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Prey capture performance in hatchlings of two sibling *Harmonia* ladybird species in relation to maternal investment through sibling cannibalism

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Abstract. 1. To elucidate the factors responsible for the evolution of host specialisation, prey capture performance in hatchlings of two sibling ladybird species, *Harmonia yedoensis* and *H. axyridis*, feeding on four aphid species was examined. *Harmonia yedoensis* is a specialist predator that preys only on pine aphids in the field, whereas *H. axyridis* is a generalist predator with a broad prey range.

2. In *H. yedoensis*, sibling cannibalism in each clutch was intense and predation against pine aphid as well as other aphid species was moderately successful. In contrast, the predation success rate of *H. axyridis* against pine aphid was quite low.

3. Moreover, it was experimentally shown that increased maternal investment enhanced prey capture performance against pine aphid in *H. yedoensis* but not in *H. axyridis* hatchlings, despite their increased body size due to maternal investment.

4. In addition, morphological and behavioural analysis showed that hatchlings of *H. yedoensis* had longer legs and a larger head capsule size and could walk faster than *H. axyridis*.

5. In summary, the interactive effects between a large amount of maternal investment and morphological specialisation of the first instars may enable *H. yedoensis* to capture the pine aphid efficiently, a highly elusive prey for ladybird hatchlings. The ability of *H. yedoensis* to utilise the three other aphid species in addition to the pine aphid suggests that a trade-off in prey capture performance is not the main factor in the host specialisation of *H. yedoensis*.

Key words. *Cinara pini*, first instars, ladybird beetle, specialisation, trade-off.

Introduction

A generalist can exploit multiple habitat types and food sources, while a specialist is limited to only one or a few. Even closely related species often differ greatly in the breadth of resources exploited (Ehrlich & Raven, 1964; Fox & Morrow, 1981; Futuyma *et al*., 1995). The question to be addressed thus becomes, under what conditions can specialisation be expected to evolve (e.g. Levins, 1968; Futuyma & Moreno, 1988)? Comparative studies of recently evolved species constitute a powerful evolutionary tool because they focus on a minimum number of uncontrolled variables that stem from separate evolutionary histories. A number of factors may promote specialisation, such as genetically based trade-offs in performance between different resources (Levins, 1968; Futuyma & Moreno, 1988; McPeek, 1996) and competition for resources among closely related species (MacArthur & Levins, 1964). Although host specialisation is a well-studied and reasonably well-understood phenomenon (Bernays & Graham, 1988; Futuyma & Moreno, 1988; Thompson, 1988), there is no consensus among evolutionary ecologists as to the primary mechanism of host specialisation (Mayhew, 2006).

The trade-off hypothesis assumes that a high level of fitness in one host species entails a decline in the fitness on other hosts (Levins, 1968). Empirical studies in herbivorous insects, however, have accumulated evidence that is inconsistent with

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the trade-off hypothesis (e.g. Jaenike, 1990). Specifically, larvae often survive as well and grow as fast and as large on host plants that are never utilised in nature (e.g. Roininen & Tahvanainen, 1989; Ohsaki & Sato, 1994; Friberg & Wiklund, 2009). A few studies have evaluated the trade-off hypothesis among closely related species of predatory insects (Tauber & Tauber, 1987; Albuquerque *et al*., 1997). Although Bristow (1988) remarked that predators tend to be generalists precisely because animal tissues are supposed to be much more nutritious than plant tissues, providing a balanced diet that does not differ greatly from species to species, entomologists and ecologists are well aware that a high degree of specificity is shown by many if not most carnivorous insects, especially parasitoids but also many predators (Gilbert, 1990). In predators, it is argued that morphological traits as well as other functional traits that lead to more efficient exploitation of resources in each of several environments cannot be simultaneously maximised, with the result that predator species have diverged, each specialising on particular resources (Schluter, 1996). Testing for the existence of a trade-off in the evolution of a morphological novelty requires demonstrating that this novelty increases predator performance in one situation, but reduces it in another situation (Benkman, 1988; Schluter, 1995).

In any study of the evolution of host specialisation, it is also important to consider maternal investment in offspring, because (i) maternal investment in hatchlings is crucial for their feeding and foraging performance, with the small hatchlings of arthropods being particularly vulnerable to various environmental conditions (Dixon, 1959; Osawa, 1992a; Hodek & Honěk, 1996; Zalucki *et al*., 2002), and (ii) the amount of maternal investment probably reflects the quality of resources (e.g. McGinley *et al*., 1987). Clutton-Brock (1991) defined maternal investment as any characteristics or actions of mothers that increase the fitness of their offspring at a cost to any component of maternal fitness. It has been theoretically predicted that females of species specialised to poor quality food sources should invest more resources per offspring (e.g. Ito, 1980; Parker & Begon, 1986; McGinley *et al*., 1987), and some empirical studies support this prediction (Ito, 1980; Tauber & Tauber, 1987; Fox *et al*., 1997; Kishi & Nishida, 2006). For example, Tauber and Tauber (1987) found that egg size is larger in the specialist predator green lacewing *Chrysopa slossonae* Banks, which exclusively utilises highly elusive preys in nature, than in its congener generalist *Chrysopa quadripunctata* Burmeister. They proposed that the relatively larger eggs of the specialist might be related to the performance and prey specificity of its offspring. However, the adaptive significance of maternal investment with regard to host specialisation is still unclear, because in most cases egg size cannot be experimentally manipulated, thus preventing evaluation of the causal relationship between the amount of maternal investment and offspring performance.

The ladybird beetle *Harmonia yedoensis* Takisawa (Coleoptera: Coccinellidae) is a specialist predator found only on pine trees; in its natural range in central Japan, it preys only upon the giant pine aphid *Cinara pini* Linné or upon Thunberg's pine aphid *Eulachnus thunbergii* Wilson (Tanigishi, 1975; Osawa & Ohashi, 2008). In contrast, its congener, *Harmonia axyridis* Pallas, despite its close resemblance to *H. yedoensis*, is a generalist predator found in various habitats that preys on many species of aphid (Osawa, 1993, 2000). Similar to *H. yedoensis*, some species of ladybird beetles occurring in Europe and North America, such as *Anatis ocellata* Linné and *Myrrha octodecimguttata* Linné, are restricted to pine trees and thus restricted to feeding on pine aphids (Chapin, 1985; Gordon, 1985; Majerus, 1994; Sloggett, 2008). Why and how ladybirds specialise to a particular habitat and to specific resources are, however, still little understood (Sloggett & Majerus, 2000; Sloggett, 2008).

Osawa and Ohashi (2008) demonstrated that egg size is larger and sibling cannibalism occurs more frequently in *H. yedoensis* than in *H. axyridis*. In aphidophagous ladybird beetles, sibling cannibals eat both the undeveloped eggs in a cluster and those eggs that are developing but with delayed hatching (Kawai, 1978; Osawa, 1992a). Both types of eggs should be costly for the mother and are sacrificed to increase the survival rate of the larval-stage sibling (Osawa, 1992a). Therefore, sibling cannibalism can be regarded as a form of maternal investment as defined by Clutton-Brock (1991), although the precise mechanisms leading to the production of the two types of cannibalised eggs in *H. axyridis* and *H. yedoensis* are unknown. Therefore, the more frequent sibling cannibalism as well as the larger egg size in *H. yedoensis* should reflect a greater maternal investment per egg in this species than in *H. axyridis* (Osawa & Ohashi, 2008). Because it is known that species of the genus *Cinara*, including the giant pine aphid, have very long legs and walk very fast compared with other aphids (Moritsu, 1983), these aphids may be highly elusive prey for small ladybird hatchlings. Thus, it can be hypothesised that higher maternal investment may play the important role of allowing *H. yedoensis* to specialise on giant pine aphids, which are likely difficult to capture. No studies, however, have examined the effects of maternal investment on prey capture performance to elucidate the linkage between the amount of maternal investment and host specialisation.

In this study, the prey capture performance of *H. yedoensis* and *H. axyridis* hatchlings against four aphid species, including the giant pine aphid, was examined. In most insects, egg size cannot be experimentally manipulated, making it difficult to examine the effect of maternal investment on hatchling performance. Fortunately, in aphidophagous ladybird beetles, sibling cannibalism occurs frequently. Thus, by controlling the number of eggs that hatchlings could feed upon after their eclosion, the amount of maternal investment was manipulated in a step-by-step manner, and the relationship between the amount of maternal investment and the prey capture performance of the hatchlings was evaluated and compared between the two species. Additionally, morphological and behavioural traits related to the hatchlings' prey capture abilities were measured. Here we test two hypotheses: (i) Maternal investment through sibling cannibalism and morphological traits of hatchlings affect host specialisation in *H. yedoensis*. (ii) Trade-offs in prey capture performance of hatchlings are responsible for the evolution of host specialisation in *H. yedoensis*.

Materials and methods

Ladybirds

The specialist predator *H. yedoensis* and the generalist predator *H. axyridis* are sibling species with sympatric distributions in central Japan (Sasaji, 1998). Adults of the two species are difficult to distinguish because of their morphological similarity, but larvae, especially the third and fourth instars, show morphological differences (Sasaji, 1998). *Harmonia yedoensis* is an oligophagous aphid predator that preys only on *C. pini* and *E. thunbergii* (Tanigishi, 1975); it has never been found on any vegetation other than pine trees in central Japan. In contrast, *H. axyridis* is a polyphagous predator that preys upon a wide range of aphid species in numerous habitats (Osawa, 2000; Osawa & Ohashi, 2008).

Adults of *H. yedoensis* and *H. axyridis* were collected at Iwakura, Kyoto city (135°79′E, 35°09′N), central Japan, in April 2009. The habitat was young Japanese red pine (*Pinus densiflora* Sieb. et Zucc.) growing on land that had been subdivided for housing lots. Colonies of the specialist aphid *C. pini* on the *P. densiflora* shoots and branches attracted both ladybird species for foraging and mating. In the laboratory, 6 *H. yedoensis* females and 20 *H. axyridis* females were individually maintained in plastic Petri dishes (9 cm in diameter by 1.5 cm high) at 25 °C and a LD 16 : 8 h cycle, and provided each day with a surplus of frozen *Ephestia kuehniella* Zeller eggs (Beneficial Insectary, Ontario, Canada). Many egg clutches were obtained from 6 *H. yedoensis* and 14 *H. axyridis* females, and the first instars of each species derived from the egg clutches were used for the experiments.

Prey capture performance

In this experiment, sibling cannibalism was allowed to occur in each clutch similarly to field conditions. Four aphid species were used to measure the prey capture performance in the two ladybirds: *C. pini*, *Myzus varians* Davidson, *Aphis spiraecola*, and *Chaitophorus horii* Takahashi, which infest *P. densiflora*, *Prunus persica* Linné, *Spiraea thunbergii* Sieb. ex Blume, and *Salix koriyanagi* Kimura ex Goerz, respectively. *Cinara pini* was collected in Iwakura, and the other three species were collected in the Botanical Garden of Kyoto University (135°47′E, 35°02′N), where *H. axyridis* intensively forages on these aphids. Although *H. yedoensis* has been reported to prey on *E. thunbergii*, which can infest pine trees in nature (Tanigishi, 1975), this aphid was very rare at our collecting site. Thus, *E. thunbergii* was regarded at best as a minor prey item of *H. yedoensis* at the study site and not used in the experiments. Each clutch was maintained in a plastic Petri dish at 25 °C, and the number of eggs and the number of hatchlings after sibling cannibalism were counted. First, the rate of sibling cannibalism, defined as the number of eggs cannibalised by the siblings divided by the number of eggs in the clutch, was calculated. Then, four hatchlings were randomly chosen from each clutch. To standardise the hunger level, the hatchlings were used for the experiments within 12 h after dispersal from their clutches. One ladybird larva and three individuals of one of the four aphid species were placed in a 3.5-cm-diameter plastic Petri dish, the bottom of which was covered fully with filter paper as a substratum for walking. Preliminary observations showed that the first instars of neither ladybird species could capture *C. pini* adults because the adult aphids were too large (mean body size $\pm$ SE $= 6.24 \pm 0.77$ mg, $n = 10$) and their walking speed was too rapid. In consideration of the foraging ability of the first instars of *H. axyridis* and *H. yedoensis*, therefore, small, young *C. pini* instar larvae (0.41 ± 0.06 mg, $n = 9$) were used for the experiments, although their exact age in days was not determined. In contrast, apterous adults of the other three species of aphids were used because of their smaller body size (*M. varians*, 0.90 ± 0.03 mg, $n = 10$; *A. spiraecola*, 0.28 ± 0.02 mg, $n = 13$; *C. horii*, 0.26 ± 0.02 mg, $n = 10$). The foraging behaviour of the ladybird larvae was observed for 10 min at room temperature (25 °C). During the observation period, each time a hatchling attacked an aphid and captured it successfully was recorded. Then, the data for all hatchlings of each species of ladybird were combined, and predation success, defined as the proportion that could successfully prey upon each species of aphid, was calculated. The number of failed attacks was also compared among aphid species as an indicator of the relative elusiveness of each prey species to the larvae of each ladybird species. In all, 36 replicates (four hatchlings each from nine clutches) were completed for each combination of aphid species and ladybird species.

Predation success was analysed by using a generalised linear mixed effects model (GLMM; Schall, 1991) using the glmmPQL function of the MASS (Venables & Ripley, 2002) package of R software (version 2.10.1, R Foundation for Statistical Computing, Vienna, Austria), which can fit both fixed and random components to the model and thus can avoid pseudo-replication. The clutch identity and the rate of sibling cannibalism were fitted to random terms. The dependent variable was a binary term (binomial error structure) describing whether each individual successfully preyed upon an aphid (succeeded = 1, failed = 0). Tukey's HSD test was used for multiple comparisons. The number of failed attacks, because it was a positive integer, was used as a dependent variable with a Poisson error structure in the GLMM (Crawley, 2002). Some individuals that never attacked an aphid were excluded from this analysis. More than three-quarters of individuals in each treatment attacked the aphid at least once, with the exception that 12 of 36 *H. yedoensis* individuals did not attack *C. horii*.

The effect of sibling cannibalism on prey capture performance

In this experiment, the number of conspecific eggs that each larva could consume after hatching was manipulated, and then the prey capture performance of first instars against *C. pini* was measured. Eggs were removed from clutches 1 day after oviposition and placed individually in a plastic case (6.4 cm long by 3.3 cm wide by 1.7 cm high; 25 °C; LD 16 : 8 h) with a dampened soft brush. On the following day, different numbers of conspecific eggs (0, 1, 2, or 3) that had been laid on that day were put next to the first egg. Then, each hatchling was allowed to cannibalise the provided conspecific eggs. No larvae were excluded from the experiment, neither those that hatched normally nor those that consumed the provided eggs completely. About 20 replicates of each treatment were completed (see sample size in Fig. 2), and the eggs used in a treatment replication were not necessarily from the same clutch. The predation success against *C. pini* was measured in the same way as in the first experiment. In addition, whether hatchlings could moult to the second instar when they did not consume the aphids in the plastic case was recorded. Kawai (1978) reported that first instars of *H. axyridis* not provided with aphids can go through to the second instar only if they have consumed a sufficient number of conspecific eggs. The predation success of each ladybird species was then compared among the treatments by using the extended Fisher's exact test.

Morphological and behavioural traits

First instars provided with different numbers of conspecific eggs (0, 1, 2, or 3), as in the second experiment, were used to analyse the effect of sibling cannibalism on body size and walking ability. In the laboratory at 25 °C and under constant lighting, a bamboo skewer marked with two ticks, 20 cm apart, was leaned at 45° relative to the vertical on the bench. Then, with a soft brush, each larva was put on the lower end of the skewer, one at a time. Almost all individuals walked up the skewer, and their walking speed between the two ticks was measured. This behaviour is typical of predatory ladybird hatchlings, and may help them locate aphid colonies at the tips of host plant twigs or on leaf edges (Dixon, 2000; Völkl *et al*., 2007). Subsequently, each larva was preserved in 70% ethanol and its body length was measured under a digital microscope (VHX-900, Keyence, Osaka, Japan) as an indicator of maternal investment per offspring mediated by egg size and sibling cannibalism. About 10 replicates of each treatment were completed (see sample size in Fig. 3a). In addition, the head capsule width and the length of the femur and tibia of each right fore leg, middle leg, and hind leg of larvae that had not consumed conspecific eggs were measured. From these data, the total leg length (sum of the femur and tibia lengths) and the relative head capsule width and relative leg length (the ratio of each trait value divided by the body length in each individual) were calculated and compared between species.

Results

Prey capture performance

The mean egg cluster size was significantly smaller in *H. yedoensis* (mean ± SE = 21.5 ± 1.0, $n = 36$) than in *H. axyridis* (26.6 ± 1.9, $n = 36$; Student's t-test, $t_{70} = 2.4184$, $P < 0.05$). The mean rate of sibling cannibalism was significantly higher in *H. yedoensis* (0.424 ± 0.030) than in *H. axyridis* (0.265 ± 0.032; Student's t-test, $t_{70} = -3.664$, $P < 0.001$). In *H. yedoensis*, predation success did not differ significantly among the treatments with the four aphid species (GLMM, $P > 0.05$ after Tukey's HSD; Fig. 1a). By contrast, the predation success of *H. axyridis* was considerably lower against *C. pini* than against the other aphid species (GLMM, $P < 0.05$ after Tukey's HSD; Fig. 1a). In both species, the number of failed attacks was significantly higher against *C. pini* and *M. varians* than against *A. spiraecola* and *C. horii* (GLMM, $P < 0.05$ after Tukey's HSD; Fig. 1b).

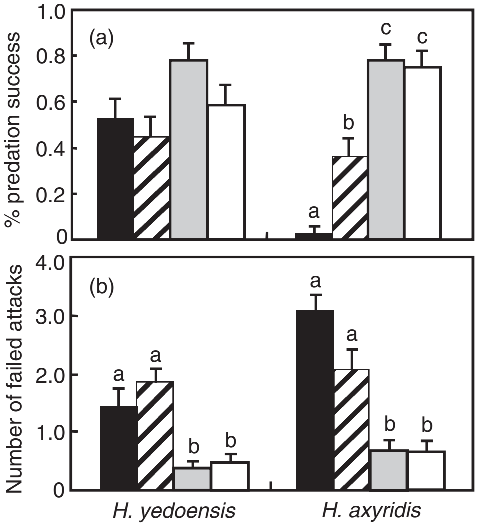

Fig. 1. (a) Predation success and (b) number of failed attacks in first instars of *Harmonia yedoensis* and *Harmonia axyridis* against four species of aphids: *Cinara pini* (black solid bars), *Myzus varians* (hatched bars), *Aphis spiraecola* (grey solid bars), and *Chaitophorus horii* (open bars). Vertical lines indicate SE and error structure is symmetrical. Different letters indicate significant differences among the treatments within a species (GLMM followed by Tukey's HSD, $P < 0.05$).

The effect of sibling cannibalism on prey capture performance

In the second experiment, the number of conspecific eggs consumed by a larva significantly affected predation success in *H. yedoensis* (extended Fisher's exact test, $\chi^2_3 = 10.44$, $P < 0.05$; Fig. 2a). Predation success of larvae that consumed more than two eggs decreased, but a high proportion of them could moult to second instar (Fig. 2a). By contrast, in *H. axyridis* the provision of conspecific eggs did not enhance predation success (extended Fisher's exact test, $\chi^2_3 = 1.03$, $P = 0.83$; Fig. 2b) and predation success was quite low. No individuals that consumed less than three conspecific eggs moulted successfully, and only some of those that consumed three conspecific eggs were able to moult successfully (Fig. 2b).

Morphological and behavioural traits

The body length of both *H. yedoensis* and *H. axyridis* increased as the number of conspecific eggs they consumed increased (one-way ANOVA, *H. yedoensis*, $F_{3,43} = 85.02$, $P < 0.0001$; *H. axyridis*, $F_{3,44} = 71.08$, $P < 0.0001$; Fig. 3a). The consumption of conspecific eggs also significantly enhanced

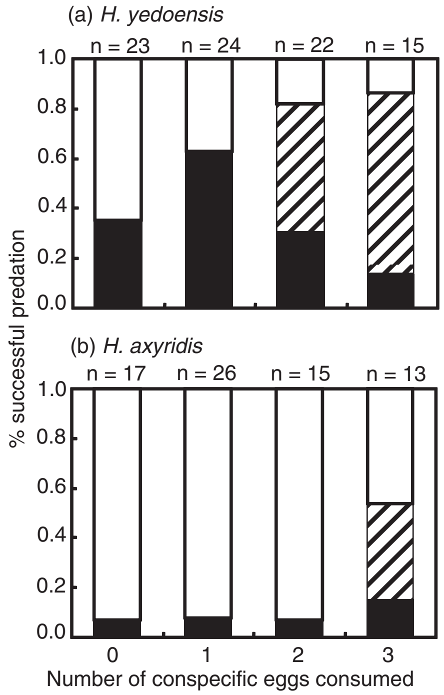

Fig. 2. Proportions of (a) *Harmonia yedoensis* and (b) *Harmonia axyridis* exhibiting successful predation against *Cinara pini* (solid segments), failed predation resulting in death from starvation (open segments), and successful moulting without successful predation or any food intake (hatched segments). The sample size of each treatment is shown above each bar.

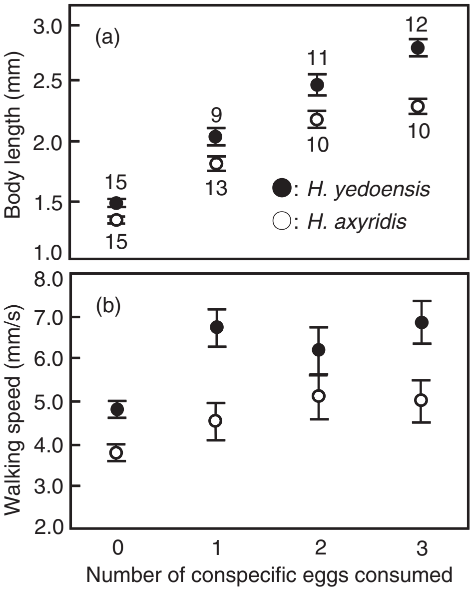

Fig. 3. (a) Body length and (b) walking speed in first instars of *Harmonia yedoensis* and *Harmonia axyridis* that consumed 0, 1, 2, or 3 conspecific eggs after eclosion. Vertical lines indicate SE. The sample size of *H. yedoensis* is shown above and that of *H. axyridis* is shown below each circle.

walking speed in *H. yedoensis* (one-way ANOVA, $F_{3,43} = 5.66$, $P < 0.01$; Fig. 3b) but not in *H. axyridis* ($F_{3,44} = 2.54$, $P = 0.07$; Fig. 3b). Head capsule width was larger in *H. yedoensis* than in *H. axyridis*, although relative head capsule width was not significantly different between them (Table 1). Both absolute and relative leg length were significantly larger in *H. yedoensis* than in *H. axyridis* (Table 1).

Discussion

This study demonstrated that *H. yedoensis* predation against *C. pini* was moderately successful, whereas *H. axyridis* predation against this species of aphid was highly unsuccessful (Fig. 1a). Moreover, *H. yedoensis* hatchlings that consumed a conspecific egg improved their prey capture performance (Fig. 2a). Although the consumption of two or three eggs decreased the rate of successful predation, a high proportion of hatchlings in these treatments could moult to second instar without any food intake (Fig. 2a), indicating that they did not need to attack the aphid because they were satiated. In addition, *C. pini* was a very elusive prey even for *H. yedoensis* hatchlings (Fig. 1b). Small *C. pini* larvae, however, which were likely to be less elusive than the apterous adults, were used in these experiments. These results suggest that a large amount of maternal investment, indirectly mediated by intense sibling cannibalism, enabled *H. yedoensis* hatchlings to hunt *C. pini*, a highly elusive prey for ladybird hatchlings, efficiently. We further showed that *H. yedoensis* hatchlings had a larger head capsule and longer legs than *H. axyridis* hatchlings (Table 1). The manipulation of maternal investment highlighted the importance of interactive effects

Table 1. Morphological traits of hatchlings of two species of *Harmonia* ladybird beetles (means ± SE).

	Head capsule width (μm)	Relative head capsule width	Length of fore leg (μm)	Length of mid leg (μm)	Length of hind leg (μm)	Total leg length (μm)	Relative length of legs
H. yedoensis	432.92 ± 3.21	0.28 ± 0.01	962.70 ± 10.18	916.35 ± 11.72	872.10 ± 10.50	2751.14 ± 28.04	1.84 ± 0.03
H. axyridis	394.00 ± 2.45	0.26 ± 0.00	772.15 ± 9.85	735.92 ± 12.59	710.85 ± 10.57	2218.92 ± 29.16	1.60 ± 0.03
t^*	−9.65	−0.673	−13.46	−10.49	−10.82	−13.16	−5.58
P^*	>0.001	0.506	>0.001	>0.001	>0.001	>0.001	>0.001

*Student's *t*-test.

Sample sizes of each species are 15. Relative head capsule width and relative leg length are the ratios of the respective trait values divided by the body length in each individual.

among multiple phenotypes including larval morphology. Specifically, given that consumption of conspecific eggs did not enhance the predation success of *H. axyridis* hatchlings against *C. pini* (Fig. 2b), morphological traits of first instars in *H. yedoensis* may also contribute to the prey capture performance against *C. pini* with long legs and high walking ability. Osawa and Ohashi (2008) showed that egg size, as well as the sibling cannibalism rate per clutch, is higher in *H. yedoensis* than in *H. axyridis*. Therefore, these results suggest that the combination of intense sibling cannibalism, larger egg size, and aggressive larval morphology is necessary for the specialisation to the giant pine aphid in *H. yedoensis*.

In contrast to *H. yedoensis*, consumption of conspecific eggs did not improve the predation success of *H. axyridis* hatchlings against *C. pini* (Fig. 2b), even though it increased their body size (Fig. 3a). This result suggests that another morphological trait other than body size of hatchlings may be needed to capture *C. pini* efficiently. In the field, females of *H. axyridis* oviposit on pine trees as well as on deciduous trees (Tanigishi, 1975; Sasaji, 1998). However, the results of this study suggest that most *H. axyridis* hatchlings in pine trees would be unable to capture *C. pini* and would die from starvation even if they cannibalised a few conspecific eggs. It is possible that middle or last instars of *H. axyridis* would be able to capture *C. pini* effectively because of their larger body size, but we think that the low capture performance of small first instars might cause high mortality in *H. axyridis*.

Morphological specialisation of the hatched larvae may also be important for the prey capture performance against *C. pini*. The morphological and behavioural analysis results of this study showed that *H. yedoensis* hatchlings have longer legs (Table 1) and can walk faster than *H. axyridis* hatchlings (Fig. 3b). Body size was larger and walking ability was higher in *H. yedoensis* than in *H. axyridis* when they were provided with the same number of conspecific eggs (Fig. 3) precisely because egg size is larger in *H. yedoensis* than in *H. axyridis* (Osawa & Ohashi 2008). In particular, *H. yedoensis* larvae that fed on conspecific eggs greatly enhanced their walking speed (Fig. 3b). Because giant aphids of the genus *Cinara* have very long legs and walk very fast compared with other aphids (Moritsu, 1983), the enhanced walking ability of *H. yedoensis* may contribute to its prey capture performance. Moreover, head capsule size, which can be interpreted as an indicator of the biting strength of hatchlings (Tauber *et al*., 1995), was larger in *H. yedoensis* than in *H. axyridis* (Table 1). Observation of hunting behaviour indicated that most *H. yedoensis* hatchlings first attacked the leg of *C. pini* with their mandibles, and then they hauled in the aphid using their fore legs, suggesting that biting strength may be important for predatory success. Indeed, hatchlings of *H. axyridis* frequently failed to capture *C. pini* even when they attacked this aphid (Fig. 1b). Therefore, morphological specialisation of hatchlings may be particularly important for a ladybird species to be able to prey successfully on *C. pini*, although the importance of morphological traits relative to the amount of maternal investment cannot be evaluated by our present data. Similar morphological and life history adaptation, that is, a larger head width and egg size, has also been reported in *C. slossonae*, a specialist lacewing that preys only upon the highly elusive alder aphid in the field (Tauber & Tauber, 1987; Tauber *et al*., 1995).

Comparison of the prey capture performance of these two ladybirds among aphid species has implications for the evolution of host specialisation. The results of this study showed that the specialist predator *H. yedoensis* could capture not only *C. pini* but also other aphid species that they never utilise in the field (Fig. 1a). Therefore, trade-offs in prey capture performance must not be the main factor explaining host specialisation in *H. yedoensis*. In contrast, the generalist predator *H. axyridis* could effectively capture various aphid species, other than *C. pini* (Fig. 1). This result suggests that even though hatchlings of *H. axyridis* receive less maternal investment and have no morphological specialisation against *C. pini*, they can utilise less elusive aphid species in various habitats in the field. In this study, we focused on the behaviour and performance of hatchlings because the hatchling stage is particularly important for host utilisation in insects (Zalucki *et al*., 2002). Sasaji (1998) reported that *H. yedoensis* can develop, pupate, emerge, and lay eggs normally if experimentally provided with aphids that it does not prey upon in nature such as *Cryptosiphum artemisiae* Buckton and *Macrosiphoniella* sp. However, further experimental study that quantitatively evaluates the nutritional quality of aphids for all stages of larval development as well as for female reproduction is needed for more complete understanding of host utilisation by *H. yedoensis.*

Our experimental results should also be interpreted with caution because the experiments were not designed to explore how spatial structure affects the prey capture performance of ladybird hatchlings. Specifically, in the experiments of this study, small and simple arenas were used to determine the prey capture performance of the two ladybirds against four aphid species. As a result, possible avoidance or emigration behaviour of aphids, which might occur in an environment with a complex spatial structure, could not be observed. For example, leaf morphology and plant structure often affect the foraging behaviour of ladybird larvae (Hodek & Honěk, 1996; Dixon, 2000). Therefore, investigation of the interaction between ladybird hatchlings and aphids under field conditions would be valuable for fully understanding host utilisation by *H. yedoensis*.

Given that trade-offs in prey capture performance are not responsible for the evolution of host specialisation, why is the prey type of *H. yedoensis* restricted to pine aphids in the field? Even though *H. yedoensis* mothers invest a large amount of resources per larva, sacrificing larger clutch size (Osawa & Ohashi, 2008), *C. pini* was more elusive than other aphids (Fig. 1b). It is possible that negative interactions between closely related species of ladybird, such as exploitative resource competition, intraguild predation, reproductive interference, and their interactions, determine their habitat types and food sources. Interspecific resource competition has been proposed as a major cause of host specialisation and phenotypic divergence among species of insects (Schluter, 2000). In fact, *H. axyridis* larvae often overexploit aphid colonies (Osawa, 1992b), causing intense intraspecific resource competition. However, in many animal taxa, interspecific competition for a common resource is very rarely intense (Strong, 1982; Schluter, 2000; Kaplan & Denno, 2007), whereas intraguild predation has been shown to be a directly harmful interaction (Polis *et al*., 1989; Holt & Polis, 1997). In particular, *H. axyridis* is reported to be an aggressive intraguild predator, and some laboratory experiments have shown that intraguild predation is asymmetric, occurring more frequently in *H. axyridis* larvae than in larvae of other ladybird species (Koch, 2003; Yasuda *et al*., 2004; Pell *et al*., 2008; Ware & Majerus, 2008). Nevertheless, it is still unknown whether interspecific larval interaction is ecologically important for host specialisation in insect communities (Polis *et al*., 1989; Schluter, 2000). Therefore, alternative hypotheses that can reliably and plausibly explain the evolution of host specialisation in *H. yedoensis* should be developed and tested.

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References

Albuquerque, G.S., Tauber, M.J. & Tauber, C.A. (1997) Life-history adaptations and reproductive costs associated with specialization in predaceous insects. *Journal of Animal Ecology*, **66**, 307–317.

Benkman, C.W. (1988) Seed handling ability, bill structure, and the cost of specialization for crossbills. *Auk*, **105**, 715–719.

Bernays, E. & Graham, M. (1988) On the evolution of host specificity in phytophagous arthropods. *Ecology*, **69**, 886–892.

Bristow, C.M. (1988) What makes a predator specialize? *Trends in Ecology & Evolution*, **3**, 1–2.

Chapin, J.B. (1985) Revision of the genus *Mulsantia* Weise (Coleoptera: Coccinellidae). *Annals of the Entomological Society of America*, **78**, 348–368.

Clutton-Brock, T.H. (1991) *The Evolution of Parental Care.* Princeton University Press, Princeton, New Jersey.

Crawley, M.J. (2002) *Statistical Computing: An Introduction to Data Analysis Using S-Plus.* John Wiley & Sons, Chichester, West Sussex.

Dixon, A.F.G. (1959) An experimental study of the searching behavior of the predatory coccinellid beetle *Adalia decempunctata* (L.). *Journal of Animal Ecology*, **28**, 259–281.

Dixon, A.F.G. (2000) *Insect Predator–Prey Dynamics*. Cambridge University Press, Cambridge, U.K.

Ehrlich, P.R. & Raven, H. (1964) Butterflies and plants: a study of coevolution. *Evolution*, **18**, 586–608.

Fox, L.R. & Morrow, P.A. (1981) Specialization: species property or local phenomenon. *Science*, **188**, 887–892.

Fox, C.W., Thakar, M.S. & Mousseau, T.A. (1997) Egg size plasticity in a seed beetle: an adaptive maternal effect. *The American Naturalist*, **149**, 149–163.

Friberg, M. & Wiklund, C. (2009) Host plant preference and performance of the sibling species of butterflies *Leptidea sinapis* and *Leptidea reali*: a test of the trade-off hypothesis for food specialization. *Oecologia*, **159**, 127–137.

Futuyma, D.J. & Moreno, G. (1988) The evolution of ecological specialization. *Annual Review of Ecology and Systematics*, **19**, 207–233.

Futuyma, D.J., Keese, M.C. & Funk, D.J. (1995) Genetic constraints on macroevolution: the evolution of host affiliation in the leaf beetle genus *Ophraella*. *Evolution*, **49**, 797–809.

Gilbert, F. (1990) Size, phylogeny and life-history in the evolution of feeding specialization in insect predators. *Insect Life Cycles: Genetics, Evolution and Co-ordination* (ed. by F. Gilbert), pp. 101–124. Springer, London, U.K.

Gordon, R.D. (1985) The Coccinellidae (Coleoptera) of America north of Mexico. *Journal of the New York Entomological Society*, **93**, 1–912.

Hodek, I. & Honěk, A. (1996) *Ecology of Coccinellidae*. Kluwer Academic Publishers, Dordrecht, The Netherlands.

Holt, R.D. & Polis, G.A. (1997) A theoretical framework for intraguild predation. *American Naturalist*, **149**, 745–764.

Ito, Y. (1980) *Comparative Ecology*. Cambridge University Press, Cambridge, U.K.

Jaenike, J. (1990) Host specialization in phytophagous insects. *Annual Review of Ecology and Systematics*, **21**, 243–273.

Kaplan, I. & Denno, R.F. (2007) Interspecific interactions in phytophagous insects revisited: a quantitative assessment of competition theory. *Ecology Letters*, **10**, 977–994.

Kawai, A. (1978) Sibling cannibalism in the first instar larvae of *Harmonia axyridis* Pallas (Coleoptera: Coccinelidae). *Kontyû*, **46**, 14–19.

Kishi, S. & Nishida, T. (2006) Adjustment of parental investment in the dung beetle *Onthophagus atripennis* (Col., Scarabaeidae). *Ethology*, **112**, 1239–1245.

Koch, R.L. (2003) The multicolored Asian lady beetle, *Harmonia axyridis*: a review of its biology, uses in biological control, and non-target impacts. *Journal of Insect Science*, **3**, 1–16.

Levins, R. (1968) *Evolution in Changing Environments.* Princeton University Press, Princeton, New Jersey.

MacArthur, R.H. & Levins, R. (1964) Competition, habitat selection and character displacement in a patchy environment. *Proceedings of the National Academy of Sciences of the United States of America*, **51**, 1207–1210.

Majerus, M.E.N. (1994) *Ladybirds.* Harper Collins, London, U.K.

Mayhew, P. (2006) *Discovering Evolutionary Ecology: Bringing Together Ecology and Evolution*. Oxford University Press, Oxford, U.K.

McGinley, M.A., Temme, D.H. & Geber, M.A. (1987) Parental investment in offspring in variable environments: theoretical and empirical considerations. *American Naturalist*, **130**, 370–398.

McPeek, M.A. (1996) Trade-offs, food web structure, and the coexistence of habitat specialists and generalists. *American Naturalist*, **148**, S124–S138.

Moritsu, M. (1983) *Aphids of Japan in Color.* Zenkoku Noson Kyoiku Kyokai Publishing, Tokyo, Japan [in Japanese].

Ohsaki, N. & Sato, Y. (1994) Food plant choice of *Pieris* butterflies as a trade-off between parasitoid avoidance and quality of plants. *Ecology*, **75**, 59–68.

Osawa, N. (1992a) Sibling cannibalism in the ladybird beetle *Harmonia axyridis* Pallas: fitness consequences for mothers and offspring. *Researches on Population Ecology*, **34**, 45–55.

Osawa, N. (1992b) A life table of the ladybird beetle *Harmonia axyridis* Pallas (Coleoptera, Coccinellidae) in relation to the aphid abundance. *Japanese Journal of Entomology*, **60**, 575–579.

Osawa, N. (1993) Population field studies of the aphidophagous ladybird beetle *Harmonia axyridis* Pallas (Coleoptera: Coccinellidae): life tables and key factor analysis. *Researches on Population Ecology*, **35**, 335–348.

Osawa, N. (2000) Population field studies on the aphidophagous ladybird beetle *Harmonia axyridis* (Coleoptera: Coccinellidae): resource tracking and population characteristics. *Population Ecology*, **42**, 115–127.

Osawa, N. & Ohashi, K. (2008) Sympatric coexistence of sibling species *Harmonia yedoensis* and *H. axyridis* (Coleoptera: Coccinellidae) and the roles of maternal investment through egg and sibling cannibalism. *European Journal of Entomology*, **105**, 445–454.

Parker, G.A. & Begon, M. (1986) Optimal egg size and clutch size: effects of environment and maternal phenotype. *American Naturalist*, **128**, 573–592.

Pell, J.K., Baverstock, J., Roy, H.E., Ware, R.L. & Majerus, M.E.N. (2008) Intraguild predation involving *Harmonia axyridis*: a review of current knowledge and future perspective. *Biological Control*, **53**, 147–168.

Polis, G.A., Myers, C.A. & Holt, R.D. (1989) The ecology and evolution of intraguild predation: potential competitors that eat each other. *Annual Review of Ecology and Systematics*, **20**, 297–330.

Roininen, H. & Tahvanainen, J. (1989) Host selection and larval performance of two willow-feeding sawflies. *Ecology*, **70**, 129–136.

Sasaji, H. (1998) *Natural History of the Ladybirds.* University of Tokyo Press, Tokyo, Japan [in Japanese].

Schall, R. (1991) Estimation in generalized linear models with random effects. *Biometrika*, **78**, 719–727.

Schluter, D. (1995) Adaptive radiation in sticklebacks: trade-offs in feeding performance and growth. *Ecology*, **76**, 82–90.

Schluter, D. (1996) Ecological causes of adaptive radiation. *American Naturalist*, **148**, S40–S64.

Schluter, D. (2000) *The Ecology of Adaptive Radiation.* Oxford University Press, Oxford, U.K.

Sloggett, J.J. (2008) Weighty matters: body size, diet and specialization in aphidophagous ladybird beetles (Coleoptera: Coccinellidae). *European Journal of Entomology*, **105**, 381–389.

Sloggett, J.J. & Majerus, M.E.N. (2000) Habitat preferences and diet in the predatory Coccinellidae (Coleoptera): an evolutionary perspective. *Biological Journal of the Linnean Society*, **70**, 63–88.

Strong, D.R. (1982) Harmonious coexistence of hispine beetles on *Heliconia* in experimental and natural communities. *Ecology*, **63**, 1039–1049.

Tanigishi, K. (1975) Comparative studies on sibling species of lady beetle, *Harmonia axyridis* and *H. yedoensis*: differences in egg numbers per cluster. *Paper Presented at the 35th Annual Meeting of the Entomological Society of Japan*, Chiba, Japan [in Japanese].

Tauber, C.A. & Tauber, M.J. (1987) Food specificity in predacious insects: a comparative ecophysiological and genetic study. *Evolutionary Ecology*, **1**, 175–186.

Tauber, C.A., Ruberson, J.R. & Tauber, M.J. (1995) Size and morphological differences among the larvae of two predacious species and their hybrids (Neuroptera: Chrysopidae). *Annals of the Entomological Society of America*, **88**, 502–511.

Thompson, J.N. (1988) Coevolution and alternative hypotheses on insect/plant interactions. *Ecology*, **69**, 893–895.

Venables, W.N. & Ripley, B.D. (2002) *Modern Applied Statistics with S*. Springer, New York, New York.

Völkl, W., Mackauer, M., Pell, J.K. & Brodeur, J. (2007) Predators, parasitoids and pathogens. *Aphids as Crop Pests* (ed. by H. F. V. Emden and R. Harrington), pp. 187–233. CABI, Wallingford, Oxfordshire, U.K.

Ware, R.L. & Majerus, M.E.N. (2008) Intraguild predation of immature stages of British and Japanese coccinellids by the invasive ladybird *Harmonia axyridis*. *Biological Control*, **53**, 169–188.

Yasuda, H., Evans, E.W., Kajita, Y., Urakawa, K. & Takizawa, T. (2004) Asymmetric larval interactions between introduced and indigenous ladybirds in North America. *Oecologia*, **141**, 722–731.

Zalucki, M.P., Clarke, A.R. & Malcolm, S.B. (2002) Ecology and behavior of first instar larval Lepidoptera. *Annual Review of Entomology*, **47**, 361–393.

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