Enhanced Carbon Enrichment in Parasitoids (Hymenoptera): A Stable Isotope Study

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ABSTRACT By considering the magnitude of isotope enrichment associated with trophic transfers in biologically important elements such as carbon ($^{13}C/^{12}C$) and nitrogen ($^{15}N/^{14}N$), it is possible to infer trophic interactions in systems where direct observations are logistically difficult. Several recent reviews have estimated that consumers become enriched in the heavy nitrogen isotope on the order of 2.3 to 3.4‰ with each trophic transfer. Furthermore, these same reviews have estimated that consumers become enriched in the heavy carbon isotope between 0.4 and 0.5‰ per trophic transfer. Although these estimates have been used to infer trophic interactions in a variety of taxa, the applicability of these estimates for studies of arthropod community ecology is poorly understood. Specifically for insect parasitoid communities, estimates of nitrogen and carbon isotope enrichment from a comprehensive study have yet to be published. Here, we present the results of nitrogen and carbon stable isotope analyses for a suite of hymenopteran parasitoids that attack the gall-making midge, *Rhopalomyia californica* Felt (Diptera: Cecidomyiidae), on coyote bush, *Baccharis pilularis* (Compositae), in northern California. Mean carbon enrichment for all parasitoids developing on *R. californica* was considerably higher than expected, based on recent reviews. In fact, discrimination among trophic levels was possible, based on carbon enrichment values alone. Mean nitrogen isotope enrichment was slightly lower than values reported in recent reviews. However, the variation associated with our estimate of nitrogen enrichment falls well within the range of values reported in the reviews. Mechanisms behind the greater than expected enrichment in carbon are currently unknown and will require further investigation.

KEY WORDS stable isotopes, ^{15}N, ^{13}C, *Rhopalomyia californica*, parasitoids

A RELATIVELY NOVEL TOOL in ecological studies involving insects, stable isotope analysis offers the potential to quickly and easily assess community structure in systems where direct observations of trophic interactions or manipulative studies are difficult. By considering natural variations in the isotopic ratios of biologically important elements such as carbon ($^{13}C/^{12}C$) and nitrogen ($^{15}N/^{14}N$), ecologists have been able to infer nutrient flow pathways among taxa. Such inferences are possible because the ratio of heavy to light isotopes of these elements changes in a relatively predictable manner due to physical, chemical, and biological processes that fractionate the isotopes (Peterson and Fry 1987, Ehleringer and Rundel 1989, Gannes et al. 1998, Robinson 2001).

Carbon and nitrogen stable isotope data are usually expressed in delta notation,

$$\delta X, = [(R_{sample}/R_{standard}) - 1] * 10^3,$$

where X is ^{13}C or ^{15}N, and R is the corresponding ratio of $^{13}C/^{12}C$ or $^{15}N/^{14}N$ in either the sample being analyzed (R_{sample}) or in a standard reference material ($R_{standard}$). The standard reference material for carbon is PeeDee limestone. Atmospheric air is the standard reference material for nitrogen.

Stable isotope ratios of carbon (e.g., $\delta^{13}C$) have been used to determine the ultimate source of energy (in the form of organic matter) as it moves among consumers (Kwak and Zedler 1997). Generally, $\delta^{13}C$ isotopes are considered poor candidates for assessing trophic interactions because of the negligible fractionation (≈0.4–0.5‰ increase in $\delta^{13}C$) associated with trophic transfers (Post 2002, McCutchan et al. 2003). Carbon isotopes are most fractionated as atmospheric CO_2 is taken in by plants and converted to carbohydrates during photosynthesis. Distinct $\delta^{13}C$ signatures among plants by using the Calvin cycle (C_3), Hatch–Slack cycle (C_4), and Crassulacean acid metabolism (CAM) for photosynthesis are due to differences in the diffusion and carboxylation of CO_2 within each pathway (Park and Epstein 1960, 1961). C_3 and C_4 plants average $\delta^{13}C$ values of −26 and −12‰, respectively (Smith and Epstein 1971, Ambrose 1986). The $\delta^{13}C$ values of CAM plants are intermediate between those of C_3 and C_4 plants (Ambrose 1986). Because the carbon isotope signal is conserved during trophic transfers, researchers have

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used $\delta^{13}C$ to estimate the relative use of C_3 versus C_4 primary productivity by both primary and secondary consumers in terrestrial ecosystems (Ostrom et al. 1997, Magnusson et al. 1999, Callaham et al. 2000).

Unlike carbon, stable isotopes of nitrogen are most fractionated during the consumption, assimilation, and excretion of organic material by consumers (DeNiro and Epstein 1981, Ambrose and DeNiro 1986). For example, during biochemical activities associated with excretion, deamination enzymes preferentially remove amine groups with ^{14}N over ^{15}N. Consequently, the excreted nitrogen of a consumer (e.g., ammonia, uric acid, or urea) is depleted in ^{15}N relative to its diet (Minagawa and Wada 1984). This means that consumers at higher trophic levels should have a higher $^{15}N/^{14}N$ and $\delta^{15}N$ than consumers or producers at lower trophic levels. Indeed, three recent reviews of stable isotope studies have shown that $\delta^{15}N$ exhibits enrichments of 2.3, 2.5, and 3.4‰ in the heavy isotope from diet to consumer (McCutchan et al. 2003, Vanderklift and Ponsard 2003, and Post 2002, respectively). Because of the stepwise enrichment in $\delta^{15}N$ in successively higher trophic levels, interpreting the $\delta^{15}N$ signal of a consumer relative to an appropriate baseline has been used to estimate trophic position (Vander Zanden and Rasmussen 1999) and infer community structure in an array of ecosystems.

Despite the abundance of published studies that have used stable isotopes to examine community structure and trophic interactions in mammals (reviewed by Kelly 2000), birds (reviewed by Kelly 2000), and fish (Pinnegar and Polunin 2000, Vander Zanden and Rasmussen 2001), current reports on the use of stable isotopes to infer community structure in insects are rare. This is most likely due to the fact that reliable carbon and nitrogen enrichment patterns have yet to be determined for most insect taxa. Instead, studies involving stable isotopes and insects have usually focused on how single-species and mixed prey diets affect the stable isotope signal of a particular predator (Ostrom et al. 1997, Collier et al. 2002, Oelbermann and Scheu 2002) or the selective use of C_3, C_4, and/or CAM resources by consumers (Magnusson et al. 1999, Callaham et al. 2000, Markow et al. 2000).

Before stable isotopes can be confidently applied to studies of arthropod community structure, it is imperative to quantify the magnitude of isotope enrichment associated with trophic transfers in insects. Furthermore, deciphering the physiological and biochemical mechanisms that yield particular enrichment values will allow the development of a more general model of isotope enrichment in insect community ecology. In this study, we analyzed the magnitude of carbon and nitrogen isotopic enrichment per trophic transfer in the *B. pilularis* food web and compared these values to estimates in recent reviews. In addition, we assessed whether taxon-based variation in $\delta^{15}N$ and/or $\delta^{13}C$ was sufficient to discriminate among trophic groups.

Materials and Methods

Natural History of System. The evergreen shrub *Baccharis pilularis* DeCandolle (Compositae), commonly known as coyote bush, grows throughout coastal ranges and in the Sierra Nevada foothills of California. The midge *Rhopalomyia californica* Felt (Diptera: Cecidomyiidae) is a specialist gall-maker on *B. pilularis.* Adult females of the midge lay clusters of eggs on terminal buds and growing tips of *B. pilularis.* Upon hatching, the larvae induce gall formation. A separate chamber forms around each midge larva, with a single gall typically containing from one to >50 chambers (Ehler and Kinsey 1991) with occasional galls containing several hundred chambers (Hopper 1984). The entire midge life cycle can be as short as 30 d under favorable conditions (Force 1970, Hopper 1984), but it can take >70 d in the field (Briggs and Latto 1996).

The midge eggs and larvae are parasitized by a suite of hymenopteran species, with total parasitism rate usually exceeding 80% in the field (Force 1970, 1974; Ehler 1982; Hopper 1984; Briggs 1993). The parasitoid *Platygaster californica* Ashmead (Platygasteridae) attacks the midge eggs and first instars while they are still on the outside of the plant. Parasitized midge eggs and larvae continue to develop and produce galls along with the unparasitized midges. *P. californica* has the highest attack rate of any of the common parasitoid species (Force 1970, Hopper 1984, Briggs and Latto 1996), but it is an inferior competitor to all of the other parasitoids (Force 1970). When another wasp oviposits on a larva in which a *P. californica* is developing, *P. californica* usually dies.

All of the other common parasitoid species oviposit into the chambers of already developed galls. These include the primary ectoparasitoids *Torymus koebelei* Huber (Torymidae) and *Torymus baccharidis* Huber (Torymidae), as well as the primary endoparasitoid *Tetrastichus* sp. (Eulophidae). *Zatropis capitis* Burks (Pteromalidae) and *Mesopolobus* sp. (Pteromalidae) are both facultative hyperparasites that develop externally on either the midge or on the larvae of the other wasp species.

Perhaps the most significant problem associated with the use of stable isotope analysis to infer species' interactions is the lack of appropriate experimental controls, in which consumers are fed known diets and their resulting isotopic signatures are measured (Gannes et al. 1997, Eggers and Jones 2000). In many studies where controls are lacking, it may be impossible to produce definitive interpretations of complex patterns in isotope enrichment, especially where omnivory "mixes" stable isotope signatures. The system we chose has the distinct advantage of acting as a "controlled diet experiment in the field," because the herbivore in this community is a confined-feeding specialist and most of the parasitoids feed on a definitively identifiable set of plant or insect tissue during their entire development. Thus, signal mixing within individual insects due to omnivory is largely absent. The pteromalids (*Mesopolobus* sp. and *Z. capitis*),

however, are facultative hyperparasitoids that are capable of feeding at more than one trophic level (Ehler and Kinsey 1991). As a result, we were not able to determine enrichment estimates for these hyperparasitoids due to the possible presence of signal mixing.

Study Sites. Galls were collected from three sites in northern California, from which, galls were repeatedly collected from 15 *B. pilularis* bushes. Within each bush, between two and eight galls were collected during each sampling period. Our first site was located just outside of Stebbins Cold Canyon Reserve (Solano County, CA). Here, galls were collected from a low clearing along Putah Creek near the Monticello Dam. At the second study site, located within Point Reyes National Seashore (Marin County, CA), galls were collected from a gently sloping ridge near the Sky Trail trailhead. The third site was located within Tomales Bay State Park (Marin County, CA). Here, galls were collected from a bayside hill just north of the town of Cypress Grove.

Galls were collected three times during the 2002 field season (collection times $t = 1, 2,$ and 3). Because very few midges or parasitoids emerged from galls collected at Stebbins Cold Canyon at time $t = 3$, only data for the first two collection dates are reported for that site. At Stebbins Cold Canyon, galls were collected on 7 May 2002 ($t = 1$) and 5 June 2002 ($t = 2$). At both Point Reyes National Seashore and at Tomales Bay State Park, galls were collected on 7 May 2002 ($t = 1$), 10 June 2002 ($t = 2$), and 8 July 2002 ($t = 3$). As much as possible, only fully formed galls without emergence holes were chosen from each bush.

Stable Isotope Analysis. Field-collected galls were brought back to the laboratory for rearing. The diameter and condition of each gall was noted before separately placing each gall in a 1 dram shell vial topped with a cotton stopper (which prevented emerging midges and parasitoids from escaping but allowed air flow). Vials were placed on the laboratory bench and reared at ambient temperature and photoperiod (≈23°C and 14:10 [L:D] h). Galls were checked every 1–2 d for emerging adults. Emerged adults (both midges and parasitoids) were taken out of the vials and frozen until they could be identified. Once identified to species, midges and parasitoids were placed in a desiccating oven for at least 48 h before being processed for stable isotope analysis. Individual, whole insects (0.01–0.06 mg) or a sample of gall tissue (2 mg) was separately loaded into tin capsules (8 by 5 mm; Elemental Microanalysis, Mason, OH). For all samples, dual isotope analysis (carbon and nitrogen) was performed on a Europa Hydra 20/20 isotope ratio mass spectrometer at the University of California Stable Isotope Facility (Davis, CA). Results for each element were expressed in delta notation (δ) as parts per thousand (‰).

Statistical Analyses. In total, 779 samples were submitted to the University of California Stable Isotope Facility for analysis. Outlier analysis revealed that 41 samples had $\delta^{13}C$ and/or $\delta^{15}N$ values >2 SD from the mean. These samples were excluded from the final analysis. Thus, all analyses represent $\delta^{13}C$ and $\delta^{15}N$ values from 738 total samples. Specifically, sample size for all carbon data ($\delta^{13}C$, C for C:N, and percent C) equals 92 for *B. pilularis*, 48 for *R. californica*, 290 for *P. californica*, 170 for *T. koebelei*, 91 for *T. baccharidis*, and 47 for *Tetrastichus* sp. Sample size for nitrogen data ($\delta^{15}N$, N for C:N, and percent N) equals 93 for *B. pilularis*, 48 for *R. californica*, 289 for *P. californica*, 169 for *T. koebelei*, 90 for *T. baccharidis*, and 47 for *Tetrastichus* sp.

To calculate the magnitude of isotope enrichment from diet to consumer, we used the simple equation $\Delta\delta X = \delta X_{consumer} - \delta X_{diet}$, where X equals the isotope (^{13}C or ^{15}N) being analyzed. Standard errors of the difference (SED) between means were calculated for each $\Delta\delta X$. To assess the possible influence of diet quality (Elser et al. 2000) on carbon and nitrogen stable isotope enrichment, the C:N ratio of both possible food sources (*B. pilularis* for the midge and *R. californica* for the primary parasitoids) was calculated.

Analysis of variance (ANOVA) (PROC GLM, SAS Institute 2001) was used to test for a significant effect of taxon on the $\delta^{15}N$ and $\delta^{13}C$ signals of members of the *B. pilularis* gall community. ANOVA was run on untransformed $\delta^{15}N$ and $\delta^{13}C$ values because the untransformed data met the assumptions of the analysis. Preplanned contrasts (SAS Institute 2001) were then used to test for significant differences in both $\delta^{15}N$ and $\delta^{13}C$ between 1) *R. californica* and the gall tissue of *B. pilularis* and 2) each of the primary parasitoids and their host *R. californica.* Based on natural history data (Force 1970, Hopper 1984, Ehler and Kinsey 1991), we expected all pairwise comparisons to be significantly different at the 5% protection level, which would suggest that organisms are feeding on different trophic levels.

Literature Review. To compare the magnitude of isotope enrichment between herbivorous, predaceous, and parasitic arthropods (insects and arachnids), we conducted a review of the literature. We confined our review to laboratory or field studies where the diet of the insect consumer was controlled. To ensure that enrichment estimates from field-collected organisms were not confounded by omnivory and isotope signal mixing, we only used estimates for specialist insects with a confined feeding habit. If isotope values were only available in graphs, the graphs were first scanned before using the freeware program Scion (http://scioncorp.com) to extract isotope means for the consumer and its diet. From 15 separate studies, we assembled a total of 39 $\Delta\delta^{13}C_{consumer\text{-}diet}$ and 31 $\Delta\delta^{15}N_{consumer\text{-}diet}$ estimates (Appendix 1). Nonparametric analysis (Kruskal–Wallis test) was used to assess whether arthropod feeding habitat (herbivorous, parasitic or predaceous) has a significant effect on $\Delta\delta^{13}C_{consumer\text{-}diet}$ and/or $\Delta\delta^{15}N_{consumer\text{-}diet}$ values.

Results

Across our three study sites, there was an increase in the $\delta^{13}C$ signals from the producer to the herbivore ($\Delta\delta^{13}C_{consumer\text{-}diet} = 1.11‰$, SED = 0.25) and from the

Table 1. $\Delta\delta^{15}N_{consumer\text{-}diet} \pm$ SED and $\Delta\delta^{13}C_{consumer\text{-}diet} \pm$ SED of four primary parasitoids that attack an herbivorous midge on *B. pilularis*

Organism or citation		$\Delta\delta^{15}N_{consumer\text{-}diet} \pm$ SED	$\Delta\delta^{13}C_{consumer\text{-}diet} \pm$ SED
Consumer	Diet		
R. californica	*B. pilularis*	3.31 ± 0.36	1.11 ± 0.25
P. californica	*R. californica*	1.84 ± 0.31	0.39 ± 0.22
Tetrastichus sp.	*R. californica*	0.44 ± 0.78	1.56 ± 0.26
T. baccharidis	*R. californica*	2.12 ± 0.43	1.04 ± 0.25
T. koebelei	*R. californica*	2.27 ± 0.34	1.22 ± 0.23
Reviews		$\Delta\delta^{15}N_{consumer\text{-}diet} \pm$ SE (Range)	$\Delta\delta^{13}C_{consumer\text{-}diet} \pm$ SE (Range)
Post 2002		3.40 ± 0.13 (0.5–5.0)	0.39 ± 0.13 (−3.0 to 3.5)
Vanderklift and Ponsard (2003)		2.54 ± 0.11 (−3.2 to 5.9)	Not Determined
McCutchan et al. (2003)		2.30 ± 0.18 (−2.4 to 5.9)	0.50 ± 0.13 (−2.7 to 5.5)

For comparative purposes, mean estimates of $\Delta\delta^{15}N_{consumer\text{-}diet} \pm$ SE and $\Delta\delta^{13}C_{consumer\text{-}diet} \pm$ SE from three review papers are also reported. The range of $\Delta\delta^{15}N_{consumer\text{-}diet}$ and $\Delta\delta^{13}C_{consumer\text{-}diet}$ values for studies within these reviews is noted in parentheses.

herbivore to the primary parasitoids ($\Delta\delta^{13}C_{consumer\text{-}diet}$ = 0.82‰, SED = 0.21). In addition, there was an increase in the $\delta^{15}N$ signals of all taxa from the producer to the herbivore ($\Delta\delta^{15}N_{consumer\text{-}diet}$ = 3.31‰, SED = 0.36) and from the herbivore to the primary parasitoids ($\Delta\delta^{15}N_{consumer\text{-}diet}$ = 1.89‰, SED = 0.29).

Specifically for each taxon, the $\Delta\delta^{13}C_{consumer\text{-}diet}$ from *R. californica* to the four described primary parasitoids was 0.39‰ (SED = 0.22) for *P. californica*, 1.04‰ (SED = 0.25) for *T. baccharidis*, 1.22‰ (SED = 0.23) for *T. koebelei*, and 1.56‰ (SED = 0.26) for *Tetrastichus* sp. These values were generally much larger than the mean values reported in recent reviews (Table 1). The $\Delta\delta^{15}N_{consumer\text{-}diet}$ from *R. californica* to the four described primary parasitoids was 1.84‰ (SED = 0.31) for *P. californica*, 2.12‰ (SED = 0.43) for *T. baccharidis*, 2.27‰ (SED = 0.34) for *T. koebelei*, and 0.44‰ (SED = 0.78) for *Tetrastichus* sp. These values were comparable with those reported in recent reviews, although $\Delta\delta^{15}N_{consumer\text{-}diet}$ is somewhat lower than expected for *P. californica* and *Tetrastichus* sp. (Table 1).

In terms of the quality of primary producers versus herbivores, the proportional protein content of the *B. pilularis* was less than that of the *R. californica.* The mean (±SE) C:N of the *B. pilularis* shrub was 40.83 ± 1.28. Mean percent carbon (±SE) of *B. pilularis* was 4.02 ± 0.001. Mean percent nitrogen 0.11 ± 0.00005. The herbivorous midge *R. californica* had a mean (±SE) C:N of 7.00 ± 0.69, a mean percent carbon of 8.77 ± 1.63, and a mean percent nitrogen of 1.38 ± 0.27.

Taxon accounted for a significant amount of variation in both $\delta^{15}N$ ($F_{5,\ 729} = 54.51$; $P < 0.0001$) and $\delta^{13}C$ signals ($F_{5,\ 729} = 46.95$; $P < 0.0001$) (Fig. 1). Preplanned contrasts of $\delta^{15}N$ and $\delta^{13}C$ values from consumer–diet pairs generally agreed with natural history data that places pairs on distinct trophic levels. Of the five pairwise comparisons conducted using $\delta^{15}N$, the herbivorous midge *R. californica* was significantly distinct from its host plant *B. pilularis* ($F_{1,\ 729} = 40.20$; $P < 0.0001$) and from the primary parasitoids *P. californica* ($F_{1,\ 729} = 16.07$; $P < 0.0001$), *T. baccharidis* ($F_{1,\ 729} = 16.24$; $P < 0.0001$), and *T. koebelei* ($F_{1,\ 729} = 22.25$; $P < 0.0001$). Nitrogen isotope signals of the herbivore *R. californica* did not differ significantly from *Tetrastichus* sp. ($F_{1,\ 729} = 0.52$; $P = 0.47$). Surprisingly, discrimination among organisms that are known to occupy different trophic levels was just as good with $\delta^{13}C$ as it was with $\delta^{15}N$. Carbon stable isotope signals differed significantly between the herbivore *R. californica* and its host plant *B. pilularis* ($F_{1,\ 729} = 21.61$; $P < 0.0001$). In addition, the parasitoids *T. baccharidis* ($F_{1,\ 729} = 19.14$; $P < 0.0001$), *T. koebelei* ($F_{1,\ 729} = 30.89$; $P < 0.0001$), and *Tetrastichus* sp. ($F_{1,\ 729} = 32.41$; $P < 0.0001$) were successfully identified as feeding on trophic levels distinct from their host *R. californica.* However, carbon isotopes did not discriminate the parasitoid *P. californica* from the midge *R. californica* ($F_{1,\ 729} = 3.33$; $P = 0.07$).

Literature Review. There was a trend toward greater carbon isotope enrichment in insect parasites ($\Delta\delta^{13}C_{consumer\text{-}diet} = 1.14 \pm 0.26$) than in either insect herbivores ($\Delta\delta^{13}C_{consumer\text{-}diet} = 0.44 \pm 0.28$) or insect/arachnid predators ($\Delta\delta^{13}C_{consumer\text{-}diet} = -0.86 \pm 0.92$) (Fig. 2A). However, this trend was not significant (Kruskal–Wallis $\chi^2 = 4.05$, $P = 0.13$). Similarly, there was no significant effect of foraging guild on $\Delta\delta^{15}N_{consumer\text{-}diet}$ values (Kruskal–Wallis $\chi^2 = 0.16$, $P = 0.92$) (Fig. 2B).

Discussion

Although the mean nitrogen enrichment associated with a single trophic transfer was similar to that observed in other taxa, carbon enrichment was greater than expected in our system. As a result, carbon proved to be as powerful as nitrogen in determining trophic level for organisms in the *Baccharis* gall community. The mean enrichment in $\delta^{15}N$ for a single trophic transfer from the herbivore to a primary parasitoid ($\Delta\delta^{15}N_{consumer\text{-}diet}$ = 1.89‰, SED = 0.29) was slightly lower than expected, based on mean values reported by recent reviews (Post 2002, McCutchan et al. 2003, Vanderklift and Ponsard 2003). However, when parasitoids were examined individually rather than as a trophic group, $\Delta\delta^{15}N_{consumer\text{-}diet}$ values for the two *Torymus* species were in agreement with mean values reported in recent reviews, whereas $\Delta\delta^{15}N_{consumer\text{-}diet}$ values for *P. californica* and *Tetrastichus* sp. were somewhat lower than the mean values

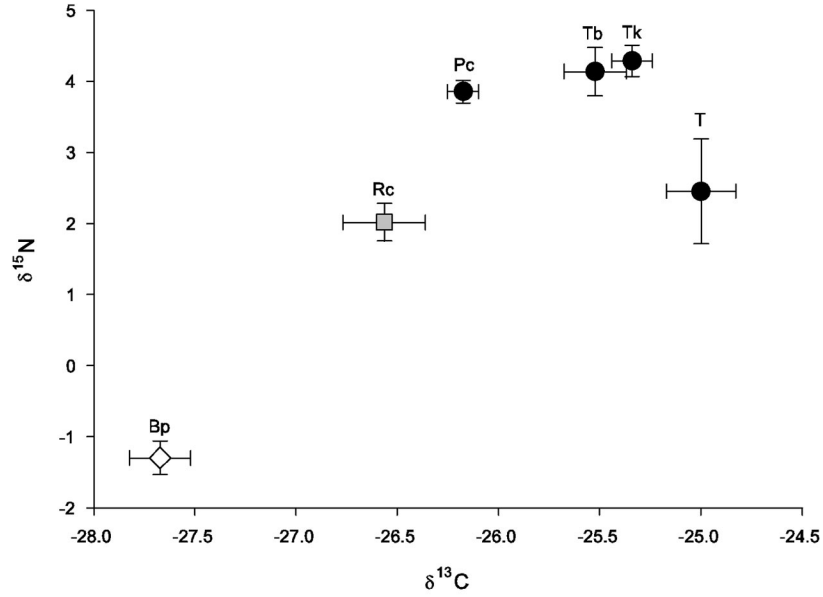

Fig. 1. $\delta^{15}N \pm SE$ and $\delta^{13}C \pm SE$ of the host plant, *B. pilularis* (Bp) and the arthropod community associated with *B. pilularis* galls. These arthropods include the herbivorous midge *R. californica* (Rc) and the primary parasitoids *T. baccharidis* (Tb), *T. koebelei* (Tk), *Tetrastichus* sp. (T), and *P. californica* (Pc).

published in the reviews (Post 2002, McCutchan et al. 2003, Vanderklift and Ponsard 2003).

For carbon isotopes, the increase in the $\delta^{13}C$ signals from the producer to the herbivore (mean $\Delta\delta^{13}C_{\text{consumer-diet}} = 1.11‰$, SED = 0.25) and from the herbivore to the primary parasitoids (mean $\Delta\delta^{13}C_{\text{consumer-diet}} = 0.82‰$, SED = 0.21, range 0.39–1.56‰) was larger than the average values reported in recent reviews (Post 2002, McCutchen et al. 2003). However, it is important to note that individual studies within these reviews exhibited a wide range of $\Delta\delta^{13}C$ values (Table 1).

Greater than expected shifts in $\delta^{13}C$ can result if all potential food sources have not been identified and the organism uses a food source that is isotopically distinct from the supposed diet. For example, shifts in $\delta^{13}C$ between 2.8 and 5.2‰ have been documented in *Drosophila mojavensis* Patterson & Crow and *Drosophila pachaea* Patterson & Wheeler in the wild (Markow et al. 2000). However, these insects are highly vagile compared with the midge and parasitoids in our study. It is possible that the enriched signals of the *Drosophila* spp. result from the utilization of multiple food sources. Previous researchers have suggested that in general, $\delta^{13}C$ shifts >1.5‰ indicate that another resource is being used by a particular consumer (Fry et al. 1978). Although one of the parasitoids in our study (*Tetrastichus* sp.) had a $\Delta\delta^{13}C_{\text{consumer-diet}} \geq 1.5‰$, its confined feeding habit minimizes the possibility of signal mixing due to the use of alternative food resources.

Recent studies suggest that increases in carbon isotope enrichment may be associated with increases in dietary protein (Pearson et al. 2003). However, increased dietary protein had no clear effect on carbon isotope enrichment in our system. Parasitoids feeding on the protein-rich midge had both higher (*Tetrastichus* sp. and *T. koebelei*) and lower (*T. baccharidis* and *P. californica*) $\Delta\delta^{13}C_{\text{consumer-diet}}$ values than the midge, which feeds on the nutritionally inferior *B. pilularis.* Furthermore, in the absence of additional studies, it is unclear whether the mean $\Delta\delta^{13}C_{\text{consumer-diet}}$ from midge to parasitoid of 0.85‰ represents a meaningful departure from expected shifts in carbon isotopes, and if so, what is the cause of the discrepancy.

Carbon isotopes have been used to determine trophic relationships in aquatic ecosystems where a common food base was assumed (McConnaughey and McRoy 1979, Gearing et al. 1984). However, that carbon was a better discriminator of trophic level than nitrogen isotopes is the first instance we are aware of in a terrestrial ecosystem. The few data points available for carbon isotope enrichment in parasitic insects suggest that our findings may not be uncommon (Petelle et al. 1979, Doucett et al. 1999, this study). In fact, analysis of 25 $\Delta\delta^{13}C_{\text{consumer-diet}}$ values from insect herbivores and seven $\Delta\delta^{13}C_{\text{consumer-diet}}$ values from insect parasites (including both strict parasites and parasitoids) reveals that parasites tend to be more enriched in carbon than their food source relative to herbivores. Although this trend is not statistically significant (Kruskal–Wallis $\chi^2 = 4.05$, $P = 0.13$; Fig. 2A) and is no doubt limited by a small sample size ($n = 7$ $\Delta\delta^{13}C_{\text{consumer-diet}}$ estimates for insect parasites), it suggests that further data collection on carbon isotope enrichment in parasitoids is warranted.

In both aquatic and terrestrial ecosystems, the use of nitrogen isotopes to determine trophic relationships has been fairly common (Kwak and Zedler 1997,

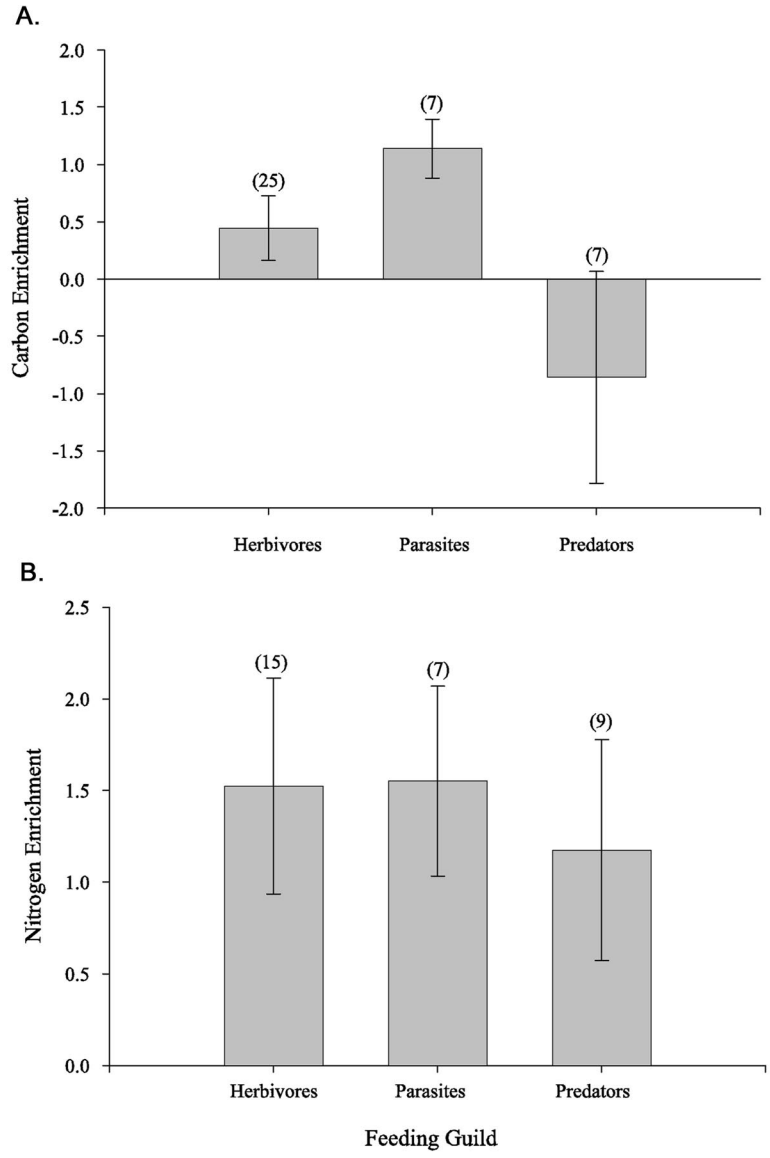

Fig. 2. Means (±SE) of herbivorous, parasitic, or predaceous insect or arachnid carbon enrichment ($\Delta\delta^{13}C_{consumer\text{-}diet}$) (A) and nitrogen enrichment ($\Delta\delta^{15}N_{consumer\text{-}diet}$) (B) estimates. Estimates of carbon and nitrogen enrichment were compiled from published studies (including this study) in which diet was either known or controlled. Numbers above each bar represent the sample size of enrichment estimates associated with each mean. There was no significant effect of foraging guild on either carbon (Kruskal–Wallis $\chi^2 = 4.05$, $P = 0.13$) or nitrogen enrichment (Kruskal–Wallis $\chi^2 = 0.16$, $P = 0.92$).

Vander Zanden and Rasmussen 1999, Herrera et al. 2003). In our system, enrichment in nitrogen isotopes was unable to distinguish the trophic level of *R. californica* relative to three of its four primary parasitoids (*P. californica*, *Tetrastichus* sp., and *T. baccharidis*). Recently, Tooker and Hanks (2004) reported similar results for the braconid parasitoid *Schizoprymnus* sp. This species attacks the beetle *Mordellistena aethiops* Smith (Coleoptera: Mordellidae). However, nitrogen isotope signals alone could not distinguish the parasitoid (feeding on the second trophic level) from its herbivorous host (Tooker and Hanks 2004). That the braconid had a nitrogen isotope signal that did not differ from its host caused the authors to question the validity of applying current stable isotope trophic transfer models (often based on data from predaceous animals) to studies of parasitoid–host interactions. The authors did not report carbon isotope values for the braconid parasitoid or its mordelid host.

This study characterized the relationship between parasitoid and midge stable isotope signals for a community of parasitoids and their herbivorous host associated with *B. pilularis* galls. The utility of stable isotope methods continues to increase as estimates of

trophic shift improve for a variety of taxa. In particular, continued studies and quantitative descriptions of carbon and nitrogen stable isotope enrichment will help to clarify trophic interactions in arthropod food webs.

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Appendix 1. Estimates of insect and arachnid $\Delta\delta^{13}C_{consumer\text{-}diet}$ and $\Delta\delta^{15}N_{consumer\text{-}diet}$, compiled from published studies or reported in this study, in which diet was either known or controlled.

Guild	Consumer	Diet	$\Delta\delta^{13}C_{consumer\text{-}diet}$	$\Delta\delta^{15}N_{consumer\text{-}diet}$	Reference
Herb	*Desmia* sp. (moth)	*Vitis vinifera* L. (grape leaves)	2.6	4.2	DeNiro and Epstein 1981
Herb	*Melanoplus sanguinipes* F. (migratory grasshopper)	*Zea mays* L. (corn seedlings)	1.6	1.7	DeNiro and Epstein 1981
Herb	*M. sanguinipes*	*Triticum aestivum* L. (wheat seedlings)	2.7	−0.8	DeNiro and Epstein 1981
Herb	*Rhopalosiphum padi* L. (biry cherry-oat aphid)	*T. aestivum*	0.2	−1.2	Oelbermann and Scheu (2002)
Herb	Aphididae	*Sorghum bicolor* L. (sorghum)	−1.1	0	Ostrom et al. (1997)
Herb	*Ceratomia catalpae* Boisdoval (catalpa sphinx)	*Catalpa bigoniodes* Walt (catalpa)	0.9		Petelle et al. (1979)
Herb	*Epilachna varivestis* Mulsant (Mexican bean beetle)	*Phaseolus lunatus* L. (bean)	−0.4		Petelle et al. (1979)
Herb	*E. varivestis*	*Solanum melongena* L. (eggplant)	0.7		Petelle et al. (1979)
Herb	*Manduca quinquemaculata* Haworth (tomato hornworm)	*Lycopersicon esculentum* Miller (tomato)	−0.6		Petelle et al. (1979)
Herb	*Murgantia histrionica* Hahn (harlequin bug)	*Brassica oleracea* L. (broccoli)	0.6		Petelle et al. (1979)
Herb	*Pseudaletia unipunctata* Haworth (armyworm)	*Z. mays*	0.1		Petelle et al. (1979)
Herb	*P. unipunctata*	*Sorghum halapense* L. (Johnson grass)	1.2		Petelle et al. (1979)
Herb	*Orchelimum fidicinium* Rehn & Hebard (tetigoniid grasshopper)	*Spartina* grass	−0.3	1.1	Peterson and Howarth (1987)
Herb	*Porthetria dispar* L. (gypsy moth)	Plant diet	−1.4	1.6	Peterson and Howarth (1987)
Herb	*Amphorophora idaei* Börner (aphid)	*Rubus idaeus* L. (raspberry)	.	2	Scrimgeour et al. (1995)
Herb	*Byturus tomentosus* DeGeer (raspberry beetle)	*R. idaeus*	.	1.4	Scrimgeour et al. (1995)
Herb	*Locusta migratoria* L. (locust)	*Z. mays*	2.8		Webb et al. (1998)
Herb	*L. migratoria*	*Triticum* sp.	−2.5	2.3	Webb et al. (1998)
Herb	*Rhopalosiphum padi* L. (aphid)	Phloem	−0.1	5.1	Yoneyama et al. (1997)
Herb	*Junonea coenia* Hübner (buckeye butterfly)	*Plantago lanceolata* L. (English plaintain)	−1.8	5.4	McCutchan et al. (2003)
Herb	*Malacosoma* sp. (tent caterpillar)	*Prunus melanocarpa* Nelson (choke cherry)	0.4	0.8	McCutchan et al. (2003)
Herb	Arctiidae	*Populus deltoides* Bartr. (cottonwood)	0.2	−0.8	McCutchan et al. (2003)
Herb	*Periphyllus* sp. (aphid)	*Capsicum frutescens* Will (Thai dragon)	0.6	−2.1	McCutchan et al. (2003)
Herb	Diaspididae	*C. frutescens*	1.0		McCutchan et al. (2003)
Herb	*Antistrophus rufus* Gilette (gall wasp)	*Silphium terebinthinaceum* Jacquin (prairie duck)		2.5	Tooker and Hanks (2004)
Herb	*Rhopalomyia californica* Felt (gall midge)	*B. pilularis* Felt (coyote bush)	1.1	3.3	This study
Herb	*Schizaphis graminum* Rondani (greenbug)	*S. bicolor*	1.1		Prasifka et al. (2004)
Herb	*Aphis gossypi* Glover (aphid)	*Gossypium hirsutum* L. (cotton)	−1.3		Prasifka et al. (2004)
Para	*Nanocladius* sp. (chironomid)	*Pteronarcys biloba* Newman (stonefly)	0.9	3.4	Doucett et al. (1999)
Para	*Apanteles* sp. (braconid wasp)	*C. catalpae*	0.4		Petelle et al. (1979)
Para	*Apanteles* sp.	*M. quinquemaculata*	2.3		Petelle et al. (1979)
Para	*Platygaster californica* Ashmead (platygasterid wasp)	*R. californica* (gall midge)	0.4	1.8	This study
Para	*Tetrastichus* sp. (eulophid wasp)	*R. californica*	1.7	0.6	This study
Para	*T. baccharidis* Huber (torymid wasp)	*R. californica*	1.0	2.4	This study
Para	*T. koebeli* Huber (torymid wasp)	*R. californica*	1.3	2.5	This study
Para	*Schizoprymnus* sp. (braconid wasp)	*Mordellistena aethiops* Smith (mordellid beetle)		0.9	Tooker and Hanks (2004)
Para	*Schizoprymnus* sp.	*M. aethiops*		−0.7	Tooker and Hanks (2004)
Pred	*Hippodamia variegata* Goeze (lady beetle)	*S. bicolor*	−1.3	2.9	Ostrom et al. (1997)
Pred	*H. variegata*	Aphids	-0.2	2.9	Ostrom et al. (1997)
Pred	*Adalia bipunctata* L. (twospotted lady beetle)	Aphids		1.2	Scrimgeour et al. (1995)
Pred	*A. bipunctata* (larva)	Aphids		0.5	Scrimgeour et al. (1995)
Pred	*Coccinella septempunctata* L. (sevenspotted lady beetle)	Aphids		1.7	Scrimgeour et al. (1995)
Pred	*Hippodamia convergens* Guérin-Méneville (convergent lady beetle)	*S. graminum*	1.1		Prasifka et al. (2004)
Pred	*Chrysoperla carnea* Stephens (lacewing)	*Drosophila melanogaster* Meigen (fruit fly)	−4.3	1.3	Patt et al. (2003)
Pred	*Pardos a. lugubris* Walckenaer (wolf spider)	*D. melanogaster*	0.4	2.2	Oelbermann and Scheu (2002)
Pred	*P. lugubris*	*Heteromurus nitidus* (Templeton)	<0.1	2.5	Oelbermann and Scheu (2002)
Pred	*P. lugubris*	*Folsomia candida* (Willem)	−4.4	−3.2	Oelbermann and Scheu (2002)
Pred	*P. lugubris*	*R. padi*	1.4	1.5	Oelbermann and Scheu (2002)

Herb, herbivore; Para, parasite; Pred, predator.