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Asymmetric larval interactions between introduced and indigenous ladybirds in North America

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Abstract Understanding the mechanisms that result in the success of introduced species will contribute to predicting future invasions and managing invaded systems. We examined interactions between larvae of two predatory ladybird species recently introduced to North America, *Coccinella septempunctata* (CS) and *Harmonia axyridis* (HA), and two indigenous ladybirds, *Coccinella transversoguttata* (CT) and *Hippodamia convergens* (HC). By pairing young and old larvae in the laboratory at low and high levels of aphid availability, we assessed the degree of asymmetry in intraguild predation (IGP), the strength of competitive effects on growth and development of larvae escaping predation, and the nature of attack and escape behavior among the species. Interactions were generally asymmetric, with larvae of introduced species acting most frequently as intraguild predators and larvae of indigenous species serving most frequently as intraguild prey (the two *Coccinella* spp., however, preyed on each other at similar rates). Because they were especially aggressive and because other larvae were least successful in escaping their attacks, larvae of HA had stronger negative effects on larvae of the two indigenous species than did larvae of CS. Such negative effects, expressed most strongly when aphid availability was low, were especially adverse for the smaller of the two indigenous species, HC. In general, older larvae interacted with each other more strongly than young larvae did, and older larvae had especially strong negative effects on young larvae when interactions occurred between age classes. Our results suggest that HA more than CS may represent a threat to indigenous ladybirds as an intraguild predator, and that IGP in turn

may play a stronger role for HA than for *C. septempunctata* in promoting the successful invasion of North America.

Keywords Coccinellidae · Competition · Indigenous species · Intraguild predation · Invasive species

Introduction

Introduced species of animals pose increasing threats for the conservation of indigenous species, with major implications for biodiversity worldwide in both natural and managed (e.g., agricultural) habitats. Thus, recent species invasions leading to replacements of indigenous species have occurred across diverse taxa (e.g., Hill and Lodge 1994; Kupferberg 1997; Holway 1999; Byers 2000; Kiesecker et al. 2001; Woodward and Hildrew 2002). An understanding of the mechanisms that result in the success of introduced species will assist in predicting future invasions and managing invaded systems. Presently, however, little experimental study has been completed to further our understanding of such mechanisms.

In recent years, introduced aphidophagous ladybirds (Coleoptera: Coccinellidae) appear to have depressed abundances of indigenous ladybird species in North America (Staines et al. 1990; Wheeler and Hoebeke 1995; Elliott et al. 1996; Brown and Miller 1998; Colunga-Garcia and Gage 1998; Michaud 2002; Brown 2003; Turnock et al. 2003; Evans 2004). Several experimental studies have focused on larval interactions between the introduced and indigenous ladybird species (Evans 1991; Cottrell and Yeargan 1998; Obrycki et al. 1998a, b; Burgio et al. 2002; Michaud 2002). Collectively, these and related studies (Yasuda and Shinya 1997; Kajita et al. 2000; Yasuda et al. 2001) suggest that asymmetric interspecific competition and intraguild predation (IGP) may favor these introduced species. One notes, however, that introduced species do not always dominate interspecific interactions with indigenous species (e.g., Sato and Dixon 2004). Furthermore, the outcomes of larval

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interactions likely depend on additional factors such as feeding specificity, size, mobility, aggressiveness, prey availability, and developmental stages (e.g., Polis et al. 1989; Lucas et al. 1998), and hence our understanding of these interactions remains incomplete.

Two introduced ladybird species, *Coccinella septempunctata* L. (CS) and *Harmonia axyridis* (Pallas) (HA), have become of great concern regarding their potential to adversely affect indigenous North American ladybirds. Common in Asia (e.g., Takahashi 1989; Yasuda and Shinya 1997), CS and HA now occur throughout North America (e.g., Maredia et al. 1992; Tedders and Schaefer 1994; LaMana and Miller 1996; Brown and Miller 1998; Colunga-Garcia and Gage 1998; Lucas et al. 2002; Musser and Shelton 2003; Nault and Kennedy 2003). HA larvae are stronger intraguild predators than CS larvae in Japan (Takahashi 1989; Yasuda and Shinya 1997; Sato et al. 2003), and HA larvae also appear more able to exploit coleopteran (vs. aphid) prey than are larvae of CS (Yasuda and Ohnuma 1999; Kalaskar and Evans 2001). Although much attention has been paid to how each of the two species may affect indigenous ladybirds in North America, there is little experimental foundation as yet to compare directly the effects of these species on indigenous ladybirds (but see the recent studies of Snyder et al. 2004).

We compare here interactions of the two species with two common species of indigenous ladybirds with which they now co-occur in alfalfa and adjacent habitats of northern Utah (e.g., Evans 1991; Evans and Richards 1997). After first appearing in 1991, CS now predominates among ladybirds in alfalfa (Evans 2000, 2004), whereas HA still occurs only in low numbers (personal observations). The two indigenous species that we have studied here, *Coccinella transversoguttata richardsoni* Brown (CT) and *Hippodamia convergens* Guerin (HC), occur commonly in Utah alfalfa fields alongside the introduced species, with all four species attacking especially the pea aphid, *Acyrtosiphum pisum* Harris (Evans 2004). We chose to study these indigenous species because of both their abundance and their body sizes. CT is larger than HC, and is similar in size to CS and HA (Gordon 1985; Gordon and Vandenberg 1991). Relative body sizes may influence outcomes of interspecific interactions (by favoring larger individuals) both in ladybirds (e.g., Obrycki et al. 1998a; Michaud 2002; Sato et al. 2003), and other predatory arthropods (e.g., Lucas et al. 1998; Rosenheim et al. 1999; Hindayana et al. 2001).

Here, we use laboratory experiments to study larval interactions between the two indigenous and two introduced ladybird species. We focus especially on the potential importance of IGP and the variation in relative frequency with which it occurs between pairs of ladybird species. This interaction is noteworthy in that the consumption of a competitor benefits a predator such as HA and CS both directly by providing key nutrients and energy and indirectly by reducing exploitative competition (e.g., Agarwala and Dixon 1992; Dixon 2000), and hence IGP may contribute importantly to the successful establishment of these introduced species in North America. In

seeking to understand the mechanistic basis for IGP, we include an experiment to examine differences in attack and escape behavior in interactions between paired larvae of various species combinations. Our experimental design includes two levels of food availability, as species interactions are affected by food availability (Evans 1991; Agarwala and Dixon 1992; Lucas et al. 1998; Obrycki et al. 1998a; Schellhorn and Andow 1999; Kajita et al. 2000; Hindayana et al. 2001). Furthermore, because changes in competitive ability occur with larval development (e.g., Cisneros and Rosenheim 1997), we focus on interactions between younger and older ladybird larvae, as well as between individuals of the same age class.

We address five key hypotheses, as suggested by previous research:

1. Interactions between larvae of the indigenous and introduced species are asymmetric, with larvae of the introduced species having greater adverse effects on the larvae of the indigenous species than vice versa.
2. Larvae of HA have stronger adverse effects than larvae of CS on the larval performance of both of the indigenous species, CT and HC.
3. Adverse effects of both introduced species are greatest on larval performance for HC (a relatively small-bodied species) than for CT.
4. High food availability weakens adverse effects of interactions on larval performance.
5. Adverse effects of the introduced species on the indigenous species are strongest when interactions occur between older rather than younger larvae, or between older introduced and younger indigenous larvae.

Materials and methods

Larvae of CS, HA, CT, and HC were obtained for experiments from eggs laid by adults that had been collected a few weeks earlier in summer 2001 from alfalfa fields near Logan, Utah. Adults were maintained to obtain eggs, and larvae were then reared to the appropriate stage (second or fourth instar), in an incubator at 22°C and 16:8 h light:dark (16L:8D) on a diet of pea aphids, *A. pisum*, taken from a laboratory colony reared on broad beans (*Vicia faba* L.). Pea aphids used in the experiments were taken from this same source.

Rates of IGP and cannibalism for larvae of introduced and indigenous ladybirds

We conducted a laboratory experiment to determine the relative frequency with which larval ladybirds eat each other when they mature under different conditions of aphid availability. Our experiment included six combinations of the two indigenous ladybirds (CT and HC) and the two introduced ladybirds (CS and HA), two levels of prey (aphid) availability, and four age combinations of

predators. For each treatment combination, we assessed rates of IGP or cannibalism for larvae reared as pairs in a Petri dish (5 cm in diameter, 1 cm in height). The Petri dishes were placed in an incubator at 22°C and 16L:8D photoperiod.

The six combinations of ladybird species that we studied were: (1) CT+CS, (2) CT+HA, (3) CT+CT, (4) HC+CS, (5) HC+HA, (6) HC+HC. We used second and fourth instars reared in all four possible pairings for mixed species combinations (e.g., a second instar of both species, or a second instar of one and a fourth instar of the other), and reared in all three possible pairings for single species combinations (e.g., CT+CT). For each type of pairing, we studied interactions at both high and low availability of aphid prey (on each day of the experiment, the pair of predators was transferred to a new Petri dish stocked with either 20 or five adult pea aphids). Thus, we studied a total of 44 treatment combinations (six species combinations \times four or three age combinations \times two food levels), each of which we replicated 30 times (yielding a grand total for sample size of 1,320 pairs of larvae).

The ladybird larvae used in the experiment had molted within the previous 12 h. We observed paired individuals daily at 0800 hours and 2000 hours until the larvae developed into the next stage (i.e., third stadium or pupal stage). At each observation, we recorded whether the larvae had molted or pupated, or whether a larva had died. In all cases in which a larva died, either the larva had disappeared completely or only chewed parts of its body remained. We therefore attributed the cause of death to predation or cannibalism (nonetheless, we recognize that a weakened condition from exploitative competition or other causes of poor health may have predisposed the larva to become a victim of IGP or cannibalism). From these observations, we determined the frequency with which IGP or cannibalism occurred between two larvae for each of the 44 treatment combinations that we studied.

Effects of introduced ladybirds on performance of surviving larvae of indigenous species

We used the results of the rearing experiment to compare also the effects of the introduced species (CS and HA) versus conspecifics on the rate of development and weight gain of larvae of the indigenous species (CT and HC) that were not eaten, but instead survived to complete either the second or fourth stadium. Thus, we weighed larvae to the nearest 0.1 mg prior to pairing them with a second individual, and then we reweighed them after they molted to the next stage. In analyzing results for weight gain and rates of development, we eliminated data for five anomalous larvae of CT that survived but remained in the fourth stadium for 2 weeks or more (the extreme lengths of these periods suggest that these individuals failed to develop normally due to factors other than those that the experiment was designed to examine). In those replicates in which CT and HC larvae were paired with conspecifics and both larvae survived, we estimated rate of develop-

ment and weight gain by taking the average of values for the two individuals.

Because larvae completed the second stadium much more rapidly than the fourth stadium, our experimental design (of rearing larvae as pairs) made it difficult to assess well the effects of second instars on rate of development and weight gain of fourth instars. Therefore, we analyzed results only for larvae that were reared with other larvae of the same age (i.e., for second instars paired with second instars, and for fourth instars paired with fourth instars).

Behavioral observations of interactions between fourth instars

In a second experiment, we studied the behavioral mechanisms underlying competitive interactions between the introduced and indigenous ladybird larvae. For each of the same six species combinations used in the first experiment, we completed 25 replicates in which we placed together two fourth instars (tested within 12 h of molting) in a 3-cm-diameter plastic Petri dish with six fourth instars of the pea aphid as food. Wet weights of individuals were estimated from representative samples as follows: CS, 9.5 mg (SE=1.6, $n=19$); HA, 11.3 mg (SE=1.1, $n=17$); CT, 10.6 mg (SE=0.8, $n=15$); HC, 7.2 mg (SE=1.1, $n=17$).

We observed the ladybirds' behavior for 10 min at room temperature (20–22°C). Prior to introducing the ladybird larvae and aphids to the experimental arena, we placed the aphids in a freezer at -18°C for 30 s (to limit their movement during the 10-min observation period), and we starved the ladybird larvae for 12 h (to induce relatively uniform levels of hunger among individuals).

During the observation period, we recorded each instance in which the larvae came into contact. For each contact, we also recorded whether one larva attacked the other, and if so, whether the second larva was killed or escaped. From these data, we calculated the rate of attack on each larva (defined as number of attacks/number of contacts), and the rate of escape (defined as number of instances in which a larva escaped/number of times it was attacked). We also calculated the rate of predation as the percentage of individuals preyed upon for a given combination of larvae (e.g., the percentage of fourth instars of CT that were killed by fourth instars of HA for the 25 pairs of these predators that were observed).

Data analyses

We used two-way ANOVA in the first experiment to test the effects of the two independent factors, prey density (high or low) and species of competitor (conspecific, CS or HA), on mean rate of development and mean weight gain of surviving second instars of CT and HC when paired with another second instar. Similar analyses were not completed for other combinations of paired larvae

because of low survival rates. Sufficient numbers of fourth instars of CT and HC survived when prey availability was high such that we used one-way ANOVA to test the effect of species of competitor on rate of development and weight gain. Mortality and survival rates in the first experiment were compared among treatments by χ^2 -test, and the sequential Bonferroni procedure was used to control for table-wide Type I error (Rice 1989). Analyses in the second experiment were conducted on attack and escape rates using ANOVA, with mean differences separated using Scheffé's multiple comparison test (the data were arcsine-square-root transformed before analysis). Differences in rate of predation among treatments were compared by χ^2 -test.

Results

Rates of IGP and cannibalism for larvae of introduced and indigenous ladybirds

Larval mortality varied depending on the age and species identities of paired predators, and on the level of prey availability. When reared with another second instar (of either CT or HC), all second instars of HA and CS developed into third instars, even when prey availability was low (Fig. 1a). Similarly, most or all second instars of CT and HC survived to the third instar when reared with another second instar (Fig. 1b). Thus, rates of survival of CT or HC larvae did not differ significantly when individuals were reared with a larva of HA or CS or with a conspecific larva (for CT at low food level, $\chi^2=5.51$, $P>0.05$; for HC at high food level, $\chi^2=1.99$, $P>0.05$). An exception occurred in the case of HC larvae at low prey density ($\chi^2=6.67$, $P<0.05$) wherein survivorship was slightly reduced for those paired with an HA larva (Fig. 1b).

Similarly, few fourth instars of either indigenous or introduced species died when reared at high prey density with either a conspecific or heterospecific fourth instar (Fig. 2; for CT, $\chi^2=4.29$, $P>0.05$; for HC, $\chi^2=2.09$, $P>0.05$; for CS, $\chi^2=1.05$, $P>0.05$; note all larvae of HA survived). The case was quite different, however, when fourth instars were reared at low prey density. Individuals of HC were eaten at very high rates by both HA and CS, and at a moderately high rate by conspecifics (Fig. 2a; for mortality of HC paired with HA vs. HC, $\chi^2=16.48$, $P<0.001$; with CS vs. HC, $\chi^2=11.92$, $P<0.01$; and with HA vs. CS, $\chi^2=0.58$, $P>0.05$). Individuals of CT were eaten at very high rates by HA and conspecifics, but only at a moderately high rate by CS (Fig. 2a; for mortality of CT paired with HA vs. CS, $\chi^2=23.72$, $P<0.001$; and with CS vs. CT, $\chi^2=23.72$, $P<0.001$). It is also noteworthy that at low prey density, fourth instars of CT cannibalized each other more frequently than did fourth instars of HC ($\chi^2=25.71$, $P<0.001$).

The interaction between fourth instars of HA and indigenous species was highly asymmetric in that few HA larvae became intraguild prey even at low prey density, when paired with either CT or HC (Fig. 2b). Thus, fourth instars of the two indigenous species were much more frequently the victims of IGP from fourth instars of HA than vice versa (HA vs. CT, $\chi^2=60.00$, $P<0.001$; HA vs. HC, $\chi^2=41.71$, $P<0.001$). The interaction between fourth instars at low prey density was also asymmetric between CS and HC, but not between CS and CT. Thus, few CS fell victim to IGP from HC while a large number did so to IGP from CT (Fig. 2b). Consequently, fourth instars of HC but not of CT were more frequently the victims of IGP from fourth instars of CS than vice versa (CS vs. CT, $\chi^2=1.07$, $P>0.05$; CS vs. HC, $\chi^2=26.07$, $P<0.001$).

Rates of mortality of second instars varied dramatically with prey availability when these individuals were paired with conspecific and heterospecific fourth instars (Fig. 3).

Fig. 1a, b Mean survival rates of second instars of *Coccinella transversoguttata* (CT), *Hippodamia convergens* (HC), *Coccinella septempunctata* (CS), and *Harmonia axyridis* (HA), when reared with another second instar (either conspecific or heterospecific, as indicated). Different letters indicate significant differences among treatments within a species, χ^2 -test after Bonferroni adjustment ($P<0.05$)

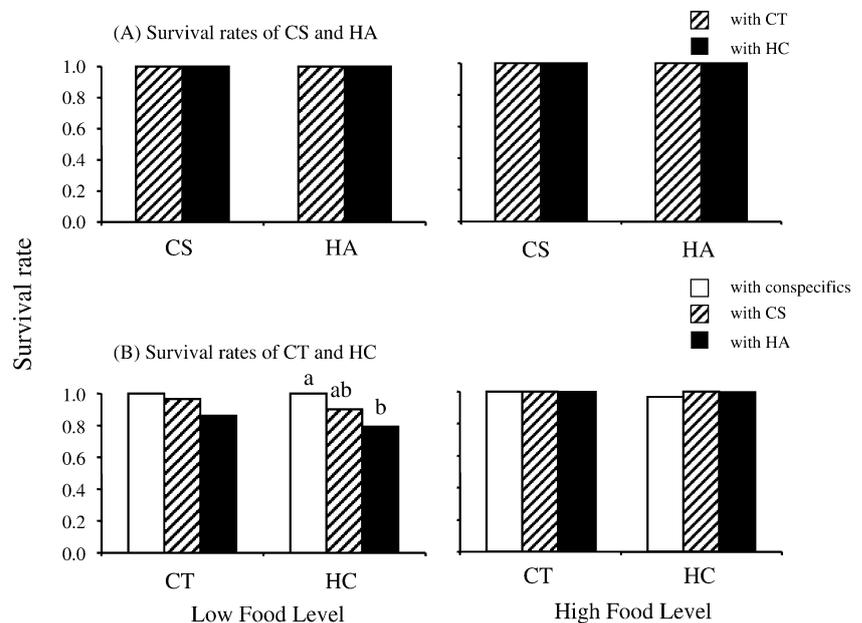
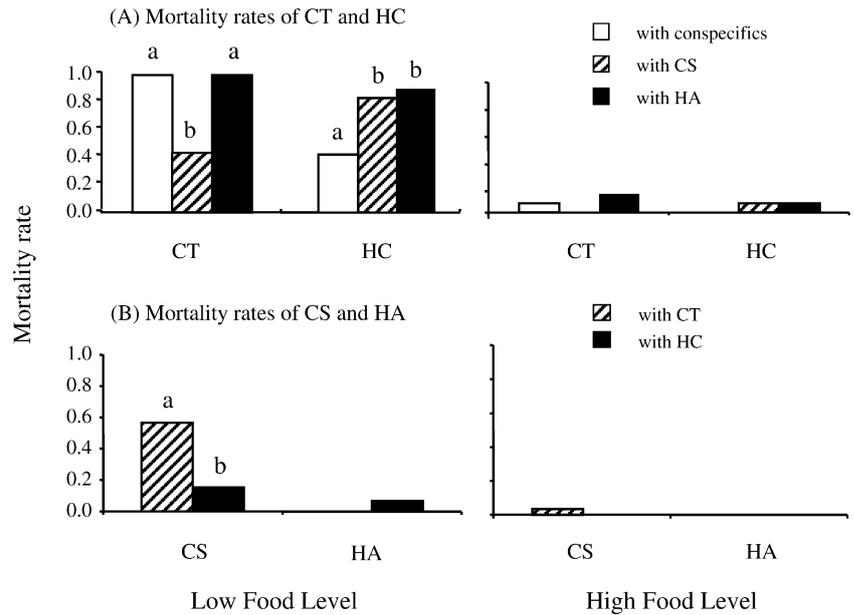


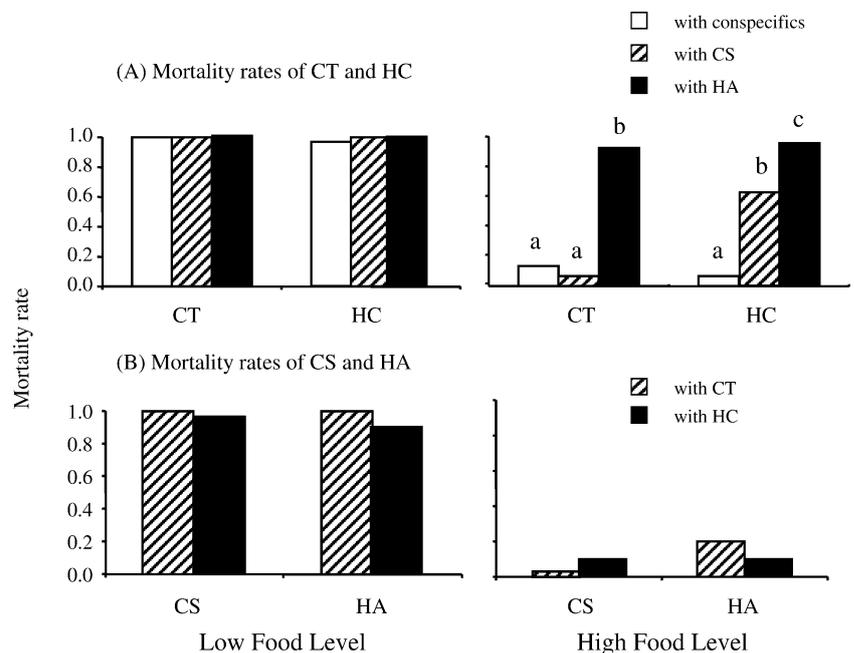
Fig. 2a, b Mean mortality rates of fourth instars of CT, HC, CS, and HA, when reared with another fourth instar (either conspecific or heterospecific, as indicated). Different letters indicate significant differences among treatments within a species, χ^2 -test after Bonferroni adjustment ($P < 0.05$). For abbreviations, see Fig. 1



When prey availability was low, second instars of all four species suffered very high mortality from predation (100% or nearly so) when paired with fourth instars (all larvae of CT were preyed upon by HA or CS or conspecifics; for mortality of HC paired with HA or CS or conspecifics, $\chi^2=2.02$, $P>0.05$; for mortality of CS paired with CT or HC, $\chi^2=1.02$, $P>0.05$; and for mortality of HA paired with CT or HC, $\chi^2=3.16$, $P>0.05$). Mortality rates of second instars were more variable at high prey density. Again, interactions between larvae of the introduced and indigenous species were markedly asymmetrical. Second instars of HA and CS had very low rates of mortality when reared with fourth instars of CT and HC, but second instars of the indigenous species were frequent victims of IGP when reared with fourth instars of the introduced species.

At high aphid density, fourth instars of HA preyed heavily upon second instars of both CT and HC whereas conspecific fourth instars killed few second instars of these two species (Fig. 3; for mortality of CT paired with HA or CT, $\chi^2=38.57$, $P<0.001$; and for mortality of HC paired with HA or HC, $\chi^2=48.65$, $P<0.001$). Fourth instars of CS preyed much less heavily on second instars of CT than did fourth instars of HA (Fig. 3a; for mortality of CT paired with HA or CS, $\chi^2=45.07$, $P<0.001$), and indeed were no more likely than fourth instars of CT to prey on second instars of CT ($\chi^2=0.74$, $P>0.05$). Fourth instars of CS also preyed significantly less often on second instars of HC than did fourth instars of HA, but significantly more often than did fourth instars of HC (for mortality of HC paired with HA or CS, $\chi^2=10.42$, $P<0.01$; and with CS or HC,

Fig. 3a, b Mean mortality rates of second instars of CT, HC, CS, and HA, when reared with a fourth instar (either conspecific or heterospecific, as indicated). Different letters indicate significant differences among treatments within a species, χ^2 -test after Bonferroni adjustment ($P < 0.05$). For abbreviations, see Fig. 1



$\chi^2=21.17$, $P<0.001$). In all of the different combinations of pairings between larvae of different age, the second instar never killed the fourth instar.

Overall, several generalizations emerge from these results. Interactions between larvae of the introduced and indigenous species were generally asymmetric, with larvae of introduced species acting most frequently as intraguild predators. In addition, the effect of HA on mortality of the indigenous species was greater than that of CS. Rates of mortality of CT and HC from IGP by HA were similar, but HC experienced greater mortality from CS than did CT. High availability of aphid prey weakened the intensity of these interactions, which were stronger in older larvae than in young larvae and which were especially strong when older larvae were paired with young larvae.

Effects of introduced ladybirds on performance of surviving larvae of indigenous species

Second instars of both indigenous species that survived to molt to the next stadium when paired with another second instar (either conspecific or HA or CS) developed more slowly when prey availability was low than when it was high (Fig. 4a, b; Table 1). The rate of development of a HC larva did not vary significantly, however, depending on the species identity of the second larva (Table 1). The rate of development of a CT larva also was similar among treatments, although CT larvae placed with conspecifics took slightly more time to develop than those placed with CS or HA larvae (Fig. 4a, b; Table 1). Similarly, both CT and HC second instars gained less weight before molting when prey availability was low than when it was high (the difference was only significant for CT, however; Table 1). No significant difference in weight gain at either level of prey availability was observed for either CT or HC second instars when these individuals were reared with another conspecific second instar or a second instar of CS or HA (Table 1).

When paired with another fourth instar and reared at low prey availability, too few fourth instars of HC and CT survived to permit analyses of rates of development and

weight gain. At high prey availability, surviving fourth instars of CT and HC did not differ significantly in their rates of development depending on the species identity of the fourth instar with which they were paired (for CT, $F_{2,106}=2.14$, $P=0.12$; for HC, $F_{2,113}=2.25$, $P=0.11$), nor did they differ significantly in weight gain (for CT, $F_{2,106}=0.63$, $P=0.53$; for HC, $F_{2,113}=2.12$, $P=0.13$).

Behavioral observations of interactions between fourth instars

The basic asymmetry of interactions between the introduced and indigenous ladybirds, and the unusually aggressive nature of HA in particular, were apparent in behavioral assays in which two fourth instars were placed together and observed for 10 min. No HC larvae were observed to attack either CS or HA larvae during these observations, nor were CT larvae ever observed to attack CS larvae. CT larvae attacked HA larvae in 16% of contacts, and in every such attack, the HA larva escaped. Consequently, no instances were observed in behavioral assays in which larvae of indigenous species killed and consumed larvae of introduced species.

Indigenous ladybird larvae were also rarely attacked by conspecifics, and every instance of conspecific attack ended in successful escape (Fig. 5). In contrast, larvae of the two introduced species CS and HA readily attacked indigenous ladybird larvae. CT larvae in particular differed significantly in the rates at which they were subject to attack depending on the species identity of the larva with which they were paired (Fig. 5a; for CT, $F_{2,53}=16.44$, $P<0.001$; for HC, $F_{2,44}=2.97$, $P=0.06$). HA was an aggressive predator of CT, attacking in >30% of instances in which larvae of the two species came into contact (in contrast, CS was never observed to attack CT upon contact).

Larvae of CT and HC did not always succeed in escaping from attacks by larvae of introduced ladybirds (Fig. 5b). Differences in escape rates depending on the species identity of the attacker were not significant, however, for either HC ($F_{2,9}=3.67$, $P=0.06$) or CT (the

Fig. 4a–d Developmental time and weight gain of a second instar of CT and HC when reared with another second instar (either conspecific or heterospecific, as indicated), at low and high levels of aphid availability. For abbreviations, see Fig. 1

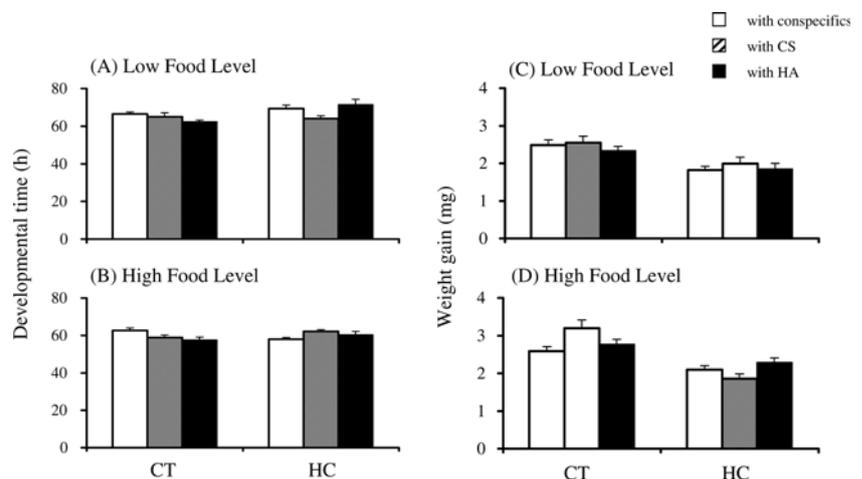


Table 1 Two-way ANOVAs for the effects of food level and competitor on weight gain and developmental time of second stadium larvae of *Coccinella transversoguttata* (CT) or *Hippodamia convergens* (HC)

Source of variation	Weight gain						Developmental time					
	CT			HC			CT			HC		
	df	F	P	df	F	P	df	F	P	df	F	P
Food level (F)	1	10.32	<0.01	1	2.81	0.09	1	23.92	<0.001	1	23.92	<0.001
Competitor (C)	2	2.08	0.13	2	1.26	0.29	2	1.33	0.27	2	7.74	<0.001
F×C	2	0.85	0.43	2	2.38	0.09	2	0.55	0.58	2	3.40	0.04
Error	167			163			167			163		

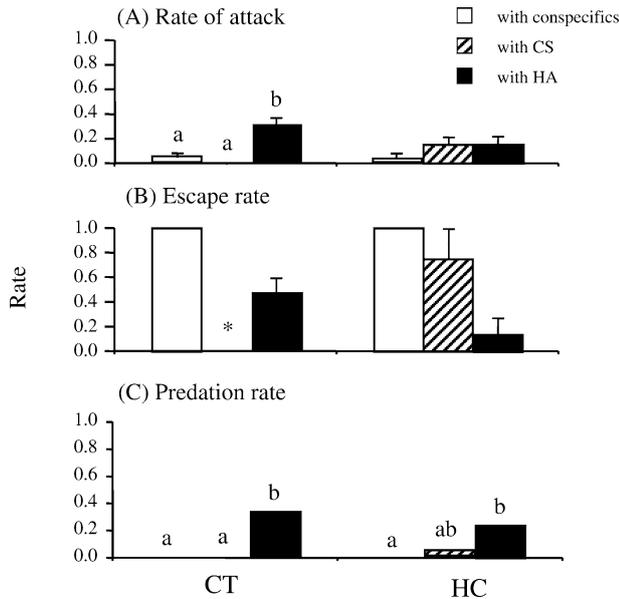


Fig. 5a–c Rates at which fourth instars of CT and HC were attacked, succeeded in escaping, or were preyed upon when paired with another fourth instar (either conspecific or heterospecific, as indicated). Different letters indicate significant differences among treatments within a species, Scheffé's test for rate of attack and χ^2 -test for predation rate after Bonferroni adjustment ($P < 0.05$). Asterisk shows that there were no escape behaviors since CS never attacked CT during a 10-min observation period. For abbreviations, see Fig. 1

F-value for CT could not be calculated because of the small sample size). Nonetheless, it is noteworthy that an especially low rate of escape (14.3%) was recorded when HC fourth instars were attacked by HA fourth instars (Fig. 5b).

These variations in attack and escape rates led to striking differences in the rates at which CT and HC larvae were killed by larvae of different species, with almost all deaths attributable to HA (Fig. 5c; rates of predation by conspecifics, CS, and HA on CT, $\chi^2 = 17.19$, $P < 0.001$; and on HC, $\chi^2 = 9.77$, $P < 0.01$). Thus, as noted above, both CT and HC larvae were attacked more readily by, and escaped less readily from, HA larvae than conspecifics. Although HC larvae were attacked with approximately equal frequency by HA and CS larvae, they escaped more often from attacks from CS larvae, and hence were much less likely to be preyed upon by CS than by HA (Fig. 5).

Discussion

We observed numerous instances of IGP and cannibalism in our experiments (interestingly, however, rates of development and weight gain of those individuals that escaped IGP appeared to be little affected by the presence of strong intraguild predators). We consider these results here in the light of the five hypotheses that we sought to test. Overall, our results supported our first hypothesis, as raised in previous studies (Cottrell and Yeagan 1998; Obrycki et al. 1998a; Kajita et al. 2000; Michaud 2002), that interactions would favor the introduced species that we studied over indigenous ladybirds. This was particularly observed in interactions between fourth instars, with HA and CS larvae eating CT and HC larvae much more frequently than vice versa (except in the case of CS and CT). Snyder et al. (2004) report a similar basic asymmetry in IGP among these species.

Our results also supported the related hypothesis that because they are particularly aggressive, larvae of HA would have stronger adverse effects than larvae of CS on the larval performance of the indigenous species. Differences in aggressiveness among species have previously been shown to be an important factor in IGP among predatory insects. For example, in interactions of three aphidophagous insects, the least aggressive species (a midge) was most often the victim in IGP (Lucas et al. 1998). In Japan, HA is a more aggressive and stronger intraguild predator than is CS (Yasuda and Shinya 1997; Yasuda and Ohnuma 1999; Kajita et al. 2000; Yasuda et al. 2001). In addition, HA is more able to escape from the attacks of CS than vice versa (Yasuda et al. 2001). In our experiments, we found that HA especially (in comparison to CS) was most frequently the predator and indigenous ladybirds were the victims in cases of IGP. The aggressive nature of HA was apparent both in the relatively high rate with which it attacked CT and in the relatively high rate with which it overpowered HC in attacks (as reflected by the relatively low escape rate of HC from such attacks).

The breadth of diet of HA in comparison to CS and the indigenous ladybirds is probably intertwined with its aggressiveness and large size in determining the results of IGP. Generalist predators may be most likely to act as intraguild predators and specialist predators may be most likely to act as intraguild prey (Polis et al. 1989; Lucas et al. 1998). In general, HA is a voracious and polyphagous species (Hodek and Honek 1988, 1996). In comparison, CS is more aphid-specific in its diet (Yasuda and Ohnuma 1999; Kalaskar and Evans 2001), as are the two indige-

nous species CT and HC (e.g., Gordon 1985). These differences in feeding specificity among the species are reflected in the nature of asymmetries in IGP that occurred in our experiments.

We also hypothesized that adverse effects on a ladybird larva would be greatest when the larva was reared with a larger competitor. Size differences among interacting species are well known to lead to asymmetric outcomes (favoring larger individuals) not only in ladybirds (e.g., Obrycki et al. 1998a; Michaud 2002; Sato et al. 2003; but see also Snyder et al. 2004), but also in other predatory arthropods (e.g., Lucas et al. 1998; Rosenheim et al. 1999; Hindayana et al. 2001). The strength of interactions also changes with the sizes of individuals during development, as shown for example in competitive interactions between two wolf spider species (Balfour et al. 2003). Size differences are likely to influence the outcome of interspecific interactions through a variety of species-specific behavioral and morphological attributes such as attack behavior, body strength, and defense and escape mechanisms (Hindayana et al. 2001). Furthermore, body size may be a significant factor in the colonization success of introduced species (Roy et al. 2002), and this in part may be related in ladybirds to the degree that IGP may enhance successful colonization and invasion.

In regard to the potential importance of relative body sizes of competitors, we hypothesized that interactions would be strongest among older (and larger) rather than younger larvae, and that younger larvae would suffer most when paired with older larvae. Our results clearly supported these conjectures, as we found much lower rates of survival for all species when two fourth instars versus two second instars were paired, and we found that second instars of indigenous species experienced very high rates of mortality when paired with fourth instars of introduced species. Similar results were also obtained in studies of interactions between a crab spider and predatory ladybird larvae (Yasuda and Kimura 2001), between HA and CS larvae (Yasuda et al. 2001), and between a pentatomid predator and ladybird larvae (De Clercq et al. 2003).

We hypothesized further that the adverse effects of HA and CS (which are both relatively large species of ladybirds) would be greater on larval performance of the relatively small indigenous species HC than on larval performance of the larger species CT. Overall, our findings supported this hypothesis in that CS affected the smaller species HC much more negatively than it affected CT. However, HA preyed heavily on both HC and CT. Thus HA's aggressive nature in addition to its large size enabled it to dominate in interactions with both species of indigenous ladybirds.

Finally, based on previous studies (e.g., Evans 1991; Agarwala and Dixon 1992; Lucas et al. 1998; Kajita et al. 2000; Hindayana et al. 2001), we hypothesized that interactions among larvae would be expressed most strongly at low food availability. We found that both exploitative competition and IGP were less strong when aphid prey were more available, as increasing the supply

of food resulted in decreased mortality rates and accelerated rates of growth of indigenous species (this was especially striking in the comparison of interactions of paired second instars at high vs. low aphid availability). Furthermore, second instars (with relatively low food requirements) interacted less strongly with each other than did fourth instars at a given level of prey availability. Our findings are consistent with those of previous laboratory studies in which HA or CS reduced survival rates of other ladybirds (Obrycki et al. 1998a; Kajita et al. 2000) or lacewings (Phoofolo and Obrycki 1998) more strongly at low than at high prey availability. Similar effects of food availability on predator mortality have also been suggested in field studies of ladybirds (Yasuda and Shinya 1997; Obrycki et al. 1998a; Musser and Shelton 2003). Interestingly, Snyder et al. (2004) did not find an effect of food availability in their laboratory studies of ladybird larval interactions.

Since its arrival in North America several decades ago (e.g., Angalet et al. 1979, Schaefer et al. 1987), CS has spread widely and increased enormously in abundance, with apparent negative consequences for abundances of a number of indigenous ladybird species (Staines et al. 1990; Wheeler and Hoebeke 1995; Elliott et al. 1996; Brown and Miller 1998; Colunga-Garcia and Gage 1998; Turnock et al. 2003; Evans 2004). HA soon followed CS in invading North America, and much attention has been paid to understanding interactions as they occur in natural settings both between HA and indigenous species, and between HA and CS (Brown and Miller 1998; Colunga-Garcia and Gage 1998; Burgio et al. 2002; Michaud 2002; Brown 2003; Musser and Shelton 2003; Nault and Kennedy, 2003). The effects of HA on other ladybird species differ among the species and habitats studied. For instance, *Cycloneda sanguinea* declined in abundance in Florida citrus groves apparently as the result of IGP by HA (Michaud 2002), but *Coleomegilla maculata* has so far coexisted with HA in cornfields of New York field crops because of high food availability and differences in microhabitat selection (Musser and Shelton 2003). Furthermore, HA still remains at low abundance in eastern North Carolina agroecosystems (Nault and Kennedy 2003). Food availability influences larval mortality and growth of ladybirds in natural populations (e.g., Yasuda and Shinya 1997), and hence it is not surprising that the effect of HA on natural populations of indigenous species may vary across time and space depending on food availability.

Brown (2003) reported changes in ladybird guild structure following the introduction of CS and HA into apple orchards of West Virginia, and hypothesized that the negative effects of CS on indigenous ladybird species might ultimately be stronger than those of HA. Our results revealed HA to have stronger direct adverse effects than CS on indigenous species, but our experimental results must be interpreted carefully. The degree of habitat and microhabitat overlap among the species, for example, and the timing of arrival and larval development of individual species in a given habitat may influence interspecific

interactions (e.g., Gagné and Martin 1968, Coderre et al. 1987, Musser and Shelton 2003). HA often tends to arrive and oviposit later in habitats than other ladybird species (e.g., Yasuda and Shinya 1997; Musser and Shelton 2003; Nault and Kennedy 2003). Such appears to be the case in Utah alfalfa fields as well (E. W. Evans, personal observations). Other species (including CS) may derive protection by co-occurring as older larvae with younger larvae of HA (e.g., Sato and Dixon 2004). As revealed in the field studies of Yasuda and Shinya (1997), however, early maturing immature ladybirds may become vulnerable during the pupal stage to IGP by later maturing larvae of other species.

Our experiments must also be interpreted with caution because they were not designed to explore the potential for indirect interactions affecting guilds of ladybird species, including those that may arise from competitive interactions between HA and CS (e.g., Sih et al. 1998; Schmitz and Suttle 2001; Okuyama 2002). Our experiments were also carried out in small and simple arenas to determine the relative frequency of IGP among different combinations (pairs) of predators. The results are thereby likely to overestimate the killing capacity of a predator in more natural settings (but see Snyder et al. 2004), and they do not reflect the potential for avoidance and emigration behavior that could occur at a larger spatial scale that included greater habitat complexity (e.g., Phoofolo and Obrycki 1998; Schellhorn and Andow 1999; Dixon 2000; Finke and Denno 2002; De Clerg et al. 2003; Sato et al. 2003). For example, in natural settings larvae of CS defend themselves from IGP by dropping from a plant when attacked (S. Sato, personal observations).

Our results, especially when combined with those of Snyder et al. (2004), nonetheless represent a valuable first step towards understanding the potential for adverse larval effects of the introduced species, HA and CS, on indigenous ladybird species of North America. Although there are exceptions (e.g., Brown 2003), there is a natural tendency among researchers to assume that CS and HA, as two recently arrived and highly successful ladybirds that often overlap in the habitats that they invade, may be similar in how they may interact with and affect indigenous ladybirds. Our results suggest, however, that because of its broad feeding habits and unusually aggressive nature, HA much more than CS may represent a threat to indigenous species as an intraguild predator. IGP, furthermore, may play a stronger role for HA than for CS in promoting successful establishment and dominance of the invader in North American ladybird assemblages.

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