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# Tracing prey origins, proportions and feeding periods for predatory beetles from agricultural systems using carbon and nitrogen stable isotope analyses

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## Abstract

Predatory beetles are an important component of the natural enemy complex that preys on insect pests such as aphids within agroecosystems. Tracing diet origins and movement of natural enemies aids understanding their role in the food web and informs strategies for their effective conservation. Field sampling and laboratory experiments were carried out to examine the changes of carbon and nitrogen stable isotope ratios ($\delta^{13}$C and $\delta^{15}$N) among crops (cotton and maize), pests (cotton and maize aphids), and between wing and abdomen of predatory beetles, *Propylea japonica*, and to test the hypothesis that prey origins, proportions and feeding periods of the predatory beetles can be deduced by this stable isotope analysis. Results showed that the $\delta^{13}$C values both in wing and abdomen of adult *P. japonica* were changing from a $C_3$- to a $C_4$-based diet of aphids reared on maize or cotton, respectively; the isotope ratio of their new $C_4$ substrates were detectable within seven days and the $\delta^{15}$N values began to reflect their new $C_4$ substrates within three days. The relationship between $\delta^{13}$C or $\delta^{15}$N values of *P. japonica* adults in wing or abdomen and diets of aphids from a $C_3$-based resource transitioning to a $C_4$-based resource were described best in linear or quadratic equations. Results suggest that integrative analysis of $\delta^{13}$C and $\delta^{15}$N values can be regarded as a useful method for quantifying to trace prey origins, proportions of diets and feeding periods of natural enemies. The results can provide quantifying techniques for habitat management of natural enemies.


**Keywords:** aphid; agricultural landscape; carbon stable isotope; nitrogen stable isotope; predatory beetles; *Propylea japonica*;

## 1. Introduction

Predatory beetles as an important component of the natural enemy complex play great roles in regulating and controlling pest insect populations such as aphids in agroecosystems. Tracing diet origins and migration or movement of natural enemies, represents a fundamental aspect for their effective conservation and a precondition for their biological control (Hobson, 1999; Hood-Nowotny and Knols, 2007). Methodologies for determining the nutritional source fed upon by a herbivore or predator include direct observation of feeding insects (Petelle et al., 1979), gut content analysis (Isely and Alexander, 1949; Marples, 1966), antigen-antibody reaction measurement (Dempster, 1960), radioisotope (Marples, 1966) or biological pigment tracer studies (Putman, 1965) and intrinsic markers (such as naturally occurring stable isotopes, molecular DNA and fatty acid profiles) in animal tissues (Hobson, 1999). Stable isotope analyses are safe since they are non-radioactive, and they can reflect the long-term feeding behavior of animals, which make them useful natural tracers (Hood-Nowotny and Knols, 2007; Peterson and Fry, 1987; Schmidt et al., 1999).

Carbon or nitrogen stable isotope ratios ($\delta^{13}C$ or $\delta^{15}N$) are commonly applied in stable isotope analysis. Determinations of $\delta^{13}C$ and $\delta^{15}N$ values in animals and their diet substrates are usually used as a mark to ascertain their position in the food webs in aquatic and terrestrial systems (Angerbjorn et al., 1994; Colborne and Robinson, 2013; Gratton and Forbes, 2006; Schoeninger and Deniro, 1984). For example, Ostrom et al. (1997) determined carbon and nitrogen stable isotope ratios in organisms of a predatory ladybird beetle, *Hippodamia variegata* (Goeze), and quantified pathways of energy flow within agroecosystems. Our previous research documented variations in stable carbon isotope ratios ($\delta^{13}C$) among crops (cotton and maize), pests (cotton and maize aphids) and the predatory beetle, *Propylea japonica* (Thunberg), in an agricultural landscape system composed of cotton and maize/aphids/lady beetles (Ouyang et al., 2012). Variations in nitrogen stable isotope ratios ($\delta^{15}N$) in *P. japonica* adults and $\delta^{13}C$ and $\delta^{15}N$ ratios in their wing or abdomen tissues remain to be elucidated.

Cotton, a $C_3$ plant and maize, a $C_4$ plant are important crops in Northern China (Ge Feng, 1995). Cotton aphid, *Aphis gossypii* (Glover), is a serious pest of cotton. Maize aphid, *Rhopalosiphum maidis* (Fitch), is a key pest of maize. We hypothesize that diet origins, proportions and prey time of a predatory beetle, *Propylea japonica* can be traced by stable isotope analysis in agricultural systems composed of cotton and maize/aphids/lady beetles. In order to test this hypothesis, field sampling and laboratory experiments were carried out to examine changes of carbon and nitrogen stable isotope ratios among crops (cotton and maize), pests (cotton and maize aphids), and wing and abdomen tissues of *P. japonica* in this study. Our goals were:

1). to quantify differences in $\delta^{13}C$ and $\delta^{15}N$ values in wing and abdomen of predatory beetles fed on $C_3$ and $C_4$-based substrates, 2). to detect their rates of change after a shift in the isotopic composition of the predators diet, or turnover time (time required to completely exchange the C or N of an organism), 3). to assess the effect of dietary sources on the $\delta^{13}C$ and $\delta^{15}N$ values in wing and abdomen of predatory beetles, 4). to determine the relationships between the $\delta^{13}C$ or $\delta^{15}N$ values in wing and abdomen of predatory beetles and dietary sources of aphids from $C_3$ and $C_4$-based substrates.

## 2. Materials and Methods

### *2.1 Dietary shift experiment*

To quantify the $\delta^{13}C$ and $\delta^{15}N$ values of predatory beetles and detect their rates of change or turnover time after a shift of diets, larval beetles were fed on cotton aphids, and after emergence adult beetles were fed on maize aphids. Mature predatory beetles of parental generation were catched from crops in the field. Offsprings laid by mature predatory beetles were put into Petri dishes inside an environmental cabinet, and eighty 1st-instar larvae were fed on cotton aphids, reared on cotton leaves, until pupation occurred under the control conditions: 25 °C with relative humidity of ~80% and a photoperiod of L:D = 14:10. Once emergence, adult predatory beetles of offspring (n=6) was first sampled, removed, labeled, kept starve for three days, placed in plastic vials containing 95% ethanol for ten minutes to clear excrement, dried for 72 h at 65 °C and stored in a freezer for preservation to serve as control samples. The remaining adult beetles were changed to another diet of maize aphids that were reared on maize leaves in Petri dishes for 21 days. Subsamples of the remaining adult beetles (n=6) were sampled on 1, 3, 5, 7, 14 and 21 days after the diet was shifted to maize aphids. Procedures of preservation for these subsamples were same as control samples. To establish the $\delta^{13}C$ and $\delta^{15}N$ values of plants and aphids in the field, plant and aphid samples were also collected by referring to the methods of Ouyang et al. (2012). Ten plant samples of single individuals were cut from the upper leaves of cotton and maize plants. Plant samples were collected, labeled, cleared with distilled water, dried for 72 h at 65 °C and stored in a freezer for preservation and analysis. Ten samples of aphids were collected in groups of 20 or more individuals. Aphid samples were collected, labeled, dried for 72 h at 65 °C and stored in a freezer for preservation and analysis (Prasifka et al., 2004).

***2.2 Dietary proportion experiment***

Larval and adult predatory beetles were fed on a mixed diet of cotton and maize aphids in changing proportions to assess the influence on the $\delta^{13}C$ or $\delta^{15}N$ values. Five groups of predatory beetles were developed from eggs to adults on diets made up of five diverse proportions of cotton and maize aphids. According to the weight ratio of cotton to maize aphids, five diets were set with the following proportions: 100:0, 75:25, 50:50, 25:75 and 0:100. Each group composed of ~20 1st-instar larval beetles, each put into a Petri dish inside an environmental cabinet. The mixed diet for each predatory beetle was inspected every day, and a new diet of aphids were added after the old diet had been completely eaten up. The predatory beetles were raised in their respective treatments for 20 days. Samples of mature adult beetles from the five groups were sampled, labeled, kept starve for three days, placed in plastic vials containing 95% ethanol for ten minutes to clear excrement, dried for 72 h at 65 °C and stored in a freezer. Ten single individuals per test group were prepared for analysis.

***2.3 Stable isotope determination***

All samples, which were collected in field or laboratory stored in a freezer for analysis of stable isotope. The wing and abdomen of each adult beetle were clipped and respectively placed in a plastic vial. The vials with samples were then dried, capped and stored. Aphids were sampled from cotton and maize and respectively, collected in groups of 20 or more by disturbing aphid colonies with fine point forceps and placed in a plastic vial. Each plant sample of cotton or maize was large enough to demand homogenization. All samples of adult beetle, aphids and leaf tissue were pulverized to a powder, and then enclosed a subsample of desired mass (2-3 mg) into a sample capsule. After dried for 72 h at 65 °C, all of the samples were weighed to an accuracy of ±1 μg and packaged in tin sample capsules.

Carbon and nitrogen stable isotope ratios of the samples were determined at Stable Isotope Laboratory of the Chinese Academy of Forestry in Beijing of China, via a combustion-gas chromatography-mass spectrometry process. Stable isotope measurements were performed using an elemental analyser (Flash EA1112 HT, Thermo Finnigan, USA), and were made on a Finnigan MAT (Thermo Fisher Scientific, Inc., USA) Delta V advantage isotope ratio mass spectrometer. Carbon and nitrogen stable isotopes were analysed separately on duplicated subsamples. Abundances of stable isotope were showed as deviation from standards in parts per thousand (‰) (Caquet, 2006), according to the following equation:

$$\delta Z = \left[\left(R_{\text{sample}} / R_{\text{standard}}\right) - 1\right] \times 1000$$

Where $Z$ is $^{13}C$ or $^{15}N$ and the $R_{\text{sample}}$ and $R_{\text{standard}}$ are the ratios of $^{13}C/^{12}C$ or $^{15}N/^{14}N$ for the sample and the analytical standard. The repeatability of sample was less than ±0.1‰ and ±0.2‰ for carbon and nitrogen stable isotope analysis, respectively.

### *2.4 Statistical analysis*

Statistical analyses were executed using SPSS software (SPSS.17, 2008, SPSS Inc., Chicago, IL, USA). Independent samples t Test was used to assess the differences of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) stable isotope ratios in the wing and abdomen of predatory beetles, *P. japonica.* One-way analysis of variance (ANOVA) followed by LSD post hoc test was used to assess the effect of the treatments of diet proportions on the $\delta^{13}C$ and $\delta^{15}N$ values of predatory beetles in wing and abdomen. Regression analysis of linear and quadratic model were performed to determine the relationship between $\delta^{13}C$ and $\delta^{15}N$ values of adult predatory beetle in wing and abdomen and diet proportions of aphids from cotton aphids reared on cotton (a $C_3$-based resource) and maize aphids reared on maize (a $C_4$-based resource).

## 3. Results

### *3.1. Carbon and nitrogen stable isotope ratios and their differences*

The ranges of carbon stable isotope ratios ($\delta^{13}C$) were distinct between the two food chains of the $C_3$ and $C_4$-based substrates (Table 1). Based on the food chain of the $C_3$-based substrate, the $\delta^{13}C$ values of cotton, cotton aphids and the wing and abdomen of *P. japonica* fed on cotton aphids were in the range of -26.5‰ to -23.8‰. While in the food chain of $C_4$-based substrate, the $\delta^{13}C$ values of maize, maize aphids and the wing and abdomen of *P. japonica* reared on maize aphids were in the range of -13.8‰ to -10.7‰.

The $\delta^{13}C$ values in tissues of *P. japonica* adults fed on cotton aphids between wing and abdomen showed no significant differences ($t$ = 0.265, $df$ = 6, $p$ = 0.800, Table 1). While significant differences were observed for the $\delta^{13}C$ values in tissues of *P. japonica* adults fed on maize aphids between wing and abdomen ($t$ = 4.746, $df$ = 12, $p$ < 0.001, Table 1), the mean differences between their tissues was 0.4‰.

The nitrogen stable isotope ratios ($\delta^{15}N$) of cotton was significantly different from the $\delta^{15}N$ values of maize ($t$ = -3.150, $df$ = 8, $p$ = 0.014, Table 1). However, no significant differences were observed between the $\delta^{15}N$ values of cotton aphids and maize aphids ($t$ = 0.566, $df$ = 8, $p$ = 0.587, Table 1). The $\delta^{15}N$ values in the wing of *P. japonica* adults fed on cotton aphids was significantly different from the $\delta^{15}N$ values in the wing of *P. japonica* adults fed on maize aphids ($t$ = 16.788, $df$ = 5, $p$ < 0.001, Table 1). The $\delta^{15}N$ values in the abdomen of *P. japonica* adults fed on cotton aphids was significantly different from the $\delta^{15}N$ values in the abdomen of *P. japonica* adults fed on maize aphids ($t$ = 14.986, $df$ = 5, $p$ < 0.001, Table 1). *P. japonica* adults were 6.0 of 7.2‰ enriched in $^{15}N$ relative to the cotton aphid while being 0.3‰ enriched or 0.2 depleted in $^{15}N$ relative to maize aphid.

The $\delta^{15}N$ values in tissues of *P. japonica* adults fed on cotton aphids between wing and abdomen were significantly different ($t$ = -2.498, $df$ = 6, $p$ = 0.047, Table 1), and the mean variance between these tissues was 1.2‰. No significant differences were observed for the $\delta^{15}N$ values between wing and abdomen in tissues of *P. japonica* adults fed on maize aphids ($t$ = -2.410, $df$ = 4, $p$ = 0.074, Table 1).

***3.2. Effects of dietary shifting on carbon and nitrogen stable isotope ratios through***

***time***

The $\delta^{13}C$ values in the wing of *P. japonica* adults after cotton aphids was switched to maize aphids, changed from -24.0±0.2‰ in 0 day to -18.2±1.3‰ in 14 days (Fig. 1A), and the $\delta^{13}C$ values in the abdomen of *P. japonica* adults moved from -24.1±0.2‰ to -15.0±1.1‰ in 14 days (Fig. 1A). After feeding on cotton aphids exclusively for 21 days, the $\delta^{13}C$ values in the wing and abdomen of *P. japonica* adults reached -15.5±0.5‰ and -14.6±0.5‰ respectively, but were still fractionated in $^{13}C$ relative to their diet (maize aphid). The mean differences of the $\delta^{13}C$ values between the wing and abdomen of *P. japonica* adults and their diet were 3.7‰ and 2.8‰, respectively.

After emergence from pupation, *P. japonica* adults were switched from cotton to maize aphids; the $\delta^{13}C$ values between their wing and abdomen tissues were not significantly different when fed on maize aphids at day 0 ($t$ = 0.265, $df$ = 6, $p$ = 0.800), day 1 ($t$ = -0.479, $df$ = 4, $p$ = 0.657), day 3 ($t$ = -0.389, $df$ = 4, $p$ = 0.717), day 5 ($t$ = 0.5106, $df$ = 6, $p$ = 0.628), or day 7 ($t$ = 0.312, $df$ = 4, $p$ = 0.770) (Fig. 1A). The $\delta^{13}C$ values in the abdomen of *P. japonica* adults were higher than those in the wing when fed on maize aphids on day 14 ($t$ = 3.709, $df$ = 6, $p$ = 0.010), and day 21 ($t$ = 2.465, $df$ = 7, $p$ = 0.043) (Fig. 1A).

The $\delta^{15}N$ values in the wing of *P. japonica* adults changed after the original $C_3$-based diet (cotton aphids) was switched to a $C_4$-based resource (maize aphids), moving from 6.1±0.7‰ to 2.7±0.1‰ in 3 days (Fig. 1B), and the $\delta^{15}N$ values in the abdomen of *P. japonica* adults moved from 7.3±0.8‰ to 3.3±0.3‰ in 3 days (Fig.

1B). After feeding on cotton aphids exclusively for 21 days, the $\delta^{13}C$ values in the wing and abdomen of *P. japonica* adults reached 1.4±0.2‰ and 1.9±0.3‰ respectively, but were still enriched in $^{15}N$ relative to their maize aphid prey (-0.4±1.2‰).

After emergence from pupation, *P. japonica* adults were switched to a diet of maize aphids; the $\delta^{15}N$ values in the abdomen of *P. japonica* adults were higher than those in the wing, and significant differences were observed for the $\delta^{15}N$ values between abdomen and wing of *P. japonica* adults fed on maize aphids at day 0 ($t = 3.709$, $df = 6$, $p = 0.010$), and day 5 ($t = 2.465$, $df = 7$, $p = 0.043$).

***3.3. Effects of diet proportions on carbon and nitrogen stable isotope ratios***

Analysis of stable isotopes ratios of $\delta^{13}C$ and $\delta^{15}N$ found differences among *P. japonica* adults fed on different diets (Fig. 2A, B). Dietary substrate had a significant effect on the $\delta^{13}C$ values in the wing of adult beetles (one-way ANOVA, $F_{4,25} = 296.369$, $p < 0.001$, Fig. 2A). Multiple comparisons indicated that significant differences were observed for the $\delta^{13}C$ values of *P. japonica* adults fed on diets of cotton:maize aphids of 100:0 and 75:25 (LSD post hoc test, $p < 0.001$), 75:25 and 50:50 (LSD post hoc test, $p = 0.035$), 50:50 and 25:75 (LSD post hoc test, $p < 0.001$), 25:75 and 0:100 (LSD post hoc test, $p < 0.001$) ( Fig. 2A). Dietary substrate also had a significant effect on the $\delta^{15}N$ values in the wing of adult beetles (one-way ANOVA, $F_{4,15} = 127.649$, $p < 0.001$). Multiple comparisons showed that significant differences were observed for the $\delta^{15}N$ values of *P. japonica* adults fed on dietary substrates of cotton:maize aphids of 100:0 and 75:25 (LSD post hoc test, $p < 0.001$), 75:25 and

50:50 (LSD post hoc test, $p = 0.002$), 50:50 and 25:75 (LSD post hoc test, $p = 0.046$), 25:75 and 0:100 (LSD post hoc test, $p = 0.009$) ( Fig. 2A).

Dietary substrate had a significant effect on the $\delta^{13}C$ values in the abdomen of adult beetles (one-way ANOVA, $F_{4,23} = 644.977$, $p < 0.001$, Fig. 2B). Multiple comparisons indicated that significant differences were observed for the $\delta^{13}C$ values of *P. japonica* adults fed on dietary substrates of cotton:maize aphids of 100:0 and 75:25 (LSD post hoc test, $p < 0.001$), 75:25 and 50:50 (LSD post hoc test, $p < 0.001$), 50:50 and 25:75 (LSD post hoc test, $p < 0.001$), 25:75 and 0:100 (LSD post hoc test, $p < 0.001$) ( Fig. 2B). The $\delta^{15}N$ values in the wing of adult beetles were also affected by the dietary substrate (one-way ANOVA, $F_{4,15} = 127.649$, $p < 0.001$). Multiple comparisons showed that significant differences were observed for the $\delta^{15}N$ values of *P. japonica* adults fed on dietary substrates of cotton:maize aphids of 100:0 and 75:25 (LSD post hoc test, $p < 0.001$), 75:25 and 50:50 (LSD post hoc test, $p = 0.009$), 50:50 and 25:75 (LSD post hoc test, $p = 0.048$), 25:75 and 0:100 (LSD post hoc test, $p = 0.013$) ( Fig. 2B).

### *3.4. Relationship between stable isotope ratios and diet proportions*

In the dietary proportion experiment, the $\delta^{13}C$ values in the wing of *P. japonica* reared on maize:cotton aphid ratios of 0:100, 25:75, 50:50, 75:25, 100:0 were -24.0±0.2‰ to -10.9±0.2‰ (Fig. 3A). The $\delta^{13}C$ values in the abdomen of *P. japonica* reared on maize:cotton aphid ratios of 0:100, 25:75, 50:50, 75:25, 100:0 were -24.1±0.2‰ to -11.3±0.2‰ (Fig. 3A). Estimated linear and quadratic equations between $\delta^{13}C$ values of *P. japonica* adults in wing or abdomen and ratios of aphids from a $C_3$-based and a $C_4$-based substrate were listed in Table 2.

The $\delta^{15}N$ values in the wing of *P. japonica* reared on maize:cotton aphid ratios of 0:100, 25:75, 50:50, 75:25, 100:0 were 6.1±0.7‰ to -0.6±0.1‰ (Fig. 3A). And the $\delta^{15}N$ values in the abdomen of *P. japonica* reared on maize:cotton aphid ratios of 0:100, 25:75, 50:50, 75:25, 100:0 were 7.3±0.8‰ to -0.1±0.4‰ (Fig. 3B). Estimated linear and quadratic equations between $\delta^{15}N$ values of *P. japonica* adults in wing or abdomen and proportions of aphids from a $C_3$-based and a $C_4$-based substrate were also listed in Table 2.

## 4. Discussion

### *4.1. Prey origins of predatory beetles*

Carbon stable isotope ratios could discriminate $C_3$ from $C_4$ plants because their photosynthetic pathways differ in the ratio of $^{13}C/^{12}C$ in their constituent tissues (Teeri and Schoeller, 1979). The $\delta^{13}C$ values of cotton ($C_3$ plant) and maize ($C_4$ plant) in this study were -24.8±0.5‰ and -12.7±1.1‰, which were in the range of -22 to -27‰ for typical $C_3$ plants and of -9 to -14‰ for typical $C_4$ plants (Smith et al., 1976). Mean differences, or isotopic shifts of $\Delta\delta^{13}C$ based on $C_3$ substrates between trophic levels were -1.4‰ (cotton aphids to cotton), 2.2‰ (wing of *P. japonica* to cotton aphids), 2.1‰ (abdomen of *P. japonica* to cotton aphids), 0.8‰ (wing of *P. japonica* to cotton), 0.8‰ (abdomen of *P. japonica* to cotton), while isotopic shifts of $\Delta\delta^{13}C$ based on $C_4$ substrates between trophic levels were 0.9‰ (maize aphids to maize), 0.9‰ (wing of *P. japonica* to maize aphids), 0.5‰ (abdomen of *P. japonica* to maize aphids), 1.8‰ (wing of *P. japonica* to maize), 1.3‰ (abdomen of *P. japonica* to maize) (Table 1). Our study showed that the $\delta^{13}C$ values from cotton or maize aphids were a reliable indicator of their diet origin when they were reared on a single host (cotton or maize); similarly, the $\delta^{13}C$ values reflected the dietary origins of *P. japonica* fed on a single diet (cotton or maize aphids).

Nitrogen stable isotope ratios can serve as an indicator of the consumer's trophic level or position in the food web (Cabana and Rasmussen, 1994; Fry, 1988; Oelbermann and Scheu, 2002; Ponsard and Averbuch, 1999; Vander Zanden and Rasmussen, 1999; Webb et al., 1998) because typical consumers are enriched in $\delta^{15}N$ by ~2‰ to 3‰ relative to their diet (Deniro and Epstein, 1981; McCutchan et al., 2003; Schoeninger and Deniro, 1984). Our results showed that isotopic shifts of $\Delta\delta^{15}N$ based on $C_3$ substrates between trophic levels were 0.6‰ (cotton aphids to cotton), 6.0‰ (wing of *P. japonica* to cotton aphids), 7.2‰ (abdomen of *P. japonica* to cotton aphids), 6.6‰ (wing of *P. japonica* to cotton), 7.8‰ (abdomen of *P. japonica* to cotton), while isotopic shifts $\Delta\delta^{15}N$ based on $C_4$ substrates between trophic levels were -5.3‰ (maize aphids to maize), -0.2‰ (wing of *P. japonica* to maize aphids), 0.3‰ (abdomen of *P. japonica* to maize aphids), -5.5‰ (wing of *P. japonica* to maize), -5.0‰ (abdomen of *P. japonica* to maize) (Table 1). However, this study documented that cotton aphids were only 0.5‰ enriched in $^{15}N$ relative to their hosts (cotton) and even maize aphids were more than 5‰ depleted in $^{15}N$ relative to their hosts (maize). The result was similar to the green peach aphid, *Myzus persicae*, which was more than 6‰ depleted in $^{15}N$ relative to their hosts (cabbage seedlings) (Wilson et al., 2011). Aphids are plant sap-feeding insects, which have been frequently reported as showing no enrichment or even depletion in $^{15}N$ relative to their diet (McCutchan et al., 2003; Sagers and Goggin, 2007; Schumacher and Platner, 2009; Scrimgeour et al., 1995). The mechanism governing nitrogen stable isotopic trophic differences between the $\Delta\delta^{15}N$ of *P. japonica* adults to cotton aphids (6.0 or 7.2‰) and those of *P. japonica* adults to maize aphids (0.3 or -0.2‰) remains unclear.

### *4.2. Feeding period of predatory beetles*

Diet composition of carbon and nitrogen could affect diet-tissue isotopic discrimination and elemental turnover rate in consumers (Miron et al., 2006). In the dietary shift experiment, the $\delta^{13}C$ values in the wing and abdomen of *P. japonica* adults indicated that individual beetles shifting from a $C_3$- to a $C_4$-based diet of aphids fed on maize or cotton, respectively, would start to reflect the carbon stable isotope ratios of their new $C_4$ substrates within seven days. Following a fourteen day interval after the dietary shift, our results show the $\delta^{13}C$ values in the abdomen of the *P. japonica* adult were significantly higher than those in the wing, implying that the metabolic rate for carbohydrate in the abdomen occurred faster than that in the wing (Gratton and Forbes, 2006). Analogously, studies of two predacious beetles, *Harmonia axyridis* (Pallas) and *Coccinella septempunctata* L. (Coleoptera: Coccinellidae), found that the carbohydrate signature in their skeletal wing tissue changed more slowly over the same period as well (Gratton and Forbes, 2006). After 21 days there were still differences of the $\delta^{13}C$ values between the wing and abdomen of *P*. *japonica* adults and their diet, their differences may be related to sampling or preservation methods that the use of ethanol for cleaning specimens could probably influence sampling (Ponsard and Amlou, 1999; Tillberg et al., 2006). The *P. japonica* adult began to reflect the nitrogen stable isotope ratios of their new $C_4$ substrates within three days.

### *4.3. Effects of diet proportions on the $\delta^{13}C$ and $\delta^{15}N$ values of predatory beetles*

Diets with distinct isotope ratios, having nutritionally different compositions, can be used to study the effects of diet on animal isotope abundance among trophic levels and within the major tissues (Webb et al., 1998). For example, Teeri and Schoeller (1979) found that $\delta^{13}C$ values of whole body samples of red flour beetle, *Tribolium castaneum* (Herbst) are closely correlated with the $\delta^{13}C$ values of the plant carbon in its mixed diet ranging from 100% $C_4$ to 100% $C_3$ plant material. In this study, diets mixed with various proportions of aphids between a $C_3$-based resource (cotton aphids reared on cotton) and $C_4$-based resource (maize aphids reared on maize) significantly affected $\delta^{13}C$ in the wing and abdomen of the *P. japonica* adult. This is due to the $\delta^{13}C$ values of cotton being derived from the $C_3$ form of photosynthesis, whereas those of maize derive from the $C_4$ form. These distinctive $^{13}C/^{12}C$ ratios in the plant then transfer to aphids via the food chain with little further fractionation, and the distinctive $^{13}C/^{12}C$ ratios of aphids then transfer to *P. japonica*. This shows the stable carbon isotope composition of *P. japonica* is an important clue to what it has eaten. Simultaneously dietary mixtures of cotton and maize aphids significantly affected $\delta^{15}N$ in the wing and abdomen of the *P. japonica* adult.

### *4.4. Quantification of effects of aphid dietary mixtures on $\delta^{13}C$ and $\delta^{15}N$ values in the beetle predator*

Based on the dietary mixture experiment, linear and quadratic equations between $\delta^{13}C$ or $\delta^{15}N$ values in the wing or abdomen of *P. japonica* adults and dietary source of aphids from a $C_3$-based and a $C_4$-based substrate were proposed to be correlated. Linear and quadratic equations of stable isotope ratio were used to determine the relative contribution of C or N from different plants or animals based on the food web. The dietary origin of adult *P. japonica* in the field can be distinguished between $C_3$ and $C_4$ substrates from the linear or quadratic equations, and the proportion of $C_3$ and $C_4$ substrates ingested could be assessed when *P. japonica* preyed on both cotton and maize aphids within a period of approximately two weeks. Therefore, the linear or quadratic equations are recommended when determining the dietary sources and their proportional contribution from $C_3$ and $C_4$ substrates in the field.

## 5. Conclusion

Our study found the $\delta^{13}C$ values in the wing and abdomen of adult *P. japonica* were shifting from a $C_3$- to a $C_4$-based diet of aphids reared on maize or cotton, respectively, and begin to reflect the isotope ratio of their new $C_4$ substrate within seven days. The $\delta^{15}N$ values began to reflect their new $C_4$ substrate within three days. But, nitrogen stable isotope ratios, as a single indicator, may be not a suitable quantifier of the consumer's trophic level or position in the predatory beetle/cotton or maize aphid /host systems. Moreover, dietary mixtures of cotton and maize aphids significantly affected $\delta^{13}C$ and $\delta^{15}N$ in the wing or abdomen of the *P. japonica* adult. The relationship between $\delta^{13}C$ or $\delta^{15}N$ values in the wing or abdomen of *P. japonica* adults and dietary mixtures of aphids from a $C_3$-based and a $C_4$-based substrate were well presented in linear and quadratic equations. These results suggest that $\delta^{13}C$ and $\delta^{15}N$ ratios in tissues of the predatory beetles may provide a better indicator of their diet. Our results in this study suggest that aphid origins, proportions and turnover time of *P. japonica* adults can be determined in agricultural systems consisting of $C_3$ and $C_4$ crops based on integrative analysis of $\delta^{13}C$ and $\delta^{15}N$ values, which can be regarded as useful methods in quantifying to trace dietary substrates, prey origins used by natural enemies, and the predators feeding history. The results can provide quantifying techniques for habitat management of natural enemies.

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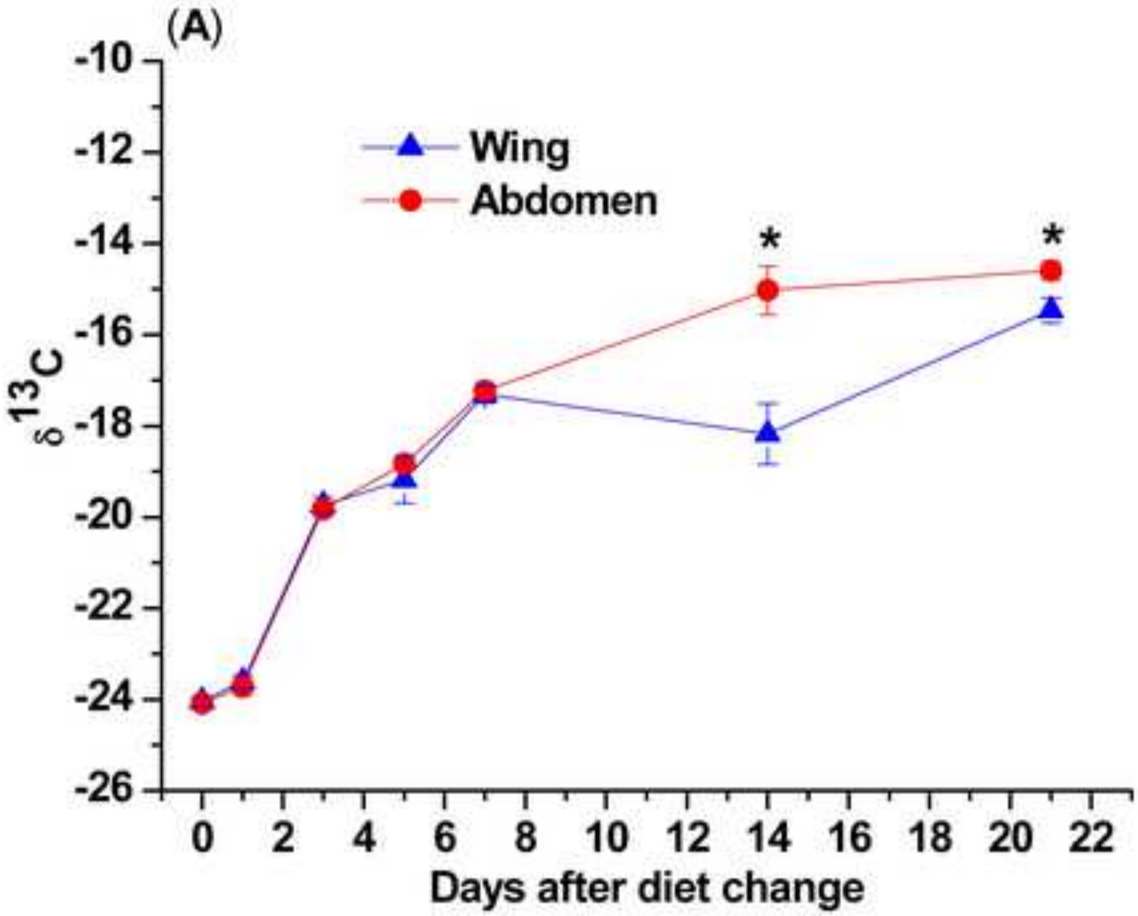
**Table 1.**

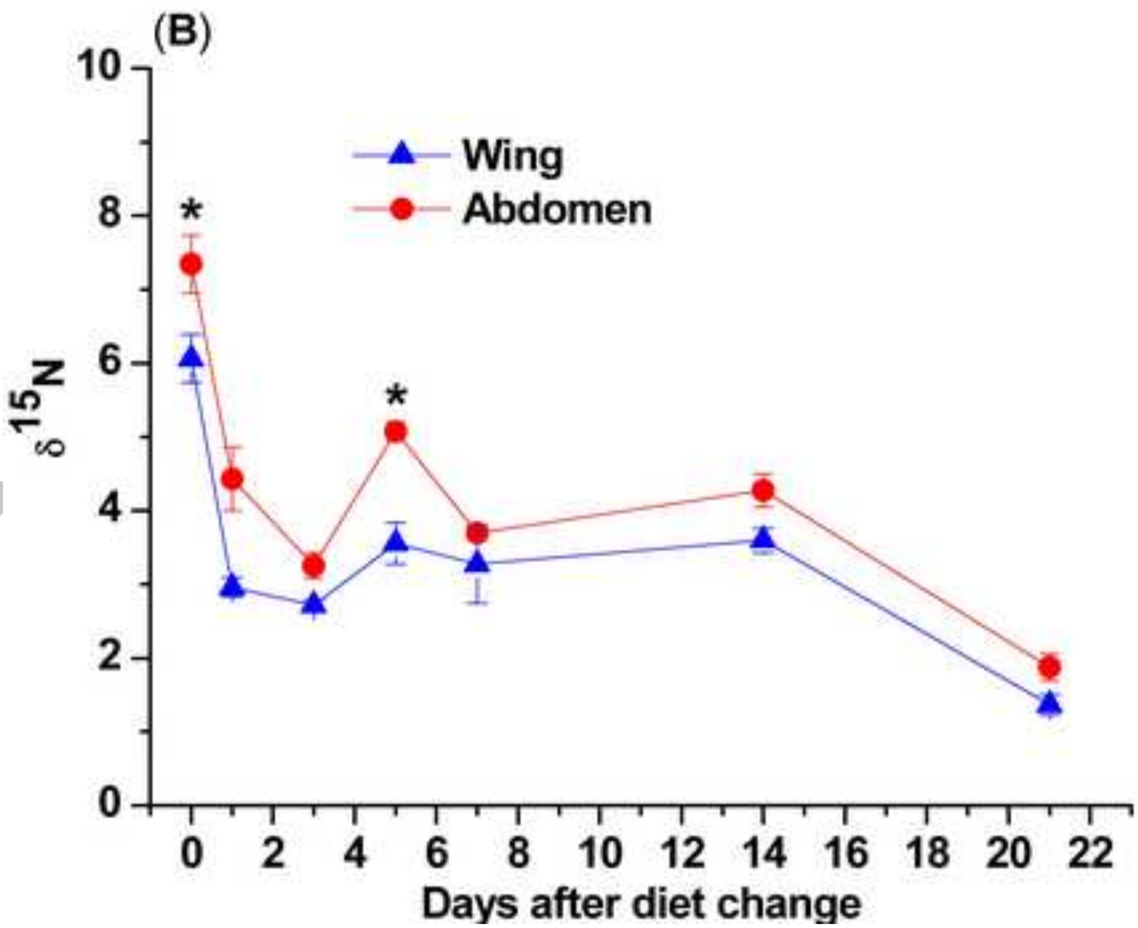
| **Organisms** | **$\delta^{13}C$±SD (‰)** | **n** | **$\Delta\delta^{13}C$ (‰)** | **$\delta^{15}N$±SD (‰)** | **n** | **$\Delta\delta^{15}N$ (‰)** |
|---|---|---|---|---|---|---|
| **$C_3$ plant** | | | | | | |
| Cotton | -24.8±0.5 | 6 | | -0.5±0.8 | 5 | |
| Cotton aphids | -26.2±0.3 | 4 | | 0.1±0.9 | 5 | |
| *P. japonica* (Wing) | -24.0±0.2 | 4 | | 6.1±0.7 | 4 | |
| *P. japonica* (Abdomen) | -24.1±0.2 | 4 | | 7.3±0.8 | 4 | |
| Cotton aphids-Cotton | | | -1.4 | | | 0.6 |
| *P. japonica* (Wing)-Cotton aphids | | | 2.2 | | | 6.0 |
| *P. japonica* (Abdomen)-Cotton aphids | | | 2.1 | | | 7.2 |
| *P. japonica* (Wing)-Cotton | | | 0.8 | | | 6.6 |
| *P. japonica* (Abdomen)-Cotton | | | 0.8 | | | 7.8 |
| **$C_4$ plant** | | | | | | |
| Maize | -12.7±1.1 | 9 | | 4.9±3.3 | 5 | |
| Maize aphids | -11.8±0.4 | 4 | | -0.4±1.2 | 5 | |
| *P. japonica* (Wing) | -10.9±0.2 | 7 | | -0.6±0.1 | 3 | |
| *P. japonica* (Abdomen) | -11.3±0.2 | 7 | | -0.1±0.4 | 3 | |
| Maize aphids-Maize | | | 0.9 | | | -5.3 |
| *P. japonica* (Wing)-Maize aphids | | | 0.9 | | | -0.2 |
| *P. japonica* (Abdomen)-Maize aphids | | | 0.5 | | | 0.3 |
| *P. japonica* (Wing)-Maize | | | 1.8 | | | -5.5 |
| *P. japonica* (Abdomen)-Maize | | | 1.3 | | | -5.0 |

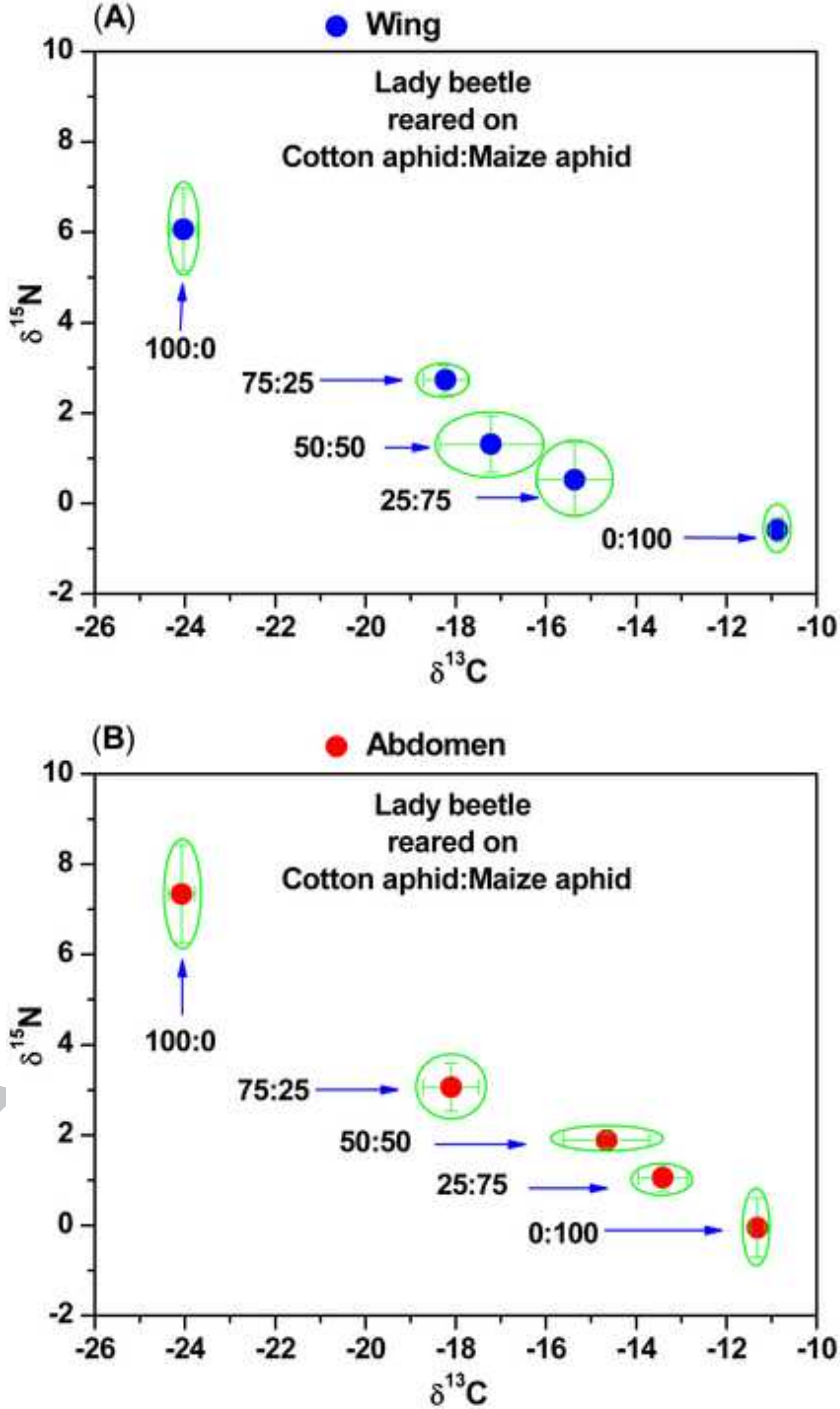
**Table 2.**

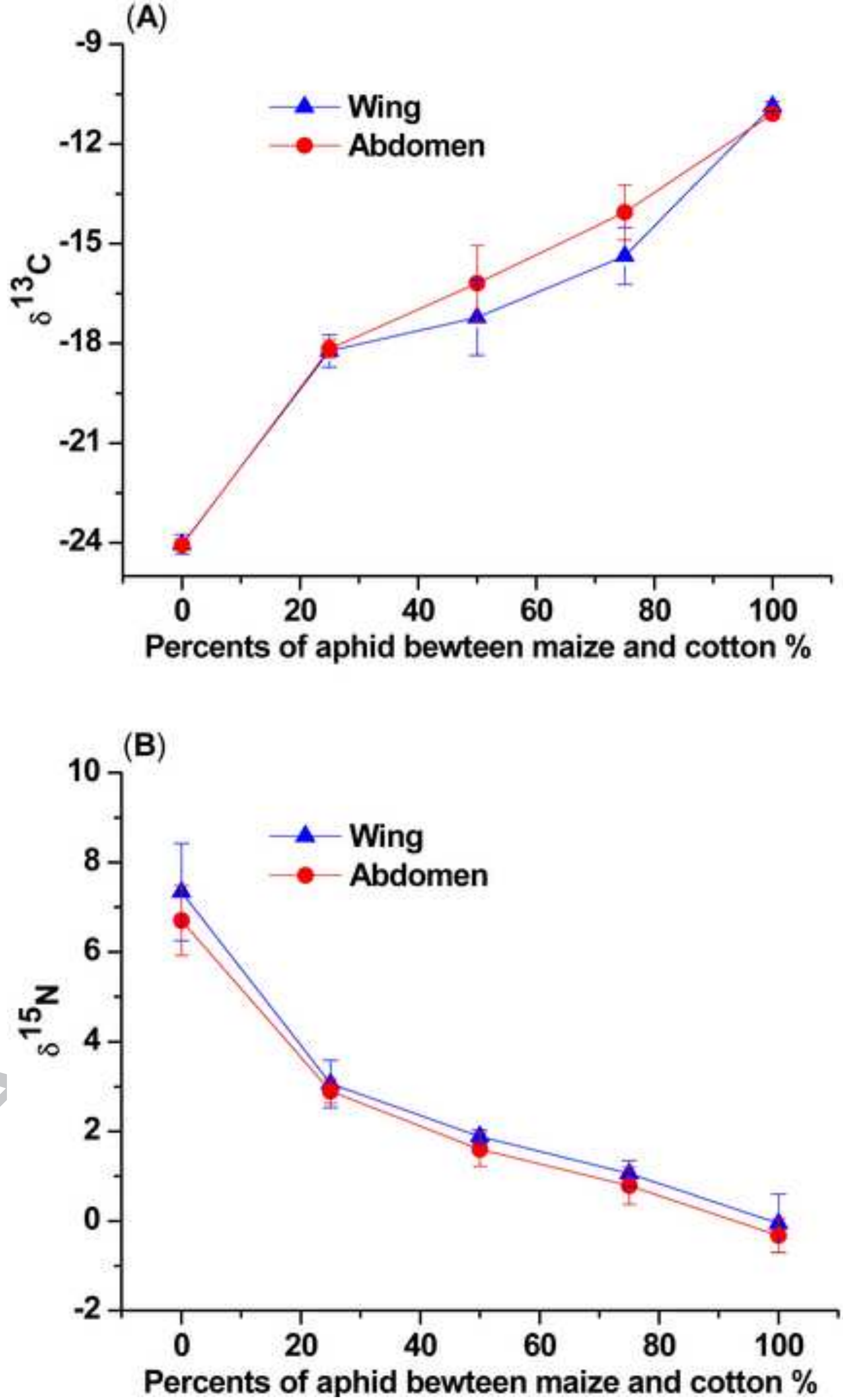
| **Isotope ratios** | **Sampled organisms** | **Model** | **Equation [a]** | **$R^2$** | **MS** | **F** | ***P*** |
|---|---|---|---|---|---|---|---|
| $\delta^{13}C$ | Wing | Linear | Y=0.1168X-22.9848 | 0.9343 | 85.3 | 42.7 | 0.0073 |
| | | Quadratic | $Y=0.1372X-0.0002X^2-23.2397$ | 0.9368 | 42.8 | 14.8 | 0.0632 |
| | Abdomen | Linear | Y=0.1209X-22.3582 | 0.9164 | 91.3 | 32.9 | 0.0105 |
| | | Quadratic | $Y=0.2347X-0.0011X^2-23.7812$ | 0.9875 | 49.2 | 79.2 | 0.0125 |
| $\delta^{15}N$ | Wing | Linear | Y=-0620 X+5.1120 | 0.9115 | 24.0 | 30.9 | 0.0115 |
| | | Quadratic | $Y=-0.1200X+0.0006X^2+5.8360$ | 0.9810 | 12.9 | 51.7 | 0.0190 |
| | Abdomen | Linear | Y=-0672 X+6.0176 | 0.8646 | 28.2 | 19.2 | 0.0221 |
| | | Quadratic | $Y=-0.1438X+0.0008X^2+6.9752$ | 0.9629 | 15.7 | 26.0 | 0.0371 |

[a] Y is stable isotope ratios of *P. japonica* adults, X is proportion of aphids from a $C_3$ to a $C_4$-based substrate.


(A)
-10
-12
-14
-16
-18
-20
-22
-24
-26
δ13C
Wing
Abdomen
*
*
0 2 4 6 8 10 12 14 16 18 20 22
Days after diet change


(B)
10
8
6
4
2
0
δ15N
Wing
Abdomen
*
*
0 2 4 6 8 10 12 14 16 18 20 22
Days after diet change


(A)
Wing
Lady beetle
reared on
Cotton aphid:Maize aphid
100:0
75:25
50:50
25:75
0:100
$\delta^{15}N$
$\delta^{13}C$
10
8
6
4
2
0
-2
-26
-24
-22
-20
-18
-16
-14
-12
-10
(B)
Abdomen
Lady beetle
reared on
Cotton aphid:Maize aphid
100:0
75:25
50:50
25:75
0:100
$\delta^{15}N$
$\delta^{13}C$



(A)
Wing
Abdomen
δ 13C
-9
-12
-15
-18
-21
-24
0
20
40
60
80
100
Percents of aphid bewteen maize and cotton %
(B)
Wing
Abdomen
δ 15N
10
8
6
4
2
0
-2
0
20
40
60
80
100
Percents of aphid bewteen maize and cotton %

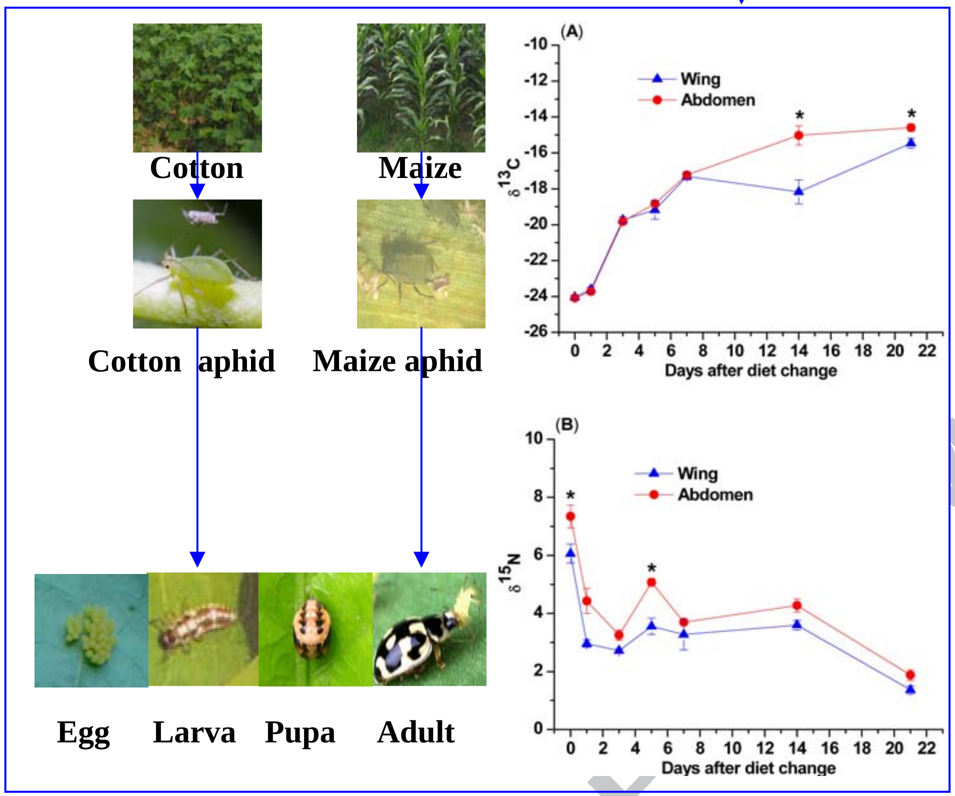
**Highlights**

- We examine the changes of $\delta^{13}C$ and $\delta^{15}N$ among crops, pests and predators.
- $\delta^{13}C$ or $\delta^{15}N$ values of predators related to proportions of diets with equations.
- Values of $\delta^{13}C$ or $\delta^{15}N$ can trace prey origins, proportions of diets.
- Integrative values of $\delta^{13}C$ and $\delta^{15}N$ can trace feeding period of natural enemies.
- Provide quantifying techniques for habitat management of natural enemies.

# Tracing prey origins, proportions and feeding periods for predatory beetles from agricultural systems using carbon and nitrogen stable isotope analyses

## Dietary shift experiment

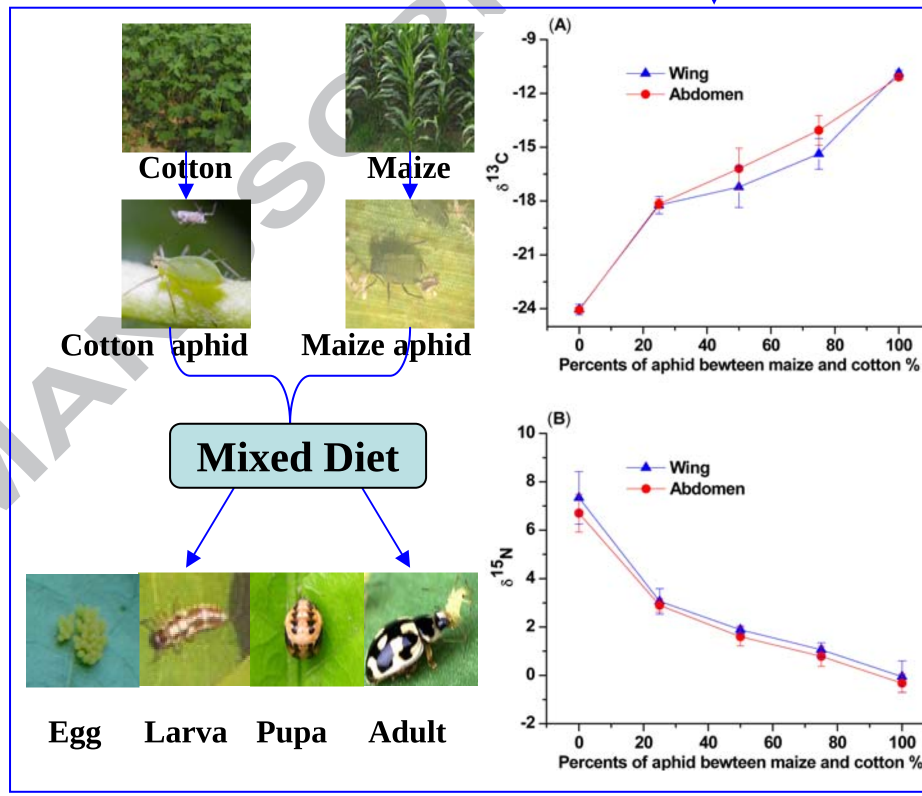
| Crop | Cotton | | Maize | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Insect pest | Cotton aphid | | Maize aphid | | | | | | |
| Natural enemy | Larva | Pupa | Adult | | | | | | |
| Day | | | 0 | 1 | 3 | 5 | 7 | 14 | 21 |




Feeding Period ← Turnover Time

## Dietary proportion experiment

| Natural enemy from larva to adult | | | | | |
|---|---|---|---|---|---|
| Prey origin | Five varying proportions % | | | | |
| Cotton aphid | 100 | 75 | 50 | 25 | 0 |
| Maize aphid | 0 | 25 | 50 | 75 | 100 |




Prey Origin and Proportion ← Linear or Quadratic Equation