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Changes in δ^{13}C stable isotopes in multiple tissues of insect predators fed isotopically distinct prey

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Abstract Traditionally, researchers have used measurements of carbon stable isotopes to infer the composition of consumers' diets. However, since consumer's tissues may process carbon isotopes differently, particularly following a diet shift, it is possible to use measurements of carbon isotopes in multiple tissues to determine not only the composition of an individual's diet, but also the temporal dynamics thereof. This study examined how stable isotopes of carbon ($^{13}C/^{12}C$, expressed as $\delta^{13}C$) changed in different adult tissues of two predacious beetles, *Harmonia axyridis* and *Coccinella septempunctata* (Coleoptera: Coccinellidae). In the laboratory, we switched ladybeetles from a C3-based diet (soybean aphids, *Aphis glycines*) to a C4-based one (corn leaf aphids, *Rhopalosiphum maidis*). The $\delta^{13}C$ of metabolically active tissues such as the body fat and reproductive organs changed rapidly (≤ 5 days) following the diet shift. Tissues expected to be more metabolically inert, such as wings, changed more slowly over the same period. Although these general patterns were largely similar between males and females, females had more rapid changes in $\delta^{13}C$ in fat and reproductive tissues. However, females showed a significant depletion in $\delta^{13}C$ after 10 days, while males' $\delta^{13}C$ continued to increase. Given the results of this experiment, it is now possible to distinguish between ladybeetles eating a mixed diet (beetles with multiple tissues at similar, intermediate, equilibrial $\delta^{13}C$ signatures) from those that have shifted diets (beetles with different tissues at distinctly different $\delta^{13}C$ values). Thus, this approach can be used broadly to infer not only what constitutes the diet of a consumer, but also the temporal history of dietary intake.

Keywords Tissue turnover · Diet-switch experiment · Diet-mixing · Temporal dynamics

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Introduction

The abundance of naturally occurring stable isotopes in the tissues of consumers has been increasingly used by ecologists to resolve trophic interactions in terrestrial and aquatic food webs (Peterson and Fry 1987; Vander Zanden et al. 1999). Stable isotopes offer many advantages over other methods used for determining diets, particularly when feeding interactions are difficult to directly observe (Ponsard and Arditi 2000; Scheu and Falca 2000). Such is often the case with consumers such as insects or other arthropods, which may have cryptic feeding habits (e.g., inside plant tissues or in the soil), feed on host fluids (e.g., spiders, sap-feeding insects), or whose small size makes gut-content identification difficult. Stable isotopes reflect a time-averaged assimilation of different foods by a consumer and indicate what a consumer has actually assimilated, rather than what was merely ingested (Cabana and Rasmussen 1994; Post 2002). Moreover, since different tissues incorporate isotopes at different rates, they can provide dietary information over different temporal scales (Hobson and Clark 1992; Tieszen et al. 1983). Interpreting patterns of stable isotopes in consumers to determine diet sources requires an understanding of the isotopic signature of potential food items, how those molecules fractionate once they are assimilated, and how isotopes become incorporated into different tissues over time. Laboratory experimentation needed for interpreting isotopes' patterns has advanced rapidly in vertebrate systems (Ayliffe et al. 2004; Hobson and Clark 1992; Ogden et al. 2004; Tieszen et al. 1983) and is increasing in intensity for invertebrates (Chamberlain et al. 2004; Oelbermann and

Scheu 2002; Ostrom et al. 1997; Rothe and Gleixner 2000; Ruess et al. 2004; Scheu and Folger 2004) although few studies have examined isotope changes in more than one tissue (Webb et al. 1998).

Due to their small size, researchers typically choose to study the stable isotope composition of insects by assaying either entire individuals, or for the sake of simplicity a single body part (e.g., sections of wings) (Ostrom et al. 1997; Ponsard and Arditi 2000; Prasifka et al. 2004). Such analyses assume that these tissues represent an adequate approximation of the effect of diet on isotopic compositions—whether this assumption is valid for small arthropods is not known.

Moreover, inferences regarding the diet composition of a consumer derived using linear mixing models assume that consumers and the diets are at isotopic equilibrium (Phillips 2001). For example, in a simple case of a consumer feeding on two isotopically distinct resources, the isotopic values of the consumer are assumed to be the mass-weighted average of the assimilation of the two resources (Fig. 1A). However, since not all tissues incorporate available pools of assimilate at equal rates and tissues may be in a state of non-equilibrium with the diet, a one time measurement of a composite of tissues (e.g., whole body) may result in a poor estimate of consumer diet at a particular point in time (Fig. 1B).

The observation that tissue isotope signatures do not instantly equilibrate with a resource and that tissues do so at different rates can give us information about the feeding history of a consumer (Hobson and Wassenaar 1999). Thus it should be possible to identify isotope pools within a consumer that have either been recently acquired, as would be the case with tissues with rapid metabolic or turnover rates (e.g., breath, Hatch et al. 2002; plasma, Podlesak et al. 2005), versus isotope pools that reflect a long-term integration of diet and have slow turnover rates (e.g., bone collagen, Hobson and Clark 1992; hair, Tieszen et al. 1983).

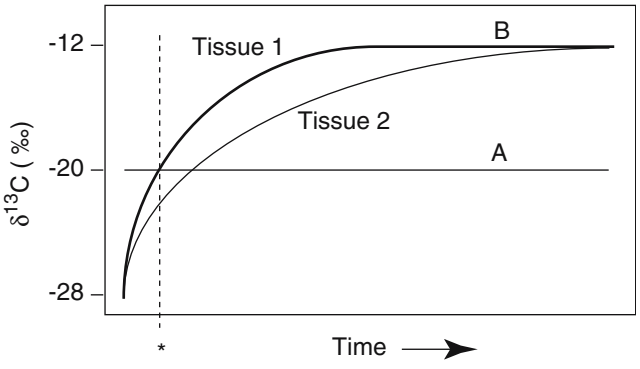

Fig. 1 Hypothetical changes in $\delta^{13}C$ over time in a consumer feeding on isotopically distinct diets. If a consumer is measured at a single point in time (*Asterisk*), an intermediate $\delta^{13}C$ value (e.g., −20‰) is typically interpreted to indicate a mixing of diet with two resources (A) in this case hypothesized to be approximately 50% each of the two diets of −28‰ and −12‰. However, a similar conclusion can be drawn if a consumer has switched from feeding exclusively on one diet (−28‰) to a second diet (−12‰) since there will be a period of time during which tissues are not at isotopic equilibrium with the new diet (B). By taking a single sample from a consumer at a certain point in time "*Asterisk*", it is not possible to distinguish between diet-mixing (A) and diet-switching (B). Nevertheless, since different tissues (*lines 1 and 2*) incorporate the new isotope signature at different rates, there will be a period of time during which it is possible to determine if all tissues are at equilibrium with the diets (model A) or are in a state of transition (model B) as revealed by differences in isotope values in the two tissues measured

In this study, we performed a controlled laboratory diet-switch experiment to examine how stable isotopes of C (ratio of $^{13}C/^{12}C$ as $\delta^{13}C$) changed within the adult tissues of two insect ladybeetle predators, *Harmonia axyridis* (Pallas) and *Coccinella septempunctata* L. We hypothesized that tissues that are expected to be more metabolically active such as fat body and reproductive tissues would show a more rapid change in $\delta^{13}C$ signature toward their new diet compared to tissues that are expected to turnover less rapidly, such as the insect cuticle.

Moreover, because of the differential turnover rates of tissues it should be possible to distinguish if consumers have recently switched from one diet to another (since different tissues will have isotopically different signatures, Fig. 1, line 1 vs. 2), or whether consumers are mixing diets (different tissues should have equilibrated to the same isotopic values in this hypothetical example). Adjustments for isotopic fractionation between tissues would need to be made if tissues are equilibrating at different values. Hence, by measuring isotopic changes over time in multiple tissues it will be possible to reconstruct *what* diet items are utilized by a consumer and *when* a consumer has switched to a particular diet.

Materials and methods

Diet-switch experiment

To determine how stable isotopes of C change over time within different tissues of an insect predator, we performed a diet-switch experiment with two common and dominant predators in agroecosystems, the multicolored Asian ladybeetle, *H. axyridis* (hereafter *Harmonia*, Colunga-Garcia and Gage 1998) and the seven-spotted ladybeetle, *C. septempunctata* (Colunga-Garcia et al. 1997). These ladybeetles are voracious generalist predators of soft-bodied insects, principally aphids. *Harmonia* is implicated as being one of the key predators feeding on the newly established Asian soybean aphid, *Aphis glycines* Matsumura in North America (Fox et al. 2004). Ladybeetle adults initially feeding on aphids from a C3-based diet (*A. glycines* fed on soybean) were either (1) maintained on the same diet or (2) switched to a C4-based diet (corn leaf aphid, *Rhopalosiphum maidis* Fitch fed on corn). As C3 and C4 photosynthetic pathways generate isotopically distinct $\delta^{13}C$ signatures

within plant tissues (≈−28‰ vs. −12‰, respectively) and there is generally little fractionation of $\delta^{13}C$ between resources and consumers (McCutchan et al. 2003; Post 2002), we expected $\delta^{13}C$ in the tissues of the ladybeetles to reflect a balance between current and historical sources of C in the diet that could be isotopically distinguished.

We collected *Harmonia* and *C. septempunctata* pupae from soybean fields at the University of Wisconsin's Arlington Agricultural Research Station (Columbia Co., WI, USA) in July 2003. Collection of pupae ($n \approx 100$) ensured that beetles had been residents within the field in which they were collected and likely had a homogeneous diet source (i.e., soybean prey) as larvae. In 2003 there was a significant outbreak of *A. glycines* in Wisconsin ensuring that there was plenty of prey available for these predators. Emerging adults were kept in large colony cages for 3 days where they could feed on *A. glycines* on soybean (*Glycine max* cv. BSR101) and mate. Beetles were then randomly assigned to feeding treatments: one half of the beetles (approximately 50 and 15 *Harmonia* and *C. septempunctata* adults, respectively) were maintained on soybean aphids as a C3-prey treatment and the other half was switched to a C4-prey treatment of *R. maidis*. At the onset of the experiment (day 1), adult ladybeetles were individually placed in Petri dishes (9 cm diameter) with moistened filter paper to which aphid prey (>20) on excised host leaves were added in excess of beetle consumption. We acquired soybean aphids from the laboratory colony while we collected corn leaf aphids from corn ears and leaves at the UW Arlington ARS. We replaced prey every 2 days at which time the filter paper was also changed to avoid fungal and pathogen contamination. Petri dishes with beetles and aphids were maintained at 22°C at 16:8 L:D within a growth chamber for the duration of the experiment.

Five *Harmonia* from each treatment were randomly removed from the experiment on day 0 (pre-diet-switch), 1, 2, 5, 10, and 14 and immediately frozen (−20°C) to kill the beetles. Additional *Harmonia* were collected on days 8 ($n = 4$) and 12 ($n = 5$) on the corn diet. *C. septempunctata* was only harvested on days 0 and 14 of the experiment (beginning and end). To determine the isotope signature of the beetle diet resources, bulked samples of aphid prey and leaf tissue were frozen on days 0, 5, and 10 of the experiment.

Beetles harvested over the 2-week course of the experiment from the two diet treatments were then thawed and dissected into six parts: (1) elytra (forewings), (2) membranous hind wings, (3) legs, (4) remaining cuticular integument, (5) flight muscles, and (6) reproductive and fatty tissues. Legs, elytra, and hind wings were first removed and placed separately into sample vials. Under a stereomicroscope at 10–30× magnification beetles were dissected in water. The insect gut was immediately removed and discarded to minimize potential contamination from the recent diet. We subsequently removed as much reproductive and fatty tissues as possible by excising the ovaries or testes and pipetting any loose fat body into a sample vial. Flight musculature was carefully cut away from the thorax with a small scalpel. The remainder of the beetle material (excluding head and prothorax) was abdominal and thoracic integument from which we attempted to remove all visible soft tissue. For *Harmonia* harvested on days 8 and 12, only elytra and reproductive tissues were dissected. All samples were dried at 50°C for a minimum of 48 h, weighed to 0.5–1.5 mg and placed into tin capsules for isotope analysis.

Samples were analyzed for stable isotopes of C using a Thermo-Finnigan DELTA-plus Advantage Mass Spectrometer coupled to a Carlo Erba NC2100 Elemental Analyzer (EA) at the Colorado Plateau Stable Isotope Lab (Northern Arizona University, Flagstaff, AZ, USA). Ratios of $^{13}C/^{12}C$ are expressed relative to a known standard (VPDB) in per mil (‰) notation ($\delta^{13}C_{sample} = (^{13}C/^{12}C_{sample})/(^{13}C/^{12}C_{standard}) - 1] \times 1{,}000$). Data were normalized using four International Atomic Energy Association reference standards (CH6, CH7, N1, and N2). An internal laboratory standard (National Institute of Standards and Technology, NIST 1547—peach leaves) was run every ten samples, and 10% of samples were run in duplicate. Measurement errors on the laboratory standard were approximately $\delta^{13}C = \pm 0.05‰$ (±SD) while measurement errors on duplicates were approximately $\delta^{13}C = \pm 0.15‰$.

Statistical analysis

We modeled $\delta^{13}C$ changes over time in the different tissues of male and female *Harmonia* using linear mixed models (SAS/STAT software, PROC MIXED, SAS Institute 2000). The beetle to which a given tissue belonged was treated as a random effect and tissue type and time were nested within beetle to account for the non-independence of body parts collected from each beetle on a particular harvest date. The fixed effects included time (treated as a continuous variable), body part (elytra, hind wings, legs, flight muscles, reproductive and fatty tissues, and integument), diet type (soy and corn aphid), sex (male and female) and their interactions. Except for beetles maintained on soybean diet, there was a significant effect of sex on the rate of change of $\delta^{13}C$ over time in different tissues. Therefore for all analyses involving beetles switched to corn-based diet, we examined males and females separately. For each sex and diet treatment separately (soybean and corn), we fit a model to describe how $\delta^{13}C$ changed over time in each tissue type ($\delta^{13}C = \beta_0 + \beta_1 day + \beta_2 day^2$). We used Akaike's Information Criterion (AIC) to determine whether constant, linear or quadratic time terms provided a more useful fit of the model to the data (lower values indicate relatively better fit to data). Because of the lack of saturation and/or downturn in $\delta^{13}C$ isotope values at the end of the experiment it was not possible to fit more traditional exponential decay models to the data (Tieszen et al. 1983). Estimated regression coefficients

and 95% CI were used to compare changes in isotope values over time across the different body parts. Subsequently, we examined whether the diet treatments resulted in differences in δ^{13}C among tissues (soybean vs. corn). For *C. septempunctata*, we analyzed δ^{13}C differences among tissues and for the different diets as a one-way mixed model ANOVA (soybean day 0; soybean day 14 and corn day 14) since beetles were only harvested on day 0 and 14 of the experiment. Sexes were pooled in this analysis.

Results

Diet isotope signatures

Aphids feeding on corn and soybean had distinct δ^{13}C isotope ratios that were reflective of signatures typical of C4 and C3 plants, respectively. Moreover, there was little indication that isotope values of plants or aphid prey changed substantially over the course of the 2 weeks. δ^{13}C values for *A. glycines* were -28.4 ± 3.7‰ (mean ± SD, $n = 3$ dates) and was similar to that of the host plant, *G. max* (-29.21 ± 1.01‰). *R. maidis* had δ^{13}C values around -12.2 ± 0.18‰ and was likewise similar to that of corn, -12.06 ± 1.35‰.

δ^{13}C change over time in *Harmonia*

Beetles collected just before the diet-switch occurred (Figs. 2, 3, day 0) had signatures that were not significantly different among the different tissue types (-26.2 ± 0.52‰, $F_{5,10} = 0.58$, $P = 0.72$), but were slightly more enriched relative to their aphid diet (+2.2‰). For *Harmonia* maintained on a soybean-based diet

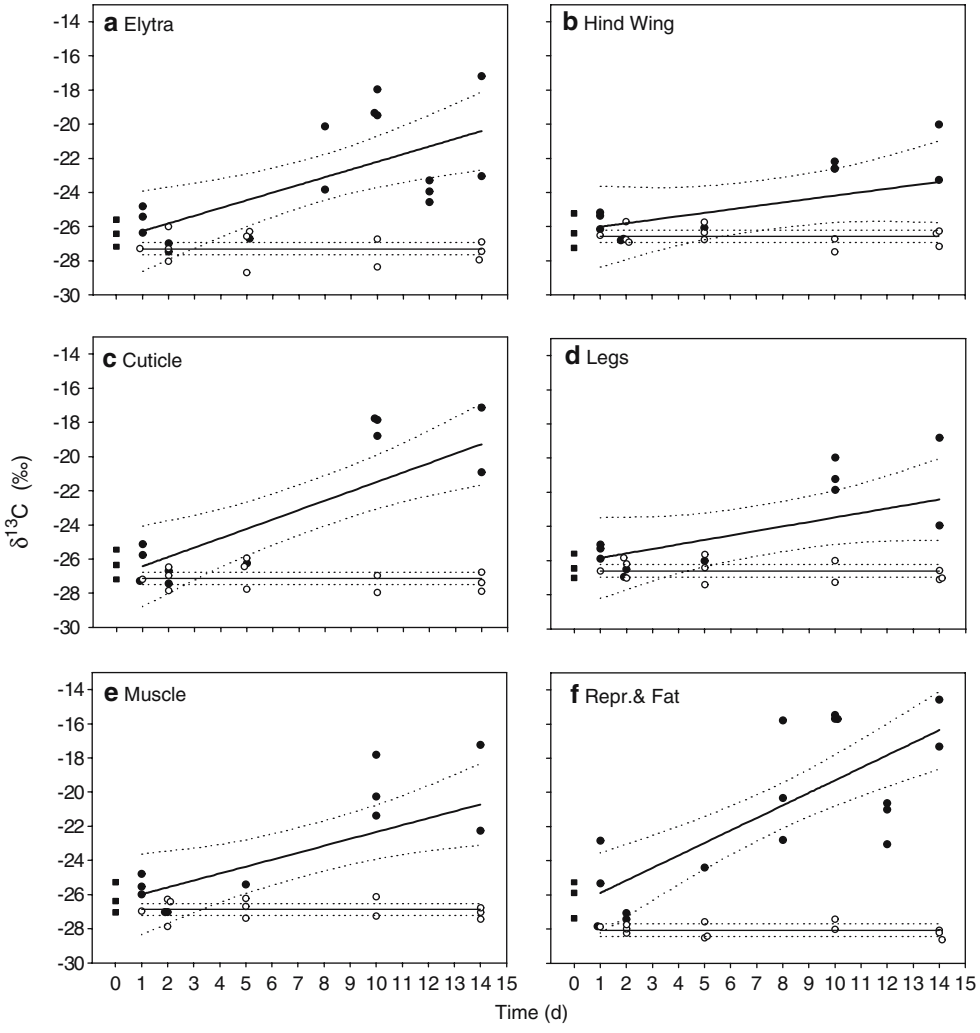

Fig. 2 Changes of male *H. axyridis* δ^{13}C (‰) over time (days) since start of diet-switch for different body parts, **a** elytra (forewings), **b** hind wings, **c** abdominal and thoracic integument ("cuticle", excluding prothorax and head), **d** legs, **e** thoracic flight muscles, **f** reproductive and fat tissues from abdomen. *Filled circles* are values for individual beetles switched to corn-based diet (*R. maidis*), while *open circles* are beetles maintained on soybean-based diet (*A. glycines*). *Squares* are beetles harvested before the diet-switch occurred (day = 0). *Points* are jittered to minimize overlap. *Lines* are the best-fit linear regressions (*dotted lines* are 95% CI)

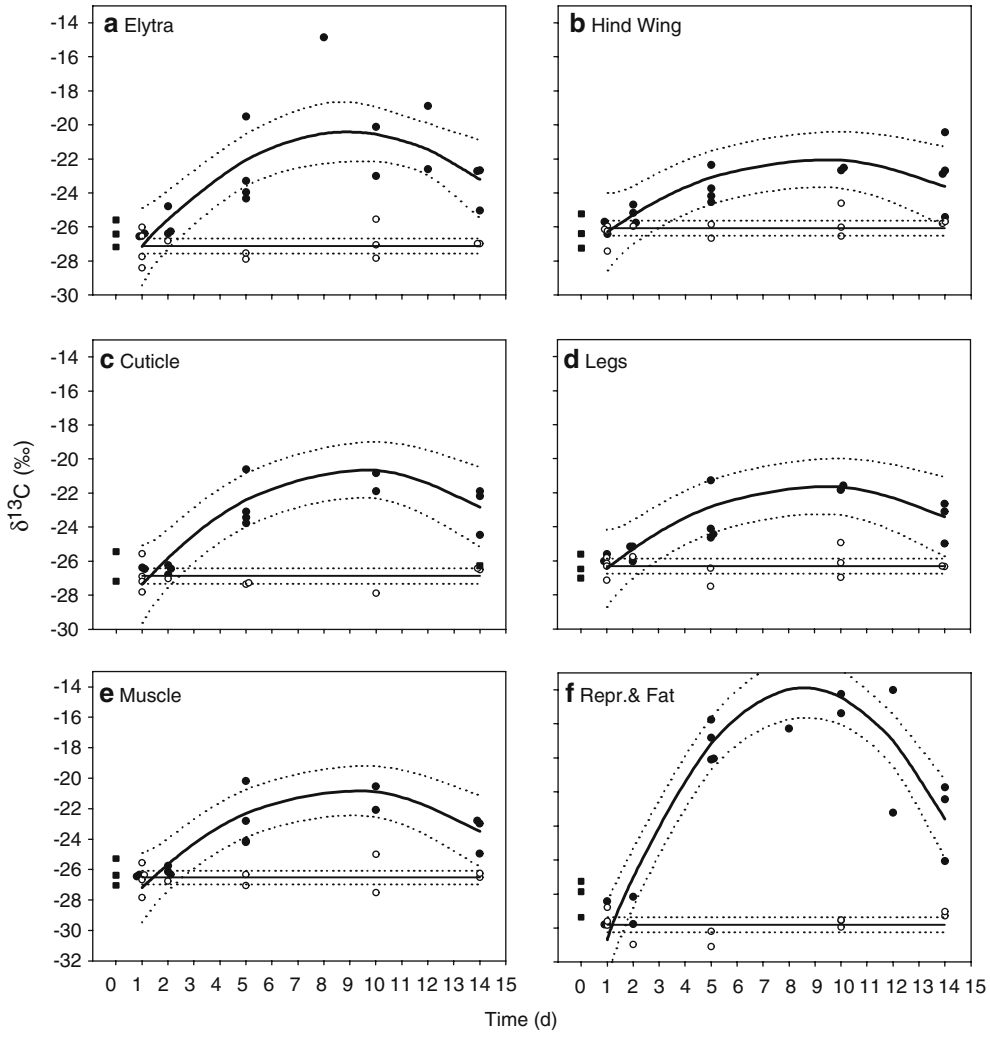

Fig. 3 Changes of female *H. axyridis* δ^{13}C (‰) over time (days) since start of diet-switch for different body parts, **a** elytra (forewings), **b** hind wings, **c** abdominal and thoracic integument ("cuticle", excluding prothorax and head), **d** legs, **e** thoracic flight muscles, **f** reproductive and fat tissues from abdomen. *Filled circles* are values for individual beetles switched to corn-based diet (*R. maidis*), while *open circles* are beetles maintained on soybean-based diet (*A. glycines*). *Squares* are beetles harvested before the diet-switch occurred (day = 0). *Points* are jittered to minimize overlap. *Lines* are the best-fit linear regressions (*dotted lines* are 95% CI)

(*A. glycines*) there was no difference in δ^{13}C between males and females (Sex, $F_{1,19.8} = 0.03$, $P = 0.86$, Sex × Part, $F_{5,97} = 0.10$, $P = 0.99$) but there were significant differences between tissues (Part, $F_{5,97} = 17.3$, $P < 0.0001$). Reproductive and fatty tissues were the most depleted (−27.9‰) while legs and hind wings were the most enriched (≈−26.4‰, Table 1). Moreover, beetles maintained on a soybean-based diet remained unchanged over the course of the experiment (Figs. 2, 3, Date effect, $F_{1,19.9} = 0.02$, $P = 0.90$ and no higher-order interactions with sex or part, $P = 0.23$–0.97) and a model with only a constant term for each tissue type best described the data (Table 1).

For beetles switched to a corn-based diet (*R. maidis*), the pattern of δ^{13}C isotope change over time differed among tissues of males and females (Sex × Part, $F_{5,100} = 3.30$, $P = 0.009$ and interactions with Date and Date^2, $P < 0.0001$) and was analyzed separately for each sex. Male δ^{13}C signatures increased linearly over the course of the experiment (Fig. 2, constant, linear, quadratic models, AIC = 312.6, 276.2, 302.7, respectively). Moreover, δ^{13}C of different tissues changed at different rates (Part × Date, $F_{5,49.9} = 13.93$, $P < 0.0001$), with reproductive and fatty tissues having the greatest linear slope (0.73, Fig. 2f) and hind wings changing the slowest (0.20, Fig. 2b). However, there was a great deal of overlap in the rates of change between different tissues (Table 1).

Female δ^{13}C changed in a curvilinear fashion with a significant depletion in δ^{13}C occurring at the end of the experiment (Fig. 3, linear, quadratic model, AIC = 376.9, 319.9, respectively). Tissues of females changed at different rates (Part × Date, $F_{5,54.6} = 33.97$, $P < 0.0001$, Part × Date^2, $F_{5,54.6} = 30.29$, $P < 0.0001$), with reproductive and fatty tissues changing significantly more rapidly than all other tissues (Fig. 3f, Table 1).

Table 1 Coefficients (β's and 95% CIs) of best linear regression fit for δ^{13}C change over time in different body parts of male and female *Harmonia axyridis* fed either soybean or corn diets (see Figs. 2, 3)

Sex	Part	Soybean		Corn					
		β_0	(95% CI)	β_0	(95% CI)	β_1	(95% CI)	β_2	(95% CI)
Female	Elytra	−27.11	(−27.83, −26.40)	−28.97	(−32.03, −25.91)	1.92	(0.90, 2.94)	−0.11	(−0.17, −0.04)
	Hind wings	−26.07	(−26.79, −25.36)	−27.42	(−30.49, −24.34)	1.19	(0.16, 2.23)	−0.07	(−0.13, 0.00)
	Cuticle	−26.87	(−27.60, −26.14)	−29.08	(−32.16, −26.01)	1.83	(0.79, 2.87)	−0.10	(−0.17, −0.03)
	Legs	−26.31	(−27.03, −25.60)	−27.72	(−30.80, −24.65)	1.35	(0.31, 2.39)	−0.08	(−0.14, −0.01)
	Muscle	−26.53	(−27.25, −25.81)	−28.93	(−32.01, −25.85)	1.84	(0.81, 2.88)	−0.10	(−0.17, −0.04)
	Repr&Fat[a]	−27.81	(−28.52, −27.09)	−32.88	(−35.96, −29.80)	4.44	(3.42, 5.47)	−0.26	(−0.33, −0.19)
Males	Elytra	−27.20	(−27.87, −26.73)	−26.71	(−29.30, −24.11)	0.45	(0.16, 0.74)		
	Hind wings	−26.57	(−27.14, −26.00)	−26.21	(−28.81, −23.61)	0.20	(−0.09, 0.50)		
	Cuticle	−27.13	(−27.70, −26.56)	−26.97	(−29.57, −24.37)	0.55	(0.25, 0.85)		
	Legs	−26.60	(−27.18, −26.03)	−26.11	(−28.71, −23.51)	0.26	(−0.03, 0.56)		
	Muscle	−26.88	(−27.45, −26.31)	−26.39	(−29.00, −23.79)	0.40	(0.11, 0.70)		
	Repr&Fat[a]	−28.06	(−28.63, −27.49)	−26.62	(−29.21, −24.02)	0.73	(0.44, 1.02)		

Regression equations were of the form $\delta^{13}C = \beta_0 + \beta_1 day + \beta_2 day^2$. Beetles maintained on soybean showed no change over time (only β_0 estimated), while male beetles on a corn-based diet showed a linear change of δ^{13}C (β_0 and β_1), and females on corn-based diet had a curvilinear fit over time (β_0, β_1, and β_2)

[a]Reproductive tissues and fat body

Changes of other tissues also varied with hind wing (Fig. 3b) and leg tissues (Fig. 3d) changing more slowly than other tissues. However, only reproductive and fat tissues changed significantly faster than other tissues (Table 1).

For all tissue types and for both sexes, δ^{13}C became significantly more enriched when beetles were switched to a corn-based diet when compared to those maintained on a soybean-based diet (Figs. 2, 3, $P < 0.01$ after day 5). The slowest changing tissues were male hind wings (Fig. 2b), which were significantly different than those of beetles on soybean diet after 8 days. On average (pooling across males and females), at the end of the 14-day feeding experiment, beetles fed on a corn-based diet were +9.3‰ and +3.8‰ enriched, respectively, for reproductive and fat tissue and all other tissues combined, relative to those maintained on a soybean-based prey. In addition, randomly selected egg clutches laid by *Harmonia* under the two different diet regimes during the experiment were significantly different from each other (−27.10 ± 1.18‰ vs. −19.9 ± 6.52‰, $n = 4$, soybean vs. corn, respectively, Kruskall–Wallis, $\chi^2 = 5.33$, $df = 1$, $P = 0.021$).

δ^{13}C changes in *C. septempunctata*

δ^{13}C isotope values in *C. septempunctata* collected in soybean as pupae and reared on *A. glycines* for 2 days after adult emergence were around −26 to −27‰ and similar to values of *Harmonia* before the diet-switch experiment. There were no significant differences in δ^{13}C between tissues, although fat and reproductive tissues were slightly more depleted than other tissues (Fig. 4). After 14 days on exclusively corn-based prey, *C. septempunctata* tissues were significantly enriched (≈ +3.8‰) relative to beetles maintained on soybean-basFed diet (Diet treatment effect, $F_{2,12} = 28.37$, $P < 0.0001$) and reproductive and fat tissues changed even more (+9.1‰) compared to other tissues (Diet × Part, $F_{10,58.1} = 28.94$, $P < 0.0001$, Fig. 4).

Discussion

Changes in δ^{13}C in tissues of predatory beetles

Switching the predatory coccinellid beetles *Harmonia* and *C. septempunctata* from a C3 (soybean)- to a C4 (corn)-based diet resulted in rapid shift in their δ^{13}C

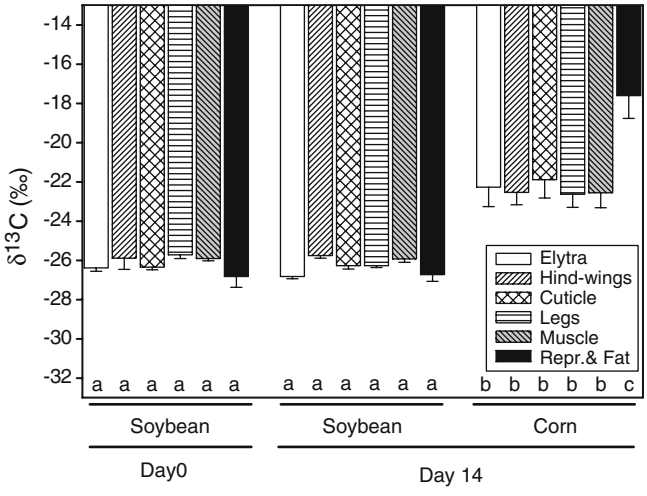

Fig. 4 δ^{13}C (‰) in different tissues of *C. septempunctata* on a soybean-based diet (*A. glycines*) sampled before the diet switch (day = 0), and beetles harvested after 14 days of continuous feeding on either soybean (*A. glycines*)- or corn (*R. maidis*)-based diet. *Letters* beneath each bar are different if tissues are significantly different from each other (Tukey's HSD, $P \leq 0.05$)

isotope signatures. Similar patterns were observed in assays of insects switched between C3 and C4 diets (Ostrom et al. 1997; Prasifka et al. 2004; Webb et al. 1998). However, in this study, we found that rate of δ^{13}C change of ladybeetles was not the same in all tissues. As has been observed in other animals (Hobson and Clark 1992, 1993; Podlesak et al. 2005; Tieszen et al. 1983), tissues that are hypothesized to be more metabolically active, such as reproductive and fat tissues, changed most rapidly. In general, reproductive and fatty tissues changed more rapidly than all other tissues (Figs. 2f, 3f), a pattern that was particularly pronounced in females (Fig. 3f). Within 5 days after a switch to corn leaf aphids, reproductive and fatty tissue δ^{13}C values in female beetles were enriched by $+10‰$ relative to beetles maintained on soybean-based diet. In contrast, over the same period tissues considered less metabolically labile, such as the wings, changed only $\approx +4‰$. This pattern was consistent across two unrelated beetle species, suggesting that for predatory arthropods reproductive and fatty tissues could be a useful and general indicator of recent diet, while tissues containing significant amounts of cuticle (wings) are more long-term integrators of predator diet.

Fatty and reproductive tissues within young ladybeetle adults are expected to be metabolically active as beetles feed and mature to reproduction. The abundant food supply would allow excess carbohydrates to be converted and stored in the fat body in the hemolymph. Accounting for the fact that fat-containing tissues are typically depleted in δ^{13}C ($-2‰$ in *Harmonia*, DeNiro and Epstein 1977) relative to other tissues, isotope signatures of these organs reached that of their new prey after 8–10 days (Fig. 3f, $-14‰$). Maturation of reproductive organs (eggs and testes) in adults could also result in a large pool of isotopically distinct C being assimilated into these tissues. In fact, the isotopic signature of eggs deposited over the course of the experiment for beetles on the corn-based diet were significantly enriched relative to those on soybean diet, suggesting that some fraction of this pool of carbon is incorporated into reproductive tissues (O'Brien et al. 2000).

The downturn of isotope values in *Harmonia* females after 10 days on the corn-based diet was unexpected (Fig. 3). Since this downturn was not observed in males (Fig. 2), we speculate that increased allocation of egg production during vitellogenesis in females may be responsible for the isotopic depletion of these tissues. The δ^{13}C signature of eggs from *Harmonia* feeding on corn leaf aphids was significantly higher ($-19.9‰$) than that of eggs from beetles on soybean aphids ($-27.1‰$), but was nevertheless lower than that of the diet. These results suggest that there may be two pools of C that are utilized to assemble eggs, one of larval origin having a depleted C3 signature, while the other is derived from adult resources (C4). Depleted δ^{13}C in reproductive organs or the fat body could be either due to synthesis of protein or lipid-rich tissues that are significantly depleted in δ^{13}C (DeNiro and Epstein 1977) or through the utilization of larval pools of C (Rivero et al. 2001) that were not measured in this experiment (e.g., head, prothorax). Resource allocation to reproduction (eggs) from resources derived from larval feeding has been shown for both parasitoids (Rivero et al. 2001) and Lepidoptera (Boggs 1997; O'Brien et al. 2004). Larval resource contributions to reproduction are typically highest at the onset of egg maturation, but decrease over time (Rivero et al. 2001). Since this experiment ended after 14 days and encompassed only the initial reproductive output of beetles, it is uncertain how long the depletion in δ^{13}C in reproductive and fat tissues will last in the face of enriched δ^{13}C being assimilated. Moreover, it is unknown if the isotopic depletion is specific to a particular part the organs assayed (eggs, ovaries, fat body). A mass-balance approach measuring multiple tissues as well as fecal material (frass) could be useful in reconstructing the origin and fate of larval and adult derived C and could explain the nature of the downturn in δ^{13}C in insect reproductive and fat tissues.

Changes in isotope values in tissues can be due to either growth or metabolic turnover (Fry and Arnold 1982). In a growth-only model, the initial isotope pool is simply diluted by the addition of novel tissue with different isotopic signature. In the growth-turnover model, in addition to tissue dilution, an additional amount of initial material is lost via metabolic turnover allowing for a more rapid change in overall isotope values (Hesslein et al. 1993; Jardine et al. 2004). In vertebrates, the relative contribution of growth and isotope dilution is generally thought to be small for animals that have reached maturity (Hesslein et al. 1993; Hobson and Clark 1992; Tieszen et al. 1983). For adult ladybeetles, the relative importance of tissue turnover versus growth is generally unknown. Insect tissues made of mostly integument (e.g., wings, body integument, legs, Figs. 2a–d, 3a–d) are unlikely to add significant additional mass since these insects have reached their maximal size. Hence, that these tissues change, albeit slowly, suggests that insect cuticle is turned over and added to with the novel pool isotopically different δ^{13}C. Webb et al. (1998) found that *Locusta migratoria* chitin δ^{13}C was the most rapidly changing component of immature insects, suggesting that chitin in the insect cuticle is continuously digested and reassembled over the course of repeated molts. For holometabolous insect adults, such as the ladybeetles in this study, it is unknown whether the entire pool of carbon in chitin can be turned over even if given enough time since these insects will no longer molt.

In contrast, growth and development of insect reproductive organs could be significant and may account for the rapid change in their isotope values. Other studies have found that variation of stable isotopes' values in rapidly growing organisms, such as young-of-year fish (Jardine et al. 2004; Maruyama et al. 2001), is associated with gains in relative weight. Thus the presence of tissues with both slow and rapid growth and/or turnover in adult lady beetles offers the opportunity to examine long-term and recent dietary intake. Similar

patterns are likely to hold with other insects as well. How rapidly isotope values change in different tissues of immature insects remains to be tested, but is expected to be greater than in adults simply due to their relatively greater growth rates (e.g., Maruyama et al. 2001; Webb et al. 1998).

Diet-switching versus diet-mixing

Interpretation of stable isotope patterns in consumers has largely relied on the simplifying assumption that a consumer's tissues have come to equilibrium with its diet. For example, if a consumer is known to have two potential food items each with distinct stable isotope signatures, possession of an isotopic signature between (e.g., midway) those of the two items is often interpreted as the result of a consumer mixing of the food items in the diet (e.g., 50%A and 50%B, Fig. 1A). An alternative explanation could be that a consumer has recently switched from using one diet to utilizing exclusively a second diet with a different signature (e.g., 100%A → 100%B, as was the case in our diet-switch experiment, Fig. 1B). In this latter scenario, an intermediate signature is due to a non-equilibrium state between the tissues and the new diet, rather than a mixing of diets which would be inferred using a two-endmember mixing model. Time-averaging of diets in tissues or organisms with different turnover rates could lead to misleading interpretations regarding diet composition (O'Reilly et al. 2002). On the other hand, by examining multiple tissues within an organism with different turnover rates, it should be possible to differentiate between these two alternative scenarios (Podlesak et al. 2005).

Differential $\delta^{13}C$ turnover rates can be visualized by plotting the $\delta^{13}C$ signature of two tissues of an individual (Fig. 5). A consumer feeding on a diet of constant composition for a long time (relative to the slowest tissue turnover rate) is expected to have tissues with $\delta^{13}C$ at equilibrium with carbon assimilated from various sources in the constant diet. Such was the case with beetles that were collected in soybean fields and maintained on soybean aphids for the duration of the experiment (Fig. 5). The cloud of points in the lower left (Fig. 5, numbers) are centered on the soybean-diet equilibrium $\delta^{13}C$ values for hind wings (−26‰) and reproductive and fat tissues (−28‰) reflecting the fractionation of C between these two tissues. Points along a 1:1 line centered on this baseline (Fig. 5) would indicate that both tissues are at equilibrium with the $\delta^{13}C$ of the diet (adjusting for fractionation).

If a consumer switches to a diet with a different isotopic signature, there will be a period of time during which tissues will have different isotopic values (Fig. 1, Podlesak et al. 2005). Relative to more refractory or slow-growing tissues, metabolically active or rapidly growing tissues will change more rapidly and would be seen as a deflection away from a 1:1 line. Deflection from the 1:1 line is analogous to observing differences between tissues after a diet-switch (e.g., the difference between the lines 1 and 2, Fig. 1). The magnitude and rate of deflection will be a function of the relative rates

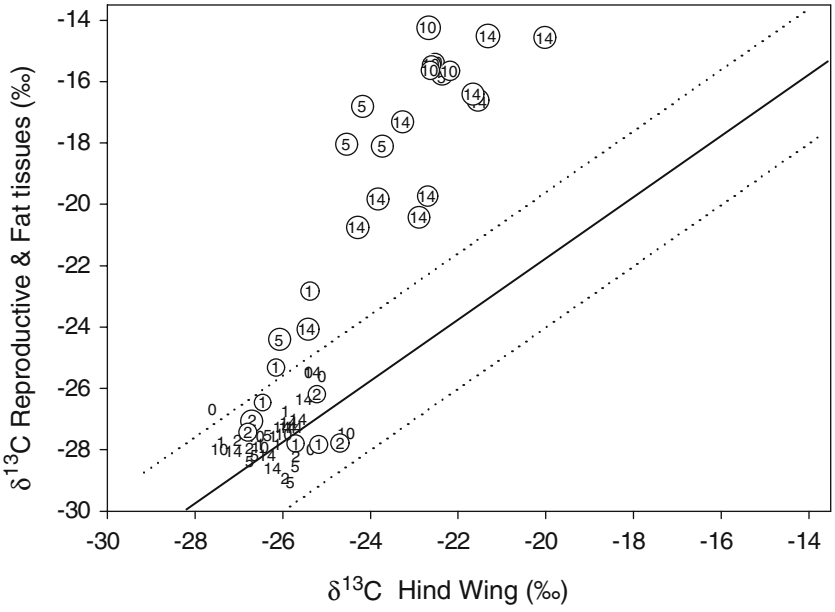

Fig. 5 Plot of $\delta^{13}C$ (‰) in hind wings versus reproductive and fat tissues of individual beetles. *Harmonia* and *C. septempunctata* and sexes are combined. *Numbers* refer to the date after the diet-switch when beetles were sampled. *Open circles* represent beetles switched to a corn-based diet ($\delta^{13}C \approx -12‰$), whereas *numbers with no circles* are beetles maintained on soybean-based diet. The *line* has a slope of 1 and is centered on the soybean-diet equilibrium $\delta^{13}C$ values for hind wings (−26‰) and reproductive and fat tissues (−28‰) reflecting the fractionation of C between these two tissues (see text for Discussion). The *dotted lines* represent hypothetical confidence limits around the 1:1 equilibrium line, $\varepsilon = \pm 2.0‰$

of change of the different tissues, the differences in isotopic signature of the diets, and the time since switch. For fast and slow tissues in *Harmonia* this difference occurs after 5 days and has not reached equilibrium after 14 days (Fig. 5). Moreover, the ability to detect if tissues are not at equilibrium with a diet will also depend on the variability of the signature within tissues and among individuals. Thus significant deviations (outside of a confidence band, arbitrarily set at $\varepsilon = \pm 1$–2‰, e.g., pooled error of hind wing and reproductive and fat tissues) from a 1:1 line of tissue equilibrium is an indication of diet-switching, rather than diet-mixing. If the relationship between the difference in isotope values over time was known for different tissues, it is also possible to back-calculate the time since the diet-switch occurred, further enhancing the utility of measuring isotope values in multiple tissues.

General assumptions and caveats

Whether a one-time measurement of isotopes from multiple tissues can be utilized broadly to infer temporal changes in diets will depend on whether the organism being studied has (a) diets with isotopically distinct signatures and (b) variation in isotope turnover rates between tissues. The essence of detecting evidence of diet-switching lies in having a large enough difference between the isotope values obtained from the two tissues (e.g., the difference between lines 1 and 2, Fig. 1). This difference must be larger than the sampling error and among-individual variation around these curves. Therefore, the larger the difference between the two diet end-members, the more likely it will be to detect a difference between the two lines. Moreover, if nothing is known regarding differences in fractionation between tissues, caution must be used before inferring diet-switching. Inherent differences in fractionation between tissues and the original and/or new diets (i.e., shifting the curves up or down, Fig. 1) can alter the distance between the isotope change curves. Isotope values that appear different between tissues due to mere fractionation could thus be erroneously interpreted as being the result of diet-switching. However, if fractionation differences between tissues and the diet is known a priori, this can be used to adjust the origin of the 1:1 line (as in Fig. 5) and will not affect the ability to determine the occurrence of diet-switching.

Secondly, how fast isotopes change in different tissues (i.e., changing the slopes of lines 1 and 2, Fig. 1) will influence the ability of detecting diet-switching. If there is a large difference in the turnover rates between two tissues, then there will be a relatively long period of time during which we can detect a diet-switch with a one-time sample. Alternatively, if the difference in tissue turnover rates is small (i.e., both tissues change slowly or quickly), it may not be possible to determine if a consumer has just switched between two resources since this will be indistinguishable from the scenario of diet-mixing with all tissues appearing at equilibrium with a two-component diet.

Given the above assumptions, the ability to infer diet-mixing, and the timescale over which this can be done, will be continent upon the biology and physiology of a consumer. Vertebrates for example have large differences in isotope turnover rates among different tissues ranging from days and weeks (liver, breath) to months and years (bone collagen, hair, muscle) (Ayliffe et al. 2004; Hobson and Clark 1992; Tieszen et al. 1983). This variation has been used to infer changes in diets in migratory birds over a time period of 2–3 weeks (Podlesak et al. 2005). In contrast, studies of invertebrates, such as beetles, springtails, and moths, have often found rapid changes in isotope values (5–10 days for 75% isotope change) in whole-body assays (Ostrom et al. 1997) as well as more specific fractions (Chamberlain et al. 2004; O'Brien et al. 2000, this study). It remains to be seen if there are any tissues within arthropods that reflect dietary intake over longer periods (e.g., months). Thus, the temporal scale over which isotope values from different tissues will be useful for inferring diet-switching will depend on the species in question. It is possible that differences between invertebrates and vertebrates are related to the sizes of the organisms rather than biology and the importance of dilution versus tissue turnover, and its effect on rates of isotope change, may therefore be a function of organism or tissue growth rates (Fry and Arnold 1982).

Conclusions

For the ladybeetle predators examined in this study, tissues with rapid turnover rates (or increasing mass) integrate dietary intake on a finer scale and are indicative of recent C sources in the diet. In contrast, tissues with slower turnover have more isotopic "momentum" and integrate dietary C over longer periods. Incorporating tissue-specific changes in models of diet-mixing could help understand with finer resolution the temporal dynamics of resource use in consumers. We can learn not only what diets a consumer has been utilizing, but also potentially when (Podlesak et al. 2005). In addition, if resources are spatially segregated in the environment (e.g., upland vs. lowland habitats, Hobson et al. 2003; sorghum vs. cotton fields, Prasifka et al. 2004), isotope data could give clues about the dynamics of movement among habitats (Rubenstein and Hobson 2004).

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