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Intraguild predation of immature stages of British and Japanese coccinellids by the invasive ladybird *Harmonia axyridis*

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Abstract Declines in native aphidophages in North America have been linked to intraguild predation (IGP) by the invasive coccinellid *Harmonia axyridis* (Pallas) (Coleoptera: Coccinellidae). It is feared that many British species will face a similar fate following the recent establishment of *H. axyridis* in the UK. Meanwhile, *H. axyridis* exists in apparent ecological equilibrium with other members of its guild in Japan. The impact of *H. axyridis* on British coccinellids is uncertain but intraguild predatory interactions do occur, particularly amongst immature stages. This study investigates IGP between immature stages of *H. axyridis* and various British and Japanese coccinellids. The only asymmetric IG predator of *H. axyridis* at first instar was *Anatis ocellata* (Linnaeus). *Harmonia axyridis* engaged in symmetric IGP with *Coccinella septempunctata* Linnaeus, *Calvia quatuordecimguttata* (Linnaeus), *Harmonia quadripunctata* (Pontoppidan) and *Eocaria muiri* Timberlake, but was the asymmetric IG predator of all other species studied. The level of IGP was high between fourth instar larvae, and frequently biased towards *H. axyridis*, except in the case of *A. ocellata*, which again was the only IG predator of *H. axyridis.* In interactions between fourth instar larvae and pre-pupae, IGP was unidirectional towards *H. axyridis* for all species except *A. ocellata*, which acted as both IG predator and IG prey. Pupae were better protected against IGP than pre-pupae but most species were still susceptible to attack by *H. axyridis*, although IGP was symmetric with *A. ocellata*, and *H. quadripunctata* pupae were never attacked. The differences in susceptibility of the various species and developmental stages to IGP by *H. axyridis* are discussed in relation to physical defence structures. We find no evidence that Japanese species have superior defences to British ones and suggest that behavioural strategies may enable co-existence in the native range. We discuss the relevance of IGP by *H. axyridis* to the species it is likely to encounter in Britain.

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Introduction

The establishment and spread of the invasive coccinellid *Harmonia axyridis* (Pallas) (Coleoptera: Coccinellidae) in Britain (Brown et al. 2007) has provoked concerns for native ecosystems centred on aphids (Majerus et al. 2006). An increasing body of evidence from North America suggests that *H. axyridis* has the potential to negatively impinge on a wide range of species through both resource competition and interspecific predation, including intraguild predation (Koch and Galvan 2007). Such interactions are accepted to be a common cause of declines in native species following the arrival of an invasive alien (Williamson 1996). The observation that *H. axyridis* coexists with many other aphidophages in its natural Asian range (Kuznetsov 1997; R. Ware and M. Majerus, pers. obs.), might suggest that species in its introduced range are at a disadvantage through their lack of co-evolved defensive strategies.

Intraguild predation (IGP) is well reported from aphidophagous guilds (Rosenheim et al. 1995; Lucas 2005). It occurs when two species that share a host or prey, and may therefore compete, also interact trophically (Polis et al. 1989). There is controversy over whether the killing of a potential competitor constitutes IGP if the victim is not consumed. Some authors prefer to classify such instances as ‘interspecific killing’, while others accept that, with the exception of nutritive gain for the predator, the same benefits and costs are involved here as in IGP sensu stricto and therefore both situations should be included when considering IGP (Lucas 2005). In the context of this paper we are primarily interested in the vulnerabilities of different species as intraguild prey to *H. axyridis*: i.e. whether an individual is killed or not. Thus we adopt Lucas’ (2005) revised definition when referring to IGP.

The size and mobility of the IG predator and the IG prey are known to have considerable influence on the outcome of their interaction (Şengonca and Frings 1985; Phoofolo and Obrycki 1998; Lucas et al. 1998; Rosenheim et al. 1995; Felix and Soares 2004). Thus, in coccinellids, eggs, younger larvae, ecdysing larvae, pre-pupae and pupae constitute particularly vulnerable stages (Agarwala and Dixon 1992; Majerus 1994; Dixon 2000; Sato and Dixon 2004). Most coccinellids possess some form of defence against predation, which may be chemical, physical or behavioural, and many show such adaptations throughout their life cycle.

Chemical defence has been particularly well studied at the egg stage, where both internal toxins (Pasteels et al. 1973; Agarwala and Dixon 1992) and external feeding deterrents (Hemptinne et al. 2000; Omkar et al. 2004) have been reported. Indeed, we know that the eggs of at least one Japanese species, *Eocaria muiri* Timberlake, and one British species, *Calvia quatuordecimguttata* (Linnaeus), are particularly well defended from IGP by *H. axyridis* (Ware et al. in press; Ware et al. 2007). Coccinellid larvae, pre-pupae and pupae are also equipped with a chemical armoury and contain similar defensive alkaloids to those present within eggs (Pasteels et al. 1973). In addition, adults, larvae and pre-pupae will ‘reflex bleed’ when disturbed (Majerus 1994). The exudate contains alkaloids and pyrazines and is known to be an effective deterrent to many predators (Majerus and Kearns 1989; de Jong et al. 1991; Holloway et al. 1991).

When IGP between larvae does occur, it is usually the larger that attacks the smaller (Dixon 2000; Felix and Soares 2004). Thus, being larger than average at each developmental stage may itself be an adaptation for success in IGP. However, there are a variety of other physical defences employed by coccinellid immature stages. Larvae of many species possess hairs or spines which render them unpalatable or difficult to 'handle' or subdue (R. Ware, pers. obs.), while others, such as *Scymnus* spp., are covered in a waxy coating, which provides an effective defence against ants and syrphid larvae (Pope 1979; Völkl and Vohland 1996; Agarwala and Yasuda 2001). It is notable, however, that ecdysing or newly ecdysed larvae of all species are particularly vulnerable to attack, as it takes time for their integument to harden or waxes to develop fully (Lucas et al. 2000; Agarwala and Yasuda 2001). Before the larval skin splits in preparation for pupation, the physical defences of a coccinellid pre-pupa are largely the same as those of a final instar larva. However, some species are reported to use 'gin-traps' at the pupal stage as an effective defence against enemies such as mites (Hinton 1955) and ants (Eisner and Eisner 1992).

Most coccinellids also have some form of behavioural defence against predation. For mobile stages, such as larvae, a first response to a perceived threat would simply be to run away (R. Ware, pers. obs.). Further defensive tactics of aphidophagous larvae include dropping from a host plant if a potential predator approaches (Sato et al. 2005) or migrating to a new site before aphid populations decline (Sato et al. 2003). Predation of vulnerable sessile stages, such as eggs, ecdysing larvae and pupae, may be avoided by species-specific oviposition sites (Schellhorn and Andow 1999) and selection of moulting and pupation sites (Lucas et al. 2000) respectively. Although coccinellid pre-pupae and pupae appear to be extrinsically quiescent, they are not completely immobile and are able to flick up their anterior end rapidly when disturbed, which is thought to be a defence against parasitoid attack (Disney et al. 1994).

Variability in defences of members of an aphidophagous guild to IGP is likely to have a marked influence on the guild's overall structure. Intraguild predation of immature stages is considered a likely scenario between *H. axyridis* and British coccinellids (Majerus et al. 2006; Ware et al. in press) but its precise nature and impact is uncertain. Here we investigate the level and symmetry of IGP between *H. axyridis* and British coccinellids at four different stages of development: first instar larvae, fourth instar larvae, newly formed pre-pupae and 3-day-old pupae. British species were chosen from a range of different habitats on which *H. axyridis* is likely to encroach (Table 1). Four species of Japanese coccinellid were also investigated with a view to comparing the vulnerabilities and defences of species from the native range of *H. axyridis* with those from part of its invaded range.

Materials and methods

General protocol

The coccinellid species used in this study and the areas of origin of their stock cultures are listed in Table 1. All individuals used in experiments were F1 or F2 offspring from field collected samples. Mating pairs of all species were housed in 9 cm Petri dishes and maintained in a constant environment room at 22°C, 35% humidity and 14 hL:10 hD. Adults were supplied with excess pea aphids, *Acyrthosiphon pisum* (Harris), daily, and eggs were collected daily prior to transfer of adults to a clean dish to induce oviposition (Majerus et al. 1989).

Table 1 Details of species (Coleoptera: Coccinellidae) used in this study

Species	Code	Origin	Habitat in UK or Japan
Harmonia axyridis (Pallas 1773)	HA-B	Battersea, London, UK	Generalist
Coccinella septempunctata Linnaeus 1758	C7	Thetford, Norfolk, UK	Herbaceous generalist
Adalia bipunctata (Linnaeus 1758)	A2	Cambridge, Cambridgeshire, UK	Generalist
Adalia decempunctata (Linnaeus 1758)	A10	Thetford, Norfolk, UK	Broad-leaved deciduous woodland specialist
Propylea quatuordecimpunctata (Linnaeus 1758)	P14	Cambridge, Cambridgeshire, UK	Generalist
Calvia quatuordecimguttata (Linnaeus 1758)	C14	Thetford, Norfolk, UK	Broad-leaved deciduous woodland specialist
Anatis ocellata (Linnaeus 1758)	AO	Thetford, Norfolk, UK	Host plant restricted specialist: needled conifers
Harmonia quadripunctata (Pontoppidan 1763)	H4	Thetford, Norfolk, UK	Host plant restricted specialist: needled conifers
Coccinella quinquepunctata Linnaeus 1758	C5	Aberystwyth, Wales, UK	Generalist with environmental constraints: unstable river shingle
Harmonia axyridis (Pallas 1773)	HA-J	Fuchu, Honshu, Japan	Generalist
Coccinella septempunctata brucki Mulsant 1866	CSB	Fuchu, Honshu, Japan	Generalist
Cheilomenes sexmaculatus (Fabricus 1781)	CHS	Fuchu, Honshu, Japan	Generalist
Propylea japonica (Thunberg 1781)	PJ	Fuchu, Honshu, Japan	Herbaceous generalist
Eocaria muiri Timberlake 1943	EM	Fuchu, Honshu, Japan	Broad-leaved deciduous woodland specialist

Habitat classes for British species amended from Majerus (1994, pp. 141–143); information for Japanese species from R. Ware and M. Majerus (pers. obs.)

All experiments were carried out in a constant environment room at 22°C, 35% humidity and 14 hL:10 hD.

First instar larval contests

Newly hatched larvae were given excess aphids for 24 h and those used in first instar contests were age-matched based on the date of hatching. A single *H. axyridis* larva was transferred to one pole of a clean 5 cm Petri dish using a single-bristle paintbrush. A single larva of a non-conspecific species was then added to the opposite pole of the dish. No other food or water was provided thereafter. For all interactions, except those between *H. axyridis* and *Harmonia quadripunctata* (Pontoppidan), dishes were monitored twice daily until both larvae had died, and times of death from the time of set-up were recorded. Larval identification was carried out under a dissecting microscope on the basis of spine morphology (R. Ware, pers. obs.). Due to the difficulty in distinguishing *H. axyridis* and *H. quadripunctata* at first instar, the larva that survived longest in each contest was reared to fourth instar when identity could be established by use of abdominal markings. Causes of death of larvae were classified as: ‘unknown’ (where no obvious injury was visible but larvae may have appeared somewhat shrivelled due to desiccation), ‘attacked, but not eaten after 48 h’ (evidence of injury, e.g. bite marks, reflex blood, but corpse otherwise intact) or ‘attacked, and eaten within 48 h’ (body parts missing, evidence of fluid being sucked). First instar larvae of British *H. axyridis* were matched with first instar larvae of each of the eight British species, while first instar larvae of Japanese *H. axyridis* were matched with those of each of the four Japanese species. Each trial was replicated 20 times.

Fourth instar larval contests

First instar larvae not used in the above experiments were reared on aphids in 9 cm Petri dishes (five larvae per dish) to generate material for fourth instar contests. Larvae used were age-matched based on the date of third ecdysis. A single fourth instar *H. axyridis* larva that had ecdysed that day was transferred to one pole of a clean 9 cm Petri dish using a fine bristled paintbrush. A single fourth instar larva of a non-conspecific species was then added to the opposite pole of the dish. No other food or water was provided thereafter. Dishes were observed regularly over the next 2 days (eight checks in 48 h) and once daily thereafter. Evidence of IGP, time of larval deaths and pupations were noted. The time elapsed from the set-up of the experiment until death of one protagonist was analysed as an indication of the ease with which an IG predator could subdue its prey. All species were identifiable by eye at fourth instar (R. Ware, pers. obs.). Again, British *H. axyridis* larvae were matched with larvae of the British species, and Japanese *H. axyridis* were pitted against larvae of the Japanese species. Twenty replicates were performed for each contest.

IGP of pre-pupae by fourth instar larvae

Fourth instar larvae not used in the above experiments were isolated in 9 cm Petri dishes, provided with excess aphids daily, and allowed to pupate in the dish. Following formation of the pre-pupa, the dish was cleaned with an alcohol swab to remove any aphid debris and waste material, and a fourth instar *H. axyridis* larva, which had undergone third ecdysis that day, was added using a fine bristled paintbrush. No other food or water was provided thereafter. Dishes were observed regularly over the next 2 days (eight checks in 48 h) and once daily thereafter. Pre-pupae were observed for attack/consumption by the larva or eclosion. Larvae were observed for death or pupation. *Harmonia axyridis* larvae were presented with sympatric pre-pupae of all British and Japanese species in this manner, and larvae of all these species were presented with pre-pupae of *H. axyridis*. Cannibalism of *H. axyridis* pre-pupae by non-sibling conspecific larvae was also tested. Twenty replicates were performed for each trial, with the exception of *H. axyridis* larvae versus *Coccinella quinquepunctata* Linnaeus pre-pupae, in which only ten replicates were achieved due to low culture output.

IGP of pupae by fourth instar larvae

Pupal trials followed the same protocol as above, but using 3-day-old pupae rather than pre-pupae. Again, problems with producing enough *C. quinquepunctata* meant reduced replicates for *H. axyridis* larvae versus *C. quinquepunctata* pupae ($N = 13$) and *C. quinquepunctata* larvae versus *H. axyridis* pupae ($N = 8$).

Statistical analysis

All statistical manipulations were performed using Minitab 14, apart from Fisher's exact test and the Fisher-Freeman-Halton test, which were carried out using StatXact 7. British species are considered separately from Japanese species throughout.

Levels and symmetry of IGP

Outcomes of larval fights were classified as: *H. axyridis* acted as the IG predator, *H. axyridis* acted as the IG prey, or no IGP event occurred. For each species-pair comparison, the level of IGP between *H. axyridis* larvae and larvae of another species is determined as the proportion of replicates in which IGP occurred out of the total number of replicates for that species-pair, and an index of symmetry is given by the proportion of replicates in which *H. axyridis* was the IG predator out of the total number of replicates in which IGP occurred (Lucas et al. 1998). Thus, a symmetry index of >0.5 shows that IGP was in favour of *H. axyridis*, while an index of <0.5 indicates IGP was in favour of the other species. For first and fourth instar larvae, symmetry of IGP was analysed using a one-sample χ^2 goodness of fit test with an expected symmetry of 50%. Comparisons of levels and symmetry between species were made using a fully orthogonal analysis of contingency tables using Fisher's exact test (for 2 × 2 tables) (FEt) or the Fisher-Freeman-Halton test (for $r \times 2$ tables) (FFHt).

The concept of 'symmetry' is not applicable to trials involving larvae versus (pre)pupae as the latter can never be the IG predator. Instead, we considered the relative level of IGP when *H. axyridis* is the larva, compared to when *H. axyridis* is the (pre)pupa, in 2 × 2 contingency tables of numbers of (pre)pupae 'killed' versus 'not killed' for each species, using Fisher's exact test. Levels of IGP of (pre)pupae of different species by *H. axyridis* were compared using a fully orthogonal analysis of contingency tables with Fisher's exact test or the Fisher-Freeman-Halton test as appropriate. Similar analysis was conducted to compare the levels of IGP of *H. axyridis* (pre)pupae by larvae of different species.

Time until death of IG prey

For fourth instars, pre-pupae and pupae, the time until death of the IG prey (measured from time of experimental set-up) was compared non-parametrically as the Kolmogorov–Smirnov tests revealed a significant difference from normality in some cases ($P < 0.05$). The Kruskal–Wallis test and multiple pairwise Mann–Whitney U tests adjusting for ties were used. Alpha values were adjusted according to the Bonferroni correction for multiple comparisons (referred to in text as αB).

Results

First instar larval contests

Levels and symmetry of IGP

Both larvae scored as attacked but not eaten after 48 h and those scored as attacked and eaten after 48 h were considered to be the subject of IGP. The level and symmetry of IGP between *H. axyridis* and all British and Japanese species at first instar is shown in Table 2. The number of replicates in which the pairing of *H. axyridis* with a non-conspecific competitor resulted in IGP was similar for all British species (FFHt = 10.32, 7 d.f., $P = 0.167$). When IGP does occur between two species, A and B, it is clear that there are three possibilities: IGP may be symmetric (with an equal likelihood of either species acting as predator or prey), asymmetric in favour of species A (species A is the IG predator and

Table 2 The number of replicates in which *H. axyridis* acted as the IG predator or IG prey in pairings with British and Japanese species at first and fourth instar. Levels and indices of symmetry of IGP are also shown

Species	First instar				Fourth instar			
	HA acts as IG predator	HA acts as IG prey	Level of IGP	Symmetry of IGP	HA acts as IG predator	HA acts as IG prey	Level of IGP	Symmetry of IGP
British								
C7	6	4	0.50	0.60	20	0	1.00	1.00
A2	10	3	0.65	0.77	20	0	1.00	1.00
A10	18	0	0.90	1.00	20	0	1.00	1.00
P14	13	0	0.65	1.00	20	0	1.00	1.00
C14	6	6	0.60	0.50	11	3	0.70	0.79
AO	2	13	0.75	0.13	2	17	0.95	0.11
H4	7	9	0.80	0.44	16	4	1.00	0.80
C5	12	1	0.65	0.92	17	0	0.85	1.00
Japanese								
CSB	17	2	0.95	0.89	20	0	1.00	1.00
CHS	13	2	0.75	0.87	20	0	1.00	1.00
PJ	18	0	0.90	1.00	20	0	1.00	1.00
EM	9	3	0.67	0.75	19	1	1.00	0.95

$N = 20$ for each pairing. For species codes, see Table 1

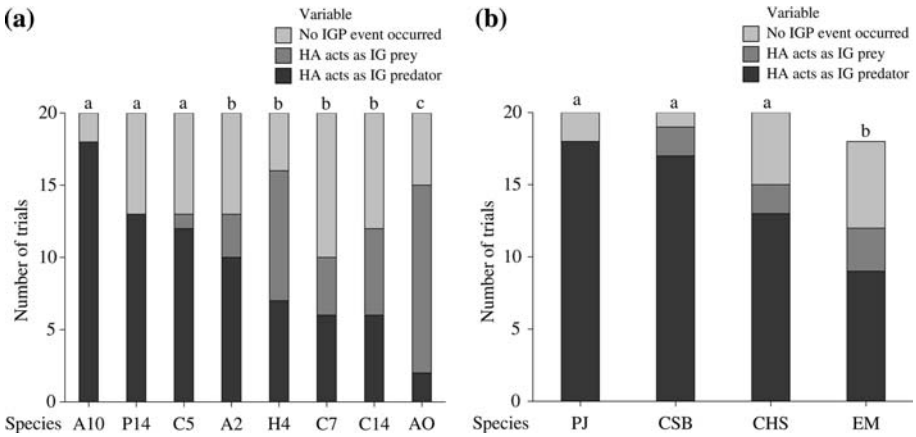

Fig. 1 Symmetry of IGP between *H. axyridis* and (**a**) British coccinellids and (**b**) Japanese coccinellids at first instar. Analysis shows that with species marked a, IGP was asymmetric in favour of *H. axyridis* (referred to as ‘class 1’ in text); with species marked b, IGP was symmetric (‘class 2’); and with species marked c, IGP was asymmetric in favour of the other species (‘class 3’). Species are placed along the *x*-axes from left to right in order of decreasing susceptibility to IGP by *H. axyridis*. For species codes, see Table 1

species B is the IG prey) or asymmetric in favour of species B (species B is the IG predator and species A is the IG prey).

In respect of the relationship of *H. axyridis* with British coccinellids at first instar, this study provides examples of all three (Fig. 1a). In contests with *Adalia decempunctata* (Linnaeus), *Propylea quatuordecimpunctata* (Linnaeus) and *C. quinquepunctata*, *H. axyridis* was predominantly the IG predator, hereafter referred to as ‘class 1’. *Harmonia axyridis* engaged in symmetric IGP with *Coccinella septempunctata* Linnaeus, *Adalia bipunctata* (Linnaeus), *C. quatuordecimguttata* and *H. quadripunctata*, hereafter referred to as ‘class 2’. Only one British species, *Anatis ocellata* (Linnaeus) acted as an asymmetric IG predator of *H. axyridis*, hereafter referred to as ‘class 3’. The validity of grouping species into classes 1 and 2 is shown by homogeneity within each class (FFHt $= 2.14$, 2 d.f., $P = 0.591$ for class 1 and FFHt $= 3.518$, 3 d.f., $P = 0.348$ for class 2). *Harmonia axyridis* acted as the IG predator significantly more often in class 1 than in class 2 (FEt $= 24.22$, 1 d.f., $P < 0.001$) and acted as the IG prey significantly less often in class 2 than in class 3 (FEt $= 9.04$, 1 d.f., $P = 0.003$).

The level of IGP was similar for all Japanese species studied (FFHt $= 6.40$, 3 d.f., $P = 0.082$). IGP was symmetric with *E. muiri*, but asymmetric in favour of *H. axyridis* in interactions with all other species (Fig. 1b). However, there was no significant difference in symmetry found between Japanese species (FFHt $= 4.72$, 3 d.f., $P = 0.160$).

Fourth instar larval contests

Levels and symmetry of IGP

The level and symmetry of IGP between *H. axyridis* and all British and Japanese species at fourth instar is shown in Table 2 and Fig. 2. Intraguild predation occurred on 100% of occasions when *H. axyridis* was paired with *C. septempunctata*, *H. quadripunctata*,

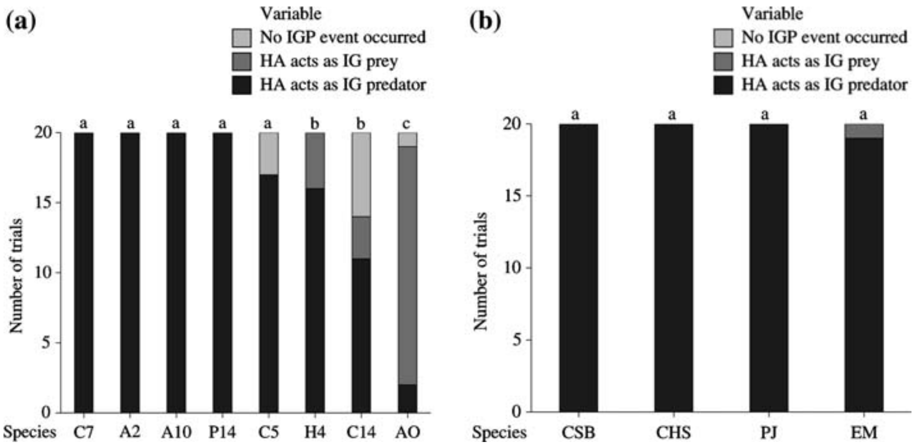

Fig. 2 Symmetry of IGP between *H. axyridis* and (**a**) British coccinellids and (**b**) Japanese coccinellids at fourth instar. Analysis shows that with species marked a, IGP was asymmetric in favour of *H. axyridis* ('class 1'); with species marked b, IGP was also asymmetric in favour of *H. axyridis* but significantly less so than for species marked a ('class 2'); and with species marked c, IGP was asymmetric in favour of the other species ('class 3'). Species are placed along the *x*-axes from left to right in order of decreasing susceptibility to IGP by *H. axyridis*. For species codes, see Table 1

A. bipunctata, *A. decempunctata* and *P. quatuordecimpunctata*, but less frequently with *C. quatuordecimguttata*, *A. ocellata* and *C. quinquepunctata* (FEt $= 18.00$, 1 d.f., $P < 0.001$), for which the level of IGP was similar (FFHt $= 4.27$, 3 d.f., $P = 0.130$). *Harmonia axyridis* was universally the IG predator of *C. septempunctata*, *A. bipunctata*, *A. decempunctata*, *P. quatuordecimpunctata* and *C. quinquepunctata* larvae ('class 1'). Intraguild predation was asymmetric in favour of *H. axyridis* in contests with *H. quadripunctata* and *C. quatuordecimguttata* ('class 2'), but asymmetric in favour of *A. ocellata* in interactions with this species ('class 3'). *Harmonia axyridis* acted as the IG predator significantly more often in class 1 than class 2 (FEt $= 17.8$, 1 d.f., $P < 0.001$), and significantly more often in class 2 than class 3 (FEt $= 24.36$, 1 d.f., $P < 0.001$). There was no difference in symmetry within a class (IGP was 100% asymmetric in class 1; FEt $= 0.095$, 1 d.f., $P \sim 1$ for class 2).

All replicates involving fourth instars of *H. axyridis* and Japanese species resulted in an IGP event. Intraguild predation was fully asymmetric towards *H. axyridis* for *Coccinella septempunctata brucki* Mulsant, *Cheilomenes sexmaculatus* (Fabricus) and *Propylea japonica* (Thunberg) and significantly so for *E. muiri* (Fig. 2b). There was no significant difference in symmetry between Japanese species (FFht $= 2.84$, 3 d.f., $P \sim 1$).

Time until death of IG prey

When H. axyridis *is the IG predator*. In considering attacks made by *H. axyridis* on British species, *A. ocellata* was excluded from analysis as only two individuals were killed. There was significant variation in the time until death of other British larvae (Kruskal–Wallis test: $H = 30.04$, 6 d.f., $P < 0.001$) (Fig. 3a). Multiple pairwise Mann–Whitney U tests (αB (21 tests) $= 0.002$) revealed the following significant results: *C. septempunctata* larvae were killed more quickly than *H. quadripunctata* and *C. quatuordecimguttata* larvae ($W = 262.0$,

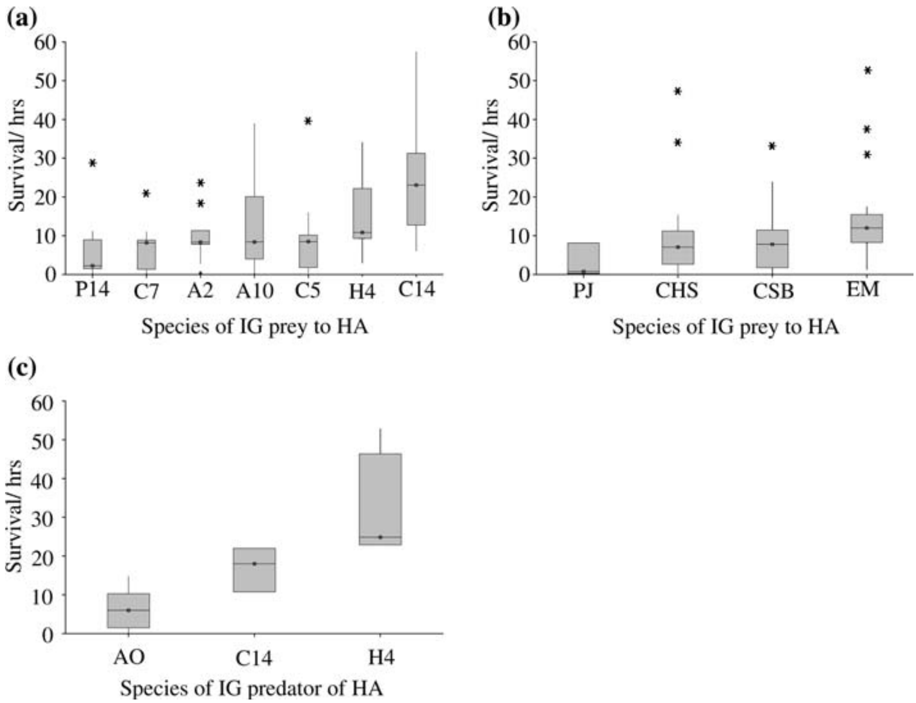

Fig. 3 Time until death of fourth instar larvae of (**a**) British and (**b**) Japanese coccinellids when acting as IG prey to *H. axyridis* fourth instar larvae, and (**c**) time until death of fourth instar *H. axyridis* larvae when acting as IG prey to *C. quatuordecimguttata*, *A. ocellata* and *H. quadripunctata* fourth instar larvae. Species are placed along the *x*-axes from left to right in order of increasing survival time. Medians (circles with stars), interquartile ranges (boxes), ranges (vertical lines) and outliers (stars) shown. For species codes, see Table 1

$P < 0.001$ and $W = 238.0$, $P < 0.001$ respectively); and *P. quatuordecimpunctata* larvae were killed more quickly than *H. quadripunctata* and *C. quatuordecimguttata* larvae ($W = 258.0$, $P < 0.001$ and $W = 228.5$, $P < 0.001$ respectively). All other species-pairs were not significantly different from each other at the corrected α value (all $P > 0.002$), but see Fig. 3a for order of increasing times.

Japanese fourth instar larvae also differed in how quickly they were attacked and killed by *H. axyridis* (Kruskal–Wallis test: $H = 19.96$, 3 d.f., $P < 0.001$) (Fig. 3b). Multiple pairwise Mann–Whitney *U* tests (αB (6 tests) $= 0.008$) showed that *P. japonica* larvae were killed more quickly than *C. sexmaculatus* larvae ($W = 514.4$, $P = 0.005$) and *E. muiri* larvae ($W = 246.5$, $P < 0.001$). All other species-pairs were not significantly different from each other at the corrected α value (all $P > 0.008$).

When H. axyridis *is the IG prey. Harmonia axyridis* larvae were killed significantly more quickly by *A. ocellata* than by *H. quadripunctata*, (Mann–Whitney *U* test: αB (3 tests) $=$ 0.017, $W = 153.0$, $P = 0.003$) but there was no difference in the timing of deaths caused by *A. ocellata* and *C. quatuordecimguttata* (Mann–Whitney *U* test: αB (3 tests) $= 0.017$, $W = 53.0$, $P = 0.026$) or between *C. quatuordecimguttata* and *H. quadripunctata* (Mann–Whitney *U* test: αB (3 tests) $= 0.017$, $W = 6.0$, $P = 0.052$) (Fig. 3c).

IGP of pre-pupae by fourth instar larvae

Levels of IGP

The levels of IGP between *H. axyridis* and British coccinellids in interactions between fourth instar larvae and pre-pupae are shown in Table 3 and Fig. 4a. Intraguild predation occurs significantly more often when *H. axyridis* is the larva compared to when it is the pre-pupa in interactions with *C. septempunctata*, *A. bipunctata*, *A. decempunctata*, *P. quatuordecimpunctata*, *C. quatuordecimguttata* and *C. quinquepunctata*. Intraguild predation between *H. axyridis* and *H. quadripunctata* is also significantly unidirectional towards *H. axyridis*. *Anatis ocellata* larvae preyed upon *H. axyridis* pupae at a similar level to the reverse.

There was no difference in the susceptibility of different British species' pre-pupae to IGP by *H. axyridis* (FFHt = 5.60, 7 d.f., $P = 0.585$) and the level of IGP of heterospecific pre-pupae was similar to that of British conspecific pre-pupae (FEt = 2.82, 1 d.f., $P = 0.148$). The susceptibility of *H. axyridis* pre-pupae to IGP by larvae of different species did vary (FFHt = 72.97, 7 d.f., $P < 0.001$). *Anatis ocellata* and *H. quadripunctata* larvae showed higher levels of IGP of *H. axyridis* pre-pupae than the other British species (FEt = 70.65, 1 d.f., $P < 0.001$), who all showed similarly low levels (FFHt = 2.96, 5 d.f., $P = 0.871$). There was no statistically significant difference in the level of predation by *A. ocellata* and *H. quadripunctata* (FEt = 4.67, 1 d.f., $P = 0.065$).

Table 3 The number of British and Japanese (pre)pupae killed by fourth instar *H. axyridis* larvae (and level of IGP), and the number of *H. axyridis* (pre)pupae killed by fourth instar larvae of those species

Species	Fourth instar larvae versus pre-pupae				Fourth instar larvae versus pupae			
	HA as larva		HA as pre-pupa		HA as larva		HA as pupa	
	Number pre-pupae killed	Level of IGP	Number pre-pupae killed	Level of IGP	Number pupae killed	Level of IGP	Number pupae killed	Level of IGP
British								
HA-B	18	0.90	N/A	N/A	0	0	N/A	N/A
C7	20	1.00	2	0.10	4	0.20	0	0.00
A2	18	0.90	2	0.10	3	0.15	0	0.00
A10	20	1.00	0	0.00	10	0.50	0	0.00
P14	20	1.00	1	0.05	18	0.90	0	0.00
C14	19	0.95	2	0.10	15	0.75	0	0.00
AO	20	1.00	18	0.90	7	0.35	11	0.55
H4	19	0.95	12	0.60	0	0.00	2	0.10
C5	10	1.00	1	0.05	4	0.31	1	0.08
Japanese								
HA-J	20	1.00	N/A	N/A	2	0.10	N/A	N/A
CSB	19	0.95	2	0.10	7	0.35	0	0.00
CHS	19	1.00	0	0.00	18	0.90	0	0.00
PJ	20	1.00	0	0.00	19	0.95	0	0.00
EM	20	1.00	1	0.05	16	0.80	0	0.00

$N = 20$ for each pairing (except those involving *C. quinquepunctata*, for which N is indicated in Materials and methods). For species codes, see Table 1

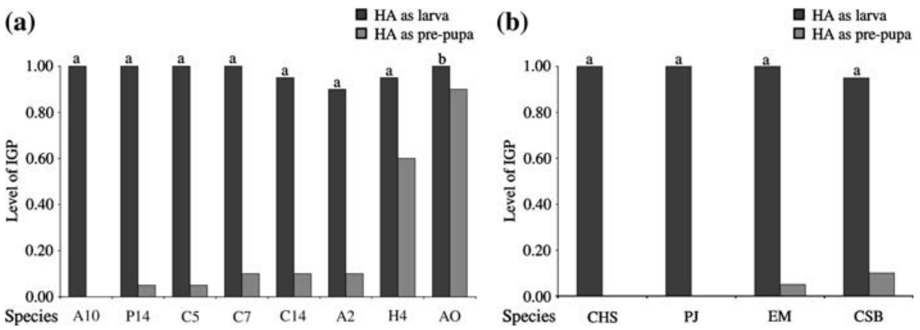

Fig. 4 Levels of IGP between *H. axyridis* and (**a**) British and (**b**) Japanese coccinellids in interactions between fourth instar larvae and pre-pupae. Analysis shows that with species marked a, IGP was significantly unidirectional towards *H. axyridis*; while with species marked b, IGP was bidirectional. Species are placed along the *x*-axes from left to right in order of decreasing susceptibility to IGP by *H. axyridis*. For species codes, see Table 1

The levels of IGP between *H. axyridis* and Japanese coccinellids in interactions between fourth instar larvae and pre-pupae are shown in Table 3 and Fig. 4b. Intraguild predation is significantly unidirectional towards *H. axyridis* for all species, in that *H. axyridis* larvae predate other species' pre-pupae at a much higher level than those species' larvae predate *H. axyridis* pre-pupae.

There was no difference in the susceptibility of different Japanese species' pre-pupae to IGP by *H. axyridis* (FFHt $= 2.16$, 3 d.f., $P \sim 1$) and the level of IGP of heterospecific pre-pupae was similar to that of Japanese conspecific pre-pupae (FEt $= 0.45$, 1 d.f., $P \sim 1$). The level of IGP of *H. axyridis* pre-pupae was similarly low for all species of Japanese larvae (FFHt $= 3.00$, 3 d.f., $P = 0.611$).

Time until death of IG prey

When H. axyridis *is the IG predator.* The time elapsed from a *H. axyridis* larva being placed in a dish containing a British pre-pupa until the larva killed the pre-pupa is shown in Fig. 5a. This time showed significant variation between species (Kruskal–Wallis test: $H = 38.05$, 8 d.f., $P < 0.001$). Multiple pairwise Mann–Whitney U tests (αB (36 tests) $= 0.0014$) showed that *P. quatuordecimpunctata* pre-pupae were killed more quickly than pre-pupae of *H. axyridis* ($W = 489.0$, $P < 0.001$), *A. bipunctata* ($W = 489.0$, $P < 0.001$), *H. quadripunctata* ($W = 230.0$, $P < 0.001$), *A. ocellata* ($W = 275.0$, $P < 0.001$) and *C. quatuordecimguttata* ($W = 277.5$, $P < 0.001$). All other pairwise comparisons were not statistically significant at the Bonferroni-corrected α value.

The different species of Japanese pre-pupae also differed with respect to how quickly they were killed (Kruskal–Wallis test: $H = 29.49$, 4 d.f., $P < 0.001$) (Fig. 5b). Multiple pairwise Mann–Whitney U tests (αB (10 tests) $= 0.005$) revealed that *C. sexmaculatus* and *P. japonica* pre-pupae were killed more quickly than *H. axyridis* and *C. septempunctata brucki* pre-pupae (CHS-HA: $W = 504.0$, $P = 0.004$; PJ-HA: $W = 570.0$, $P < 0.001$; CHS-CSB: $W = 480.5$, $P = 0.001$; PJ-CSB: $W = 530.0$, $P < 0.001$). No other species-pair comparisons gave significant values (all $P > 0.005$).

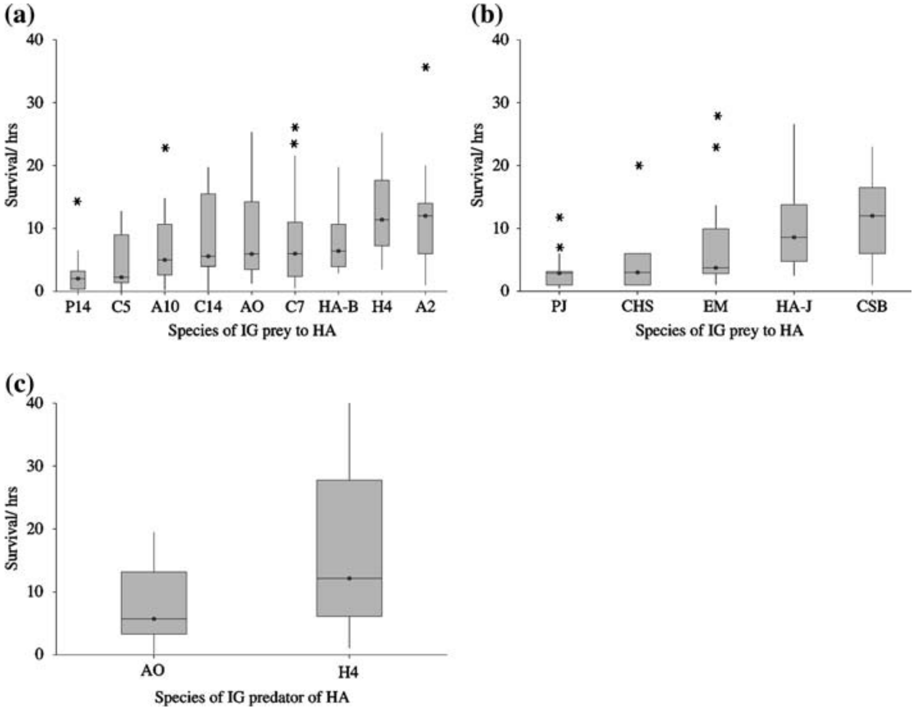

Fig. 5 Time until death of pre-pupae of (**a**) British and (**b**) Japanese coccinellids when acting as IG prey to fourth instar *H. axyridis* larvae, and (**c**) time until death of *H. axyridis* pre-pupae when acting as IG prey to *A. ocellata* and *H. quadripunctata* fourth instar larvae. Species are placed along the *x*-axes from left to right in order of increasing survival time. Medians (circles with stars), interquartile ranges (boxes), ranges (vertical lines) and outliers (stars) shown. For species codes, see Table 1

When H. axyridis *is the IG prey*. The only British species that acted as IG predators of *H. axyridis* pre-pupae at appreciable frequency were *H. quadripunctata* and *A. ocellata* ($N \leq 2$ for all other species). There was no significant difference in how quickly *H. axyridis* pre-pupae were killed by larvae of these two species (Mann–Whitney *U* test: $W = 230.0$, $P = 0.066$) (Fig. 5c).

IGP of pupae by fourth instar larvae

Levels of IGP

The levels of IGP between *H. axyridis* and British coccinellids in interactions between fourth instar larvae and pupae are shown in Table 3 and Fig. 6a. Intraguild predation of pupae of *C. septempunctata*, *A. bipunctata* and *C. quinquepunctata* by *H. axyridis* larvae occurred at low levels and therefore showed no significant difference from the reciprocal interactions, which occurred rarely, or not at all. No *H. axyridis* larvae preyed upon *H. quadripunctata* pupae, and the reciprocal interaction only occurred on two occasions, again resulting in no significant difference in the level of IGP. Intraguild predation of *A. ocellata* pupae by *H. axyridis* occurred at a similar frequency to the reverse interaction.

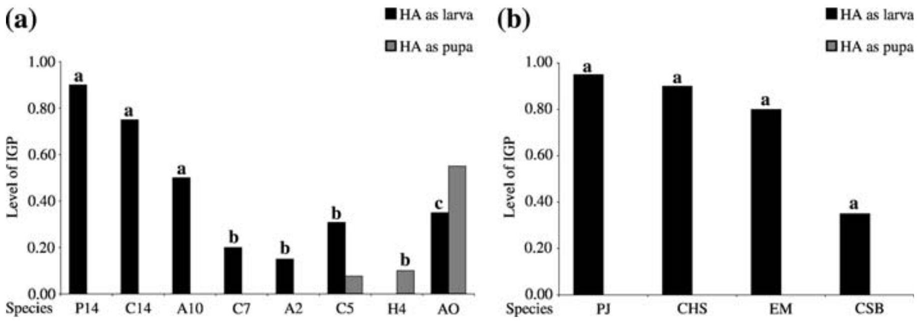

Fig. 6 Levels of IGP between *H. axyridis* and (**a**) British and (**b**) Japanese coccinellids in interactions between fourth instar larvae and pupae. Analysis shows that with species marked a, IGP was significantly unidirectional towards *H. axyridis*; with species marked b, levels of IGP were too small to reveal the directionality of IGP; while with species marked c, IGP was bidirectional. Species are placed along the *x*-axes from left to right in order of decreasing susceptibility to IGP by *H. axyridis*. For species codes, see Table 1

Intraguild predation of pupae by larvae was significantly unidirectional towards *H. axyridis* with respect to *A. decempunctata*, *P. quatuordecimpunctata* and *C. quatuordecimguttata*.

The level of IGP of British conspecific and non-conspecific pupae by *H. axyridis* larvae differed with respect to the species of pupa (FFHt = 74.03, 8 d.f., $P < 0.001$). *Propylea quatuordecimpunctata* and *C. quatuordecimguttata* pupae were preyed upon at similar levels (FEt = 1.49, 1 d.f., $P = 0.408$) and at a higher rate than pupae of *C. septempunctata*, *A. bipunctata*, *A. decempunctata*, *A. ocellata* and *C. quinquepunctata* (FEt = 31.90, 1 d.f., $P < 0.001$), which showed similar levels of IGP by *H. axyridis* (FFHt = 6.88, 4 d.f., $P = 0.143$). Finally, *H. quadripunctata* and conspecific pupae were never killed by *H. axyridis* larvae. The susceptibility of *H. axyridis* pupae to IGP by larvae of different species also varied (FFHt = 72.97, 7 d.f., $P < 0.001$), with *A. ocellata* larvae showing significantly higher levels of IGP of *H. axyridis* pupae than the other British species, including conspecifics (FEt = 40.09, 1 d.f., $P < 0.001$), which all showed similarly low levels (FFHt = 7.23, 7 d.f., $P = 0.062$).

The levels of IGP between *H. axyridis* and Japanese coccinellids in interactions between fourth instar larvae and pupae are shown in Table 3 and Fig. 6b. Intraguild predation of pupae by larvae was significantly unidirectional towards *H. axyridis* with respect to all Japanese species studied, as no non-conspecific larvae killed *H. axyridis* pupae.

The level of IGP of Japanese conspecific and non-conspecific pupae by *H. axyridis* larvae differed with respect to the species of pupa (FFHt = 47.79, 4 d.f., $P < 0.001$). *Cheilomenes sexmaculatus*, *P. japonica* and *E. muiri* pupae fell victim to IGP by *H. axyridis* larvae at a similar level to each other (FFHt = 1.52, 2 d.f., $P = 0.77$) but more often than did conspecific pupae and pupae of *C. septempunctata brucki* (FEt = 45.84, 1 d.f., $P < 0.001$), which were killed with similar frequency (FEt = 3.45, 1 d.f., $P = 0.127$).

Time until death of IG prey

When H. axyridis *is the IG predator*. The time elapsed from a *H. axyridis* larva being placed in a dish containing a British pupa until the larva killed the pupa is shown in

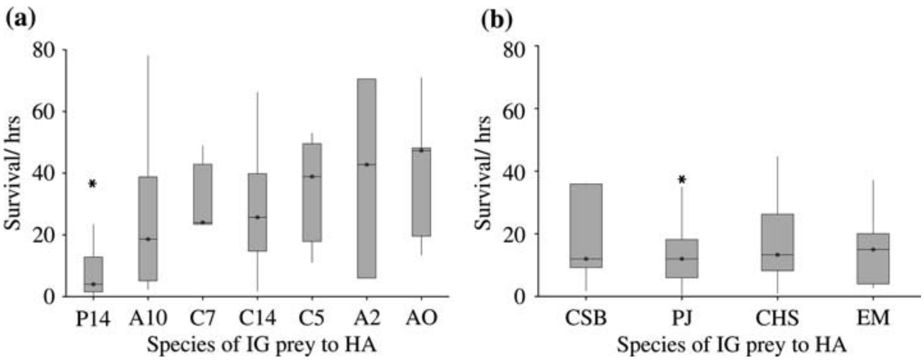

Fig. 7 Time until death of pupae of (**a**) British and (**b**) Japanese coccinellids when acting as IG prey to *H. axyridis*. Species are placed along the *x*-axes from left to right in order of increasing survival time. Medians (circles with stars), interquartile ranges (boxes), ranges (vertical lines) and outliers (stars) shown. For species codes, see Table 1

Fig. 7a. Note that the sample sizes being analysed for *A. bipunctata*, *C. septempunctata* and *C. quinquepunctata* were low, at $N = 3$, $N = 4$ and $N = 4$ respectively. The time until death of pupae showed significant variation with respect to species (Kruskal–Wallis test: $H = 23.06$, 6 d.f., $P = 0.001$). It is clear from Fig. 7a that *P. quatuordecimpunctata* pupae were killed very quickly, and significantly more quickly than pupae of *C. septempunctata*, *A. ocellata* and *C. quatuordecimguttata* (Mann–Whitney *U* tests: αB (6 tests) = 0.008; $W = 174.5$, $P = 0.006$; $W = 178.0$, $P = 0.001$ and $W = 207.5$, $P = 0.004$ respectively) but not significantly more quickly than *A. bipunctata*, *A. decempunctata* and *C. quinquepunctata* pupae (all $P > 0.008$). There was no significant difference in the time until death of pupae of the other species if *P. quatuordecimpunctata* is excluded (Kruskal–Wallis test: $H = 3.64$, 5 d.f., $P = 0.603$).

There was no significant variation in the time until death of non-conspecific Japanese pupae (Kruskal–Wallis: $H = 0.83$, 3 d.f., $P = 0.840$) (Fig. 7b).

When H. axyridis *is the IG prey*. Sample sizes of pupae killed by *H. quadripunctata* and *C. quinquepunctata* were too low to analyse. *Anatis ocellata* larvae killed *H. axyridis* pupae within a median time of 19.5 h.

Discussion

Introduced species often seem to perform better than conspecifics in their native range (Williamson 1996). A prominent hypothesis explaining this success is that introduced species are typically free of, or are less affected by, the natural enemies (competitors, predators, and parasites) that they would normally encounter in their native range (Torchin et al. 2003). Furthermore, while sympatric competitors might be expected to possess co-evolved defence strategies for dealing with detrimental interactions, such as competition and intraguild predation, such co-evolved strategies are unlikely between taxa whose ranges do not overlap. Indeed, the ability of an alien species to exploit native members of a guild, while avoiding exploitation itself, is thought to be important in determining the success of establishment and spread (Sato and Dixon 2004). With respect to *H. axyridis*, it is hypothesised that superior defensive strategies of native aphidophages, compared to those inhabiting the introduced range of the species, may explain why it exists in apparent ecological equilibrium with a range of species in Asia but has had serious detrimental impacts where it has established as an invasive alien.

This study examined the nature of IGP between immatures of *H. axyridis* and a selection of coccinellids from parts of its native and introduced ranges. It is not apparent from the results presented here that Japanese species are any better protected against IGP by *H. axyridis* than are their British counterparts, at least in a laboratory setting. Intraguild predation events between larvae of *H. axyridis* and all other species were common at first and fourth instar. At first instar, the British species *C. septempunctata*, *C. quatuordecimguttata* and *H. quadripunctata*, and the Japanese species *E. muiri*, were found to engage in symmetric IGP with *H. axyridis*, and the only species to act as an asymmetric intraguild predator of *H. axyridis* was the British coccinellid *A. ocellata*. Meanwhile, all other species were the IG prey in such interactions. For many species, IGP seemed more one-sided at fourth instar, and was actually asymmetric towards *H. axyridis* for all species except *A. ocellata*, which again emerged as the only IG predator of *H. axyridis.* In interactions between fourth instar larvae and pre-pupae, IGP was considered unidirectional towards *H. axyridis* for all Japanese species and all British species with the exception of *A. ocellata*, which was both IG predator and IG prey of *H. axyridis.* Pupae were clearly much better defended than pre-pupae, as the level of IGP was noticeably lower. However, the same patterns emerged, with the only bidirectional interaction occurring between *H. axyridis* and *A. ocellata.* Conspecific pupae and pupae of *H. quadripunctata* were never attacked, and *H. quadripunctata* larvae only attacked *H. axyridis* on two occasions.

The susceptibility of different species to IGP is likely to be affected by the nature of any physical and/or chemical defences they possess. Although there was no obvious demarcation between Japanese and British species in this regard, there was some general variation between species. The outcome of IGP between larvae is known to be influenced by the relative size of the protagonists (Lucas et al. 1998; Felix and Soares 2004) with the larger species generally acting as the IG predator and the smaller as the IG prey. Thus, large size may itself be considered as an adaptation against IGP. Fourth instar *H. axyridis* larvae are considerably larger than fourth instar larvae of most other species studied, which may partly explain their dominance as IG predators. *Anatis ocellata* larvae are larger than *H. axyridis* (this species is the largest British coccinellid) and are therefore more likely to act as the IG predator. However, size was clearly not the only factor determining the susceptibility of a larva to IGP, as larvae of *C. septempunctata* and *C. septempunctata brucki* consistently acted as the IG prey in interactions with *H. axyridis*, despite being roughly comparable in size. Such size differences between larvae were less pronounced at first instar (R. Ware, pers. obs.), which may explain both the apparently lower level of IGP at this stage, and the fact that more species were able to avoid predation by, or even kill, *H. axyridis*.

Variation in physical defensive structures is also likely to play a role in governing the outcome of IG interactions. Larvae of *H. axyridis* are extremely well protected by a covering of thick dorsal spines present at all instars. The only other species which possessed similar structures were *A. ocellata*, *H. quadripunctata* and *C. quatuordecimguttata*. Fourth instar larvae of these species were the least commonly attacked by *H. axyridis*, and when *C. quatuordecimguttata* and *H. quadripunctata* larvae were killed, it was after a much longer time period than the other species, indicating they were more difficult to subdue. *Harmonia quadripunctata* larvae are structurally very similar to *H. axyridis*, so the fact that fourth instar *H. axyridis* still emerged as asymmetric IG predators of fourth instar *H. quadripunctata* larvae and pre-pupae may be a function of the smaller size of *H. quadripunctata*, or of greater aggression by *H. axyridis*, or both. *Calvia quatuordecimguttata* larvae were able to fight back against *H. axyridis* at first instar but this ability was not upheld at fourth instar, presumably due to the larger size difference at this stage. The dorsal spines of *A. ocellata* are clearly an effective defence as this species rarely succumbed to IGP by *H. axyridis*. In addition, it was able to overpower the aggressive and well protected fourth instar *H. axyridis* larvae within a relatively short time period. Species which consistently succumbed to IGP by *H. axyridis* at the larval stages were *P. quatuordecimpunctata*, *A. decempunctata*, *C. quinquepunctata*, *A. bipunctata*, *C. septempunctata* and all Japanese coccinellids. All of these species are physically less well protected than *H. axyridis*, *H. quadripunctata*, *C. quatuordecimguttata* and *A. ocellata*, in that their dorsal surface is covered in fine hairs rather than sclerotised spines. Note that the least well defended of these species, *P. quatuordecimpunctata* and *P. japonica*, have almost smooth surfaces, and were extremely susceptible to IGP by *H. axyridis* and the most rapidly subdued of all species studied. Although not studied here, we suspect that the smooth larvae of *Myzia oblongoguttata* (Linnaeus) would be similarly vulnerable, despite the larger size of this species.

The pre-pupae of a particular species are likely to be similarly, if not more, vulnerable to IGP than their fourth instar larvae. Their physical defence structures are essentially the same, since they possess the fourth instar larval skin, but they are practically immobile and so potentially represent an easy meal for an IG predator. Indeed, immobility is often cited as a significant risk factor in the context of IGP (Lucas et al. 1998). This is reflected in the results presented here, with *H. axyridis* pre-pupae being extremely well protected and only attacked to a significant extent by *A. ocellata* and *H. quadripunctata* larvae, while pre-pupae of all other species were readily attacked by *H. axyridis* larvae. Again, *P. quatuordecimpunctata* and *P. japonica* were the most quickly killed. Pre-pupae of *C. quinquepunctata* and *C. sexmaculatus* were also killed relatively quickly. It is interesting that cannibalism of *H. axyridis* pre-pupae occurred at high frequency, indicating that the physical defences of *H. axyridis* pre-pupae are more easily overpowered by larvae of the same species. Once the larval skin has split and the pupa is formed, most coccinellids are notably less susceptible to IGP, as the tough pupal integument affords better protection than the soft larval skin. The results presented here clearly show reduced levels of IGP of pupae compared to pre-pupae for all British species except *P. quatuordecimpunctata* and *C. quatuordecimguttata*, which were still highly vulnerable and killed with relative ease. All Japanese species' pupae were highly vulnerable to IGP by *H. axyridis*. *Harmonia axyridis* pupae are clearly very well defended, even more so than their pre-pupae, as IGP by conspecific larvae, *H. quadripunctata* larvae and *A. ocellata* larvae was considerably reduced.

The results of this work support the perceived status of *H. axyridis* as a top intraguild predator of immatures of other coccinellids (Dixon 2000; Pell et al. 2007, and references therein). However, we found no evidence that native sympatric species have better defences against IGP by *H. axyridis* than species in its introduced range. This was also found to be the case for IGP of eggs (Ware et al. in press). Indeed, the three species that did emerge as being the best defended were all British. It therefore seems that another explanation of the discrepancy in the intraguild interactions of *H. axyridis* between its native and introduced range must be invoked. It is possible that *H. axyridis* is subject to more rigid population control in its Asian range due to the higher incidence of natural enemies such as male-killers (Majerus et al. 1999), parasitoids (LaMana and Miller 1996) and fungi (Riddick and Schaefer 2005). This warrants further investigation. Behavioural adaptations of sympatric species may also provide an explanation, whereby particular life history strategies make encounters with *H. axyridis* less likely. One obvious limitation of this study is that interactions were confined to Petri-dish arenas. Englund (1997) highlights the importance of spatial scale and prey movements in predation experiments. In natural conditions, escape behaviour is a likely possibility and may reduce the risk of IGP. For example, *P. quatuordecimpunctata* and *P. japonica* larvae were extremely vulnerable to IGP by *H. axyridis* in our experiments but the larvae of both are long-legged and mobile and may therefore be able to escape more easily in the wild. Many species are also known to exploit species-specific oviposition sites (Schellhorn and Andow 1999) and *P. japonica* females are reported to reduce oviposition in response to faecal cues from *H. axyridis* (Agarwala et al. 2003). *Coccinella septempunctata brucki* is known to lays its eggs earlier in spring than *H. axyridis* (Takahashi 1989), which means that *H. axyridis* rarely encounters eggs and younger larvae of *C. septempunctata brucki* (Sato and Dixon 2004). Sato et al. (2003, 2005) also report the dropping behaviour and emigration of *C. septempunctata* larvae as defensive tactics against IGP. Particularly vulnerable stages such as newly ecdysed larvae and pupae may be protected from IGP if species-specific moulting and pupation sites are selected (Lucas et al. 2000). The possibility of such behavioural avoidance tactics highlights the need for realistic field studies to be conducted when evaluating the risk and impact of IGP.

Results from the laboratory experiments conducted in this study show that a range of British coccinellids face considerable risk from the recent establishment of invasive *H. axyridis*. These findings must be viewed in conjunction with knowledge of the likely extent of habitat overlap between these species and *H. axyridis* (Ware et al. 2005; Table 1). *Harmonia axyridis* is an extremely generalist species (Roy et al. 2006) and is therefore likely to encroach on the ranges of both other habitat generalists and of more habitat-specific species. The susceptibility of *C. quinquepunctata* to IGP by *H. axyridis* is of particular concern as this species is considered endangered in Britain (Majerus 1994) and could be severely threatened if *H. axyridis* invades unstable river shingles. *Coccinella septempunctata*, *A. bipunctata* and *P. quatuordecimpunctata* are all considered to be fairly generalist species (Majerus and Kearns 1989; Majerus 1991) and therefore likely to interact with *H. axyridis* commonly. *Adalia decempunctata* and *C. quatuordecimguttata* are mainly deciduous tree specialists and so also potentially at risk, although the results presented here show some level of protection for *C. quatuordecimguttata* larvae. *Anatis ocellata* and *H. quadripunctata* are coniferous tree specialists so could encounter *H. axyridis* where it invades coniferous woodland (*H. axyridis* has already been found breeding on pine trees, *Pinus sylvestris*, in Britain, R. Ware and M. Majerus, pers. obs.) but have superior defence against *H. axyridis* than other British species. We have also shown that *H. axyridis* could even be at risk from *A. ocellata.* Overall it is clear that further research into the co-existence of British species with *H. axyridis* is needed, in particular focussing on the different timings of reproduction, in order to assess the full impact of Britain's newest invasive insect.

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