Does nitrogen limitation promote intraguild predation in an aphidophagous ladybird?

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Abstract

Reciprocal intraguild predation occurs between the two aphidophagous ladybird beetles *Coccinella septempunctata* L. and *Harmonia axyridis* Pallas (Coleoptera: Coccinellidae). However, its direction is asymmetrical; *H. axyridis* generally acts as an intraguild predator, and *C. septempunctata* as an intraguild prey. According to Denno and Fagan's prediction that nitrogen shortages in predators may promote intraguild predation, it was hypothesized that growth of intraguild predator *H. axyridis* is more limited by nitrogen than that of intraguild prey *C. septempunctata*, and that *H. axyridis* growth is enhanced by feeding on *C. septempunctata* compared to feeding on aphids.

To determine nitrogen-limited growth in *H. axyridis*, the following two predictions were examined. First, it was predicted that the nitrogen content of *H. axyridis* would be higher than that of *C. septempunctata* when both feed on aphids. However, nitrogen content did not differ between the two ladybirds. Second, it was predicted that nitrogen-use efficiency of *H. axyridis* would be lower than that of *C. septempunctata*. However, there was no significant difference between species. These results did not support the hypothesis that growth of *H. axyridis* is more limited by nitrogen than that of *C. septempunctata*. In addition, the present study showed that dry mass and nitrogen growth of *H. axyridis* were not enhanced, but rather decreased, by eating high-nitrogen *C. septempunctata*, compared to eating low-nitrogen aphids. Overall, the present study did not support the hypothesis that nitrogen shortages in predators may promote intraguild predation.

Introduction

Intraguild predation is a widespread feeding behaviour observed in diverse animal taxa (Polis et al., 1989). Since these authors pointed out the ecological and evolutionary implications of intraguild predation, several studies have demonstrated its important consequences for prey–predator interactions (Lucas et al., 1998; Finke & Denno, 2003), community structure (Arim & Marquet, 2004), biological control (Burgio et al., 2002; De Clercq et al., 2003), and success in invasion of exotic species (Snyder et al., 2004; Yasuda et al., 2004). On the other hand, factors promoting intraguild predation have also been discussed, and two adaptive benefits of intraguild predation were suggested: (1) the higher nutritional and energetic gain from eating intraguild members instead of eating primary food items and (2) decreased exploitative competition by eating potential competitors (Polis et al., 1989).

Denno & Fagan (2003) developed the nutritional benefits of intraguild predation based on ecological stoichiometry. It is known that the nitrogen content of herbivorous insects is generally lower (mean $\pm$ SE = 9.7 $\pm$ 0.2% dry weight) than that of predatory insects (11.0 $\pm$ 0.2%) in terrestrial ecosystems (Fagan et al., 2002). According to stoichiometric theory, this imbalance of nitrogen content may result in nitrogen-limited growth in predators (Fagan et al., 2002). In other words, predators may compensate for nitrogen shortage by feeding on other predators with higher nitrogen content than herbivores. Thus, the higher nitrogen content of predators may favor intraguild predation (Denno & Fagan, 2003). However, there are few studies that measured nitrogen stoichiometry in prey–predator interactions in the context of intraguild predation (but see Matsumura et al., 2004).

Intraguild predation is frequently observed in aphidophagous guilds (e.g., Lucas et al., 1998; Yasuda et al., 2004). In Japan, two ladybird beetles, *Coccinella septempunctata* L. and *Harmonia axyridis* Pallas (Coleoptera: Coccinellidae),

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are widely distributed, major members of aphidophagous guilds. They eat, or are eaten by, each other during the larval period, although the direction is asymmetrical; *H. axyridis* mostly eats *C. septempunctata* (Yasuda et al., 2001). Moreover, it is known that *H. axyridis* is a generalist predator, while *C. septempunctata* specializes on aphids (Yasuda & Ohnuma, 1999; Kalaskar & Evans, 2001). These feeding habits indicate that in the relationship between the two species, *H. axyridis* generally acts as an intraguild predator and *C. septempunctata* as an intraguild prey (Yasuda et al., 2001). According to Denno and Fagan's prediction (Denno & Fagan, 2003) that nitrogen shortages in predators may promote intraguild predation, it is expected that strong intraguild predation in *H. axyridis* may have been promoted by strong nitrogen limitation due to its primary food items, i.e., aphids. Thus, it was hypothesized that the growth of the intraguild predator *H. axyridis* is more limited by nitrogen than that of intraguild prey *C. septempunctata* when they fed on aphids, and that the growth of *H. axyridis* is enhanced by feeding on *C. septempunctata*, compared to feeding on aphids.

The present study tested the above two hypotheses by comparing growth, carbon and nitrogen content, and carbon- and nitrogen-use efficiency of the two species of ladybird beetles. To determine whether nitrogen limitation in *H. axyridis* is greater than in *C. septempunctata*, the following two questions were addressed. (1) Is the nitrogen content of *H. axyridis* higher than that of *C. septempunctata*? Higher nitrogen content of *H. axyridis* can result in a larger difference in nitrogen content between *H. axyridis* and aphids than between *C. septempunctata* and aphids, indicating nitrogen limitation. (2) Is the nitrogen-use efficiency of *H. axyridis* lower than that of *C. septempunctata*, when both ladybird species fed on aphids? In general, it is known that high nitrogen-use efficiency is an adaptation for feeding on nitrogen-poor diets (Slansky & Feeny, 1977). Hence, *C. septempunctata*, which specializes on aphids having poor nitrogen, is predicted to have higher nitrogen-use efficiency than the intraguild predator *H. axyridis*. If so, *H. axyridis* would need more nitrogen for growth than does *C. septempunctata*, when feeding on aphids. This also indicates nitrogen limitation in *H. axyridis*. In addition, we examined whether the growth and nitrogen intake of *H. axyridis* is enhanced by feeding on intraguild prey *C. septempunctata*, compared to feeding on aphids.

Materials and methods

Insect rearing

Adults of the two ladybird species, *H. axyridis* and *C. septempunctata*, were collected from the experimental plots at the Center for Ecological Research, Kyoto University, and nearby areas. The ladybirds collected were reared to obtain eggs in Petri dishes (9 cm in diameter) lined on the bottom with wet paper in an environmental chamber at 23 °C, L16:D8, and 70% r.h. The ladybirds were provided with aphids, *Acyrthosiphon pisum* Harris and *Aphis craccivora* Koch (Homoptera: Aphididae). These aphids were from a laboratory population of Saga University and were reared on potted broad beans, *Vicia faba* L. (Leguminosae) under laboratory conditions. Egg masses laid by the ladybirds were placed in separate Petri dishes, and hatched larvae were reared in the environmental chamber until they were used in the experiment. The larvae were provided with *A. pisum* every day. Larvae of both species of ladybirds pass through four instars and have similar developmental times under the laboratory rearing conditions, i.e., approximately 2, 2, 2, and 3 or 4 days for the first, second, third, and fourth instar, respectively (H Kagata & N Katayama, unpubl.).

Experimental design

Less than 24-h-old third-instar larvae of the two ladybird species (i.e., 4 days after hatching) were used in the experiment. Each ladybird larva was weighed to determine fresh body weight, put in a Petri dish (9 cm in diameter) lined on the bottom with wet paper, and reared for 6 days in the environmental chamber at 23 °C, L16:D8, and 70% r.h. We set up the following three treatments: (1) CS (AP) = *C. septempunctata* reared on primary prey, *A. pisum*; (2) HA (AP) = *H. axyridis* reared on primary prey, *A. pisum*; and (3) HA (CS) = *H. axyridis* reared on intraguild prey, *C. septempunctata*. For the CS (AP) and HA (AP) treatments, *H. axyridis* or *C. septempunctata* larvae were provided with 70 ± 3 mg (fresh weight) of mixed-age *A. pisum* for the first 2 days of the experiment (corresponding with the third-instar period) and 140 ± 3 mg of *A. pisum* every day thereafter (corresponding with the fourth instar period). For the HA (CS) treatment, *H. axyridis* larvae were provided with 70 ± 3 mg of mixed-age *A. pisum* for the first 2 days of the experiment and 100 ± 3 mg of second-instar *C. septempunctata* larvae (i.e., 2 days after hatching, but several first-instar larvae were included) every day thereafter. The amount of prey was such that food shortages did not occur during the experiment. Larval body weight and consumed prey biomass were measured every day. The consumed prey biomass was determined as the difference between the weight of prey supplied and what remained. After the experiment finished (6th day of the experiment), the ladybird larvae were kept in a refrigerator at −20 °C until carbon and nitrogen contents were measured. Twenty replicates were conducted for CS (AP) and HA (AP) treatments, and 14 replicates for the HA (CS) treatment. However, three *H. axyridis* larvae in the HA (CS) treatment died during the experiment, presumably due to starvation

Table 1 Initial larval traits, prey quality, and final larval traits in the experiment. Means ± SE are presented. Different letters in the same column in the final larval traits indicate significant differences (Tukey HSD test, P<0.05). Prey–predator combinations in the experimental treatments are shown as predator (prey), and species abbreviations are CS = *Coccinella septempunctata*, HA = *Harmonia axyridis*, and AP = *Acyrthosiphon pisum*

	Fresh mass (mg)	Dry mass (mg)	% water (f.w.)[1]	% carbon (d.w.)[1]	% nitrogen (d.w.)	C : N ratio
Initial larval traits						
C. septempunctata	3.77 ± 0.19	0.86 ± 0.04	77.22 ± 0.23	47.52 ± 0.23	11.20 ± 0.24	4.27 ± 0.11
H. axyridis	3.89 ± 0.17	0.90 ± 0.04	76.90 ± 0.34	47.29 ± 0.26	12.05 ± 0.13	3.93 ± 0.06
F	0.19	0.41	0.39	0.46	10.15	7.61
P	0.66	0.52	0.54	0.51	0.0051	0.0129
Prey quality						
A. pisum	3.15 ± 0.11[2]	0.70 ± 0.03[2]	77.91 ± 0.13	49.71	7.70 ± 0.04	6.46 ± 0.04
C. septempunctata	1.21 ± 0.04	0.27 ± 0.01	77.77 ± 0.21	46.86	11.15 ± 0.05	4.21 ± 0.05
F	256.98	240.40	0.34	309.02	813.08	1160.17
P	<0.0001	<0.0001	0.57	<0.0001	<0.0001	<0.0001
Final larval traits						
CS (AP)	44.06 ± 0.97a	11.90 ± 0.26a	72.98 ± 0.17a	52.21 ± 0.14a	10.09 ± 0.12	5.19 ± 0.07ab
HA (AP)	43.40 ± 1.12a	12.97 ± 0.42a	70.19 ± 0.28b	52.65 ± 0.18a	9.85 ± 0.18	5.38 ± 0.11a
HA (CS)	34.56 ± 1.61b	9.53 ± 0.51b	72.47 ± 0.56a	51.16 ± 0.30b	10.33 ± 0.10	4.96 ± 0.07b
F	15.39	16.80	27.20	13.37	2.07	4.65
P	<0.0001	<0.0001	<0.0001	<0.0001	0.14	0.0142

[1]f.w. = fresh weight, d.w. = dry weight.
[2]Data are for wingless adult females.

or reverse predation by *C. septempunctata*, and these were excluded from the analysis.

Initial larval traits and prey quality

To determine the initial larval traits of the two ladybird species and prey quality in the experiment, water, carbon, and nitrogen content were examined. The fresh weight of groups of individuals of less than 24-h-old third-instar larvae of each ladybird species (larvae at initial day of the experiment, second-instar larvae of *C. septempunctata* (intraguild prey), and mixed-instar *A. pisum* (primary prey) was calculated; approximately 10–15, 40–60, and 500–1000 individuals were pooled as a single group, respectively. In addition, wingless adult females of *A. pisum* were weighed individually. After weighing, they were kept in a refrigerator at −20 °C until carbon and nitrogen content was measured. Ten replicates were conducted for each measurement.

Carbon and nitrogen analysis

Before measuring carbon and nitrogen content, the insects were dried in an oven at 60 °C for 72 h and weighed. The dried insects were then ground up, and carbon and nitrogen content was measured using an elemental analyzer (JM 1000CN, J-Science Co. Ltd, Kyoto, Japan).

Data analysis

Data of initial and final larval traits and prey quality were tested by ANOVA. Dry mass growth efficiency, carbon-use efficiency and nitrogen-use efficiency, and carbon–nitrogen allometric growth were analysed using ANCOVA (Raubenheimer & Simpson, 1992; Horton & Redak, 1993). These parameters were analysed separately for the third and fourth instars. For the third instar, the two treatments of HA (AP) and HA (CS) were pooled in the analysis, because these treatments were identical during the third instar (i.e., *H. axyridis* larvae in both treatments were provided with aphids for the third instar). A Tukey HSD test (P<0.05) was performed as post hoc test after ANCOVA.

Results

Initial larval traits

There were no significant differences in fresh and dry weight, and water and carbon content between third-instar larvae of *C. septempunctata* and *H. axyridis* on the initial day of the experiment (Table 1). On the other hand, the nitrogen content of *C. septempunctata* was significantly lower than that of *H. axyridis*, and therefore the C : N ratio of *C. septempunctata* was higher than that of *H. axyridis*.

Prey quality

The body weight of *A. pisum* wingless adult females was greater than that of *C. septempunctata* second-instar larvae (Table 1). However, the body weight of *A. pisum* does not correspond with the actual weight of *A. pisum* that was used in the experiment, because mixed-age *A. pisum* were

Table 2 ANCOVA table for dry-mass growth efficiency, carbon-use efficiency, nitrogen-use efficiency, and carbon–nitrogen allometric growth, of third and fourth instars of *Coccinella septempunctata* and *Harmonia axyridis*

	Third instar				Fourth instar			
	d.f.	SS	F	P	d.f.	SS	F	P
Mass growth efficiency								
Treatment	1	0.641	3.40	0.07	2	40.046	10.78	0.0001
Consumed (covariate)	1	23.257	123.62	<0.0001	1	45.079	24.28	<0.0001
Treatment*consumed	1	4.034	21.44	<0.0001	2	9.291	2.50	0.09
Carbon-use efficiency								
Treatment	1	0.169	3.31	0.08	2	9.339	9.25	0.0004
Consumed (covariate)	1	6.381	124.71	<0.0001	1	12.050	23.96	<0.0001
Treatment*consumed	1	1.073	20.96	<0.0001	2	2.318	2.30	0.11
Nitrogen-use efficiency								
Treatment	1	0.005	2.84	0.10	2	0.607	16.37	<0.0001
Consumed (covariate)	1	0.243	134.25	<0.0001	1	0.482	25.98	<0.0001
Treatment*consumed	1	0.037	20.60	<0.0001	2	0.166	4.46	0.0171
C–N allometric growth								
Treatment	1	<0.001	0.09	0.77	2	0.011	771.49	<0.0001
C growth (covariate)	1	0.310	3931.71	<0.0001	1	1.071	151,624.30	<0.0001
Treatment*C growth	1	<0.001	0.65	0.43	2	0.001	48.82	<0.0001

used. Water content of *A. pisum* and *C. septempunctata* did not differ significantly (Table 1). On the other hand, *A. pisum* had a significantly higher carbon and lower nitrogen content than *C. septempunctata*, and therefore the C : N ratio of *A. pisum* was 1.5 times higher than that of *C. septempunctata* (Table 1).

Final larval traits

When the two ladybird species fed on *A. pisum*, their fresh and dry weight, carbon and nitrogen content, and the C : N ratio did not differ significantly between *C. septempunctata* and *H. axyridis* (Table 1). However, water content differed between the two ladybird species, being higher in *C. septempunctata* than in *H. axyridis*.

When *H. axyridis* were fed on intraguild prey *C. septempunctata*, their body weight was significantly less than when fed on *A. pisum* (Table 1). Water content of *H. axyridis* was also affected by prey species, and it was higher when fed on *C. septempunctata* than when fed on *A. pisum*. Carbon content of *H. axyridis* fed on *C. septempunctata* was significantly lower than that fed on *A. pisum*, while nitrogen content did not differ between the two treatments. Hence, the C : N ratio of *H. axyridis* fed on *C. septempunctata* was lower than that fed on *A. pisum*.

Growth efficiency on primary prey: *Coccinella septempunctata* vs. *Harmonia axyridis*

During the third instar, while dry mass growth, C growth, and N growth were differentially affected by consumed dry mass, consumed C, and consumed N, respectively, between

ladybird species (Table 2 and Figure 1A–C), they did not differ significantly between *C. septempunctata* and *H. axyridis* (Table 2). In addition, carbon–nitrogen allometric growth did not differ between the two ladybird species, and there was no significant difference in relative N growth to C growth between the two species of ladybirds (Table 2 and Figure 2A).

In the fourth instar, dry mass growth and C growth were affected by consumed dry mass and consumed C, respectively, in a same manner independent of the ladybird species (Table 2 and Figure 1D–F). Dry mass growth, C growth, and N growth did not differ significantly between *C. septempunctata* and *H. axyridis* (Table 2). On the other hand, carbon–nitrogen allometric growth differed between the two ladybird species, and relative N growth to C growth was slightly, but significantly, higher in *C. septempunctata* than *H. axyridis* when both fed on aphids (Table 2 and Figure 2B).

Growth efficiency of *Harmonia axyridis*: primary prey vs. intraguild prey

Dry mass growth and C growth of *H. axyridis* were affected by consumed dry mass and consumed C, respectively, in a same manner independent of the prey species (Table 2 and Figure 1D–F). Dry mass growth, C growth, and N growth were significantly lower in the larvae fed on intraguild prey *C. septempunctata* than those fed on primary prey aphids (Figure 1B). In addition, carbon–nitrogen allometric growth of *H axyridis* differed between the prey species, and relative N growth to C growth was significantly higher in the larvae
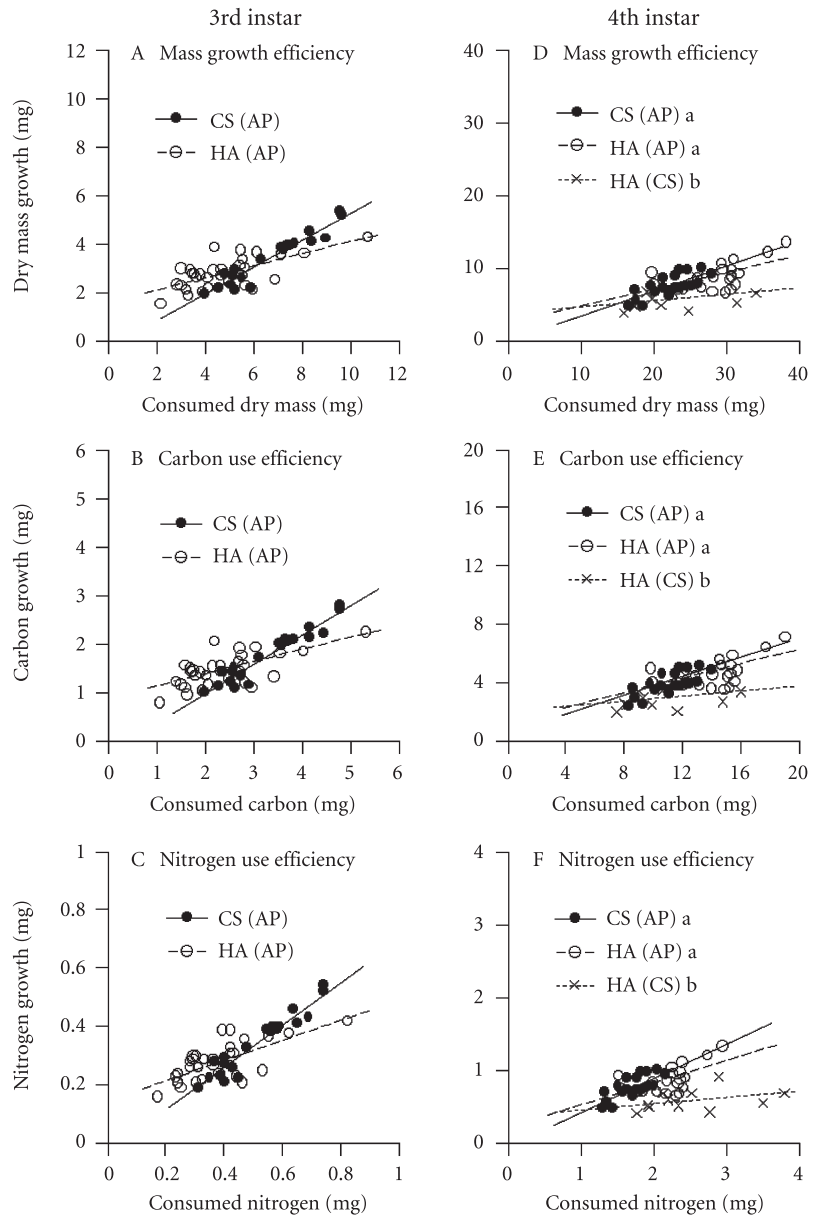
Figure 1 (A,D) Dry mass growth efficiency, (B,E) carbon-use efficiency, and (C,F) nitrogen-use efficiency, during the third instar (left) and fourth instar (right) of *Coccinella septempunctata* (CS) and *Harmonia axyridis* (HA); AP, *Acyrthosiphon pisum*. Different letters in the fourth instar indicate significant differences (P<0.05).

fed on *C. septempunctata* than those fed on aphids (Table 2 and Figure 2B).

Discussion

Is growth of the intraguild predator more nitrogen limited than that of the intraguild prey?

Denno & Fagan (2003) argued that the stoichiometric imbalance for nitrogen between herbivores and predators (i.e., $N_{predators} > N_{herbivores}$; Fagan et al., 2002) may result in nitrogen limitation for predators when they feed exclusively on herbivores, which then may promote intraguild preda-

tion to enhance nitrogen intake. The present study showed that the nitrogen content of both *C. septempunctata* and *H. axyridis* was higher than that of prey aphids. The aphid–ladybird system could be one system in which there is a great imbalance in nitrogen between prey and predators, because aphids are especially low in nitrogen (Fagan et al., 2002). We tested the hypothesis that the growth of the intraguild predator *H. axyridis* is more limited by nitrogen than that of the intraguild prey *C. septempunctata*, when they feed exclusively on aphids.

First, it was predicted that the nitrogen content of the intraguild predator *H. axyridis* is higher than that of the
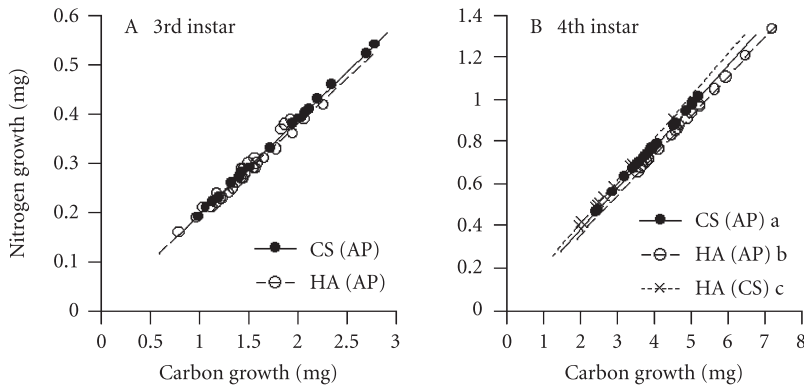
Figure 2 Carbon–nitrogen allometric growth during the (A) third instar and (B) fourth instar of *Coccinella septempunctata* (CS) and *Harmonia axyridis* (HA); AP, *Acyrthosiphon pisum*. Different letters in the fourth instar indicate significant differences (P<0.05).

intraguild prey *C. septempunctata*, resulting in a larger difference in nitrogen content between *H. axyridis* and aphids than between *C. septempunctata* and aphids. This prediction was true for third-instar larvae, where *H. axyridis* had higher nitrogen than *C. septempunctata*. However, the nitrogen content of the ladybirds varied with larval instars, and the nitrogen content did not differ between the two ladybird species for fourth-instar larvae, the critical stage for larval mass gain, when intraguild predation occurs frequently (Yasuda et al., 2001; Yasuda et al., 2004). Second, it was predicted that nitrogen-use efficiency of the intraguild predator *H. axyridis* is lower than that of the intraguild prey *C. septempunctata*. Lower nitrogen-use efficiency would necessitate more nitrogen intake for growth. However, there was no significant difference in nitrogen-use efficiency between the two species of ladybirds for third- and fourth-instar larvae. On the other hand, carbon–nitrogen allometric growth differed between the ladybird species, and relative nitrogen growth to carbon growth was slightly lower in *H. axyridis* than *C. septempunctata* during the fourth-instar period. This result indicates that relative nitrogen-use efficiency to carbon-use efficiency was higher in *C. septempunctata* than in *H. axyridis*. Therefore, *C. septempunctata* may better adapt to intake of nitrogen from aphids having rich carbon and poor nitrogen than does *H. axyridis*. Thus, this result partly supported the second prediction that nitrogen-use efficiency of the intraguild predator *H. axyridis* is lower than that of the intraguild prey *C. septempunctata*, when considering allometric growth of carbon and nitrogen. Overall, however, the present study did not support the hypothesis that growth of the intraguild predator *H. axyridis* is more limited by nitrogen than that of intraguild prey *C. septempunctata* when they feed on aphids.

Does intraguild predation enhance growth of the intraguild predator?

Denno & Fagan (2003) also predicted that predators enhance their performance by eating more nitrogen-rich prey via intraguild predation. Matsumura et al. (2004) were the first to test this prediction, by evaluating a relationship between predator performance and prey nitrogen content. They examined the performance of the spider *Pardosa littoralis* as an intraguild predator, when it fed on the planthopper *Prokelisia dolus* (primary prey), or when fed on another spider *Grammonota trivittata* (intraguild prey). However, predator performance rather decreased as a result of intraguild predation despite the fact that intraguild prey spiders were higher in nitrogen than planthoppers. Hence, they concluded that predator performance was mainly determined by foraging efficiency rather than by prey nitrogen.

Similarly, the present study demonstrated that growth of intraguild predator *H. axyridis* was not enhanced, but rather decreased, by eating *C. septempunctata* (intraguild prey), compared to eating aphids (primary prey), despite that *C. septempunctata* had higher nitrogen content than the aphids. The decreased growth due to intraguild predation can be ascribed to several factors. First, intraguild predation is risky because of the chance of being inversely attacked by intraguild prey, and therefore, there are costs involved in capturing intraguild prey. Three larvae of *H. axyridis* (21.4%) died due to starvation or predation in the present study, and even the surviving larvae decreased their prey consumption, when *C. septempunctata* larvae were supplied as an intraguild prey. Yasuda & Ohnuma (1999) showed, using the same system as in our experiment, that survival rate, growth rate, and developmental time of *H. axyridis* did not differ between when fed on *C. septempunctata* and when fed on aphids. Their experiment was designed to eliminate the capturing costs in intraguild predation by cutting off the legs of intraguild prey. Hence, Yasuda & Ohnuma (1999) indicated that intraguild predation would not be more beneficial for growth of *H. axyridis* than feeding on primary prey, even when capturing costs in intraguild predation were eliminated. Second, nitrogen-use efficiency of *H. axyridis* decreased when fed on *C. septempunctata* compared with

feeding on the aphids. This is probably because *H. axyridis* may release nitrogen in excess of their requirements in order to maintain nitrogen homeostasis, if *C. septempunctata* larvae have more nitrogen than *H. axyridis* requires. Such a low nutrient-use efficiency when fed on a nitrogen-rich diet has been reported in other predators (Furrer & Ward, 1995) and herbivores (Slansky & Feeny, 1977; Lee et al., 2004; Raubenheimer & Simpson, 2004). In addition to the total nitrogen content, specific amino acids, carbohydrates, or micronutrients may be also important for insect growth (Mattson & Scriber, 1987; Cole, 1997; Mayntz & Toft, 2001). Because *H. axyridis* is also primary aphidophagous, it may require such nutrients that are only encountered in aphids (Evans et al., 1999; Nielsen et al., 2002). On the other hand, we found that carbon–nitrogen allometric growth of *H. axyridis* was affected by prey species, and relative nitrogen-use efficiency to carbon-use efficiency was higher when feeding on *C. septempunctata* than when feeding on aphids. This would result in decreasing carbon content and change in C : N ratio in *H. axyridis* when feeding on intraguild prey (see Table 1). Because body C : N ratio is expected to maintain the optimal ratio for growth by physiological process for homeostasis (Sterner & Elser, 2002), the difference from optimal element composition may be inadequate for growth. This may also be the reason why growth of *H. axyridis* was not enhanced by feeding on intraguild prey rich in nitrogen. Thus, the present study did not find any nutritional benefits of intraguild predation on predator growth. Nevertheless, our results must be interpreted with caution because we did not examine a mixed diet of primary and intraguild prey (Evans et al., 1999; Oelbermann & Scheu, 2002). In nature, intraguild predators in aphidphagous guilds would generally consume both aphids and intraguild prey, not only intraguild prey. Feeding mixed diet of aphids and intraguild prey may have extra nutritional or energetic benefits for intraguild predator. Hence, our experimental design that intraguild predator was provided with only intraguild prey might not be representative of the true benefits of intraguild predation in terms of mixed diet.

Promoting intraguild predation

Several studies have argued that size, aggressiveness, diet range, and defensive chemicals and morphology are important in determining the direction of intraguild predation, i.e., which species acts as an intraguild predator and which as an intraguild prey (Polis et al., 1989; Lucas et al., 1997; Lucas et al., 1998; Sato & Dixon, 2004). From a series of studies examining the relationship between *H. axyridis* and *C. septempunctata*, Yasuda et al. (2004) concluded that strong intraguild predation by *H. axyridis* would occur due to its high aggressiveness, defensive bristles, and wide diet range,

but not due to its size and toxicity (Yasuda & Ohnuma, 1999; Yasuda & Kimura, 2001; Yasuda et al., 2001; Yasuda et al., 2004). In addition, it was demonstrated that intraguild predation among aphidophagous ladybirds occurred more frequently when aphid availability was low (Kajita et al., 2000; Yasuda et al., 2004; but see Snyder et al., 2004). It is furthermore known that in the field *H. axyridis* is more limited by aphid availability than *C. septempunctata* due to their difference in appearance phenology (Sato et al., 2005). Therefore, strong intraguild predation in *H. axyridis* may be promoted by a quantitative food shortage, but not by nutritional limitation. Yet, our results would represent a valuable first step towards understanding the nutritional ecology of intraguild predation in aphidphagous ladybirds from a stoichiometric approach. Further nutritional benefits of intraguild predation, such as benefits from mixed diet, should be examined to explain the frequent and asymmetrical intraguild predation in aphidophagous guilds.

Acknowledgements

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