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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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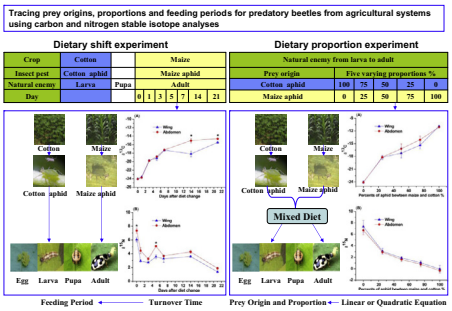
HIGHLIGHTS

- 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.

GRAPHICAL ABSTRACT



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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 7 days and the $\delta^{15}N$ values began to reflect their new C_4 substrates within 3 days. The relationship between $\delta^{13}C$ and $\delta^{15}N$ 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.

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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, *P. 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 3 days, placed in plastic vials containing 95% ethanol for 10 min 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 3 days, placed in plastic vials containing 95% ethanol for 10 min 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