

Intrinsic prey suitability in specialist and generalist *Harmonia* ladybirds: a test of the trade-off hypothesis for food specialization

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Abstract

The trade-off hypothesis posits that increased performance on a given resource comes at the cost of decreased performance on other resources, and that this trade-off is a driving force of food specialization in both predators and herbivores. In this study, we examined larval survival and performance in two sibling ladybird species, *Harmonia yedoensis* Takizawa and *Harmonia axyridis* Pallas (Coleoptera: Coccinellidae), fed on one of four prey species. *Harmonia yedoensis* is a specialist predator that preys mostly on pine aphids in the field, whereas *H. axyridis* is a generalist predator with a broad prey range. We experimentally showed in the laboratory that larval survival and performance were not higher when *H. yedoensis* was fed on pine aphids, compared with the other prey species. Rather, prey suitability was similar in both ladybird species, and *H. yedoensis* larvae developed as well or even better on prey species that they never utilize in nature. These results suggest that the host range in *H. yedoensis* may not be limited by the intrinsic suitability of the aphid species per se. Moreover, as shown by our previous study, the pine aphid is a highly elusive prey that is difficult for small ladybird hatchlings to capture, which means that the cost of utilizing this prey is high. Therefore, we conclude that some factor other than prey suitability is responsible for the observed food specialization in *H. yedoensis*.

Introduction

Understanding how ecological specialization occurs and is maintained is a fundamental problem in ecology and evolution, because it is closely associated with mechanisms of species coexistence and divergence in resource use (e.g., Futuyma, 2001; Mayhew, 2006). A large proportion of insects specialize on only one or a few related food sources, whereas even closely related species have a broad food and habitat range (Bernays & Graham, 1988; Schluter, 2000; Schoonhoven et al., 2005). In many cases, generalist insects can maintain a stable population by tracking

multiple resources in highly heterogeneous food patches, whose quality and quantity vary unpredictably within and among years (Jaenike, 1978; Courtney & Forsberg, 1988; Osawa, 2000; Wiklund & Friberg, 2009). Therefore, as generalization should be advantageous in unpredictably variable environments, the loss of such flexibility in resource use by specialization requires explanation (Futuyma, 2001; Mayhew, 2006). It is frequently assumed with regard to food specialization that a high level of fitness on one host species entails a decline in fitness on other hosts (trade-off hypothesis; Levins, 1968). However, this assumption often fails empirical tests in herbivorous insects (Jaenike, 1990; Hereford, 2009), whose larvae can often develop as well or even better on host plants that are less or never utilized in nature (e.g., Wiklund, 1975; Smiley, 1978; Roininen & Tahvanainen, 1989). In view of these findings, recent studies on herbivore host selection have investigated factors other than plant nutritional quality, such as the effects of natural enemies on larval survival (e.g., Ohsaki & Sato, 1994; Murphy, 2004; Wiklund & Friberg, 2008), and adult

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foraging behaviour, that can shape decision making on oviposition preference (Scheirs et al., 2000; Scheirs & De Bruyn, 2002; Janz et al., 2005).

Although the literature on host-plant specialization in herbivorous insects is extensive, less is known about food specialization in predatory insects (Tauber & Tauber, 1987; Albuquerque et al., 1997; Sadeghi & Gilbert, 2000). In fact, a high degree of specificity is shown by many, if not most, carnivorous insects, especially parasitoids, but also many predators (Gilbert, 1990). In predators, morphological and behavioural adaptations in which functional efficiency is increased in one situation, but reduced in another, are considered to be the main driving force of food specialization through trade-offs (Schluter, 1996). For example, an extremely elongate, small-headed morphotype of the malacophagous carabid beetle *Damaster blaptoides* Kollar achieves a high feeding performance on snails with a large aperture by pushing its head through the aperture, but it cannot efficiently penetrate the shells of snails with a small aperture (Konuma & Chiba, 2007). However, as in herbivorous insects, the nutritional quality and toxin contents of food items may also influence host selection in predatory insects. Even though Bristow (1988) reported that animal tissues of different species may be similarly able to provide predators with a balanced diet, plant-derived chemical compounds in a herbivore's tissues can decrease the survival and growth rate of a predator feeding on that herbivore (e.g., Hodek & Honěk, 1996; Sadeghi & Gilbert, 2000; Francis et al., 2001). Therefore, confirmation of the trade-off hypothesis in predatory insects should generally require measurements of the intrinsic prey suitability as well as the functional efficiency of multiple prey items.

Two sibling species of predatory ladybirds, *Harmonia yedoensis* Takizawa and *Harmonia axyridis* Pallas (Coleoptera: Coccinellidae), have highly different food preferences and habitat ranges in central Japan. *Harmonia yedoensis* preys exclusively upon the giant pine aphid *Cinara pini* L. (Homoptera: Aphididae) in a pine tree habitat (Sasaji, 1998), whereas *H. axyridis* is a generalist predator that utilizes multiple prey species that occupy numerous habitats (Osawa, 2000). *Harmonia yedoensis* exhibits both morphological and behavioural specialization to *C. pini*, which has both a large body size and high vagility. So that they can hunt this highly elusive prey efficiently, *H. yedoensis* hatchlings have a larger head capsule, longer legs, and a higher vagility than *H. axyridis* hatchlings (Noriyuki et al., 2011). Moreover, *H. yedoensis* hatchlings are provided with maternal resources through large egg size and intense sibling cannibalism in a clutch (Osawa & Ohashi, 2008). Importantly, *H. yedoensis* hatchlings are able to capture various kinds of aphids provided experimentally as

well or even better than its usual prey, although they never prey on these species in nature (Noriyuki et al., 2011). Therefore, the specialization of *H. yedoensis* on *C. pini* cannot be explained solely by trade-offs in prey capture performance against different aphid species. Alternatively, aphid nutritional quality and toxin contents, which affect survival and growth rate of larvae in some species of ladybird (Okamoto, 1978; Hodek & Honěk, 1996; Sugiura & Takada, 1998; Francis et al., 2001; Provost et al., 2006), may shape the food specificity of *H. yedoensis*. To date, however, larval developmental performance has not been quantitatively examined in *H. yedoensis*.

In this study, we fed *H. yedoensis* and *H. axyridis* larvae four different prey (three aphid species and the eggs of a moth), and examined larval survival and performance in terms of larval stage duration, pupal weight, and growth rate. The trade-off hypothesis predicts that survival and performance of *H. yedoensis* larvae should be higher when they feed on *C. pini* than when they feed on other species of aphids, with the benefit of this intrinsic prey suitability compensating for the fitness reduction caused by the capture difficulty of this prey. Furthermore, if prey suitability is an important determinant of host range, then the performance of *H. yedoensis* fed on aphid species that it never utilizes in nature, but are normally consumed by *H. axyridis*, should be lower than the performance of *H. axyridis* fed on those species. We also examined larval performance on an artificial 'prey' [frozen eggs of a pyralid moth, *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae)] to assess whether some specific dietary component obtained exclusively from the aphids is essential for the development of *H. yedoensis*. We then evaluated whether the trade-off hypothesis could explain food specialization in *H. yedoensis* and explored other factors, such as intra-guild predation and interspecific sexual interactions, which might determine host range in these two ladybird species.

Materials and methods

Ladybirds

Adults of *H. yedoensis* and *H. axyridis* were collected at Iwakura, Kyoto city (135°79'E, 35°09'N), central Japan, in April 2011. 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). In the laboratory, more than 15 egg clutches were obtained from each *H. yedoensis* and *H. axyridis* female, which were individually maintained in plastic Petri dishes (9 cm in diameter, 1.5 cm high) at 25 °C, with a L16:D8 photoperiod, and provided every day with a surplus of pea aphids, *Acyrtosiphon pisum*

Harris (Hemiptera: Aphididae). *Harmonia yedoensis* (N Osawa & S Noriyuki, unpubl.) and *H. axyridis* females (Majerus et al., 1998; Nakamura et al., 2005) can carry male-killing bacteria that are transmitted from mother to daughter, and male embryos killed by the bacteria look like infertile eggs. Therefore, because we aimed to examine the larval performance of both males and females and also to exclude any confounding effects of symbiont bacteria on the development of its host (e.g., Elnagdy et al., 2011), we excluded offspring of *H. yedoensis* and *H. axyridis* adult females that produced clutches with more than 40% non-developing eggs on average from our analysis. The threshold of 40% was chosen as a conservative criterion for exclusion (Perry & Roitberg, 2005). As a result, we used offspring of seven *H. yedoensis* and five *H. axyridis* females for the experiments. Moreover, cannibalism of undeveloped sibling eggs at the time of hatching (i.e., trophic egg consumption) can promote more rapid development and larger adult size in *H. axyridis* (Osawa, 2002) as well as in the other ladybird species (Michaud & Grant, 2004; Roy et al., 2007). Therefore, to exclude the effects of sibling cannibalism on larval performance, eggs were removed from clutches 1 day after oviposition and placed individually in plastic cases (6.4 × 3.3 × 1.7 cm) with a dampened soft brush. Individual hatchlings of each species derived from these eggs were used for the experiments.

Experiments

We measured larval survival, developmental time (from hatching to pupation), pupal weight, and growth rate (mg/day) fed on one of three aphid species or an artificial food item, *E. kuehniella* eggs (Beneficial Insectary, Ontario, Canada). We used the following aphid species (Hemiptera: Aphididae) as prey in the experiments: *C. pini* from Japanese red pine, *Pinus densiflora* Sieb. et Zucc. (Pinaceae), *Lachnus tropicalis* (van der Goot) from Japanese chestnut, *Castanea crenata* Sieb. et Zucc. (Fagaceae), and *A. pisum* from broad bean, *Vicia faba* L. (Fabaceae). *Cinara pini* was collected in Iwakura, and the other two species were collected on the Northern Campus and Botanical Garden of Kyoto University (135°47'E, 35°02'N), where *H. axyridis* forages on these aphids (Osawa, 2000; S Noriyuki, unpubl.). As our objective was to evaluate intrinsic prey suitability, the food items used in each test were first frozen at -50 °C to exclude the effects of prey mobility on larval performance. Aphids mummified by parasitoids were never used in our experiments because they substantially affect larval performance of ladybirds (e.g., Takizawa et al., 2000). Frozen prey have been demonstrated to be suitable for *H. axyridis* (Kalaskar & Evans, 2001; Provost et al., 2006), and preliminary tests showed that the frozen moth eggs and frozen individuals of the three aphid species were

readily attacked by *H. yedoensis* and *H. axyridis* hatchlings. Newly emerged hatchlings of each ladybird species were individually maintained in plastic Petri dishes (9 cm in diameter, 1.5 cm high) at 25 °C and L16:D8 photoperiod, and each hatchling was randomly assigned to receive one of the four food items. As the larvae grew, the quantity of aphids or eggs offered each day was increased to keep pace with their requirements (10–500 mg per day for a larva); thus, food was always available ad libitum. *Harmonia* pupae were detached from the wall of the Petri dish 1 day after pupation and weighed to the nearest 0.01 mg using an electronic balance. Unfortunately, some individuals were injured when they were detached, causing them to be unable to emerge (*H. yedoensis*, 6 out of 84 individuals; *H. axyridis*, 3 out of 72 individuals). These individuals could not be sexed, and thus, we excluded them from our analysis of larval stage duration, pupal weight, and growth rate.

Statistical analysis

The binomially distributed response variable survival (death during development = 0, survival to pupa = 1) was tested using logistic regression analysis with the categorical factors, ladybird species and prey species (used for both the moth eggs and the three aphid species). A Three-way ANOVA was performed on larval stage duration, pupal weight, and growth rate with ladybird species, prey species, and sex as independent fixed factors. As the effect of prey species on the three performance parameters was highly significant, Tukey's HSD test was used to compare ladybird performance among prey for each sex and species. All statistical tests were conducted using JMP Discovery Software (SAS Institute, Cary, NC, USA).

Results

The survival rates of the ladybirds fed on the various prey species were not significantly different between species ($\chi^2 = 0.00015$, d.f. = 1, $P = 0.99$; Figure 1). In particular, the survival rates of *H. yedoensis* and *H. axyridis* fed on *C. pini* were identical. The survival rate of both *H. yedoensis* and *H. axyridis*, however, varied among prey species (prey: $\chi^2 = 9.18$, d.f. = 3, $P = 0.03$; ladybird*prey interaction: $\chi^2 = 8.55$, d.f. = 3, $P = 0.04$; Figure 1). The average survival rates of both *H. yedoensis* and *H. axyridis* fed on *C. pini* were lowest, compared with ladybirds fed on the other prey species. In general, however, survival rates of *H. yedoensis* and *H. axyridis* were high, varying between 80 and 100%, regardless of the prey on which they were fed.

Larval stage duration differed significantly depending on ladybird species and prey species (Table 1). In particular, the duration of the larval stage was significantly longer in both sexes of both species when fed on *C. pini* than

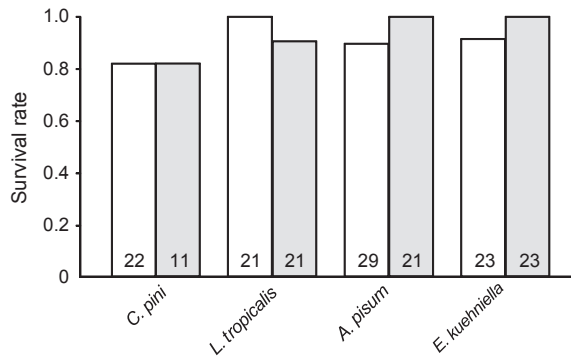


Figure 1 Survival rates of *Harmonia yedoensis* (white bars) and *H. axyridis* (grey bars) fed on one of four prey species (*Cinara pini*, *Lachnus tropicalis*, *Acyrtosiphon pisum*, and *Ephestia kuehniella*). The survival rates were significantly different among the prey species, but not between the ladybird species (logistic regression analysis: $P < 0.05$). Numbers in bars indicate sample sizes.

when they were fed on the other three prey species (Figure 2A–D). In both sexes of *H. axyridis*, larval stage duration was significantly different between *A. pisum* and *E. kuehniella* prey (Figure 2C and D). Pupal weight was significantly affected by ladybird species, prey, sex, and ladybird*prey interaction (Table 1). In contrast with larval stage duration, pupal weight did not differ significantly between *C. pini* and *E. kuehniella* in either sex of *H. yedoensis* (Figure 2E and F). However, pupal weight of male and female *H. yedoensis* fed on *C. pini* was significantly lower than that of those fed on *L. tropicalis* or *A. pisum* (Figure 2E and F). Similarly, there was a significant difference in pupal weight of both male and female *H. axyridis* between *C. pini* and *L. tropicalis*, but no significant difference among *C. pini*, *A. pisum*, and *E. kuehniella* was found (Figure 2G and H). The growth rates of the ladybirds fed on the different prey species did not vary between species, but varied significantly depending on prey and sex, and the ladybird*prey interaction was significant (Table 1). In both male and female *H. yedoensis*, growth rates were sig-

nificantly lower in those fed on *C. pini* than in those fed on the other three prey species (Figure 2I and J). The growth rate of female but not male *H. axyridis* fed on *C. pini* was significantly lower than that of those fed on the other three prey species (Figure 2K and L).

Discussion

Our results demonstrated that in *H. yedoensis* larval survival and performance, assessed by larval stage duration, pupal weight, and growth rate, were not higher when the ladybirds were fed on *C. pini* compared with the three prey species (Table 1; Figures 1 and 2). In fact, *H. yedoensis* larvae performed as well or better on the factitious diet and on aphid species that it never utilizes in nature. Moreover, the developmental time of both *H. yedoensis* and *H. axyridis* was significantly longer when they were fed on *C. pini* than when they were fed on the other prey species (Figure 2A–D). Furthermore, food quality of *C. pini* typically resulted in lower body weight in both *H. yedoensis* and *H. axyridis*, especially when compared with the other two aphid species (Figure 2E–H). As a result, larvae of both species suffered a slower growth rate when fed on *C. pini* compared with those fed on the other prey species (Figure 2I–L). These results suggest that the host range of *H. yedoensis* is not determined solely by the intrinsic suitability of aphids. Noriyuki et al. (2011) showed that *C. pini* is a highly elusive prey and difficult for even *H. yedoensis* hatchlings to capture, despite their specialized foraging morphology with a larger head capsule and longer legs. Considering those results together with the findings of this study, we conclude that some factor other than prey suitability is responsible for the host specialization of *H. yedoensis* on *C. pini*.

Our results showed that *H. yedoensis* does not require any nutrition unique to *C. pini* for larval development (Figures 1 and 2). Indeed, the larvae pupated normally even when fed on the factitious diet. To the contrary, the results suggest that the nutritional quality of *C. pini* may

Table 1 ANOVA results for the three linear models, each consisting of three factors and their first-order interactions

	d.f.	Larval stage duration			Pupal weight			Growth rate		
		SS	F	P	SS	F	P	SS	F	P
Ladybird species (L)	1	24.15	21.46	<0.0001	432.23	32.68	<0.0001	0.18	0.85	0.36
Prey species (P)	3	255.81	75.77	<0.0001	1128.60	28.44	<0.0001	24.47	37.76	<0.0001
Sex (S)	1	2.32	2.06	0.15	255.41	19.31	<0.0001	0.99	4.60	0.03
L*P	3	8.72	2.58	0.06	160.41	4.04	0.01	2.05	3.16	0.03
P*S	3	2.74	0.81	0.49	54.56	1.38	0.25	0.92	1.42	0.24
S*L	1	0.79	0.70	0.40	2.98	0.23	0.64	0.10	0.47	0.50
Residuals	134	150.80			1772.40			28.95		

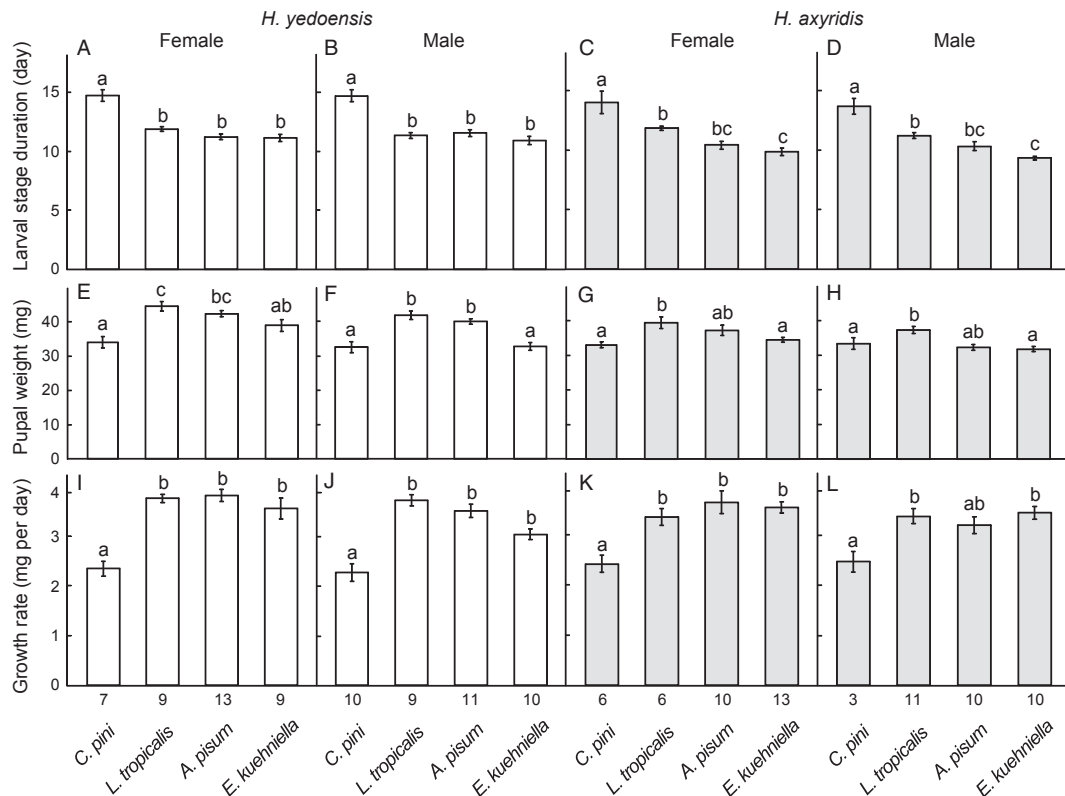


Figure 2 (A–D) Mean (\pm SE) larval stage duration, (E–H) pupal weight, and (I–L) growth rate of female and male *Harmonia yedoensis* (white bars) and *H. axyridis* (grey bars) fed on each of the four prey species (*Cinara pini*, *Lachnus tropicalis*, *Acyrtosiphon pisum*, and *Ephesthia kuehniella*). Different letters indicate significant differences among the prey species within a sex for each ladybird species (Tukey's HSD test: $P < 0.05$). The sample size of each unique experimental group is shown along the bottom horizontal axis.

be relatively low and/or this aphid may contain some growth-inhibiting substance that adversely affects the growth rate of both *H. yedoensis* and *H. axyridis*. Phloem saps generally lack toxins and chemical feeding deterrents, because plant secondary compounds tend to be localized in the apoplast and cell vacuole, not in the cytoplasmic compartment (Douglas, 2006). However, phloem-mobile secondary compounds have been reported in some plant species (Botha et al., 1977; Merritt, 1996; Brudenell et al., 1999; Hartmann, 1999). Especially in pine trees, secondary metabolites, such as monoterpenes, which are biologically active against herbivores and bacteria, are typically contained in needles and tree resin (Gershenson & Dudareva, 2007; Jason et al., 2011). Thus, it is possible that *C. pini* ingests a growth-inhibiting substance from pine trees that reduces the growth rates of *H. yedoensis* and *H. axyridis*. In fact, the chemical defences of pine sawfly larvae are known to originate from their ability to exploit the chemical defences of pine trees (Ikeda et al., 1977; Codella & Raffa, 1995). Bioassays need to be performed to evaluate the

effects of nutritional quality and plant secondary metabolites of *C. pini* on developmental performance in ladybirds.

Given that trade-offs in prey suitability are not responsible for the host specialization of *H. yedoensis*, why is its prey type restricted to pine aphids in the field? Importantly, *H. yedoensis* mothers sacrifice number of offspring by investing a large amount of maternal resources per hatchling (i.e., offspring size-number trade-offs; Smith & Fretwell, 1974) to enable the larvae to utilize their elusive prey efficiently (Osawa & Ohashi, 2008), even though, as shown in this study, *C. pini* is a nutritionally favourable prey for neither *H. yedoensis* nor *H. axyridis* (Figure 2). In contrast with *H. yedoensis*, *H. axyridis* is a generalist predator that utilizes various less elusive and nutritionally more suitable aphids in nature (Osawa, 2000). As these closely related predatory ladybird species are sympatric, negative interactions between them may determine their food sources and habitat types. Resource competition, intra-guild predation, and reproductive interference have been proposed as possible drivers of habitat partitioning

(Schluter, 2000). Indeed, *H. axyridis* has been reported to be an aggressive intraguild predator, and some laboratory experiments have shown that intraguild predation in ladybirds is asymmetric, with larvae of *H. axyridis* often experiencing advantages over other ladybird species (e.g., Yasuda et al., 2004; Pell et al., 2008; Ware & Majerus, 2008). However, *H. axyridis* larvae may engage in symmetric intraguild predation with *H. yedoensis* larvae, mainly because of their similar body size (H Yasuda & Y Yamada, unpubl.). Alternatively, adults of the two species engage in interspecific mating behaviour, and mating success substantially decreased in *H. yedoensis* not in *H. axyridis* when they were experimentally housed together (Noriyuki et al., 2012). This asymmetric reproductive interference may force *H. yedoensis* to become a specialist predator that exclusively utilizes less preferred prey in nature. Therefore, relative importance of alternative mechanisms should be investigated to adequately explain ecological specialization and generalization in *Harmonia* ladybirds.

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