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Tracing dietary origins of aphids and the predatory beetle *Propylea japonica* in agricultural systems using stable isotope analyses

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Abstract

Tracing dietary origins of the predatory beetle *Propylea japonica* (Thunberg) (Coleoptera: Coccinellidae) aids understanding their roles in the food web and provides information to develop strategies for effective conservation in agroecosystems comprised of wheat [*Triticum aestivum* L. (Poaceae)], cotton [*Hirsutum* spp. (Malvaceae)], and maize [*Zea mays* L. (Poaceae)]. Intrinsic markers of carbon and nitrogen stable isotope ratios ($\delta^{13}C$ and $\delta^{15}N$) in *P. japonica* need to be developed to ascertain the source(s) of diet. Experiments were carried out to examine the changes of $\delta^{13}C$ and $\delta^{15}N$ among the three crops, pests (wheat, cotton, and maize aphids; all Hemiptera: Aphididae), and *P. japonica* fed on aphids of each of the three crops. Results indicated that $\delta^{13}C$ values in *P. japonica* fed on wheat, cotton, and maize aphids were −27.2 to −26.5‰, −24.2 to −23.9‰, and −11.0 to −10.7‰, respectively, whereas their $\delta^{15}N$ values were 1.1 to 2.9‰, 6.0 to 7.4‰, and −0.6 to 0.1‰, respectively. $\delta^{13}C$ and $\delta^{15}N$ plots clearly identify the three crops, the dietary origins of the aphids, and the host origins of the aphid prey consumed by the ladybird beetles, as each pathway displays a non-overlapping pattern. Based on the values of $\delta^{13}C$ and $\delta^{15}N$ of the three food webs, dietary origins can be traced in the predatory beetle *P. japonica* derived from wheat, cotton, and maize crops.

Introduction

Predatory beetles are an important component of the natural enemy complex (Landis et al., 2000), and contribute to the stability of predator–prey interactions and community organization in both natural and managed systems (Tscharntke et al., 2002). These predatory beetles fly back and forth in complex agricultural landscapes to search for food (Ostrom et al., 1997). A biological control strategy to address pest problems is the promotion of earlier or greater colonization by natural enemies through specific habitat management techniques (Landis et al., 2000; Prasifka et al., 2004). The predatory beetle *Propylea japonica* (Thunberg) (Coleoptera: Coccinellidae) is a prevalent mobile predator of aphids in wheat, cotton, and maize, and moves among these crops in agricultural systems (Ge Feng, 1995; Liu et al., 2004; Gao et al., 2010; Zhang et al., 2014). Tracing dietary origins and the movement of natural enemies aids understanding their role in the food web and provides information to develop strategies for their effective conservation or control efforts within the agricultural landscape.

Stable isotope analyses have been used to trace the nutritional source fed upon by natural enemies and then track their movement in their crop or non-crop habitat (Lubetkin & Simenstad, 2004; Schallhart et al., 2009). Stable isotopes are non-radioactive and intrinsically preserved labels that can reflect the long-term feeding behavior of animals, which favor their use in food web and diet studies (Peterson & Fry, 1987; Schmidt et al., 1999; Hood-Nowotny & Knols, 2007; Birkhofer et al., 2011). Stable isotope signa-

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tures can be used directly using natural differences in the isotopic abundances in the landscape, or in enrichment studies, where isotope-labeled supplements are added to the system. Natural abundance studies use naturally occurring differences in isotopic signatures to follow flows and processes (Hood-Nowotny & Knols, 2007; Girard et al., 2011; Mollot et al., 2012). For example, prey origins and colonization behavior of the lady beetle *Hippodamia convergens* (Guérin-Méneville) in agricultural systems were examined by analysis of carbon stable isotope values (Prasifka et al., 2004). Analysis of carbon and nitrogen stable isotope ratios ($\delta^{13}C$ and $\delta^{15}N$) was used to track dietary origins and the movement of soil-living herbivores—*Agriotes obscurus* (L.) larvae—between plots harboring isotopically different plants (Schallhart et al., 2011).

Wheat [*Triticum aestivum* L. (Poaceae)], cotton [*Gossypium* spp. (Malvaceae)], and maize [*Zea mays* L. (Poaceae)] are important crops and form the primary agricultural landscape in Northern China. Wheat, a C_3 plant, is planted in the winter and harvested in the summer. The wheat aphid complex (Hemiptera: Aphididae), including *Schizaphis graminum* Rondani, *Sitobion* (*Macrosiphum*) *avenae* Fabricius, and *Rhopalosiphum padi* L., are serious pests of wheat. Cotton, a C_3 plant, and maize, a C_4 plant, are planted in the summer and harvested in the autumn in China. Cotton aphid, *Aphis gossypii* Glover (Hemiptera: Aphididae), is a key pest of cotton (Wu & Guo, 2005). Maize aphid, *Rhopalosiphum maidis* Fitch (Hemiptera: Aphididae), is a worldwide pest of maize.

Using carbon stable isotope ratios ($\delta^{13}C$), our previous research determined dietary origins and contribution in the predatory beetle, *P. japonica*, in an agricultural landscape system composed of cotton and maize, aphids, and lady beetles (Ouyang et al., 2012). However, intrinsic markers of carbon and nitrogen stable isotope ratios ($\delta^{13}C$ and $\delta^{15}N$) in *P. japonica* remain to be elucidated to trace dietary origins and their proportions in multiple crops systems comprised of wheat, cotton, and maize. To trace food origins in *P. japonica* by stable isotope analysis, our goals were: (1) to examine $\delta^{13}C$ and $\delta^{15}N$ or N% of beetles fed on aphids grown on wheat, cotton, and maize; (2) to identify the crop (wheat, cotton, or maize); (3) to identify the crop source of the aphids; and (4) to identify the prey diet of *P. japonica*.

Materials and methods

Field sampling and collecting

Wheat, cotton, and maize were planted at the Langfang Experiment Station (39.53°N, 116.70°E) in Hebei Province, China, grown without pesticides, and watered when necessary. Aphid and crop samples were collected at the station to establish $\delta^{13}C$ and $\delta^{15}N$ of the three crops and their aphids in the field. Crop samples were cut from the upper leaves of wheat, cotton, and maize plants. Aphids were collected in groups of 50 or more by disturbing aphid colonies and collecting fallen or walking aphids with fine-point forceps. *Propylea japonica* were reared in the laboratory on either field-collected wheat, cotton, or maize aphids.

Laboratory experiment

Carbon and nitrogen stable isotope ratios of adult beetles fed on various aphid prey were examined. Eggs were placed into Petri dishes inside an environmental chamber, and 30 first instars were fed on either wheat aphids reared on wheat leaves, cotton aphids reared on cotton leaves, or maize aphids reared on maize leaves. The beetles were maintained on each diet until 14 days after reaching the adult stage. The experiment was carried out at 25 °C, L14:D10 photoperiod, and ca. 80% r.h. Adult beetles were removed, coded, placed in plastic vials, and preserved in a freezer at -20 °C pending later analyses.

Sample preparation and stable isotope analysis

All field and laboratory samples collected and stored in a freezer were washed twice in reverse-osmosis filtered water upon collection. Accurate isotope analysis usually requires homogenization by grinding larger samples of solids into a fine powder and subsampling the latter (Prasifka et al., 2004). Plant samples were large enough to require homogenization. After drying, leaf tissue was pulverized to a powder before enclosing a subsample of desired mass (2–3 mg) into a sample capsule. Aphids were separated from the plant material with a scalpel and used in groups of ca. 50 individuals from ca. 20 colonies. All of the samples were dried for 72 h at 65 °C before being weighed to an accuracy of ± 1 μg and packaged in tin sample capsules. Each adult *P. japonica* was placed in an open 2.5-ml plastic vial. The vial was then dried, capped, labeled, and stored. Of each sample, $\delta^{13}C$, $\delta^{15}N$, and N% were determined at the Chinese Academy of Forestry's Stable Isotope Laboratory via a combustion–gas chromatography–mass spectrometry process. Stable isotope measurements were performed using an elemental analyzer (Flash EA1112 HT), and were made on a modified Finnigan MAT Delta V advantage isotope ratio mass spectrometer (Thermo Fisher Scientific, Waltham, MA, USA). Data were normalized using internationally accepted isotope reference standards (Pee Dee Belemnite for carbon and atmospheric N_2 for nitrogen) in parts per thousand (‰) according to the following equation: $\delta X = [(R_{sample}/R_{standard}) - 1] \times 1\ 000$, where X is ^{13}C or

^{15}N and R_{sample} and $R_{standard}$ are the ratios of ^{13}C/^{12}C or ^{15}N/^{14}N for an individual sample and the analytical standard.

Statistical analysis

All statistical analyses were conducted using SPSS v. 17, 2008 (SPSS, Chicago, IL, USA). One-way ANOVA followed by least significant difference (LSD) post-hoc tests were used to assess differences in δ^{13}C, δ^{15}N, and N% among trophic levels in each of the three food chains and among the three crops, pest insects (aphids from wheat, cotton, or maize), and lady beetles fed on either of these aphids.

Results

Carbon stable isotope ratios and differences among food chains

Analysis of stable isotope ratios of δ^{13}C found no significant differences in the wheat-based food chain ($F_{2,12}$ = 0.136, P = 0.87; Figure 1, Table 1). Although significant differences were observed for the δ^{13}C values in the cotton-based food chain ($F_{2,32}$ = 61.707, $P<0.0001$) and in the maize-based food chain ($F_{2,11}$ = 9.656, P = 0.0038), mean differences, or isotopic shifts, between trophic levels in these food chains were no more than 2.2‰ (viz., shift of δ^{13}C value from cotton aphid-fed lady beetle to cotton aphids; Table 1).

The δ^{13}C values differed among crops ($F_{2,15}$ = 267.617, $P<0.0001$); δ^{13}C of maize was higher than that of cotton and wheat (LSD tests: both $P<0.0001$). The δ^{13}C values also differed among aphids ($F_{2,22}$ = 2 476.90, $P<0.0001$), being higher for maize aphid than for cotton and wheat aphids (LSD tests: both $P<0.0001$), and among lady beetles ($F_{2,18}$ = 6 812.45, $P<0.0001$), being higher for maize aphid-fed than for cotton or wheat aphid-fed beetles (LSD tests: $P<0.0001$) (Figure 1, Table 1).

Nitrogen stable isotope ratios and differences among food chains

Analysis of stable isotopes ratios of δ^{15}N indicated significant differences in the wheat-based food chain ($F_{2,12}$ = 66.108, $P<0.0001$; Figure 2, Table 1). δ^{15}N of wheat aphid-fed lady beetles was higher than that of wheat aphids (LSD test: P = 0.010), and that of wheat aphids was higher than that of wheat (LSD test: $P<0.0001$). Mean differences of δ^{15}N values were 5.0‰ (isotopic shift from wheat aphid-fed beetles to wheat aphids) and 2.2‰ (shift from wheat aphid to wheat). Similarly, differences were also observed for δ^{15}N values in the cotton-based food chain ($F_{2,12}$ = 87.029, $P<0.0001$). δ^{15}N of cotton aphid-fed beetles was higher than that of cotton aphids (LSD test: $P<0.0001$). Mean differences of δ^{15}N values were 6.5‰ (shift from cotton aphid-fed beetles to cotton aphids) and 1.8‰ (shift from cotton aphid to cotton). In the maize-based food chain, the δ^{15}N value of beetles was significantly higher than that of maize (LSD test: P = 0.042) (Figure 2, Table 1).

The δ^{15}N values differed among crops ($F_{2,6}$ = 26.714, P = 0.0010); δ^{15}N of wheat was lower than that of maize (LSD test: P = 0.0017) and of cotton (LSD test: P = 0.0004). δ^{15}N values also differed among the three aphid types ($F_{2,11}$ = 9.298, P = 0.0043). δ^{15}N of wheat aphid was significantly lower than that of cotton aphid (LSD test: P = 0.0020) and maize aphid (LSD test: $P<0.012$). Similarly, δ^{15}N differed among aphid-fed beetles ($F_{2,17}$ = 133.379, $P<0.0001$), being lower for beetles fed on maize aphids than for beetles fed on cotton aphids (LSD test: $P<0.0001$) or wheat aphids (LSD test: P = 0.0001).

Nitrogen percentage and differences among food chains

Nitrogen percentage differed in each of the food chains (wheat: $F_{2,9}$ = 128.491; cotton: $F_{2,32}$ = 67.711; maize: $F_{2,10}$ = 29.166, $P \leq 0.0001$; Figure 3, Table 1). Mean differ-

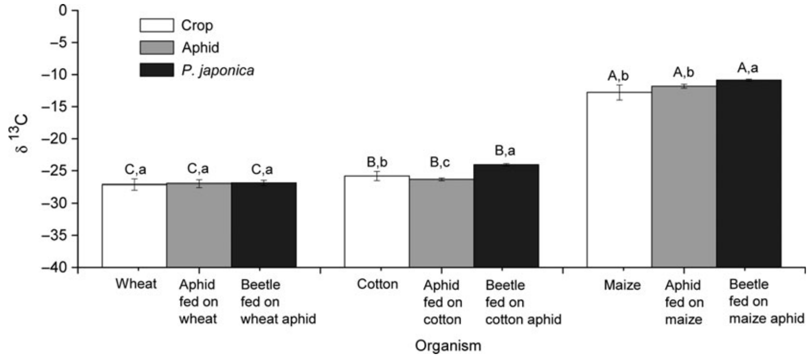

Figure 1 Mean (± SD) carbon stable isotope ratios (δ^{13}C) at the various trophic levels of three food chains, consisting of crops (wheat, cotton, and maize), aphids from each of the crops, and the aphid-feeding lady beetle *Propylea japonica*. Means within a food chain capped with different lower case letters (comparing trophic levels), and means within a trophic level capped with different upper case letters (comparing food chains), are significantly different (LSD tests: $P<0.05$).

Table 1 Mean (± SD) carbon and nitrogen stable isotope ratios ($\delta^{13}C$, $\delta^{15}N$, and N%) and isotope ratio shifts ($\Delta\delta^{13}C$, $\Delta\delta^{15}N$, and ΔN%) among trophic levels of three food chains, consisting of crops (wheat, cotton, and maize), aphids from each of the crops, and the aphid-feeding lady beetle *Propylea japonica*

	Organism	$\delta^{13}C$ (‰)	n	$\Delta\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)	n	$\Delta\delta^{15}N$ (‰)	N%	n	ΔN%
Wheat-based food chain	Wheat	−27.1 ± 0.9	3		−5.2 ± 0.6	3		1.5 ± 0.2	3	
	Wheat aphid	−27.0 ± 0.6	6		−3.0 ± 1.0	6		3.8 ± 0.3	3	
	P. japonica	−26.9 ± 0.4	6		2.0 ± 0.9	6		9.8 ± 0.9	6	
	Wheat aphid – wheat			0.1			2.2			2.3
	P. japonica – wheat aphid			0.1			5.0			6.0
	P. japonica – wheat			0.2			7.2			8.3
Cotton-based food chain	Cotton	−25.8 ± 0.7	12		−0.8 ± 0.8	12		4.4 ± 1.5	12	
	Cotton aphid	−26.3 ± 0.2	15		0.2 ± 1.3	4		5.9 ± 1.4	15	
	P. japonica	−24.1 ± 0.2	8		6.7 ± 0.9	8		11.4 ± 0.7	8	
	Cotton aphid – cotton			−0.5			1.8			1.5
	P. japonica – cotton aphid			2.2			6.5			5.5
	P. japonica – cotton			1.7			8.3			7.0
Maize-based food chain	Maize	−12.8 ± 1.2	3		−1.8 ± 0.3	3		2.8 ± 1.1	3	
	Maize aphid	−11.8 ± 0.3	4		−0.6 ± 1.3	4		4.8 ± 0.8	4	
	P. japonica	−10.9 ± 0.2	14		−0.3 ± 0.3	6		8.5 ± 1.0	6	
	Maize aphid – maize			1.0			1.2			2.0
	P. japonica – maize aphid			0.9			0.3			3.7
	P. japonica – maize			1.9			1.5			5.7

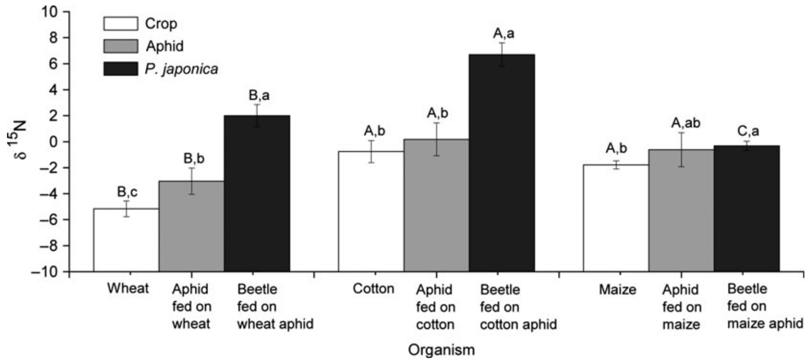

Figure 2 Mean (± SD) nitrogen stable isotope ratios ($\delta^{15}N$) at the various trophic levels of three food chains, consisting of crops (wheat, cotton, and maize), aphids from each of the crops, and the aphid-feeding lady beetle *Propylea japonica*. Means within a food chain capped with different lower case letters (comparing trophic levels), and means within a trophic level capped with different upper case letters (comparing food chains), are significantly different (LSD tests: $P<0.05$).

ences of N% in the first food chain were 6.0% (isotopic shift from wheat aphid-fed lady beetle to wheat aphid) and 2.3% (shift from wheat aphid to wheat), whereas in the second food chain, they were 5.5% (shift from cotton aphid-fed lady beetle to cotton aphid) and 1.5% (shift from cotton aphid to cotton), and in the third food chain, they were 3.7% (shift from maize aphid-fed lady beetle to maize aphid) and 2.0% (shift from maize aphid to maize) (Table 1). Differences of N% were seen among crops ($F_{2,15} = 5.685$, $P = 0.015$), among aphids ($F_{2,19} = 3.739$, $P = 0.043$), and among lady beetles ($F_{2,17} = 16.528$, $P = 0.0001$; Figure 3, Table 1).

Differentiation among crops, aphids, and natural enemies based on $\delta^{15}N$, $\delta^{13}C$, and N%

Based on the non-overlapping 95% confidence intervals of $\delta^{15}N$ vs. $\delta^{13}C$ values (Figure 4) and of N% vs. $\delta^{13}C$ values (Figure 5), differentiation could be made among the three crops, the three aphid types, and the three lady beetle lines (Table 2).

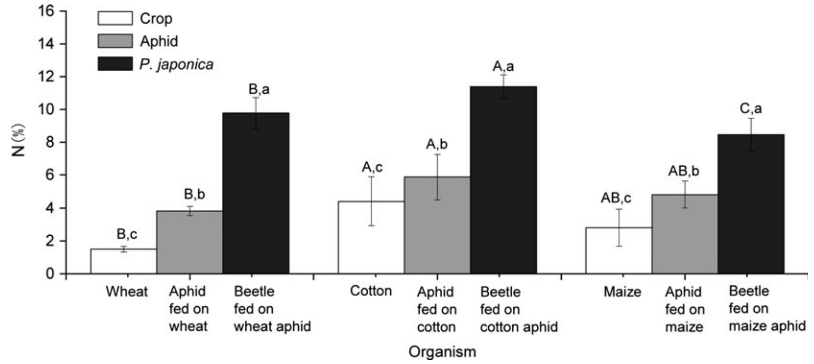

Figure 3 Mean (± SD) nitrogen percentage at the various trophic levels of three food chains, consisting of crops (wheat, cotton, and maize), aphids from each of the crops, and the aphid-feeding lady beetle *Propylea japonica*. Means within a food chain capped with different lower case letters (comparing trophic levels), and means within a trophic level capped with different upper case letters (comparing food chains), are significantly different (LSD tests: $P<0.05$).

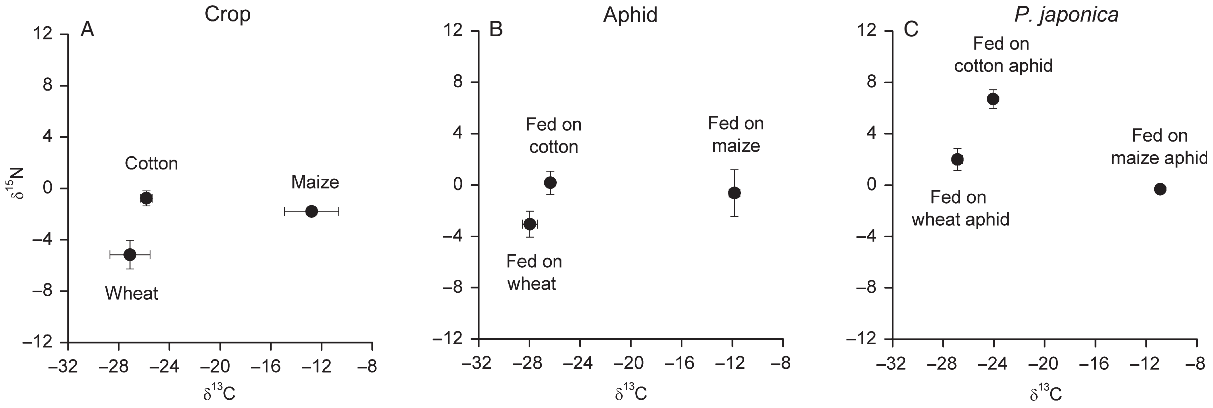

Figure 4 Mean (± 95% confidence intervals) δ^{15}N vs. δ^{13}C values of (A) crops (wheat, cotton, maize), (B) aphids from each of the crops, and (C) the lady beetle *Propylea japonica* feeding on aphids from each of the crops.

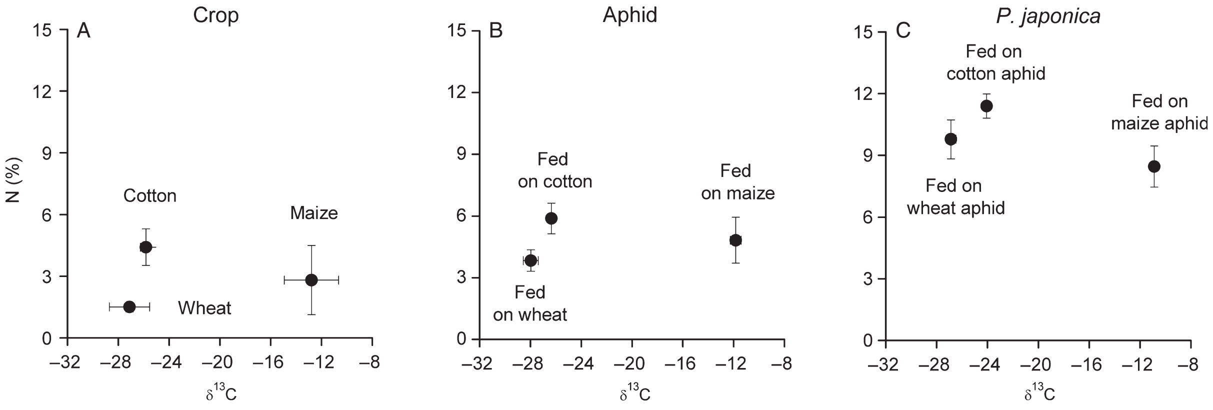

Figure 5 Mean (± 95% confidence intervals) nitrogen percentage vs. δ^{13}C values of (A) crops (wheat, cotton, maize), (B) aphids from each of the crops, and (C) the lady beetle *Propylea japonica* feeding on aphids from each of the crops.

Table 2 The 95% confidence intervals of δ^{15}N, δ^{13}C, and nitrogen percentage in crops, aphids, and *Propylea japonica*

	δ^{13}C (‰)	δ^{15}N (‰)	N (%)
Crop			
Wheat	−28.7 to −25.5	−6.3 to −4.0	1.3–1.7
Cotton	−26.3 to −25.4	−1.3 to −0.2	3.5–5.3
Maize	−14.9 to −10.6	−2.3 to −1.2	1.1–4.5
Aphid			
Wheat aphid	−28.6 to −27.4	−4.0 to −2.0	3.3–4.4
Cotton aphid	−26.5 to −26.2	−0.7 to 1.1	5.1–6.6
Maize aphid	−12.3 to −11.4	−2.4 to 1.2	3.7–5.9
P. japonica			
Fed on wheat aphid	−27.2 to −26.5	1.1 to 2.9	8.8–10.7
Fed on cotton aphid	−24.2 to −23.9	6.0 to 7.4	10.8–12.0
Fed on maize aphid	−11.0 to −10.7	−0.6 to 0.1	7.5–9.5

Discussion

δ^{13}C value among trophic levels in food chains

Isotopes in organisms reflect what a particular individual has consumed and their analysis can offer information about trophic relationships in a food web (Hood-Nowotny & Knols, 2007). Isotopic shifts for carbon between trophic levels were reported to range from −2.7 to +3.3‰ (Sagers & Goggin, 2007). The isotopic shifts in δ^{13}C values between trophic levels found in this study are within this range. Values of δ^{13}C in crops are transferred with little distortion to aphids and to the predator *P. japonica* that consume these plant-feeding pests. Consumers are usually similar to their hosts for δ^{13}C (Deniro & Epstein, 1978). This suggests that the δ^{13}C values can differentiate maize aphids from wheat aphids and cotton aphids, because maize aphids originated from maize with a C_4 form of photosynthesis, whereas wheat and cotton aphids came from C_3 hosts. Likewise, the δ^{13}C values can differentiate *P. japonica* fed on maize aphids from those fed on wheat or cotton aphids. Similar results were obtained in a laboratory experiment, where δ^{13}C values could differentiate aphids fed on sorghum (C_4) from aphids fed on alfalfa (C_3), as well as lady beetles—*Hippodamia variegata* (Goeze)—fed on sorghum aphids from beetles fed on alfalfa aphids (Ostrom et al., 1997). Based on their δ^{13}C values, the C_3 dietary substrates wheat and cotton cannot differentiate wheat aphid from cotton aphid, nor *P. japonica* fed on wheat aphids from beetles fed on cotton aphids.

δ^{15}N and N% values among trophic levels in food chains

Variability in the degree of enrichment between trophic levels has been reported to range from −2.1 to +5.4‰ for nitrogen (Sagers & Goggin, 2007). In our study, $\Delta\delta^{15}$N values between trophic levels were beyond this range. For instance, mean differences of $\Delta\delta^{15}$N were 2.2‰ (wheat aphids to wheat), 5.0‰ (*P. japonica* to wheat aphids), 1.8‰ (cotton aphids to cotton), 6.5‰ (*P. japonica* to cotton aphids), −4.2‰ (maize aphids to maize), and 0.3‰ (*P. japonica* to maize aphids). Our study showed that aphids from wheat, cotton, and maize were enriched in ^{15}N relative to their diets. Aphids feed on plant sap and they have often been reported to demonstrate large, little, or no enrichment or even depletion in ^{15}N relative to what they have eaten (Scrimgeour et al., 1995; McCutchan et al., 2003; Sagers & Goggin, 2007; Schumacher & Platner, 2009). Trophic depletion in fluid-feeder/host systems may be due to isotopic separation of aphid and host, depending on nitrogen availability; aphid colonization alters host nitrogen metabolism and subsequently affects the host nitrogen stable isotope signature (Wilson et al., 2011). However, the mechanism governing stable isotopic trophic differences between the $\Delta\delta^{15}$N values of *P. japonica* adults and aphids, and between aphids and crops, remains unclear. This study indicated that nitrogen percentage in all the consumers was higher than in their diets. This may be explained by protein synthesis of consumers, resulting from increased protein intake from the diet (Ostrom et al., 1997).

Distinguishing crops, aphids, and lady beetles based on δ^{13}C, δ^{15}N, and N%

Analysis of carbon and nitrogen isotope ratios can reveal distinct differentiation among host diets (Hood-Nowotny & Knols, 2007). For example, four host plants of *Drosophila* spec. can be distinguished using isotopic signals (Markow et al., 2000). The food source of the lady beetle *Coleomegilla maculata lengi* (Timberlake) collected from an agroecosystem was determined by carbon and nitrogen isotope ratios (Ostrom et al., 1997). In this study, isotope signatures were clearly different among wheat, cotton, and maize, segregating into a non-overlapping pattern on plots of $\delta^{13}C$ vs. $\delta^{15}N$ and $\delta^{13}C$ vs. N%. These distinctive $\delta^{13}C$ values in crops then transfer to aphids following the food chain, with little fractionation, and further to lady beetles. Simultaneously, different $\delta^{15}N$ values or N% values in diets pass on to the consumers with isotopic shifts among trophic levels.

Quantifying contribution of diets of *Propylaea japonica* using mass balance equations

Using carbon stable isotope ratios ($\delta^{13}C$), our previous research showed that 80–100% of the diet of *P. japonica* adults in maize originated from a C_3-based resource in June, July, and August, whereas ca. 80% of the diet originated from a C_4-based resource in September in agricultural ecosystem composed of cotton and maize (Ouyang et al., 2012). Some studies rely on two isotopes ($\delta^{13}C$ and $\delta^{15}N$); for example, Ostrom et al. (1997) analyzed C and N stable isotope ratios of the predatory lady beetle *C. maculata lengi* collected from an agroecosystem and estimated the relative amount of C and N derived from agricultural plants including alfalfa, wheat, and maize. By integrating $\delta^{13}C$ and $\delta^{15}N$ values of laboratory experiments in our study with mass balance equations derived from the field with multiple crops, comprising wheat, cotton, and maize, the dietary origin and their proportions to the predatory lady beetle *P. japonica* can be traced. Based on the values of $\delta^{13}C$ and $\delta^{15}N$ of the three food chains and the mass balance equations (Appendix 1), the relative quantity of C or N originating from wheat, cotton, or maize to the food web of *P. japonica* can be ascertained in the field in a further study. Such results can offer quantifying methods for habitat management of natural enemies in agricultural systems.

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Appendix 1
The mass balance equations

$$\delta^{13}C_p = f_w\delta^{13}C_w + f_c\delta^{13}C_c + f_m\delta^{13}C_m \text{ or}$$
$$\delta^{13}C_p = \delta^{13}C_{Pjap} - \Delta\delta^{13}C$$

$$\delta^{15}N_p = f_w\delta^{15}N_w + f_c\delta^{15}N_c + f_m\delta^{15}N_m \text{ or}$$
$$\delta^{15}N_p = \delta^{15}N_{Pjap} - \Delta\delta^{15}N$$

where f_w = fractional contribution of C or N from wheat to the diet of *P. japonica*; f_c = fractional contribution of C or N from cotton to the diet of *P. japonica*; f_m = fractional contribution of C or N from maize to the diet of *P. japonica* ($fw + fc + fm = 1$); $\delta^{13}C_w = \delta^{13}C$ of wheat, $\delta^{15}N_w = \delta^{15}N$ of wheat; $\delta^{13}C_c = \delta^{13}C$ of cotton, $\delta^{15}N_c = \delta^{15}N$ of cotton; $\delta^{13}C_m = \delta^{13}C$ of maize, $\delta^{15}N_m = \delta^{15}N$ of maize; $\delta^{13}C_p$ = the average $\delta^{13}C$ value of the mixture of plants that provide C to the base of the food web of *P. japonica* weighted by the fractional contribution of each plant; $\delta^{15}N_p$ = the average $\delta^{15}N$ value of the mixture of plants that provide N to the base of the food web of *P. japonica* weighted by the fractional contribution of each plant; $\delta^{13}C_{Pjap}$ = the $\delta^{13}C$ value of *P. japonica*; $\delta^{15}N_{Pjap}$ = the $\delta^{15}N$ value of *P. japonica*; $\Delta\delta^{13}C$ = estimate of the C isotopic effect between *P. japonica* and $\delta^{13}C_p$; $\Delta\delta^{15}N$ = estimate of the N isotopic effect between *P. japonica* and $\delta^{15}N_p$