ORIGINAL ARTICLE

Toxicity of the aphid *Aulacorthum magnoliae* to the predator *Harmonia axyridis* (Coleoptera: Coccinellidae) and genetic variance in the assimilation of the toxic aphids in *H. axyridis* larvae

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Abstract

Larvae of the ladybird *Harmonia axyridis* are reported to suffer high mortality when they are fed with the aphid *Aulacorthum magnoliae* associated with the elder *Sambucus sieboldiana*. In the present study we first examined whether aphid toxicity to *H. axyridis* was altered when the aphids were reared on different host plants, and second whether some ladybird families could adapt specially to the toxic aphids. Ladybird larvae from each egg batch were divided into two groups; one group was fed with *A. magnoliae* reared on potato, and the other group was fed with *A. magnoliae* reared on elder. The ladybird larvae fed with elder aphids suffered significantly higher mortality and grew more slowly than did larvae fed with potato aphids. This result indicates that *A. magnoliae* aphids absorbed toxic substances or their precursors from *S. sieboldiana*. We suggest that host association of *A. magnoliae* with the primary host plant *S. sieboldiana* has been maintained because of the advantage of deterring predation. Significant and positive correlation was detected across *H. axyridis* sib families between the mean weight of larvae fed with elder aphids and the mean weight of larvae fed with potato aphids. The hypothesis that some ladybird families adapt specially to the toxic aphids was not supported. However, the available evidence showed that a large amount of genetic variance in performance is maintained in a wild population of the ladybird *H. axyridis*.

Key words: allelochemical, Aphididae, indirect effect, interaction, *Sambucus*, secondary compound.

INTRODUCTION

A number of plant species produce a variety of secondary compounds to protect themselves from herbivorous insects (Paul & Peter 1964; Lukefahr & Martin 1966; Feeny 1970; Bernays 1978; Nielsen 1978; Isman & Duffey 1982). Likewise, an increasing number of studies have demonstrated that herbivorous insects protect themselves against general predators by accumulating toxic substances in their bodies (Rothschild *et al.* 1970; Marples *et al.* 1989; Evans & Schmidt 1990). Larvae of the ladybird *Harmonia axyridis* Pallas, a general predator of aphid species, suffer from reduced growth rate and increased mortality when particular aphid species are provided as food (Okamoto 1978); when ladybird larvae were fed with *Rhopalosiphum maidis* Fitch, 92% of them developed into adulthood, whereas ladybird larvae completely failed to develop when they were fed with *Aphis craccivora* Koch. Aphids are known to absorb secondary metabolites from their host plants to produce substances toxic to predators (Hodek 1956; Okamoto 1966; Rothschild *et al.* 1970; Wink *et al.* 1982; Obatake & Suzuki 1985; Nishida & Fukami 1989; Malcolm 1990; Francis *et al.* 2000, 2001). Furthermore, several studies have indicated that the level of aphid toxicity to predators changes depending on the portions of the host plants the aphids colonize (Bristow 1991) and on the season in which the evaluation is made (Takeda *et al.* 1964). However, no studies have revealed whether aphids that are known to be poisonous can synthesize toxic compounds on a wide range of host plants, or whether aphid toxicity is completely...
dependent on the host plants. Whether aphid toxicity changes substantially depending on the species of host plant or is stable is an issue that critically affects aphids’ host selection. For testing these hypotheses, monophagous and oligophagous aphid species are not suitable because there is no opportunity to examine the aphid potential to produce toxic substances for a wide range of plant species.

*Aulacorthum magnoliae* Essig et Kuwana, which is found on the elder *Sambucus sieboldiana* Schwer, is reported to be toxic to *H. axyris* larvae when this aphid is given as food (Okamoto 1978). *Aulacorthum magnoliae* has been recorded from more than 20 families of plants, including a number of crops (Blackman & Eastop 2000). In the present study, we first aimed to test whether *A. magnoliae* that have been reared on plants other than *Sambucus sieboldiana* var. *miqueli* have a different effect on the viability and performance of larvae of the ladybird *H. axyris* when they are given as food. The second purpose of the present study was to examine whether the sib families of *H. axyris* exhibit different developmental norms of reaction to prey aphids on different host plants. Using a general aphid predator, *Episyrphus balteatus* (Diptera: Syrphidae), Sadeghi and Gilbert (1999) observed that individual females showed different oviposition preferences for two prey aphid species, and that their larvae performed better when fed with aphid species for which the mothers showed a preference than when fed with the non-preferred aphid species. Preliminary field observations showed that egg batches and larvae of *H. axyris* occurred around *A. magnoliae* colonies on *S. sieboldiana* var. *miqueli*, suggesting that some females have a preference for *A. magnoliae* colonies. Because oviposition site selection by *H. axyris* females critically affects the offspring’s performance, the females’ preference for *A. magnoliae* might be positively connected to their offspring’s potential to assimilate the aphids. Thus, the present study focuses both on the acquisition of toxicity in aphids as an antipredator adaptation and on the possibility of any families of the predator *H. axyris* adapting specially to unpalatable prey species.

**MATERIALS AND METHODS**

**Focal species**

*Harmonia axyris* is an aphidophagous ladybird beetle that is distributed in northern and temperate Asia, including Japan (Sasaji 1998). In Hokkaido, northern Japan, this species is bivoltine (Y. Fukunaga, pers. obs.). *Aulacorthum magnoliae*, a host-alternating aphid, is distributed throughout Japan, Korea and China to India. In early spring, first instar fundatrices hatch from overwintered eggs on the elder *Sambucus sieboldiana*, the primary host. Part of the second generation and the majority of the third generation develop into alates that migrate to secondary hosts. In autumn, gynoparvae (winged females) return from the secondary hosts to *S. sieboldiana*, where they parthenogenetically deposit oviparae, which mature on *S. sieboldiana* and mate with winged males flying from the secondary hosts.

**Aphid and ladybird larva experiments (2003)**

To estimate the effect of food quality on the performance of *H. axyris* larvae, newly hatched larvae of *H. axyris* were fed with *A. magnoliae* reared on *S. sieboldiana* var. *miqueli* or on potato *Solanum tuberosum* (L.). In mid-May 2003, *A. magnoliae* fundatrices were collected from *S. sieboldiana* var. *miqueli* from the botanical garden, Field Science Center for the Northern Biosphere, Hokkaido University. The fundatrices were transplanted onto growing potato shoots (approximately 20 cm tall). Although potato has not been recorded as the primary host (only as a secondary host), a preliminary examination revealed that *A. magnoliae* fundatrices develop and reproduce on potato as well as on *S. sieboldiana* var. *miqueli*. A greenhouse was used for sprouting potato (Irish cobbler), onto which *A. magnoliae* was transplanted, and colonies reared. The second generations were used for the diet of *H. axyris* larvae. Rearing *A. magnoliae* colonies on *S. sieboldiana* var. *miqueli* was conducted in the field by netting whole colonies.

Adults and egg batches of *Harmonia axyris* were collected from several tree species, including *S. sieboldiana* from the same botanical garden as *A. magnoliae* from mid-May to mid-June 2003. *Harmonia axyris* females were kept individually in Petri dishes to induce oviposition under a photoperiod of 16 h light : 8 h dark (LD 16:8) at 18°C. Because *H. axyris* larvae are cannibalistic, individual eggs in each egg batch were separated with a fine wetted paintbrush, and they were individually placed in a polystyrene container (9 mL) lined with one sheet of filter paper for hatching. The eggs were incubated at 18°C, under an LD 16:8 photoperiod. Eggs in an egg batch hatched within 12 h. *Harmonia axyris* larvae that hatched from each egg batch were divided into two groups; one group was fed with *A. magnoliae* reared on potato (potato aphids), and the other group was fed with...
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A. magnoliae reared on S. sieboldiana (elder aphids). In 2003, H. axyridis larvae were fed daily with more aphids than they could eat (for first instars five aphids, for second instars ten aphids, for third instars approximately 40 aphids and for fourth instars approximately 80 aphids) and kept individually for 7 days. The prey aphids mainly consisted of third and fourth instar larvae. Seven days after hatching, H. axyridis larvae were, on average, in the fourth stadium when fed with potato aphids, whereas they were in the second stadium when fed with elder aphids. Live larvae were weighed to an accuracy of 0.01 mg using a microbalance (Sartorius H110, Sartorius, Goettingen, Germany) 7 days after hatching, in order to evaluate the initial growth rate of H. axyridis larvae. When H. axyridis larvae died before 7 days, their wet mass was weighed within 12 h after death. Weight on day 7 is a good index of the later performance of the larvae, as shown in the Results section.

For the experiments in 2003, a χ² test was used to compare the mortality of H. axyridis larvae fed with potato aphids with the mortality of the larvae fed with elder aphids. The mortality was evaluated 7 days after the start of the experiment. The weight of H. axyridis larvae on day 7 was analyzed by using mixed model ANOVA, with the females (mothers) and food types (potato/elder aphids) treated as the main effects. The ANOVA model also included the female × food type interaction. The females and the interaction were specified to random effects. For H. axyridis females that produced two or more egg batches, each of the egg batches was divided into the two treatment groups, and the results were pooled for each treatment.

For each of the food types, the among- and within-family variance components of larval weight were calculated using the REML option in JMP version 5 (SAS Institute 2002). These variance components were used for estimating the broad sense heritability (h²), which was computed as

\[ h^2 = 2\sigma^2 \text{ (among-family)}/[\sigma^2 \text{ (among-family)} + \sigma^2 \text{ (within-family)}]. \]

To calculate the variation in the estimated h² for both food types, the estimate was jack-knifed with one family omitted in each of the iterations, as in Ueno (2003). Based on the pseudo-values of the estimate, the difference in h² between the food types was tested using two-way ANOVA with the families omitted and food types treated as the main effects. For the families of siblings, the relationship between the mean weight of H. axyridis larvae fed with elder aphids and the mean weight of the larvae fed with potato aphids was analyzed by Pearson’s correlation in order to estimate the genetic correlation for the two food types (Via 1985).

Preliminary field observations showed that some H. axyridis larvae were feeding on A. magnoliae aphids on S. sieboldiana. This observation may suggest that a fraction of H. axyridis females have a preference for A. magnoliae on S. sieboldiana and that their offspring are capable of utilizing the fundamentally poisonous aphids. To test whether or not ladybird larvae found around A. magnoliae colonies on S. sieboldiana exhibit a special adaptation to A. magnoliae aphids, the weight of H. axyridis larvae on day 7 was analyzed by ANOVA, with oviposition place (S. sieboldiana) and food types (potato/elder aphids) as the main effects. The food type × oviposition place interaction was also included in the model. In this analysis, we used the same data sets as in the previous mixed model ANOVA.

**Aphid and ladybird larva experiments (2004)**

From mid-May to mid-June, 2004, we maintained H. axyridis larvae up to eclosion under the same conditions as in 2003 to test whether H. axyridis larvae that were provided with elder aphids could complete development. In this experiment, we used H. axyridis eggs produced by females that were collected on plants other than S. sieboldiana. We fed 46 larvae from four females with elder aphids and 46 larvae from another four females with potato aphids. All the larvae were reared separately. Each H. axyridis larva was weighed 3, 7, and 9 days after hatching, as well as at the time of pupation in order to compare the effects of aphids reared on different plants.

For the experiments in 2003, pooling the different sexes of H. axyridis larvae might have affected the results. Thus, in the experiment in 2004, the possibility of sexual differences in weight was examined by initially weighing larvae on day 7 and subsequently sexing them after eclosion.

To quantify the effect of different food types on the growth pattern of H. axyridis larvae, the weight of individual H. axyridis larvae on days 3, 7, and 9, and at the time of pupation was analyzed using repeated-measures ANOVA, with females and food types being treated as between-subjects effects, and time as a within-subject effect (Von Ende 2001). In this analysis, we used individuals that successfully reached adulthood, removing individuals that died during various larval stages. All statistical analyses were performed using JMP version 5 (SAS Institute 2002).
RESULTS

Aphid quality as food, and growth of ladybird larvae

Experiments in 2003 indicated that the mortality of *H. axyridis* larvae fed with elder aphids was significantly higher than the mortality of larvae fed with potato aphids ($\chi^2 = 8.06$, d.f. = 1, $P = 0.0045$; Fig. 1). ANOVA for the weight of *H. axyridis* larvae on day 7 indicated that the effect of the aphid’s host plant (food types) on larval weight was highly significant (Table 1). The marginal mean weight of the larvae fed with elder aphids was significantly lower than that of the larvae fed with potato aphids ($F = 952$, d.f. = 1, $P < 0.0001$). The effect of the females (mothers) yielded significant differences in larval weight. The female × food type interaction was also significant (Table 1). This result may show that some families had a relatively high ability to assimilate elder aphids. Nevertheless, there were no sib families in which the mean weight of larvae fed with elder aphids exceeded the mean weight of larvae fed with potato aphids.

Variation in the weight of the larvae fed with elder aphids was significantly smaller than variation in the weight of the larvae fed with potato aphids ($F = 3.24$, d.f. = 297,279, $P < 0.0001$). The broad-sense heritability of larvae fed with potato aphids and elder aphids was estimated to be 0.578 [95% confidence interval, 0.566–0.590] and 0.445 [0.430–0.468], respectively. Two-way ANOVA for jack-knifed pseudo-values of $h^2$ showed that $h^2$ for larvae fed with potato aphids was significantly larger than that for larvae fed with elder aphids (for food types, d.f. = 1, $F = 160.6$, $P < 0.0001$; for families excluded, d.f. = 26, $F = 1.29$, $P = 0.259$).

When comparison was made across sib families, the mean weight of *H. axyridis* larvae fed with potato aphids was significantly correlated with the mean weight of larvae fed with elder aphids (Pearson’s correlation, $r = 0.532$, $P = 0.004$; Fig. 2).

The experiments in 2004 indicated that the mortality of *H. axyridis* larvae fed with elder aphids up to pupation (37.0%) did not significantly differ from the mortality of larvae fed with potato aphids (19.6%; $\chi^2 = 3.47$, d.f. = 1, $P = 0.062$), partly because of a small sample size ($n = 46$ for each food type). However, the

![Figure 1](image1)

**Figure 1** Mortality of *Harmonia axyridis* larvae 7 days after rearing began, when they were fed with *Aulacorthum magnoliae* reared on potato ($n = 243$) or elder ($n = 243$).

![Figure 2](image2)

**Figure 2** Relationships between the mean weight of *Harmonia axyridis* larvae fed with potato aphids and the mean weight of larvae fed with elder aphids in sib families. Each line connects the mean weights of larvae fed with the two types of aphids for each sib family.

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Mixed-model ANOVA for weight on day 7 of <em>Harmonia axyridis</em> larvae fed with different food types (elder or potato aphids)</th>
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<td>Female (random)</td>
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<td>Food type</td>
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<tr>
<td>Female × Food type (random)</td>
<td>26</td>
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<td>Error</td>
<td>524</td>
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repeated-measures ANOVA for larval weight detected a significant day × food type interaction (Table 2), suggesting that the growth rate of *H. axyridis* larvae varies significantly depending on the host plants given as food to the aphids. The growth of *H. axyridis* larvae fed with elder aphids was significantly slower than that of larvae fed with potato aphids (Table 2 and Fig. 3). This experiment also detected significant differences in the rate of larval growth among the females (mothers).

In the experiment in 2003, no attempt was made to distinguish the sexes of *H. axyridis* larvae. In the experiment in 2004, ANOVA detected no significant difference between the sexes of *H. axyridis* larvae on day 7 (for male larvae, $m = 15.3 \pm 0.8$ mg and for female larvae, $m = 14.4 \pm 0.9$ mg; $F = 0.51$, d.f. = 1, $P = 0.48$). This implies that any distortion in sex ratio between the treatments would not have affected the results.

**Specialization of ladybird larvae to elder aphids**

ANOVA for the weight of *H. axyridis* larvae on day 7 detected no significant food type × oviposition place interaction, nor detected a significant effect of oviposition place (Table 3). These results refuted the hypothesis that the offspring of the *H. axyridis* females found on elder can assimilate the poisonous *A. magnoliæ* more efficiently as food.

**DISCUSSION**

Host plants of herbivorous insects sometimes have indirect effects on the performance of the predators and parasites of the herbivores (Price *et al.* 1980; Ohgushi 2005). The amount of fatty acids in the aphid *Acyrtosiphon pisum* Harris varies depending on its host plant species, and this variation affects the performance of predators and parasites of the aphid (Giles *et al.* 2000, 2002). It has been reported that herbivorous insects absorb toxic compounds from their host plants, so that the predators and parasites that consume the herbivores...
have lowered performance (Weisser & Stamp 1998; Francis et al. 2001; Harvey et al. 2003). The aphid *Aphis nerii* Boyer de Fonscolombe accumulates cardenolides that are contained in the host plant, oleander, so that it is poisonous to several predators (Rothschild et al. 1970). Similarly, the cabbage aphid *Brevicoryne brassicae* absorbs glucosinolates from brassicaceous plants (Francis et al. 2001), thereby decreasing the performance of their predators. Francis et al. (2001) reported that larvae of the ladybird *Adalia bipunctata* (L) always failed to develop when they were fed with *B. brassicae* reared on *Brassica nigra* (L), but that the mortality of *A. bipunctata* larvae reduced to 40% when they were fed with *B. brassicae* reared on *Brassica napus* (L) with a lower concentration of glucosinolates. On their host plants, aphids feed on the phloem sap (Pollard 1973; Tjallingii 1988, 1995), which contains allelochemicals in addition to amino acids and sugars (Molyneux et al. 1990; Montllor 1991; Merritt 1996).

The present study clearly demonstrated that the aphid *A. magnoliae* has the potential to absorb toxic substances, probably allelochemicals from the host plant. Hodek and Honek (1996) inferred that *A. magnoliae* accumulates sambunigrin or a related allelochemical, which is toxic to general predators. In the present study, the difference between the host plants of *A. magnoliae* indirectly affected the viability and growth of larvae of its predator, *H. axyridis*, thus corroborating the finding that *A. magnoliae* absorbs toxic substances from *S. sieboldiana* but not from *S. tuberosum*. In conclusion, aphids that are poisonous on a host plant species are not always poisonous on other plant species.

This variation in aphid toxicity among host plants may affect the host selection of the aphid species. The colonization of the primary host *S. sieboldiana* by *A. magnoliae* in spring is obviously adaptive because *A. magnoliae* can defend themselves against predators by accumulating any poisonous compounds. Therefore, even though *A. magnoliae* is capable of feeding on a number of plant species, the association of *A. magnoliae* with *S. sieboldiana* might be maintained primarily because it confers the advantage of escaping from predators. Osawa (1991) observed conspicuously lower densities of *H. axyridis* beetles on colonies of *A. magnoliae* than on colonies of other aphid species in the same season. This observation suggests that accumulating toxic substances in *A. magnoliae* actually contributes to a reduction in predation pressure by ladybirds. The association of some aphid species with the primary host plant in spring and their migration to the secondary hosts in early summer has been explained in terms of nutritive complementarity (Dixon 1985) or constraints of the fundatrix to the primary host (Moran 1992). The findings of the present study emphasize that the use of the secondary metabolites could be mainly responsible for the association of *A. magnoliae* with the primary host *S. sieboldiana*.

Rearing of *H. axyridis* larvae in 2003 was continued for only 7 days after the start of experiments; this procedure ensured a large sample size of *H. axyridis* families. This method is justified because larval weight on day 7 is a good indicator of later growth, as shown in the 2004 experiments. The growth of *H. axyridis* larvae fed with elder aphids was slower than that of larvae fed with potato aphids. Aphidophagous ladybirds tend to oviposit before the density of the aphid population peaks on the host plant (Dixon 2000; Osaka 2000). The lowered development of *H. axyridis* larvae may cause them to suffer increased predation risk and a serious deficiency of prey. In addition, the reduced body sizes of *H. axyridis* larvae fed with elder aphids may lead to a reduction in fecundity. All lines of evidence indicate that *A. magnoliae* feeding on *S. sieboldiana* are unsuitable prey for *H. axyridis* larvae. Ueno (2003) reported that *H. axyridis* larvae fed with *Aphis craccivora* Koch had longer developmental time and lighter pupal weight than larvae fed with *Acyrthosiphon pisum* Harris in all the *H. axyridis* families examined. The present study differs from the previous study in that it focuses on the indirect effects of different host plants on a single aphid species.

We found large genetic variance in larval performance in *H. axyridis* larvae fed with both types of aphids. A significant amount of genetic variation in larval performance has been detected among families of the herbivorous ladybirds *Epilachna pustulosa* Kono and *E. vigintioctomaculata* Motschulsky on their respective host plants (Ueno et al. 1997, 2001, 2003). Similarly, Sadeghi and Gilbert (1999) reported that for the aphidophagous syrphid *Epyisyrphus balteatus* DeGeer, there are significant differences among families in developmental responses to different aphid species. In general, life-historical traits that are strongly related to fitness (such as growth rate) tend to have lower heritability than do other traits that are not strongly related to fitness (Mousseau & Roff 1987). Although further experiments are needed, such a large amount of genetic variance for larval performance could be maintained in the ladybird population if there is a genetic trade-off between larval performance and other fitness-related traits (Chippindale et al. 1997). Interestingly, in the present study there was a positive genetic correlation.
between the performance of ladybird larvae fed on different aphid types. This result implies that ladybird larvae use the same physiological mechanism to assimilate both suitable prey aphids and unsuitable aphids.

When fed with elder aphids, the *H. axyridis* larvae had reduced variance in growth rate. The broad-sense heritability for larvae fed with elder aphids was also significantly smaller than that for larvae fed with potato aphids. This result was opposite to that observed when *H. axyridis* larvae were reared on artificial diets, when performance in *H. axyridis* larvae had a higher heritability. Ueno (2003) ascribed this high heritability to the fact that no selective pressures have been exerted on adaptation of *H. axyridis* larvae to the novel diet. In contrast, it has been reported that in *Drosophila melanogaster*, the heritability of some morphological traits is lower under conditions of nutritional stress than under conditions of good nutrition (Bubliy et al. 2000). Although we cannot fully explain the reason for the reduced heritability observed under adverse food conditions, the results obtained in the present study imply that evolutionary adaptation to unsuitable aphid preys is difficult.

One prediction of the present study was that if some *H. axyridis* females have the capacity to utilize elder aphids, they are more likely to oviposit on *A. magnoliae* colonies when they cannot detect suitable oviposition sites. In the field, a small proportion of *H. axyridis* females actually oviposited on *A. magnoliae* colonies. However, this prediction was not supported, and the offspring of *H. axyridis* females that had selected *A. magnoliae* colonies as oviposition sites did not assimilate elder aphids more effectively than did the offspring of *H. axyridis* females that had selected sites other than elder. This result suggests that *H. axyridis* females do not have a strong ability to distinguish the quality of aphids during their choice of oviposition sites. Although ovipositing aphidophagous syrphids prefer particular aphid species, older syrphid females exhibit a weaker oviposition preference for aphid species than do young females (Sadeghi & Gilbert 2000). Females of the aphidophagous ladybird *A. bipunctata* also tend to oviposit on aphid colonies of poor quality as they age (Frechette et al. 2004). In the present study, older *H. axyridis* females may have oviposited on *A. magnoliae* colonies, even though they are poor in quality. *Harmonia axyridis* females lay a number of egg batches (about 50) over a long period, ranging from 1 month to 2 months (Okada et al. 1973). Therefore, even if *H. axyridis* females deposit a few egg batches on aphid colonies of poor quality, they may suffer only a minimal impact on their reproductive success. Further studies are required to assess to what extent oviposition preference varies genetically among *H. axyridis* females.

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