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Resource allocation to reproduction and soma in *Drosophila*: A stable isotope analysis of carbon from dietary sugar

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Abstract

Metabolic resources in adults of holometabolous insects may derive either from larval or adult feeding. In *Drosophila melanogaster*, reproduction and lifespan are differently affected by larval vs. adult resource availability, and it is unknown how larval vs. adult acquired nutrients are differentially allocated to somatic and reproductive function. Here we describe the allocation of carbon derived from dietary sugar in aging female *D. melanogaster*. Larval and adult flies were fed diets contrasting in sucrose $^{13}C/^{12}C$, from which we determined the extent to which carbon acquired at each stage contributed to adult somatic tissue and to egg manufacture. Dietary sugar is very important in egg provisioning; at every age, roughly one half of the carbon in eggs was derived from sugar, which turned over from predominantly larval to entirely adult dietary sources. Sucrose provided ~40% of total somatic carbon, of which adult dietary sucrose came to supply ~75%. Unlike in eggs, however, adult acquired sucrose did not entirely replace the somatic carbon from larvally acquired sucrose. Because carbon from larval sucrose appears to be fairly "replaceable", larval sucrose cannot be a limiting substrate in resource allocation between reproduction and lifespan.

Keywords: Drosophila; Resource allocation; Carbon turnover; Sucrose; Stable isotopes

1. Introduction

When nutritional resources are limiting, metabolic trade-offs can constrain the evolution of growth, reproduction and somatic maintenance. How animals acquire and allocate nutrients is therefore considered to be a key determinant of their life history (Reznick, 1985; Stearns, 1992; Roff, 1992; Rose and Bradley, 1998). Many natural factors will limit resources, including the broad features of animal ecology as well as the structure of the life cycle.

In insects, nutrient acquisition and allocation may take place in different lifestages, and the analysis of nutrient routing provides a powerful way to understand how resources affect life histories (Boggs, 1997; Stevens et al., 2000; Zera and Brink, 2000; Rivero et al., 2001; O'Brien et al., 2004). Constraints on how nutrients from different life stages can be used may arise from changes in diet, anatomy, digestive physiology or metabolism, and may mediate the way nutrients affect the life history (Rose and Bradley, 1998; Zera et al., 1998; Zera and Harshman, 2001; Ricklefs and Wikelski, 2002; Zera and Zhao, 2003).

We have recently used dietary stable isotope signatures to experimentally investigate nutrient allocation in Lepidoptera, quantifying the contribution of larval and adult diets to egg manufacture in a variety of species (O'Brien et al., 2000, 2002, 2003, 2004; Fischer et al., 2004). In these studies, adult nectar feeding increased fecundity by contributing nutrient precursors for egg synthesis. By tracking larval and adult dietary carbon isotopically we measured the contribution of resources from each life stage to the production of eggs, and described how this contribution changed with adult age. One concept that

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emerged from this research was that of "renewable" and "nonrenewable" resources: some nutrients were interchangeable between larval and adult dietary sources, whereas others were not. For insects on amino acid depauperate adult diets, essential amino acids are an example of a non-renewable resource (O'Brien et al., 2002). Even in insects with more similar larval and adult diets, aspects of larval nutrition may prove to be limiting or "non-renewable". These may include biochemical constrains on nutrient synthesis, but may also include aspects of diet handing or digestive constraints that make nutrients less accessible to the adult stage.

Our present objective is to investigate the relationship between nutrition and life history using the methods of nutrient-specific isotopic analysis in an insect amenable to molecular genetic dissection—*Drosophila melanogaster*. In contrast to the lepidopteran model, *D. melanogaster* feeds on the same diet as larva and adult: in the field, rotting fruit, and in the lab, an agar–water matrix containing sugar and yeast. Since the fly diet is uniform across its life cycle, the extent to which flies use larval acquired resources to produce eggs is not obvious. Many insects synthesize storage proteins from larval-acquired amino acids for use in metamorphosis or reproduction (Burmester, 1999; Wheeler et al., 2000; Telang et al., 2002), including *Drosophila* (Mousseron-Grall et al., 1997), and restriction of dietary yeast in both larval and adult stages of *Drosophila* dramatically reduces reproduction (Good and Tatar, 2001; Tu and Tatar, 2003). Likewise, restricting adult dietary yeast extends lifespan while reducing egg production (Chippindale et al., 1993; Chapman and Partridge, 1996; Mair et al., 2005). However, despite these and other records of nutrient mediated phenotypes in *Drosophila*, little is actually known about how metabolites acquired at different points in the life cycle are allocated to reproduction relative to other aspects of the adult life history.

Here we apply stable isotope analysis of carbon to better understand the acquisition and allocation of resources deriving from dietary sugar in *D. melanogaster*. Separate experiments addressing the yeast component of fly diets are currently underway. The sugars in fly diets fuel energy metabolism and are stored as glycogen or fat when consumed in excess of demand (Candy, 1989). Sucrose is thus a very simple form of carbon currency, which nonetheless can contribute the majority of carbon used in egg manufacture in other insects (O'Brien et al., 2000, 2004). In contrast, dietary yeast supplies essential and nonessential amino acids and fatty acids (including essential fatty acids); it also provides all nitrogen, vitamins and micronutrients. We expect the sucrose-derived carbons in eggs to initially originate from the larval diet but to eventually derive from adult dietary sugar. In somatic tissues we anticipate a different pattern of carbon turnover. The adult body is formed from larval resources during metamorphosis, and the degree to which it retains a signature of larval carbon should depend on the extent of metabolic turnover in each tissue. Even if sucrose-carbon is fully renewable as an energy resource, the turnover rate of adult somatic tissue will govern the extent to which somatic carbon from larval dietary sucrose will be retained or replaced.

To test these expectations, we used naturally occurring differences in ^{13}C composition between cane and beet sugar to follow the fates of sucrose from the larval and adult diets. Plants with C4 photosynthesis (e.g. sugar cane) contain markedly more ^{13}C than plants with C3 photosynthesis (e.g. sugar beet). By feeding larvae and adults on diets contrasting only in the carbon isotope ratio of sucrose, we show that sugar-derived carbon in eggs is initially provisioned from larval acquired resources and is replaced rapidly and completely by sugar carbon consumed by adults. Sugar-derived carbon in adult soma takes the place of sugar-derived carbon from the larval stage according to the same model of exponential replacement as with eggs, although to an incomplete extent and at a slower rate.

2. Materials and methods

2.1. Experimental diets

We prepared two experimental diets with isotopically distinct sugars. Both diets contained 1.1 g of agar, 8 g of SAF yeast and 11 g of either cane sugar (C4 plant sugar; $\delta^{13}C$: −11.74‰) or beet sugar (C3 plant sugar; $\delta^{13}C$: −24.64‰) in 100 ml water with 0.6 ml of propionic acid. Since cane sugar is more anhydrous than beet sugar we added an extra 10 ml of water to cane sugar diet to maintain the consistency of the medium.

2.2. Feeding protocol

Larvae of the wildtype Canton-S strain were reared on either C3 sugar-based diet or C4 sugar-based diet at 25 °C and 12L:12D. Upon eclosion, all adults were transferred to demography cages. Half of the adults were maintained on the same diet as they were reared on as larvae, and half were transferred to the alternate isotopic diet. This design produced four feeding treatments, two in which diets were switched between larvae and adults (C4–C3, and C3–C4), and two in which larval and adult diets were the same (C4–C4 and C3–C3). For each treatment, approximately 300 females and 100 males were housed in each of three demography cages (1L clear plastic containers modified to accept a 60 mm plastic tube that terminated to a funnel). Petri dishes (60 × 15 mm) with diet were attached to the demography cage by fitting into the inside surface of the funnel. Diets were changed daily when eggs were collected from the surface of the medium; eggs were stored at −20 °C on day 2, 4, 6, 8, 10, 14, 18, 22, 30 and 39. A sample of 4–5 female flies from each treatment was collected on day 2, 4, 8, 14, 22, 30 and 38; the somatic tissue without the ovaries was stored at −20 °C.

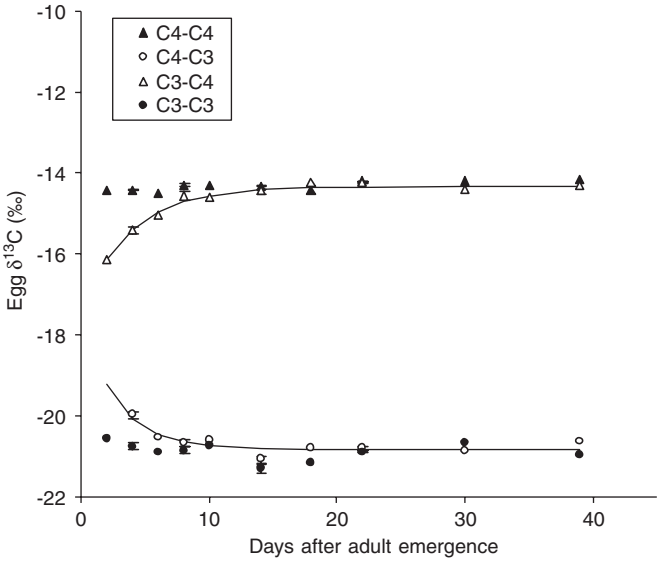

Fig. 1. The $\delta^{13}C$ of eggs laid over the lifespan of *D. melanogaster* females maintained on the four dietary treatments (given in the legend as larval-adult). Data from females on diet switch treatments (C3–C4 and C4–C3) are fitted with an exponential turnover model. Data points from days 4, 8, 12, 22 give error bars (SE; $N = 3$ replicates).

2.3. Determination of isotope ratio

Batches of eggs from one cage within each treatment were used to determine isotope ratio (averaged over a number of individuals) for each sampling day. Previous studies have shown relatively minor, consistent isotopic variation from day to day among eggs laid by replicate individuals. Collection of eggs for isotopic analysis was extremely time-consuming; therefore, eggs from each of the replicate cages were collected on a subset of days only (days 4, 8, 14, and 22) to evaluate among cage variation within treatment (error bars presented in Fig. 1). Body tissue was analyzed from samples collected on days 2, 4, 8, 14, 22, 30, and 38. Both eggs and bodies were oven-dried at 50 °C. Material within each sample was pooled to yield 0.2–0.35 mg of dry tissue (~100–200 eggs; 2–4 bodies). Samples were weighed into tin capsules and analyzed by continuous flow isotope ratio mass spectrometry at the Colorado Plateau Stable Isotope Laboratory, via a NC2100 Elemental Analyzer (Carlo Erba) connected to a DeltaPlus Advantage isotope ratio mass spectrometer (Thermo Finnigan) through the Conflo III interface (Thermo Finnigan). These analyses estimated $\delta^{13}C$, $\delta^{15}N$, and the ratio of carbon to nitrogen (C/N). The standard deviation of internal lab standards (RM1547 "peach leaf"; $N = 25$) run concomitantly were $\pm 0.09‰$ for carbon and $\pm 0.15‰$ for nitrogen. Values for acetanilide standards ($N = 3$) were $\delta^{13}C = -29.89 \pm 0.04‰$, and $\delta^{15}N = -0.10 \pm 0.09‰$.

2.4. Calculating % C from dietary sucrose

For any tissue (egg or soma), the difference in $\delta^{13}C$ between females fed isotopically contrasting sucrose reflects the proportional contribution of carbon from sucrose to that tissue (%C) according to the function

$$\%C = (\delta^{13}C_{C4\ tissue} - \delta^{13}C_{C3\ tissue})/ (\delta^{13}C_{C4\ sucrose} - \delta^{13}C_{C3\ sucrose}). \quad (1)$$

The isotope ratio $\delta^{13}C_{C4\ tissue}$ is from females fed C4 sugar and $\delta^{13}C_{C3\ tissue}$ is from females fed C3 sugar, and $\delta^{13}C_{C4\ sucrose}$ and $\delta^{13}C_{C3\ sucrose}$ are the isotopic ratio of the carbon in the dietary C4 and C3 sugar, respectively. This calculation is independent of isotopic fractionation that may occur when sucrose carbon is converted to egg or tissue (O'Brien et al., 2002). This expression can be applied to calculate the contribution of dietary sucrose acquired from the larval and adult diet to eggs or soma. We calculate the %C from adult dietary sucrose to eggs or soma through two independent comparisons: by comparing egg or somatic $\delta^{13}C$ from treatments C4–C4 and C4–C3, and from treatments C3–C3 and C3–C4. In both cases, flies have the same larval diet while they differ in their adult diet. Similarly, to calculate the percent contribution of sucrose from the larval diet to eggs or soma, we compare C4–C4 vs. C3–C4, and C3–C3 vs. C4–C3; this holds the adult diet constant and varies the isotope source of the larval diet.

We do not expect the eggs and soma of flies maintained on the same diet as both larvae and adults to exactly match the isotope signature of sugar, for two reasons. First, the yeast component of both larval and adult diets will contribute carbon with a different isotopic signature. Secondly, slight shifts in isotope ratio (fractionation) between diet and tissue are well documented when carbon is assimilated and metabolized (e.g., Spence and Rosenheim, 2005).

2.5. Turnover models

In females switched to the alternative isotope diet the change in $\delta^{13}C$ in eggs or soma as a function of age (day) can be estimated by an exponential turnover model:

$$\delta^{13}C(day) = e^{-r \cdot day}\ (\delta^{13}C_i - \delta^{13}C_f) + \delta^{13}C_f, \quad (2)$$

where r is the fractional turnover rate, and $\delta^{13}C_i$ and $\delta^{13}C_f$ are initial and final $\delta^{13}C$, respectively (Fischer et al., 2004). The fractional turnover rate can also be represented as the nutrient pool half-life $t = r/\ln(2)$, which is the time it takes for half of the replaceable pool of the larval-acquired sugar-carbon to be replaced by the adult acquired carbon sugar (also called the 'time to half-turnover'). We estimate r for eggs and for soma, and from both the C4–C3 and C3–C4 treatments.

A similar function describes the relationship of age to the origin of sucrose carbon in egg or somatic tissue

$$\%C_{(day)} = e^{-r \cdot day}(\%C_i - \%C_f) + \%C_f, \quad (3)$$

where $\%C_i$ and $\%C_f$ are initial and final %C, respectively.

2.6. Statistical analyses

All statistical analyses were conducted with JMP 5.1 statistical software (SAS Institute, Cary, NC, USA). The parameters $\delta^{13}C_f$ and r were estimated by non-linear fitting. The value for $\delta^{13}C_i$ was taken from the first $\delta^{13}C$ value in the sequence being fit (either day 2 or day 0), or the average if there were more than one. The effects of dietary treatments and of replicate lines on egg or somatic $\delta^{13}C$, $\delta^{15}N$ and C/N were evaluated using ANCOVA, with day after adult emergence as a covariate. Nonlinear fitting was done through least-squares minimization, and parameters are presented ± estimated standard error. Error bars in figures denote ±SE.

3. Results

3.1. Egg carbon isotopic ratio $\delta^{13}C$

Within a week of eclosion, adult diet provided the primary source for sucrose carbon in eggs (Fig. 1). Among diet-switch females, the isotopic ratio of carbon in the first-laid eggs was intermediate between the signature of their larval and adult diets. However, egg isotopic signatures shifted rapidly toward adult dietary sucrose, so that by age 10 days eggs reflected adult sucrose almost exclusively: −14.34‰ upon the C4 adult diet and −20.83‰ upon the C3 adult diet. Larval dietary sugar had only a slight effect on egg isotope ratio beyond day 10, and the magnitude of that effect was within analytical precision ($\delta^{13}C$: 0.09‰) (Table 1). Adult sugar as a source of carbon allocated to eggs replaced larval-acquired carbon according to an exponential turnover function, where r, the fractional turnover rate, was $0.27 \pm 0.05\,day^{-1}$ for C3–C4 flies, and 0.36 ± 0.07 for C4–C3 flies (Fig. 1 and Table 2). The estimated standard errors for these fractional turnover rates overlap, suggesting that they are not different (Table 2). The corresponding times to half-turnover were 2.6 and 1.9 days.

3.2. % sucrose carbon input into eggs

On day 2 of adult life, sucrose from the adult diet supplied ~35% of total egg carbon, whereas sucrose from the larval diet supplied ~13% (Fig. 2). The contribution from adult dietary sucrose increased over the next 8 days to a maximum of 51%, while the contribution from the larval diet dropped to zero. Overall, the percent of total egg carbon deriving from sucrose remained fairly constant at 47–51% (Fig. 2). Because diets had only two sources of digestible carbon, sucrose and yeast, yeast from either the larval or adult diet supplied the remaining 49–53% of egg carbon.

The % of egg carbon deriving from both adult and larval sucrose vs. time was fitted with an exponential turnover model (Eq. (2)): these yielded fractional turnover rates of 0.31 ± 0.4 and $0.37 \pm 0.5\,day^{-1}$, respectively. These turnover rates correspond to a time to half-turnover (half-life) of 2.2 and 1.9 days, respectively.

Table 1
Effects of diet, day, and replicate on the $\delta^{13}C$ of eggs laid after day 10 (ANOVA)

Effect	SS	df	F	P
Day	0.170	1	6.17	0.0182
Larval diet	0.138	1	2.54	0.0323
Adult diet	419	1	15,201	<0.0001
Replicate	0.023	2	0.41	0.6657
Error	0.910	33		

Table 2
Turnover model parameters (±estimated standard error) for egg and soma $\delta^{13}C$ and %C

Data set	Fractional turnover rate	Half-life (days)	Asymptote
Eggs			
C3–C4	0.27±0.05	2.6	−14.34±0.07‰
C4–C3	0.36±0.07	1.9	−20.83±0.06‰
% Adult	0.31±0.04	2.2	51.2±0.5%
% Larval	0.37±0.05	1.9	0.7±0.4%
Soma			
C3–C4	0.28±0.03	2.5	−16.5±0.1‰
C4–C3	0.29±0.05	2.4	−18.7±0.1‰
% Adult	0.15±0.05	4.6	30.6±1.0%
% Larval	0.56±0.22	1.2	12.3±0.7%

Fractional turnover rates are also converted to half-lives for ease of interpretation.

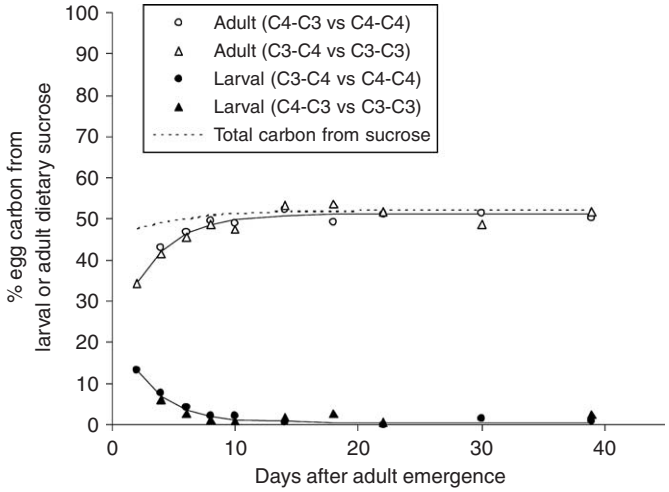

Fig. 2. The percentage of *D. melanogaster* egg carbon deriving from sucrose in the adult and larval diets. Each point was calculated by comparing eggs laid by females fed the same diet in one life stage and contrasting in the other. Open symbols indicate the contribution of sucrose from the adult diet: triangles show C3–C4 vs. C3–C3; circles show C4–C3 vs. C4–C4. Filled symbols indicate the contribution of sucrose from the larval diet: triangles show C4–C3 vs. C3–C3, circles show C3–C4 vs. C4–C4. Solid lines through the data points reflect the fitted exponential turnover model (Eq. (3)), and the dotted line indicates total egg carbon from sucrose (larval + adult).

3.3. Soma isotopic ratio $\delta^{13}C$

Flies fed with the C4 diet as larvae emerged with a somatic $\delta^{13}C$ of −16.17‰, whereas flies reared on the C3 larval diet had $\delta^{13}C$ of −19.18‰. All flies shifted slightly toward the $\delta^{13}C$ of adult dietary sucrose over time; however, the magnitude of the shift was greatest in the diet switch flies (Fig. 3). However, diet switch flies never reached the $\delta^{13}C$ of flies maintained on the same diet as larvae and as adults (Fig. 3), but remained isotopically intermediate. This incomplete replacement indicates that some body structures do not turn over with diet, or turn over only partially. The carbon that did exhibit turnover exhibited an exponential relationship, with a fractional turnover rate similar to that of egg carbon: $0.28 \pm 0.03\,day^{-1}$ for C3–C4 flies and $0.29 \pm 0.05\,day^{-1}$ for C4–C3 flies (Table 2). These turnover rates correspond to a time to half-turnover (half-life) of 2.5 and 2.4 days, respectively.

3.4. % sucrose carbon input into soma

All somatic carbon in the newly enclosed adults originates from the larval diet, and ~23% of this carbon comes from sugar. By 2 days of age sucrose from the adult diet contributed 15–17% to the total somatic carbon, and this proportion increased to 30–33% by age 38 days (Fig. 4). At age 14 days the contribution of larval-derived carbon from sucrose decreased to 11–15%, and remained at this level thereafter (Fig. 4). The total proportion of body carbon from dietary sugar was relatively consistent; varying from 35% to 42% over the flies' lifetime.

Fitting the exponential turnover model to the percent sucrose carbon data for soma yielded fractional turnover rates of 0.15 ± 0.05 and $0.56 \pm 0.22\,day^{-1}$, respectively, for adult and larval carbon; the corresponding times to half-turnover (half-lives) are 4.6 and 1.2 days (Table 2).

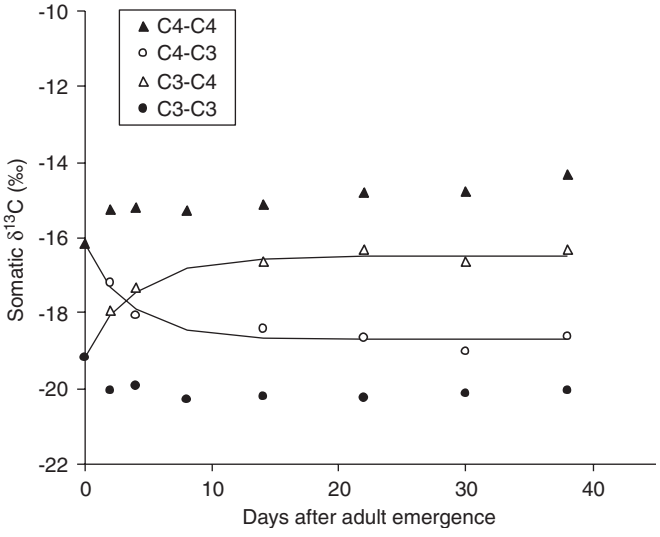

Fig. 3. The $\delta^{13}C$ of female *D. melanogaster* maintained on one of four dietary treatments (given in the legend as larval-adult) over their lifetime. Data from females on diet switch treatments (C3–C4 and C4–C3) are fitted with an exponential turnover model.

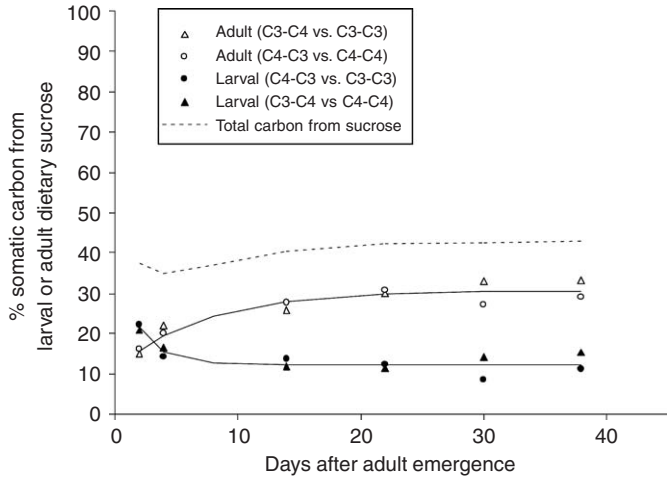

Fig. 4. The percentage of *D. melanogaster* somatic carbon deriving from sucrose in the adult and larval diets. Each point was calculated by comparing eggs laid by females fed the same diet in one life stage and contrasting in the other. Open symbols indicate the contribution of sucrose from the adult diet: triangles show C3–C4 vs. C3–C3; circles show C4–C3 vs. C4–C4. Filled symbols indicate the contribution of sucrose from the larval diet: triangles show C4–C3 vs. C3–C3, circles show C3–C4 vs. C4–C4. Solid lines through the data points reflect the fitted exponential turnover model (Eq. (3)), and the dotted line indicates total egg carbon from sucrose (larval + adult).

3.5. Nitrogen

The ratio of carbon to nitrogen (C/N) in eggs and soma were 5.0 ± 0.39 and 4.58 ± 0.15, respectively. These ratios did not vary with age or as a function of dietary sugar.

Although diets provided an isotopically uniform source of nitrogen, $\delta^{15}N$ in somatic tissue increased by 0.018 ± 0.004‰ per day and overall by ~0.8‰ in the oldest females (Table 3 and Fig. 5). Egg $\delta^{15}N$ also increased with female age, approximately 0.03‰ per day and up to 1.2‰ in late eggs (Table 3 and Fig. 5). Flies fed the C4 diet as larvae had slightly higher $\delta^{15}N$ (3.58 ± 0.064) compared to those fed with the C3 larval diet (3.35 ± 0.064).

4. Discussion

Insects with complex life cycles can exploit different resources in their larval and adult stages, either by shifting diets or through metamorphosis of their morphology and nutritional physiology. Although *D. melanogaster* larvae and adults feed on the same food, the life stages may process this diet in different ways to support reproduction and somatic maintenance. We found that dietary sucrose provided about half of the carbon in eggs at every age, but that the origin of these sugar-carbons shifted from larval to adult acquired sources. In eggs laid early in life, about 12% of the total carbon in eggs came from larval dietary sugar and 35% from adult dietary sugar. Within 10 days

Table 3
Effect of sampling day, diet treatment, and replicate line # on egg and soma δ^{15}N (ANCOVA)

Effect	Egg δ^{15}N				Soma δ^{15}N			
	SS	df	*F*	*P*	SS	df	*F*	*P*
Day	5.33	1	30.85	<0.0001	1.57	1	27.57	<0.0001
Larval diet	0.24	1	1.36	0.2474	0.38	1	6.66	0.0164
Adult diet	0.14	1	0.81	0.3723	0.0005	1	0.009	0.9251
Replicate line[a]	0.24	2	0.68	0.5094				
Error	10.54	61			1.37	24		

[a]Egg samples from replicate lines 2 and 3 were run on days 4, 8, 14 and 22.

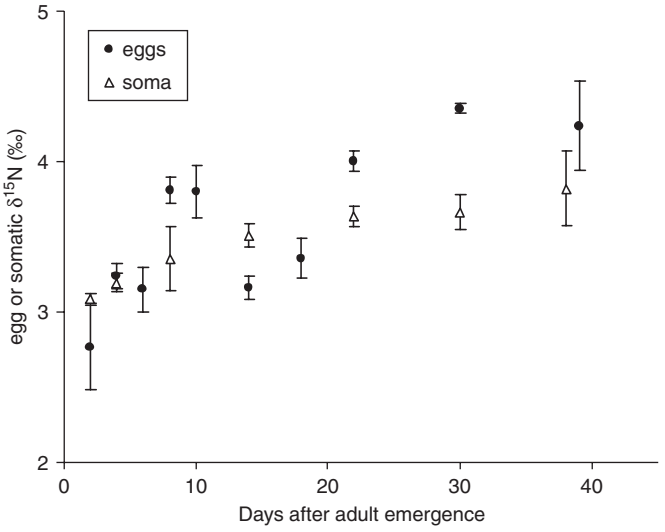

Fig. 5. Egg and somatic δ^{15}N across the lifetime of experimental females. Dietary treatment did not affect δ^{15}N in either eggs or soma, nor did the replicate line females were drawn from. Therefore, each point represents a mean of either 4 (days 2, 6, 10, 18, 30 and 39) or 12 (days 4, 8, 14, and 22) data points (±SE). The increase in both egg and somatic δ^{15}N over time is highly significant ($P<0.0001$).

the contribution from larval sucrose dropped to near zero, and sucrose in the adult diet provided all sugar-derived carbon within eggs. Sucrose, as predicted, is a relatively non-specific, interchangeable source of dietary carbon for *D. melanogaster*, which can be thought of as a "renewable resource" (O'Brien et al., 2002).

As with eggs, the somatic carbon deriving from larval dietary sucrose was replaced by carbon from adult dietary sucrose over time. However, in the soma the replacement was not complete. At early ages the sugar-derived somatic carbon originated nearly equally from larval and adult diets. About 30% of this carbon fraction retained a signature of larval origin; this is about 12% of total adult somatic carbon. This persistent larval-derived carbon is likely to reside in tissue that undergoes little or no metabolic turnover in the adult, such as chitin (Webb et al., 1998). Finally, the contribution of dietary sugar from all stages to somatic carbon increased slightly but consistently with age, from 37% at day 2 to 43% at day 38. This increase may reflect the incorporation of non-essential amino acids synthesized from adult dietary sugar into somatic tissues, as these tissues undergo protein turnover (Tieszen et al., 1983; Hawkins, 1991).

The rates by which soma and eggs shift from larval to adult carbon sources can help reveal some of the underlying physiological processes governing resource use. A simple exponential model describes the dynamics of how larval-acquired carbon is replaced by the adult resource. This suggests that females contain a metabolite pool of sugar-derived carbon for egg manufacture that is initially stocked by larval acquired resources, and upon allocation to eggs these carbons are replaced from adult acquired resources (Tieszen et al., 1983; Hobson, 1995; O'Brien et al., 2000). The time to half-turnover of this pool was approximately 2 days when estimated from the larval contribution (decreasing) or from the adult contribution (increasing). Under conditions of our study, replacement of larval-derived carbon by adult-derived carbon was nearly complete by age 10 days.

Because the carbon pool for egg manufacture resides in the adult fat body and hemolymph, we expected that the turnover in eggs should mirror the kinetics in the soma. However, the rate with which the soma as a whole adopts an adult dietary sucrose signature was markedly slower than in eggs (4.6 days vs. ~2 days to reach half-turnover). This slower replacement of total somatic carbon may occur because there are multiple carbon metabolite pools in the adult, including a "fast" pool of reproductive resources and a "slow" pool associated with somatic maintenance, e.g. protein turnover in post-mitotic tissue such as muscle or neurons (Tieszen et al., 1983; Hawkins, 1991). The present data set does not have the temporal resolution required to distinguish between models invoking a single pool vs. a multiple pools.

Use of sugar resources to support protein turnover requires a reserve of labile nitrogen for amino acid synthesis. We observed a significant increase in the nitrogen isotope ratio (δ^{15}N) with age in both eggs and soma, as has also been observed in butterflies (Fischer et al., 2004). Such increases in the ratio of ^{15}N–^{14}N can be indicative of negative nitrogen balance (Vanderklift and Ponsard, 2003; Fuller et al., 2004), suggesting that N lost in nitrogenous waste (which is typically ^{14}N enriched relative to body tissues) is not being fully replaced by the diet

(Steele and Daniel, 1978; Minagawa and Wada, 1984). That somatic $\delta^{15}N$ increased with age in our samples suggests that the loss of ^{14}N was not fully replaced by nitrogen intake, and that adult *D. melanogaster* on the diets of our trial may have been in negative nitrogen balance.

Dietary yeast is a source of carbon for both egg manufacture and for somatic development and maintenance. About half of the carbon in eggs is consistently derived from dietary yeast, while carbon from yeast provides 77% of all carbon in newly emerged flies and remains the predominant source of somatic carbon in older females. If carbon from larval-acquired yeast is a fully renewable resource for eggs, as we found for carbon of dietary sugar, excess yeast consumed by the adult is likely to enhance reproduction. How the acquisition of dietary yeast from adult diet effects survival will depend on the extent to which dietary yeast supports somatic maintenance. In future work we shall label carbon and nitrogen of dietary yeast to address how these resources function as metabolic currencies. In particular, we shall explore how resources are allocated between soma and eggs when diet (yeast) restriction extends longevity, and when longevity is extended by mutation of genes for components of nutrient-regulatory systems.

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