Interactions between introduced and native predatory ladybirds (Coleoptera, Coccinellidae): factors influencing the success of species introductions

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Abstract. 1. Why some exotic species invade successfully while others do not is poorly understood. This study focuses on the inability of the exotic predatory ladybird *Adalia bipunctata* (L.) (Coleoptera, Coccinellidae) to establish and spread in Japan. In particular, this study considers the interactions between *A. bipunctata* and three native Japanese ladybird species, *Harmonia axyridis* Pallas, *Coccinella septempunctata* Linn., and *Propylea japonica* Mulsant.

2. The number of aphids occurring on the host plants declined dramatically over the course of larval development in the presence of *H. axyridis* or *C. septempunctata*. In contrast, many aphids remained at the end of the experiment when only *A. bipunctata* or *P. japonica* was present.

3. The survivorship of *A. bipunctata* from second instar to adult was significantly reduced in the presence of *H. axyridis*, but not in the presence of *C. septempunctata* or *P. japonica*. Most deaths were the result of intra-guild predation when *H. axyridis* or *C. septempunctata* larvae were present. Larvae of *H. axyridis* and *C. septempunctata* suffered very high mortality in this experiment, but survived better when co-occurring with *A. bipunctata* than when occurring only with conspecifics. In contrast, *P. japonica* survived better when occurring with conspecifics than with *A. bipunctata*.

4. The finding that larvae of the exotic species *A. bipunctata* died at high rates from intra-guild predation by the native Japanese ladybirds *H. axyridis* and *C. septempunctata* may bear on the question of why *A. bipunctata* has not established more successfully in Japan whereas *H. axyridis* and *C. septempunctata* have been very successful in establishing themselves in North America.

Key words. Coccinellidae, interaction between introduced and native species, intra-guild predation, invasive species.

Introduction

The rapid increase in the introduction of exotic species throughout the world, and the potential of these species to become invasive, is a subject of much concern (e.g. Mack *et al.*, 2000). Ecologists have long sought to identify key factors that determine why some exotic species turn invasive and damaging to native species while others fail to do so (e.g. Williamson, 1999). Amongst biological control practitioners, these topics have recently become of great interest in regards to the establishment and spread of introduced aphidophagous ladybirds (Coleoptera: Coccinellidae). Two ladybird species in particular have been spectacular in their success as invaders in North America: *Coccinella septempunctata* Linn. (e.g. Schaefer *et al.*, 1987; Maredia *et al.*, 1992; Elliott *et al.*, 1996; Evans, 2000, 2004; Obrycki *et al.*, 2000) and *Harmonia axyridis* Pallas (e.g. Tedders & Schaefer, 1994; Brown &

Miller, 1998; Colunga-Garcia & Gage, 1998; Lucas *et al.*, 2002; Nault & Kennedy, 2003). One major factor hypothesised to be important to the success of these two invaders is their tendency to engage in the intra-guild predation (IGP) of native ladybirds (Cottrell & Yeargan, 1998; Obrycki *et al.*, 1998; Kajita *et al.*, 2000; Michaud, 2002; Snyder *et al.*, 2004; Yasuda *et al.*, 2004).

Harmonia axyridis and *C. septempunctata* are native to Japan, where they often dominate ladybird guilds (e.g. Yasuda & Shinya, 1997). Recently, the two-spotted ladybird, *Adalia bipunctata* (Linn.), an exotic to Japan, was introduced at the port town of Osaka (Sakuratani, 1994), but it did not succeed in expanding its distribution (Sakuratani *et al.*, 2000). It has been hypothesised previously that IGP by native Japanese ladybirds, especially *H. axyridis* and *C. septempunctata*, may be one of the key mechanisms of biotic resistance (Elton, 1958) responsible for the failure of *A. bipunctata* to establish in Japan (Kajita *et al.*, 2000).

This hypothesis arose from initial behavioural studies of predatory interactions between the larvae of *A. bipunctata*, *H. axyridis*, and *C. septempunctata*. When individuals were placed together in small containers without prey or plants, *A. bipunctata* larvae were most often the intra-guild prey, and *H. axyridis* larvae were stronger intra-guild predators of *A. bipunctata* than were *C. septempunctata* larvae (Kajita *et al.*, 2000). However, the experimental conditions limited the potential for escape from intra-guild predators (Dixon, 2000), and did not allow for the variation in the strength of interaction among predators that is likely to occur with the maturation of predators and variation in aphid abundance (e.g. Polis & Holt, 1992; Eklov & Werner, 2000).

The hypothesis has been more critically tested here by examining interactions between co-occurring individuals over the course of larval development in the laboratory under more realistic conditions wherein the larvae occur together with resident aphid populations on host plants. A fourth native Japanese ladybird, *Propylea japonica* Mulsant, which is also common in aphidophagous guilds in Japan (Sato, 2001), has been included in the study. This species is smaller than *H. axyridis* and *C. septempunctata*, and is similar in size to *A. bipunctata* (relative body size can be important in influencing the strength and degree of asymmetry in IGP; e.g. Moran & Hurd, 1994; Lucas *et al.*, 1998; Hindayana *et al.*, 2001). In the evaluation of the results from this study, it is discussed how the results may bear on the potentially related questions of why *A. bipunctata* has not established itself more successfully in Japan, and why *H. axyridis* and *C. septempunctata* have established themselves so successfully in North America.

Materials and methods

Rearing of ladybirds and aphids, and the subsequent completion of the experiment, occurred at constant temperature ($22 \pm 1\,°C$) and photoperiod (LD 16:8 h) in the laboratory at Yamagata University. The cowpea aphid, *Aphis craccivora* Koch, was collected from and reared on bean plants,

Vicia faba L. Adults of the ladybirds, *H. axyridis*, *C. septempunctata*, and *P. japonica*, were collected from the Yamagata University field plots at Tsuruoka in early spring, 1998. Eggs of *A. bipunctata* were obtained from the University of East Anglia, Norwich, England. The four species of ladybirds were cultured with the cowpea aphids in plastic containers (15 cm diameter × 9 cm height). Egg batches laid by females were collected daily, and placed individually in 9-cm Petri dishes. Hatched larvae were reared on cowpea aphids.

The following treatments were carried out in pots (15 cm diameter × 10 cm height). Each pot contained a single bean plant (20 ± 5 cm), enclosed in a plastic cage (12 cm diameter × 30 cm height) covered with organdie cloth. Three hundred cowpea aphids of mixed instars were placed on the bean plant and allowed to settle for 12 h. Ladybird larvae were then added, according to the seven treatments: (1) six L2 of *A. bipunctata*, (2) six L2 of *H. axyridis*, (3) six L2 of *C. septempunctata*, (4) six L2 of *P. japonica*, (5) three L2 of *A. bipunctata* and three L2 of *H. axyridis*, (6) three L2 of *A. bipunctata* and three L2 of *C. septempunctata*, and (7) three L2 of *A. bipunctata* and three L2 of *P. japonica*. Each treatment was replicated 10 times. The second instars were added to experimental cages within 12 h of moulting.

Systematic observations were carried out three times daily at 0800, 1200, and 1600 hours, to determine rates of development and causes of death of ladybird larvae. The numbers of aphids were also counted each day at 1200 hours. Observations and counts continued for 9 days until all larvae had pupated. In addition to observing larvae systematically at set times each day, the larvae were observed as often as possible in the day when they were in third and fourth stadia to detect cannibalism or IGP. An assessment was made not only of rates of the various sources of mortality, but also of rates of larval development and gain in weight (weights of larvae were measured before the experiment and at the fourth stadium). Along with increased mortality resulting directly from the interaction with other ladybirds, a slower rate of development and/or a reduction in weight gain could also potentially adversely affect ladybird performance in natural populations (e.g. through increased larval exposure to predators in general, and through reduced fecundity or survival of adults).

Mortality was attributed to (1) cannibalism, i.e. larval consumption by conspecifics, (2) IGP, i.e. larvae being eaten by heterospecifics, or (3) unknown causes, when a carcass remained (untouched by conspecifics or heterospecifics) or when individuals failed to moult to the next stage. It was possible to distinguish in all cases between cannibalism and IGP when *A. bipunctata* and *H. axyridis* were reared together. In a few instances in combination with *C. septempunctata*, it could not be determined with absolute certainty whether conspecifics or heterospecifics killed *A. bipunctata*. However, it was nonetheless possible to infer with reasonable certainty whether conspecifics or heterospecifics killed these larvae by considering the condition and behaviour of larvae when we checked cages systematically. For example, when one larva of

A. bipunctata had been killed while the two others of this species became immobile as pre-pupae, it could be inferred that a larva of *C. septempunctata* rather than a conspecific had killed the *A. bipunctata* larva.

The numbers of aphids remaining on a plant over time in the different treatments were analysed by repeated-measures ANOVA. Rates of mortality of ladybird larvae were compared among treatments using χ^2-tests. The mean developmental time of *A. bipunctata* larvae was analysed by one-way ANOVA, with significant differences between means of different treatments determined by use of the Scheffé multiple range test. Mean developmental times of *H. axyridis*, *C. septempunctata*, and *P. japonica* were analysed by ANOVA. The weight gain of *A. bipunctata* larvae was analysed by the Kruskal–Wallis test, and that of *H. axyridis*, *C. septempunctata*, and *P. japonica* was analysed by ANOVA. All statistical tests were performed using StatView 5.0. (SAS, 1998).

Results

Changes in aphid numbers

Changes in the number of aphids present over time on the host plants differed with experimental treatments (Fig. 1; Table 1). The number of aphids declined dramatically over the course of the experiment when *H. axyridis* or *C. septempunctata* larvae were present; rates of decline were similar when larvae of either of these species occurred alone or with larvae of *A. bipunctata* (Fig. 1b,c; Table 1). By the time *H. axyridis* or *C. septempunctata* larvae had reached the third stadium (i.e. 4 or 5 days after the experiment began), the number of aphids remaining on the host plant was quite small. In contrast, substantial numbers of aphids remained at the end of the experiment when only *A. bipunctata* and/or *P. japonica* larvae were present, and temporal patterns in numbers of aphids present were similar when either species occurred alone or the two species occurred together (Fig. 1a,d; Table 1).

The mortality rate of Adalia bipunctata and its causes

The survivorship of *A. bipunctata* to the adult stage was significantly reduced in the presence of *H. axyridis* ($\chi_1^2 = 30.3$, $P < 0.01$), but not in the presence of *C. septempunctata* ($\chi_1^2 = 0.19$, $P > 0.05$) or *P. japonica* ($\chi_1^2 = 0.19$, $P > 0.05$) (Table 2). Stage-specific mortality rates (i.e. rates of mortality during a given stage of development for those individuals alive at the start of that stage) generally increased for *A. bipunctata* as larvae became older, both when only conspecifics were present and when heterospecifics were also present (Fig. 2a). Mortality rates of *A. bipunctata* larvae were significantly higher in the presence of *H. axyridis* larvae during the second ($\chi_1^2 = 5.7$, $P < 0.05$), third ($\chi_1^2 = 15.7$, $P < 0.0001$), and fourth instar ($\chi_1^2 = 48.4$, $P < 0.0001$),

but were not significantly different in the pupal stage ($\chi_1^2 = 3.4$, $P = 0.06$), in comparison to mortality rates in the presence of conspecifics only. Mortality rates did not differ significantly for second and third instars of *A. bipunctata* when they occurred with *C. septempunctata* larvae vs. with conspecifics only (second instar, $\chi_1^2 = 0.2$; third instar, $\chi_1^2 = 0.2$). Mortality rates were significantly higher during the fourth instar ($\chi_1^2 = 5.4$, $P < 0.05$), and significantly lower in the pupal stage ($\chi_1^2 = 6.2$, $P < 0.05$), when individuals coexisted with *C. septempunctata* vs. with conspecifics only. Mortality rates of *A. bipunctata* did not differ significantly for either individual larval stages or pupae when they were kept with *P. japonica* larvae vs. with only with conspecifics (second instar, $\chi_1^2 = 0.2$; third instar, $\chi_1^2 = 1.8$; fourth instar, $\chi_1^2 = 2.7$; pupal stage, $\chi_1^2 = 0.1$, $P > 0.05$ in all cases).

Most *A. bipunctata* that died in the presence of conspecifics only, and all *A. bipunctata* that died in the presence of *P. japonica*, did so from causes other than cannibalism or IGP (Table 2). In contrast, relatively few *A. bipunctata* died of such causes in the presence of *H. axyridis* or *C. septempunctata*. Instead, most deaths were the result of IGP, especially when *H. axyridis* was present. IGP of *A. bipunctata* was especially common in the fourth instar.

The mortality rates of Harmonia axyridis, Coccinella septempunctata, *and* Propylea japonica *and their causes*

Harmonia axyridis and *C. septempunctata* suffered very high mortality in this experiment, but survived better when coexisting with *A. bipunctata* than with conspecifics only (Table 3; *H. axyridis*: $\chi_1^2 = 13.1$, $P < 0.01$; *C. septempunctata*: $\chi_1^2 = 3.9$, $P < 0.05$). In particular, stage-specific mortality rates (Fig. 2b,c) were significantly lower during the third and fourth instar for *H. axyridis*, and during the pupal stage for *C. septempunctata*, when *A. bipunctata* was present vs. when it was absent (third instar for *H. axyridis*: $\chi_1^2 = 16.7$, $P < 0.0001$; fourth instar for *H. axyridis*: $\chi_1^2 = 22.6$, $P < 0.0001$; pupal stage for *C. septempunctata*: $\chi_1^2 = 100.8$, $P < 0.0001$). *Adalia bipunctata* was never observed to kill individuals of the other three species (Table 3). *Harmonia axyridis* and *C. septempunctata* were especially likely to be cannibalised when they occurred only with conspecifics. The percentage of larvae falling prey to cannibalism declined when larvae of *A. bipunctata* (and correspondingly fewer conspecifics) were present. *Propylea japonica* survived to adulthood at relatively high rates, but survival was lowered when reared with *A. bipunctata* than when reared with conspecifics (Table 3; $\chi_1^2 = 8.5$, $P < 0.01$), especially during the fourth instar and in the pupal stage (Fig. 2d; fourth instar: $\chi_1^2 = 4.9$, $P < 0.05$; pupal stage: $\chi_1^2 = 12.6$, $P < 0.001$). In particular, *P. japonica* was much more likely to die of unknown causes when it co-occurred with *A. bipunctata* than with only conspecifics (Table 3).
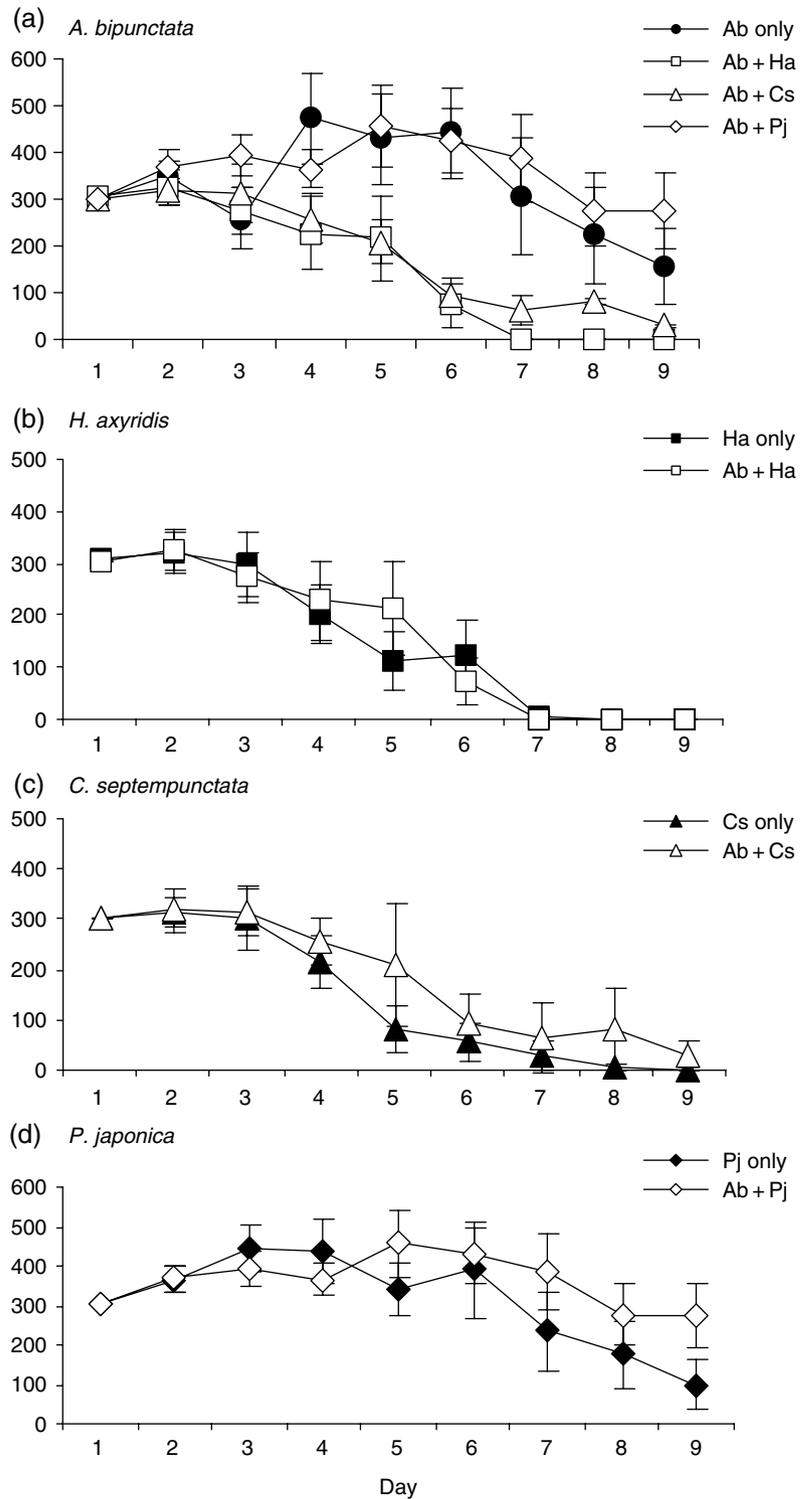
Fig. 1. The mean number of cowpea aphids present on successive days of the first experiment when larvae of *A. bipunctata* (Ab), *H. axyridis* (Ha), *C. septempunctata* (Cs), and *P. japonica* (Pj) were reared with conspecifics (e.g. 'Ab only') or with heterospecifics (e.g. 'Ab + Ha').

The developmental time and weight gain of individuals reared with conspecifics and heterospecifics

The developmental time of *A. bipunctata* from L2 to adult did not differ significantly among the treatments ($F_{3,46} = 1.095$, $P > 0.05$; mean ± standard error (SE) for individual treatments: with conspecifics, $417 ± 17.5$ h; with *H. axyridis*, $366.0 ± 6$ h; with *C. septempunctata*, $417.5 ± 18.9$ h; with *Propylea japonica*, $352.8 ± 23.9$ h). Furthermore, there was no significant difference in weight

Table 1. Repeated measures ANOVA for the comparison of number of aphids present among and between treatments illustrated in Fig. 1a–d.

Source of variation[a]	d.f.	F	P
(a) Ab only or Ab + (Ha or Cs or Pj)	3,13	12.67	0.0004
Time	8,104	14.3	<0.0001
Interaction	24,104	4.779	<0.0001
(b) Ha only or Ha + Ab	1,12	0.425	0.5268
Time	8,96	33.36	<0.0001
Interaction	8,96	0.326	0.954
(c) Cs only or Cs + Ab	1,12	0.135	0.7196
Time	8,96	31.96	<0.0001
Interaction	8,96	0.318	0.9573
(d) Pj only or Pj + Ab	1,4	2.611	0.1814
Time	8,32	1.202	0.3289
Interaction	8,32	2.838	0.0168

[a]Ab, *Adalia bipunctata*; Ha, *Harmonia axyridis*; Cs, *Coccinella septempunctata*; Pj, *Propylea japonica*.

gain of *A. bipunctata* from L2 to L4 among the treatments ($F_{3,27} = 0.894$, $P > 0.05$; mean $\pm$ SE for individual treatments: with conspecifics, 4.48 $\pm$ 0.3 mg; with *H. axyridis*, 4.14 $\pm$ 0.5 mg; with *C. septempunctata*, 4.71 $\pm$ 0.5 mg; with *Propylea japonica*, 5.18 $\pm$ 0.5 mg).

There was also no significant difference among the treatments in the developmental time of *C. septempunctata* from L2 to adult ($F_{1,5} = 1.73$, $P > 0.05$; mean $\pm$ SE for individual treatments: with conspecifics, 384 $\pm$ 13.9 h, vs. with *A. bipunctata*, 432.5 $\pm$ 29.6 h; a similar analysis could not

be completed for *H. axyridis* because of high mortality (Table 3)). The developmental time of *P. japonica* from L2 to L3 was significantly longer in the presence of *A. bipunctata* vs. only conspecifics ($F_{1,86} = 4.73$, $P < 0.05$; mean $\pm$ SE for individual treatments: with conspecifics, 58.8 $\pm$ 2.5 h, vs. with *A. bipunctata*, 68.3 $\pm$ 3.68 h). There was no significant difference, however, in developmental time thereafter, from third instar to adults ($F_{1,56} = 0.023$, $P > 0.05$; mean $\pm$ SE for individual treatments: with conspecifics, 260.5 $\pm$ 5.3 h, vs. with *A. bipunctata*, 262.5 $\pm$ 12 h). The weights gained by *H. axyridis*, *C. septempunctata*, and *P. japonica* from second instar to fourth instar were not different among the treatments (for *H. axyridis*, $F_{1,12} = 0.625$, $P > 0.05$; mean $\pm$ SE for individual treatments: with conspecifics, 11.37 $\pm$ 1 mg, vs. with *A. bipunctata*, 12.7 $\pm$ 1.5 mg; for *C. septempunctata*, $F_{1,9} = 0.301$, $P > 0.05$; mean $\pm$ SE for individual treatments: with conspecifics, 9.7 $\pm$ 1.5 mg, vs. with *A. bipunctata*, 11.9 $\pm$ 1.4 mg; for *Propylea japonica*, $F_{1,15} = 1.391$, $P > 0.05$; mean $\pm$ SE for individual treatments: with conspecifics, 3.86 $\pm$ 0.13 mg, vs. with *A. bipunctata*, 4.59 $\pm$ 0.57 mg).

Discussion

In this study, negative interactions of larvae of the exotic species, *A. bipunctata*, were observed with native Japanese ladybirds. In particular, rates of mortality increased as a result of IGP in the presence of *H. axyridis* and *C. septempunctata*, but not *P. japonica*. Interestingly,

Table 2. Percentages of *Adalia bipunctata* larvae that survived to adulthood or died from different causes when with conspecifics or heterospecifics.

	Combination of larvae reared together			
	Ab[a] only	Ab + Ha[a]	Ab + Cs[a]	Ab + Pj[a]
Survive to adults	40.0 (24)[b]	6.7 (2)	36.7 (11)	43.3 (13)
Dying as immatures (all causes combined)	60.0 (36)	93.3 (28)	63.3 (19)	56.7 (17)
From unknown causes	45.0 (27)	13.3 (4)	10.0 (3)	56.7 (17)
As second instar	1.7 (1)	10.0 (3)	3.3 (1)	3.3 (1)
As third instar	6.7 (4)	–	6.7 (2)	13.3 (4)
As fourth instar	13.3 (8)	–	–	16.7 (5)
As pupa	23.3 (14)	3.3 (1)	–	23.3 (7)
From cannibalism	15.0 (9)	0	10.0 (3)	0 (0)
As second instar	–	–	–	–
As third instar	1.7 (1)	–	–	–
As fourth instar	13.3 (8)	–	10.0 (3)	–
As pupa	–	–	–	–
From IGP	0 (0)	80.0 (24)	43.3 (13)	0 (0)
As second instar	–	–	–	–
As third instar	–	26.7 (8)	3.3 (1)	–
As fourth instar	–	50.0 (15)	30.0 (9)	–
As pupa	–	3.3 (1)	10.0 (3)	–

[a]Ab, *Adalia bipunctata*; Ha, *Harmonia axyridis*; Cs, *Coccinella septempunctata*; Pj, *Propylea japonica*.
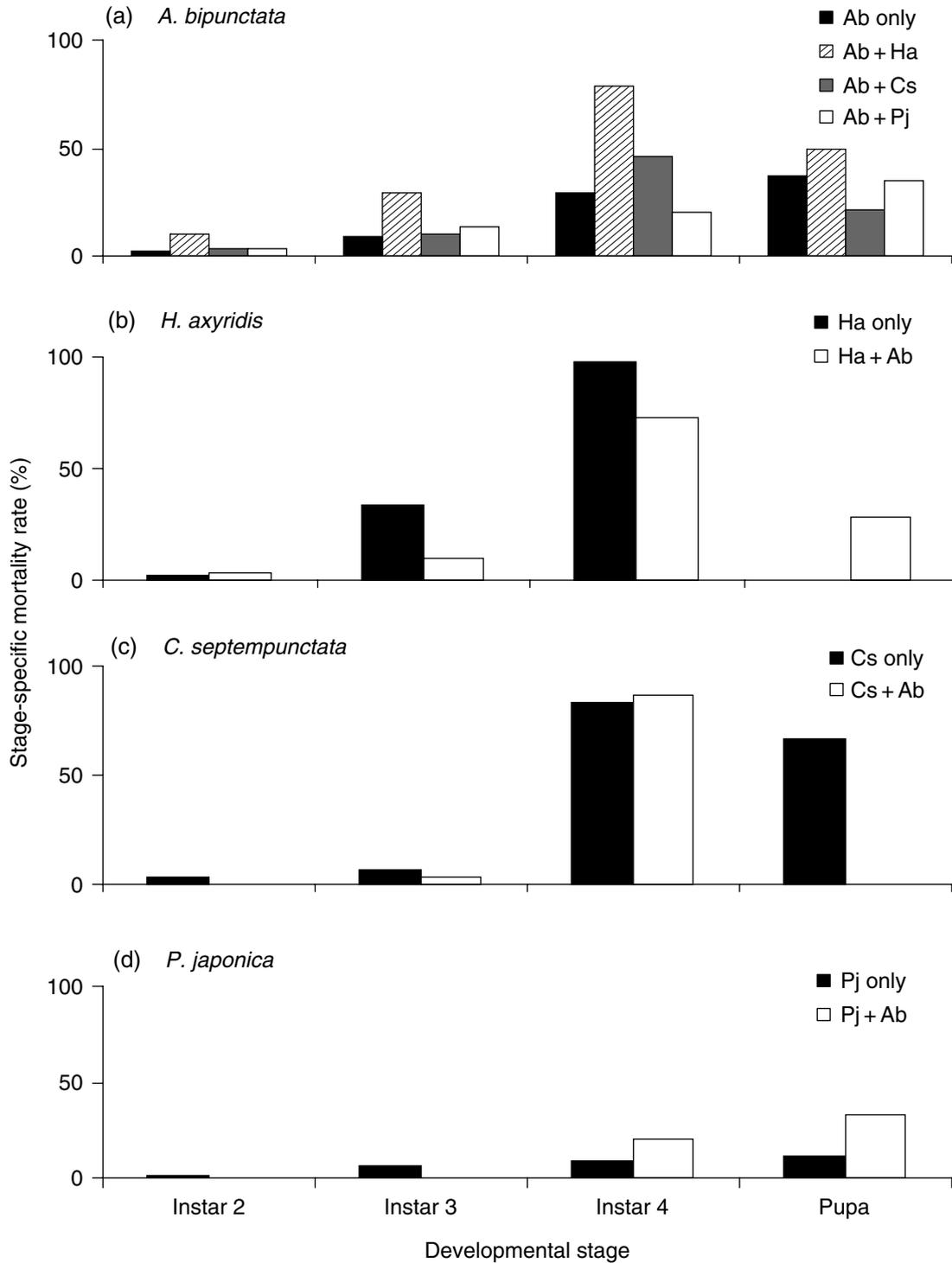[b]Values in parentheses show the number of larvae.

Fig. 2. Stage-specific mortality rates during developmental stages of *A. bipunctata*, *H. axyridis*, *C. septempunctata*, and *P. japonica* reared with conspecifics or heterospecifics.

additional negative effects of native Japanese ladybirds on rates of development and weight gain by surviving individuals of *A. bipunctata* were not observed. The high incidence of IGP of *A. bipunctata* by *H. axyridis* and *C. septempunctata* provides support for the hypothesis proposed previously (Kajita *et al.*, 2000) that interactions with native ladybirds may be important in limiting the establishment and spread of *A. bipunctata* in Japan.

Table 3. Percentages of *Harmonia axyridis*, *Coccinella septempunctata*, and *Propylea japonica* larvae that survived to adulthood or died from different causes when with conspecifics or with heterospecifics (i.e. with *Adalia bipunctata*).

	Combination of larvae reared together					
	Ha[a] only	Ha + Ab[a]	Cs[a] only	Cs + Ab	Pj[a] only	Pj + Ab
Survive to adults	1.7 (1)[b]	16.7 (5)	5.0 (3)	13.3 (4)	73.3 (44)	53.3 (16)
Dying as immatures (all causes combined)	98.3 (59)	83.3 (25)	95.0 (57)	86.7 (26)	26.7 (16)	46.7 (14)
From unknown causes	21.6 (13)	40.0 (12)	25.0 (15)	40.0 (12)	15.0 (9)	46.7 (14)
As second instar	1.7 (1)	3.3 (1)	3.3 (2)	–	1.7 (1)	–
As third instar	–	–	–	3.3 (1)	3.3 (2)	–
As fourth instar	20 (12)	33.3 (10)	13.3 (8)	36.7 (11)	–	20.0 (6)
As pupa	–	3.3 (1)	8.3 (5)	–	10.0 (6)	26.7 (8)
From cannibalism	76.7 (46)	43.3 (13)	70.0 (42)	46.7 (14)	11.7 (7)	0 (0)
As second instar	–	–	–	–	–	–
As third instar	33.3 (20)	10.0 (3)	6.7 (4)	–	3.3 (2)	–
As fourth instar	43.3 (26)	30.0 (9)	61.7 (37)	46.7 (14)	8.3 (5)	–
As pupa	–	3.3 (1)	1.7 (1)	–	–	–
From IGP	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
As second instar	–	–	–	–	–	–
As third instar	–	–	–	–	–	–
As fourth instar	–	–	–	–	–	–
As pupa	–	–	–	–	–	–

[a]Ab, *Adalia bipunctata*; Ha, *Harmonia axyridis*; Cs, *Coccinella septempunctata*; Pj, *Propylea japonica*.
[b]Values in parentheses show the number of larvae.

The results revealed high asymmetry; *A. bipunctata* was always the IGP prey and never the predator. The presence of *H. axyridis* larvae, and the high rate with which they engaged in IGP, greatly reduced the survivorship of *A. bipunctata* larvae. Interestingly, the presence of *C. septempunctata* did not result in significant change in overall survivorship of *A. bipunctata*; although many larvae of *A. bipunctata* died from IGP, fewer died from other causes when *C. septempunctata* was present vs. absent. Weights of newly moulted fourth instars of *A. bipunctata* were not significantly affected by the presence of *C. septempunctata* larvae, but during the fourth instar (when aphid numbers became very low and when most IGP occurred), larvae of *C. septempunctata* may have preyed most heavily on *A. bipunctata* that grew weak (and were especially likely to succumb to starvation in the absence of IGP). This may account in turn for the intriguing result that those larvae of *A. bipunctata* that survived to the pupal stage were less likely to die when *C. septempunctata* larvae were present than when they were absent.

Although *H. axyridis* and *C. septempunctata* are of similar size, *H. axyridis* is a more aggressive and stronger intraguild predator than *C. septempunctata* (Yasuda & Shinya, 1997; Yasuda & Ohnuma, 1999; Kajita *et al.*, 2000; Yasuda *et al.*, 2001; Snyder *et al.*, 2004; Yasuda *et al.*, 2004). In general, *H. axyridis* is a voracious and polyphagous predator (Hodek & Honek, 1996). Yasuda and Ohnuma (1999), for example, found that fourth instars of *H. axyridis* survived and grew equally well when fed on diets of aphids or other species of ladybird larvae; in contrast, larvae of *C. septempunctata* survived and grew much

better on a diet of aphids than of other ladybird larvae. Sato and Dixon (2004) found that hatching larvae of *H. axyridis* survived at relatively high rate on diets of eggs of *C. septempunctata* and *A. bipunctata*, whereas hatching larvae of these two species were unable to survive on a diet of *H. axyridis* eggs. The broad and aggressive feeding habits of *H. axyridis* likely account for its much stronger negative effects on survivorship of *A. bipunctata* through IGP than those of *C. septempunctata*.

The strength of IGP depends not only on characteristics of the predators, but also on the availability of aphids; in general, the incidence of IGP (and cannibalism) among ladybirds increases when the relative abundance of aphids to larvae is low (Takahashi, 1989; Agarwala & Dixon, 1992; Yasuda & Shinya, 1997; Lucas *et al.*, 1998; Kajita *et al.*, 2000; Yasuda *et al.*, 2004). In the present study, the intensity of IGP by *H. axyridis* and *C. septempunctata* on *A. bipunctata* increased as larvae developed and the number of prey declined. Most IGP occurred when the larvae of *H. axyridis*, *C. septempunctata*, and *A. bipunctata* were third and fourth instars. The frequent preying upon larvae of *A. bipunctata* (and the nourishment gained thereby) likely accounts for the fact that older individuals of *H. axyridis* and *C. septempunctata* survived better when reared with *A. bipunctata* than when reared only with conspecifics even though aphid numbers had become low in both cases.

The presence of *P. japonica*, which is smaller than *H. axyridis* and *C. septempunctata* and is similar in size to *A. bipunctata*, did not result in significant change in overall survivorship of *A. bipunctata*. The presence of *A. bipunctata*, however, significantly reduced the overall

survivorship of *P. japonica* larvae. Although IGP did not occur between these two similar-sized species, a much higher percentage of *P. japonica* larvae died of causes other than IGP when *A. bipunctata* larvae were present than when they were absent. Such deaths might be the result of exploitative interspecific competition for aphid prey (e.g. Evans, 1991), but it is interesting in this regard that aphid numbers remained relatively high on the host plant throughout the experiment. Thus, an alternative explanation may be that physical interference and chemical cues by *A. bipunctata* adversely affect the foraging behaviour of *P. japonica* larvae. Agarwala *et al.* (2003), for example, reported that the feeding and reproductive rates of *P. japonica* were reduced significantly in the presence of faeces of *H. axyridis* and conspecifics. It is possible that the presence of faeces had similar adverse effects on foraging and consequent survival of *P. japonica* larvae in the present experiment, and that the effects of *A. bipunctata* faeces were especially adverse.

The laboratory setting that was employed in this study enabled detailed investigation of the potential importance of IGP as it might occur when larval *A. bipunctata* and native Japanese ladybirds co-occur in exploiting natural populations of aphids. In this regard, the experimental design, wherein larvae were able to forage and interact on host plants, is an improvement over simple cage experiments because the larvae had more opportunity to avoid contact with each other (e.g. by searching at any given time in different locations on the host plants) and they were able to respond to attacks by each other as they would in natural populations (e.g. as in escaping by dropping from the host plant). Nonetheless, this experiment included only one species of host plant, and results could vary among host-plant species. Furthermore, the experimental design necessarily prevented escape from the local setting altogether by emigration, which can be an important response of ladybird larvae to shortage of prey (e.g. Schellhorn & Andow, 1999; Sato *et al.*, 2003). Because IGP is often most intense when prey become scarce, such emigration behaviour can also be important in reducing the incidence of IGP in natural populations of ladybirds (Sato *et al.*, 2003). Another shortcoming of the experimental design is that logistical constraints limited treatments to combinations at the outset of similar-sized individuals (second instars) of different species. In natural settings, not all eggs are laid at the same time at an aphid colony, and larvae of different instars co-occur; outcomes of interspecific encounters will vary accordingly (e.g. Snyder *et al.*, 2004; Yasuda *et al.*, 2004). It is therefore difficult to extrapolate from cage results such as ours, in trying to assess the frequency of IGP in the field. Field observations of larvae of *H. axyridis* preying upon pre-pupae and pupae of *A. bipunctata* (Sakuratani *et al.*, 2000), however, correlate with the laboratory results presented here in suggesting that IGP certainly has the potential to be an important larval interaction in natural populations of the exotic and native ladybirds.

Although *A. bipunctata* now occurs in Japan (Sakuratani, 1994), it remains very limited in its distribution. In contrast, *C. septempunctata* and *H. axyridis* have become very widespread and abundant following their introductions to North America, and there are many reports that these two species are negatively interacting with native ladybird species (LaMana & Miller, 1996; Brown & Miller, 1998; Colunga-Garcia & Gage, 1998; Lucas *et al.*, 2002; Michaud, 2002; Brown, 2003; Nault & Kennedy, 2003; Turnock *et al.*, 2003; Evans, 2004; Snyder *et al.*, 2004; Yasuda *et al.*, 2004), including *A. bipunctata* (Elliott *et al.*, 1996).

These observations raise the questions, why has *A. bipunctata* not established more successfully in Japan and why have *H. axyridis* and *C. septempunctata* been so successful in establishing themselves in North America? Of potential factors, this study has focused on the asymmetry in IGP, which appears to be driven by key differences in body size and behaviour among species. This favours *H. axyridis* and *C. septempunctata* over other ladybird species such as *A. bipunctata*. Additional factors may also be important. It is striking, for example, that there are often long lag times for invading species between initial introduction and subsequent spread (Mack *et al.*, 2000); in this regard, it should be noted that *A. bipunctata* has been introduced much more recently to Japan than have *C. septempunctata* and *H. axyridis* to North America. In addition, the sizes and frequencies of introductions are thought to be major factors influencing establishment and spread of exotic species (Beirne, 1995; Williamson & Fitter, 1996a, b; Williamson, 1999). In the classical biological control programmes associated with of the establishment and spread of *C. septempunctata* and *H. axyridis* in North America, large numbers of individuals were intentionally introduced widely throughout the continent (Angalet *et al.*, 1979; Schaefer *et al.*, 1987; Tedders & Schaefer, 1994). In contrast, it is likely that only a relatively few individuals of *A. bipunctata* were introduced accidentally to Japan at a single site, the port town of Osaka.

IGP may interact with other factors in influencing colonisation success of exotic species. For example, IGP may impinge especially heavily on small founder populations, limiting or preventing their establishment. Furthermore, the population of *A. bipunctata* that established at Osaka is univoltine, whereas populations of this species are bivoltine in many parts of Europe and North America (Hodek & Honek, 1996). Thus, in Japan, *A. bipunctata* may be especially vulnerable to negative interactions such as IGP with *H. axyridis* and *C. septempunctata*. These species are also univoltine in Japan, and overlap extensively with *A. bipunctata* in habitat as well as in timing of the life cycle (in contrast, the bivoltinism of *A. bipunctata* in parts of North America may provide it with some escape in time).

In summary, the results of this study reveal differing strengths of interactions among native Japanese ladybirds and the exotic species, *A. bipunctata*. Thus, although a number of factors may be important in the failure of *A. bipunctata* to establish itself and spread well in Japan, one such factor (a form of biotic

resistance; Elton, 1958) may be IGP by *H. axyridis* and (to a lesser extent) by *C. septempunctata*. Conversely, asymmetric IGP may promote the establishment of *H. axyridis* and *C. septempunctata* in North America. This laboratory study provides some useful insights and important first steps in evaluating the potential importance of IGP. As Lucas *et al.* (2002) point out, however, *in situ* studies in natural systems are now needed to more fully assess the importance of interspecific interactions such as IGP in determining the success or failure of introductions of species such as the ladybirds that we have studied here.

Acknowledgements

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