 0.05$ (Tukey's HSD post hoc contrasts). *Thr* = threonine, *Val* = valine, *Leu* = leucine, *Ileu* = isoleucine, *Phe* = phenylalanine, *Lys* = lysine, *Tyr* = tyrosine, *Arg* = arginine.

butterflies (fed sucrose solution as adults), EAAs derived exclusively from the larval host plant and were incorporated into eggs with minimal or no apparent carbon isotopic modification. In contrast, NEAAs were largely synthesized from sugar, and the extent to which de novo synthesis occurred in each amino acid was fairly consistent among species. The similarity of these patterns suggests that remodeling of larval NEAAs is common in nectar-feeding insects and depends on the metabolic role of each amino acid.

The pattern of carbon incorporation into nonessential amino acids was quite consistent among species, in that alanine, proline, and glutamate tended to reflect dietary sucrose quite closely, whereas aspartate, glycine, and serine generally had lower levels of carbon incorporation from the adult diet. This pattern is consistent with that reported previously for *Amphion floridensis* (O'Brien et al. 2002). Alanine is synthesized in a single step from pyruvate and turns over rapidly to reflect the $\delta^{13}\text{C}$ of the adult sugar diet. Glutamate is an important donor and acceptor of amine groups in amino acid metabolism (Berg et al. 2002), resulting in rapid carbon exchange with its keto acid, α -ketoglutarate, an intermediate of the Krebs cycle. Proline is synthesized from glutamate (Berg et al. 2002) and is generally similar to glutamate in $\delta^{13}\text{C}$. The fact that the carbon skeletons of these amino acids reflect dietary sugar is thus consistent with their metabolic position.

Aspartate, glycine, and serine all exhibit a lesser degree of carbon turnover with the adult diet, ranging from a maximum of $\sim 85\%$ adult carbon (with the exception of serine in *H. charitonia*) down to $\sim 30\%$ adult carbon. The keto acid of aspartate, oxaloacetate, is also a Krebs cycle intermediate, yet it undergoes a lower level of turnover with the adult diet than does glutamate. Previous data have shown both a low absolute turnover and a low turnover rate for aspartate (O'Brien et al. 2002). Aspartate is the most abundant amino acid in methionine-rich storage protein (Telfer and Kunkel 1991), a female-specific hexamerin that provides amino acids for reproduction (Ryan et al. 1985; Wheeler et al. 2000; Pan and Telfer 2001). We have previously suggested that enough larval-derived aspartate could be released as these storage proteins are broken down to result in a low apparent turnover rate (O'Brien et al. 2002). The similarity of aspartate turnover in five different species is consistent with this explanation. Glycine turnover appeared especially low in *H. charitonia* and *Speyeria mormonia*. Rates of glycine and serine synthesis via transamination have been shown to be low in some human studies (Jackson 1983), and it has been argued that glycine should be classified as essential or conditionally essential due to low synthesis rates (Jackson 1991).

A previous article reported that the extent to which each of these species used sugar carbon in egg manufacture varied, from *S. mormonia* (80% egg carbon from sugar) to *E. chalcadona* (39% egg carbon from sugar; O'Brien et al. 2004). In three out of the four species (as well as the hawkmoth previously studied), the percentage of egg carbon deriving from essential amino acids closely predicted the percentage of larval carbon being used in egg manufacture within 10% (O'Brien et al. 2004). In

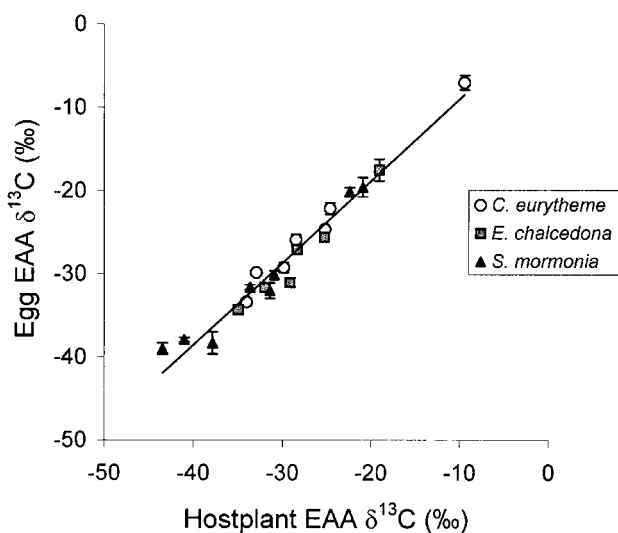


Figure 3. Mean egg essential amino acids (EAA) $\delta^{13}\text{C}$ (\pm SE) plotted against host plant EAA $\delta^{13}\text{C}$ for each species. Across all species, the relationship was described by $y = 0.98x + 0.80$; $R^2 = 0.96$.

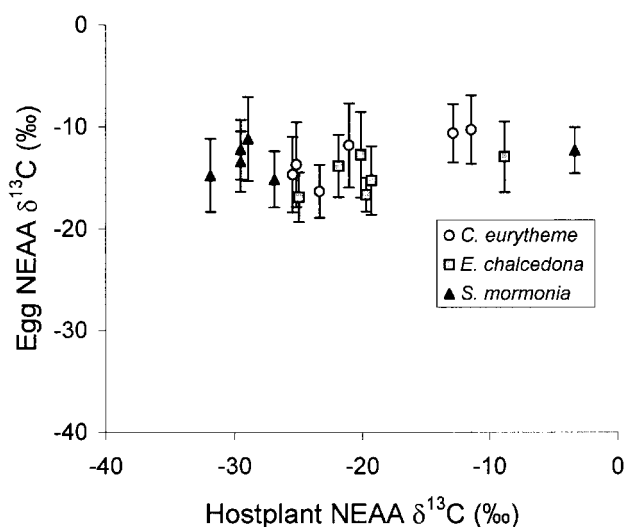


Figure 4. Mean egg nonessential amino acids (NEAA) $\delta^{13}\text{C} \pm \text{SE}$ plotted against host plant NEAA $\delta^{13}\text{C}$ for each species. Error bars reflect the data spread between C_3 - and C_4 -fed females.

contrast, *E. chalcidona* used a much greater percentage of larval carbon in egg manufacture than its egg amino acid composition would predict. The data reported here show that in *E. chalcidona* eggs, both glutamate and aspartate were considerably more “larval” in carbon composition than in the other three species. Glutamate and aspartate are the two most common amino acids in *E. chalcidona* eggs, comprising 23.5% of egg protein (mol%; O’Brien et al. 2004). Thus, the lower rates of turnover with the adult diet of these two amino acids may account for the more larval signature of *E. chalcidona* eggs overall.

The EAAs in eggs provide a high-fidelity record of the $\delta^{13}\text{C}$ of essential amino acids in the host plant. The 1 : 1 relationship between EAA $\delta^{13}\text{C}$ in eggs and larval host plant within all three species, and across species overall, suggests that EAAs in diets undergo minimal fractionation as they are incorporated into consumer proteins (and ultimately used in egg manufacture; O’Brien et al. 2002). The y -intercepts of the plots of host plant amino acid versus egg amino acid ranged among species from -0.75 to $+2.54$. Subtracting $\delta^{13}\text{C}_{\text{egg EAA}}$ from $\delta^{13}\text{C}_{\text{larval EAA}}$ gives the fractionation, Δ_1 (Eq. [3]). These values average $-1.3\text{‰} \pm 1.5\text{‰}$ (mean \pm SD). There were no patterns among amino acids or among species in these Δ_1 values. Slight offsets from $y = x$ could reflect isotope effects arising from the formation or breaking of peptide bonds (Silfer et al. 1992); however, the overall data suggest minimal metabolic remodeling. The fidelity with which the molecule in the animal reveals the dietary source makes essential amino acids powerful tracers in studies of foraging ecology and dietary reconstruction. Indeed, essential amino acid $\delta^{13}\text{C}$ has been very useful in paleontological applications (e.g., Fogel et al. 1997).

The consistent pattern of variation in plants among amino acids that are essential for animals suggests that they may serve as a useful biomarker in animals or ecosystems for plant-derived protein. Mounting evidence suggests that this pattern holds true across plants more generally (Fogel and Tuross 1999; O’Brien et al. 2002, 2003; Smallwood et al. 2003). The synthetic pathways for amino acids that are essential for animals are long (≥ 5 steps; Berg et al. 2002) or require energetic investment as ATP or NADH (Stephanopoulos et al. 1998). Thus, these pathways may be particularly conserved, a hypothesis that is supported by their consistent pattern of isotope effects. Patterns of amino acid $\delta^{13}\text{C}$ can accurately group microorganisms by their modes of central carbon metabolism (Scott et al., forthcoming). At an even finer scale, the distribution of isotopes within macromolecules can also serve as a biomarker (Brenna 2001); for example, $\delta^{13}\text{C}$ signature at the carboxyl carbon of amino acids can discern terrestrial plant material from macroalgae (Savidge and Blair 2004). These signatures appear to be passed on to the herbivores that feed on them (Savidge and Blair 2004). These mounting data point to $\delta^{13}\text{C}$ signatures as a developing tool for discerning biosynthetic origins of amino acids.

The different dietary sources and metabolic behaviors of what are ostensibly the same class of compounds highlight the complexity of nutritional requirements and their potential for constraining growth and reproduction. Insect eggs contain a diversity of compounds including glycogen (Kim et al. 1985), lipids (Chino et al. 1977), and protein (Raikhel and Dhadialla 1992). These classes of compounds are heterogeneous and are expected to exhibit different turnover dynamics depending on

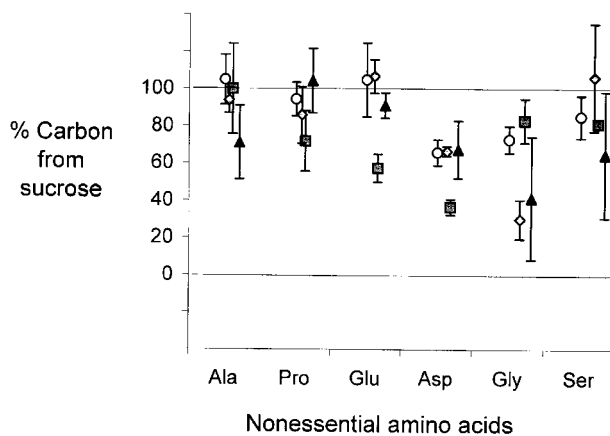


Figure 5. Percent adult dietary carbon ($\%C_{\text{adult}}$) in nonessential amino acids. Amino acids are ordered from high $\%C_{\text{adult}}$ to low, with the two more variable amino acids, glycine and serine, presented separately. Symbols are as follows: gray squares = *Euphydryas chalcidona*, open circles = *Colias eurytheme*, solid triangles = *Speyeria mormonia*, and gray diamonds = *Heliconius charitonia*. Error bars show \pm range. Ala = alanine, Pro = proline, Glu = glutamate, Asp = aspartate, Gly = glycine, and Ser = serine.

their availability and ease of synthesis. Compound-specific stable isotope analysis provides a relatively new and powerful tool for measuring the dynamics of these nutrients, including both amino acids and lipids. The wide range of naturally occurring isotopic variation among individual compounds has great potential to serve as nutrient tracers and biomarkers.

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