

Living with the enemy: parasites and pathogens of the ladybird *Harmonia axyridis*

Helen E. Roy · Emma Rhule · Susanne Harding ·
Lori-Jayne Lawson Handley · Remy L. Poland ·
Eric W. Riddick · Tove Steenberg

Received: 17 May 2011 / Accepted: 6 June 2011
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Abstract *Harmonia axyridis* is an invasive alien predator in many countries across the world. The rapid establishment and spread of this species is of concern because of the threat it poses to biodiversity as a generalist predator. Understanding the mechanisms that contribute to the success of this species as an invader is not only intriguing but also critical to our understanding of the processes governing such invasions. The enemy release hypothesis (ERH) could explain the rapid population growth of many invasive alien species. However, empirical evidence in support of the ERH is lacking. An alternative hypothesis that could explain rapid population growth

is evolution of increased competitive ability (EICA). Here we provide an overview of the parasites and pathogens of coccinellids with a particular focus on *H. axyridis* as a host. We examine the differential susceptibility of host species and highlight the resilience of *H. axyridis* in comparison to other coccinellids. We recognise the paucity and limitations of available information and suggest that studies, within a life-table framework, comparing life history traits of *H. axyridis* in both the native and introduced ranges are necessary. We predict that *H. axyridis* could benefit from both enemy release and EICA within the introduced range but require further empirical evidence.

Handling Editor: Patrick De Clercq

H. E. Roy (✉)
Centre for Ecology & Hydrology, Benson Lane,
Crowmarsh Gifford OX10 8BB, UK
e-mail: hele@ceh.ac.uk

E. Rhule
Department of Genetics, University of Cambridge,
Cambridge CB2 3EH, UK

S. Harding
Department of Agriculture and Ecology,
University of Copenhagen, Thorvaldsensvej 40,
1875 Frederiksberg C, Denmark

L.-J. Lawson Handley
Evolutionary Biology Group, Department of Biological
Sciences, The University of Hull, Kingston-Upon-Hull
HU6 7RX, UK

R. L. Poland
Clifton College, 32 College Road, Bristol
BS8 3JH, UK

E. W. Riddick
National Biological Control Laboratory,
USDA-Agricultural Research Service,
Stoneville, MS 38776, USA

T. Steenberg
Department of Agroecology, University of Aarhus,
Forsøgsvej 1, 4200 Slagelse, Denmark

Keywords *Harmonia axyridis* · Natural enemies · Enemy release hypothesis · EICA · Parasites · Pathogens

Introduction

Many theories have tried to explain the success of invasive alien species (IAS) (Keane and Crawley 2002; Allendorf and Lundquist 2003; Roy et al. 2011) and the enemy release hypothesis (ERH), also referred to as enemy escape or escape-from-enemy hypothesis, is one of the most evocative (Elton 1958; Roy et al. 2011). The ERH predicts that an alien species introduced to a new region will experience reduced impacts from natural enemies resulting in a competitive advantage for the alien species over other resident species. This theory relies on a number of assumptions including the importance of natural enemies in regulating populations and a disparity in regulatory effect on native species (stronger effect) versus alien species (weaker effect) in the introduced range, which results in increased population growth of the alien species (Roy et al. 2011).

Invaders can benefit from enemy release through either regulatory or compensatory release (Colautti et al. 2004). An alien host regulated by natural enemies in its native range may experience direct changes in various life history parameters in response to escape from natural enemies in the invaded range (regulatory release). Conversely, well-defended hosts may not benefit immediately from a reduction in enemies upon invasion, but overtime may undergo reallocation of resources from defence to population growth over ecological time (compensatory release), sometimes referred to as the evolution of increased competitive ability (EICA) hypothesis (Blossey and Notzold 1995; Roy et al. 2011).

To date, there is very little evidence for either enemy release or EICA and invasion success with respect to alien insects and other arthropods (Torchin et al. 2003). In order to demonstrate enemy release, there must be explicit evidence of that increased population growth of an alien species is directly attributable to reduced enemy impact (Colautti et al. 2004). Ceryngier and Hodek (1996) explicitly state that there is no evidence of natural enemies exerting regulatory effects on coccinellid populations. However, parasites and pathogens are undoubtedly prevalent coccinellid natural

enemies, as highlighted in a number of recent reviews (Ceryngier and Hodek 1996; Riddick et al. 2009; Ceryngier et al. 2011). Here we briefly consider the diversity of coccinellid parasites and pathogens with particular attention on their interactions with *Harmonia axyridis* (Pallas) (Coleoptera: Coccinellidae). Additionally we explore the evidence available for assessing the contribution of natural enemy release in the successful global establishment and spread of *H. axyridis* (Brown et al. 2011).

Coccinellid parasitoids

The Coccinellidae are host to a number of different hymenopteran and dipteran parasitoids (Ceryngier and Hodek 1996; Ceryngier et al. 2011). Some of these species have a worldwide distribution and are known to attack *H. axyridis* in both its native and introduced range, while others may be more limited in their distribution, attacking *H. axyridis* in Asia but not in its invasive territory, or vice versa. Here, we review the most significant groups of coccinellid parasitoids, consider those species for which *H. axyridis* already acts as a host, and discuss the potential for novel host-parasitoid interactions.

Overview of hymenopteran parasitoids

The braconid wasp *Dinocampus coccinellae* (Schrank) (Hymenoptera: Braconidae) has been recorded from adults of at least 18 species of coccinellid in Europe, all from the subfamily Coccinellinae (Ceryngier and Hodek 1996). A solitary, koinobiont endoparasitoid, *D. coccinellae* can attack all life stages, but prefers, and is most successful in adults (Obrycki et al. 1985; Hoogendoorn and Heimpel 2002), especially older females (see for example Riddick et al. 2009). It appears to be the most prevalent of coccinellid parasitoids but we know very little about fundamental aspects of the ecology and behaviour of this wasp, including host preferences and the mechanisms underlying variations in host suitability with regard to realised rates of parasitism (see for example Riddick et al. 2009). There is clearly still much to reveal with respect to the life history of *D. coccinellae*.

Coccinellid larvae and pupae are also parasitised by several species of small chalcid wasps of the genera *Homalotylus* and *Tetrastichus* (Ceryngier and

Hodek 1996). In general, females lay eggs in the third and fourth larval instars, but may occasionally parasitize pupae. Broods of the gregarious parasite, *Oomyzus scaposus*, are usually of a single sex with all adults wasps emerging from a single opening on the dorsal surface of the pupal mummy. Sexually mature upon emergence, females will mate within several minutes and so can parasitize a new host almost immediately (Ceryngier and Hodek 1996). Several species of the genus *Homalotylus* have been recorded as parasitoids of coccinellid larvae in the subfamilies Coccinellinae, Chilocorinae, and Scymninae (Ceryngier and Hodek 1996). Many have a wide host range and rates of parasitism that can reach 90–95% within a population (Ceryngier and Hodek 1996; Kenis et al. 2008).

Overview of dipteran parasitoids

Several European *Phalacrotophora* species attack coccinellid pupae (Disney and Beuk 1997). *Phalacrotophora berolinensis* Schmitz and *Phalacrotophora fasciata* Fallen (Phoridae) may parasitize up to 80% of individuals of some species of Coccinellinae and Chilocorinae (Ceryngier and Hodek 1996; Hurst et al. 1998) although rates of parasitism are variable, depending on the year, season, host or region.

Upon selection of a suitable ladybird pre-pupa, a sexually mature *Phalacrotophora* female will attract males, possibly using pheromones (Disney 1994). One or more males may arrive to mate with the female, most often on or near the host pre-pupa (Disney 1994). Copulation usually lasts several minutes before the male departs and the female oviposits, either on the surface of the pre-pupa or internally. After hatching approximately 24 h later, the parasitoid larva enters the host and develops inside the pupa. Mature larvae emerge from three to 12 days later (Filatova 1974; Disney and Chazeau 1990; Kuznetsov 1997). The number of phorids developing in a single host increases with increasing host size. In *Anatis ocellata* (L.) pupae, seven to ten larvae are usually found (Filatova 1974). The average number of phorids found in *Coccinella septempunctata* L. is similar (Semyanov 1978; Steenberg and Harding 2010a), while an average of two develops in *Adalia bipunctata* (L.) (Disney 1994; Steenberg and Harding 2010a).

The tachinid fly *Medina separata* (Meigen) parasitizes adults of 12 coccinellid species in Europe, including *A. bipunctata*, *Calvia quatuordecimguttata* (L.) and *Propylea quatuordecimpunctata* (L.). Identification of this parasitoid has been problematic, with a number of misidentifications, most commonly of *M. separata* as *M. luctuosa* (Meigen) (Tschorsnig and Herting 1994; Cerretti and Tschorsnig 2010). In Europe, many Coccinellidae are parasitized by *M. separata* whilst *M. luctuosa* is specific to adult chrysomelids of the genus *Haltica* (Kenis et al. 2008). Generalist protelean parasitoids, such as many tachinids typically begin the growth phase of the life cycle as internal parasites, eventually killing or consuming the host before emerging as free-living adults. Few tachinids are specialists and might lack tightly co-evolved relationships with their hosts.

Parasitoids of *H. axyridis* within the native range

In its native range, *H. axyridis* is attacked by several parasitoid wasps, including *D. coccinellae* (Braconidae) (Liu 1950; Kuznetsov 1997), *Homalotylus flaminus* (Dalman) (Encyrtidae) (Kuznetsov 1997), and *O. scaposus* (Thomson) (Eulophidae) (Kuznetsov 1997). It is also attacked by several parasitoid flies, including *Phalacrotophora philaxyridis* Disney, *P. berolinensis* and *P. fasciata* (Phoridae), *Strongygaster triangulifera* (Loew), *M. luctuosa*, and *M. separata* (Tachinidae).

Details of the parasitoid-host dynamics for most of these species in the native range are lacking. Research has focused on a few species notably *D. coccinellae* and *Phalacrotophora* spp. Laboratory and field studies indicate that Japanese *H. axyridis* is a less preferred host for *D. coccinellae* in comparison to *C. septempunctata brucki* (Koyama and Majerus 2008). Although *D. coccinellae* will oviposit in *H. axyridis* and *C. septempunctata* at similar frequencies, the number of emerging parasitoids is considerably less in *H. axyridis* than *C. septempunctata* (Koyama and Majerus 2008).

Phalacrotophora sp. was reared from 17.7% of 249 *H. axyridis* pupae collected in Japan (Osawa 1992). *P. philaxyridis* has been recorded from *H. axyridis* (Disney 1997) with additional reports on the congeneric species, *P. fasciata*, from *H. axyridis* pupae collected in the Russian Far East (Kuznetsov 1997).

Laboratory rearing of *M. luctuosa*-parasitised adult *H. axyridis* collected in October to November in Korea (Park et al. 1996) revealed a reduction in parasitoid life span as well as diminished egg development and oviposition. Prevalence rates varied between 0.7 and 21.1%. The true identity of the parasitoid as *M. luctuosa* is questionable though because, as previously mentioned, *M. luctuosa* is commonly misidentified as *M. separata* (Tschorsnig and Herting 1994).

Parasitoids of *H. axyridis* within the invaded range (North America)

D. coccinellae parasitism of *H. axyridis* was first reported in North America by Hoogendoorn and Heimpel (2002) and subsequently by Firlej et al. (2005). As in Europe, *H. axyridis* is considered a marginal host of *D. coccinellae*, with decreased levels of successful parasite development and emergence relative to known hosts such as *Coleomegilla maculata* DeGeer (7% in the former compared to 90% in latter), despite similar attack rates (Hoogendoorn and Heimpel 2002). Firlej et al. (2005) made similar observations in Canada and also observed that *D. coccinellae* successfully parasitized *H. axyridis* larvae, despite an apparent preference for attacking adults, however parasitoids never emerged from adult hosts. Firlej et al. (2007) suggest that this may be due, in part, to a lower number of teratocytes (cells involved in immunosuppression and nutrition) produced by parasitoids developing in *H. axyridis* compared to other hosts. This decreased ability to complete development in adults of the novel host, despite a preference for attacking adults, suggests that *D. coccinellae* does not play a significant role in regulating *H. axyridis* populations.

The chalcid wasp, *O. scaposus* has been reported from pupal collections in North Carolina, USA but at a significantly higher prevalence than in Britain; 44% ($N = 48$) (Riddick et al. 2009). The tachinid fly *S. triangulifera* has also been found on overwintering *H. axyridis* in North Carolina (Nalepa and Kidd 2002) and Oregon (Katsyannos and Aliniaze 1998). A small parasitic fly with a wide distribution across North America, *S. triangulifera* is very polyphagous, with reports of parasitized Hemiptera, Lepidoptera, Orthoptera and Dermaptera in addition to numerous reports of parasitized Coleoptera (Reeves 2004).

Rates of *H. axyridis* parasitism by *S. triangulifera* exhibit both spatial and temporal variation but, on average, only 2–4% of beetles are parasitised. Katsyannos and Aliniaze (1998) report parasitism rates of 6.7–15.4% by dissecting hosts, but only 4.8% by rearing hosts.

Parasitoids of *H. axyridis* within the invaded range (Europe)

The first record of *H. flaminus* parasitizing *H. axyridis* larvae was reported from Croatia in 2010 (Glavendekic et al. 2011). However, most research has centred on the braconid parasitoid *D. coccinellae*. Successful parasitization of *H. axyridis* by *D. coccinellae* was observed in Denmark two years after the arrival of the ladybird (Steenberg and Harding 2009a, 2010a). Successful eclosion of 3.2% was recorded at one site where parasitoids emerged from more than 91% of cocoons produced. Parasitism was observed to be highest during autumn, and up to 71% of adult *H. axyridis* sampled in September–November in one location were found parasitized when dissected, although this does not definitely imply successful parasitisation (Harding and Steenberg, unpublished data). There have been occasional sightings of *D. coccinellae* parasitizing *H. axyridis* in the field in Britain but at a low prevalence in comparison to the number of *C. septempunctata* parasitized (UK Ladybird Survey: <http://www.ladybird-survey.org>). The UK Ladybird Survey confirmed *D. coccinellae* parasitism of *H. axyridis* for the first time in South Africa in 2009.

Berkvens et al. (2010) demonstrated that when offered a choice *D. coccinellae* attacked non-melanic adults more often than melanic adults. Similarly field-collected *H. axyridis* adults were attacked less frequently than laboratory-reared adults (Berkvens et al. 2010). Intriguingly parasitized *H. axyridis* showed diminished reproductive capacities and lower aphid consumption rates. However, Berkvens et al. (2010) conclude that, despite these negative effects, *D. coccinellae* may only have a marginal impact on the population growth of *H. axyridis* in Europe.

Work on *Phalacrotophora* parasitization is ongoing and is providing preliminary insights into the dynamics of a parasitoid adapting to a novel host. Both *P. fasciata* and *P. berolinensis* have been recorded on *H. axyridis* in Denmark, with the former

being apparently the most common. Pupal parasitism was recorded shortly after the establishment of *H. axyridis* with an overall prevalence of 2.7% ($N = 4,508$), but reaching up to 23% in some locations. The high number of puparia developing per coccinellid pupa (mean number of puparia 4.6 with range 1–24), and the high emergence rate (48.8%) from puparia suggests that *Phalacrotophora* spp. seem to be well adapted to this novel host (Steenberg and Harding 2010a). In Britain, field surveys have revealed much lower levels of parasitisation by *Phalacrotophora* but over the last few years, there has been a slow increase.

Ware et al. (2010) also reported the emergence of *O. scaposus* from wild collected *H. axyridis* pupae. However, the prevalence of this was extremely low (0.08%, $N = 2,574$). To date, there are no reports of *Medina* spp. utilising *H. axyridis* as a novel host. However, the wide range of coccinellid hosts currently parasitized by the flies coupled with the generalist tendencies of this parasite, suggest that such a host switch may be possible.

Overall, it seems that although *H. axyridis* may have 'left behind' many of its natural parasitoids, evidence from both North America and Europe suggests that parasitoids of native coccinellids in these regions are starting to utilise *H. axyridis* as a novel and abundant host. However, whether or not *H. axyridis* populations will ultimately be regulated by parasitoids is uncertain.

Ectoparasitic mites

Parasitic mites in the family Podapolipidae are biotrophic and commonly attack Coleoptera. Most species function as ectoparasites living underneath the elytra of their hosts (Hajiqanbar et al. 2007). Fourteen species (all within the genus *Coccipolipus*) attack coccinellid adults (Riddick et al. 2009). The host spectrum might be limited to species in the same subfamily or genus. Husband (1984) stated that some *Coccipolipus* species might be specific within a host clade. For example, *Coccipolipus chilocori* Husband has been recorded from five central African *Chilocorus* ladybird beetles, but not other sympatric coccinellids.

Coccipolipus hippodamiae McDaniel and Morrill is a mite with a wide host range. It was first recorded in South Dakota, USA on the convergent lady beetle

Hippodamia convergens Guérin-Ménéville (McDaniel and Morrill 1969), then found in New Jersey, USA on *A. bipunctata* followed by a later discovery in Poland (Webberley et al. 2004) on *A. bipunctata*.

Laboratory studies indicate that the fecundity and egg hatch rate of three native hosts (*A. bipunctata*, *Adalia decempunctata* (L.), *Synharmonia conglobata* (L.)) and one invasive host (*H. axyridis*) are reduced by *C. hippodamiae* in Europe (Webberley et al. 2006; Rhule et al. 2010). *C. hippodamiae* was responsible for reduced winter survival of *A. bipunctata* adults, especially males, in Europe (Webberley et al. 2004, 2006).

Although *Coccipolipus* spp. attack coccinellids throughout the world, only recently has any species been found attacking *H. axyridis*. *C. hippodamiae* attacks *H. axyridis* in the USA (Riddick 2010) and in Poland (Rhule et al. 2010), but has not yet been recorded from *H. axyridis* in its native range. A field survey to assess the occurrence of this mite in Asia would be valuable. Social contact between mating and overwintering conspecific adults provides opportunities for horizontal transmission of *C. hippodamiae* (Webberley et al. 2002; Knell and Webberley 2004). Mating (or attempted mating) between *A. bipunctata* and *H. axyridis* occasionally occurs in the field (Roy et al. 2011; UK Ladybird Survey: <http://www.ladybird-survey.org>). The interspecific transfer of the mite from *A. bipunctata* to *H. axyridis* is possible under laboratory conditions (Rhule et al. 2010) and it is likely to occur at a low frequency in the field. Preliminary data indicates that *C. hippodamiae* can reduce winter survival of *H. axyridis* (males more often than females) in the USA (Riddick 2010).

Nematodes

Most studies of nematode infection of insect populations relate to entomopathogenic species from the families Steinernematidae and Heterorhabditidae used for biological control of insect pests. Laboratory studies have demonstrated that coccinellids may be susceptible to these nematodes (Abdel-Moniem and Gesraha 2001; Shapiro-Ilan and Cottrell 2005), but reports of natural infection of wild populations have been limited to the Allantonematidae (Iperti 1964) and the Mermithidae (Delucchi 1953; Iperti 1964;

Rhamhalinghan 1986). Very few studies describe natural nematode infection in Coccinellidae.

Allantonematidae (Tylenchomorpha)

Parasitylenchus coccinellinae Ipert & van Waerebeke parasitises *P. quatuordecimpunctata*, *A. bipunctata*, *S. conglobata*, *Hippodamia variegata* Goeze, *Hippodamia undecimnotata* Schneider and *Harmonia quadripunctata* Pontoppidan in Europe (Ipert 1964), and *Cheilomenes sexmaculata* and *Illeis indica* Timberlake in India (Narsi and Narayan 1984). Parasitism levels vary with host species. With the exception of the univoltine *H. undecimnotata*, a species in which nematode development rarely occurs, parasitisation by *P. coccinellinae* occurs only in multivoltine coccinellids.

Mermithidae (Mermithida)

The immature stages of several members of the Mermithidae are solitary endoparasites of adult ladybirds (Ceryngier and Hodek 1996). However, Delucchi (1953) recorded parasitisation of *Aphidecta oblitterata* larvae. Identification to species level is difficult because only the juvenile stages are parasitic. Nematodes of the genus *Mermis* occur in four species of aphidophagous coccinellids in south-east France (Ipert 1964). Prevalence of infection in the field is low.

Nematode infection of *H. axyridis*

There is little published information on nematode infection of *H. axyridis*, either in the native or in the introduced range. Shapiro-Ilan and Cottrell (2005) found lower susceptibility for both the exotic *H. axyridis* and *C. septempunctata* than for the native *C. maculata* and *Olla v-nigrum* to the entomopathogenic nematodes *Steinernema carpocapsae* and *Heterorhabditis bacteriophora*, but this study involved artificial infection in the laboratory. Until recently, the only records of natural nematode infection were from populations in the native range (Filatova 1974; Kuznetsov 1997). Nematodes of the families Mermithidae and Aphelenchidae attack several coccinellid species, including *H. axyridis*, in the Primorsky Territory in the Russian Far East, with *Aphelenchoides* sp. stated to be the most common.

In 2009, parasitic nematodes attacking *H. axyridis* were recorded outside its native range. In Denmark, infected adults were detected on lime trees (*Tilia* sp.) at several locations in the core area of colonization (Copenhagen), only two years after the establishment of *H. axyridis* (Harding et al. 2011). Both females and males were parasitized. Parasitism levels were low (1–11.6%), but field prevalence was uncertain because the ladybirds were not kept individually before dissection and nematode transmission between specimens may have occurred. During supplementary surveys on shrubs in the same area in early autumn 2010, field prevalence of up to 33.3% was detected. Based on morphological characters of gravid females and body shape of juveniles, the nematodes were identified as *Parasitylenchus* sp., slightly different from *P. coccinellinae* (Harding et al. 2011). Juvenile and adult nematodes occurred in the whole body cavity of the host in varying densities, but usually several hundred individuals.

In 2009, Poland and co-workers (unpublished data) inadvertently discovered large masses of nematodes in the abdomen of several *H. axyridis* specimens found in soybean fields in Minnesota, USA. Almost every specimen out of a sample of approximately 500 was infected, but it was not possible to determine true prevalence, as the beetles had been stored together after collection, giving the potential for cross-infection. Even so, this putative prevalence was remarkably high. Species identification is ongoing, using DNA barcoding. Although identification to the species level is not yet confirmed, the sequence falls within the Allantonematidae.

Together, these two reports show that nematodes are parasitizing *H. axyridis* in two distant parts of the invaded range (Europe and North America). Although the parasitic nematodes in both areas belong to the Allantonematidae, it is unclear whether the species are the same, and whether the nematodes found infecting *H. axyridis* in the invaded areas are identical to the nematodes infecting this ladybird in its native range. *Aphelenchoides* typically attack plants and fungi, not insects, and therefore the nematodes from the Primorsky Territory may have been misidentified. The identity of these parasitic nematodes is thus uncertain, but it may well be *P. coccinellinae* as the juveniles are very similar in appearance. It is noteworthy that the infection of Danish populations of *H. axyridis* occurred shortly

after the first record of single specimens and establishment of *H. axyridis* in Denmark (Steenberg and Harding 2009a). The impact of nematode infection on host fitness certainly warrants further investigation, because the nematodes may offer some level of natural control of invasive *H. axyridis* populations.

Fungal pathogens

Three taxonomic groups within the fungal kingdom contain species that are pathogenic or parasitic to Coccinellidae. A range of hypocrealean fungi (Ascomycota) infect and kill ladybirds especially during overwintering (Iperti 1964; Bjørnson 2008; Roy and Cottrell 2008; Steenberg and Harding 2009b, 2010b; Ormond et al. 2010). In contrast, species of obligate ectoparasitic fungi of the genus *Hesperomyces* (Laboulbeniales, Ascomycota) may have little deleterious effects on their coccinellid hosts although rhizoids penetrate the cuticle of the host body (Weir and Beakes 1996). However, heavy *Hesperomyces* infections with numerous thalli covering the insect cuticle may hamper flight, feeding and mating of its host (Nalepa and Weir 2007). Microsporidia are intracellular pathogens that invade insect tissues. Based on molecular evidence, microsporidia are members of the kingdom Fungi rather than Protozoa (Lee et al. 2008). To some degree, entomologists have overlooked microsporidia as pathogens of Coccinellidae because diagnosis involves histological examination. Here we provide a brief review of the current knowledge of these three groups of fungal pathogens and their interactions with coccinellids, with special emphasis on *H. axyridis*.

Hypocreales: Ascomycota

Hypocrealean fungi within the genera *Beauveria*, *Lecanicillium* (syn. *Verticillium*) and *Isaria* (syn. *Paecilomyces*) cause mortality of overwintering ladybirds (Lipa et al. 1975; Bjørnson 2008; Steenberg and Harding 2009b; Ormond et al. 2010). *Beauveria bassiana* occurs on coccinellids in overwintering sites and is often the most important biotic factor causing winter mortality (Iperti 1964; Lipa et al. 1975; Ormond et al. 2010). The prevalence of this fungus can reach high levels in some years, for example, Cottrell and Shapiro-Ilan (2003) found 40% infection

in *O. v-nigrum* sampled in October and Mills (1981) reported 33% infection in *A. bipunctata* sampled in March. In contrast, there are only very few reports of fungus infections in coccinellid larvae and pupae (Roy et al. 2008; Steenberg and Harding 2009b, 2010b), due to a lack of field studies focusing on this group of natural enemies as pathogens of immature life stages.

Hypocrealean infection of *H. axyridis*

In the Russian Far East, *Beauveria* spp. infect *H. axyridis*, but the impact of the fungus is minimal (Kuznetsov 1997). In North America, Cottrell and Shapiro-Ilan (2003) failed to find *Beauveria*-infected *H. axyridis* (but did find *Beauveria*-infected *O. v-nigrum*) in pecan orchards. In contrast, shortly after the arrival of *H. axyridis* in Denmark, entomopathogenic fungi in the field infected larvae, pupae and overwintering adults (Steenberg and Harding 2009b). *B. bassiana*, *Isaria farinosa* and species of *Lecanicillium* occur throughout the year, *H. axyridis* larvae seem more susceptible to infection by these fungal pathogens than adults, and mortality due to such fungal pathogens among overwintering *H. axyridis* adults reaches levels comparable to those published for coccinellid species native to Europe. Laboratory experiments have demonstrated that adult *H. axyridis* are significantly less susceptible to infection by *B. bassiana* than native coccinellids (Cottrell and Shapiro-Ilan 2003; Roy et al. 2008). More research will determine whether differential susceptibility of adults and larvae of *H. axyridis* and native coccinellids to hypocrealean fungi occurs in the field.

Laboulbeniales: *Hesperomyces* spp.

Approximately 80% of the 2,000 described species in the order Laboulbeniales use Coleoptera as hosts (Weir and Beakes 1996; Nalepa and Weir 2007). Four Laboulbeniales species in the genus *Hesperomyces* attack coccinellids (Riddick et al. 2009). Two species, *Hesperomyces chilomenis* (Thaxter) and *Hesperomyces hyperaspidis* Thaxter, have only a single reported host. The remaining two parasites, *Hesperomyces coccinelloides* (Thaxter) and *Hesperomyces virescens* Thaxter, have four and 13 reported hosts, respectively (Riddick et al. 2009). Very little information is available on the biology and ecology

of these fungi and their interactions with hosts. All species require a living host for continued survival and reproduction.

H. virescens is probably native to North America. Thaxter (1931) first discovered it in the USA on two native coccinellids, *H. convergens* and *Chilocorus stigma* (Say). Currently *H. virescens* occurs globally on entomophagous and phytophagous coccinellids (Riddick et al. 2009). Within the last two decades, *H. virescens* has attacked the coccinellid *A. bipunctata* in southern Europe (Weir and Beakes 1996; Welch et al. 2001).

Most Laboulbeniales species cause little harm to their hosts (Weir and Beakes 1996). Available records indicate that *H. virescens* has limited potential to suppress host populations when acting alone (Riddick et al. 2009). However, Kamburov et al. (1967) found that *H. virescens* caused premature mortality of *Chilocorus bipustulatus* L. when it infected up to 95% of the adult population in Israel.

H. virescens infection of *H. axyridis*

H. virescens does not attack *H. axyridis* in its native range, in central and eastern Asia. However, discovery of infected *H. axyridis* in the USA (Garces and Williams 2004; Riddick and Schaefer 2005; Riddick 2006, 2010) and in Germany (Steenberg and Harding 2010b) coupled with the rapid expansion of *H. axyridis* globally suggests that this parasite will continue to spread throughout the rest of the world. Indeed one individual *H. axyridis* in Britain has obvious signs of infection but molecular analysis is required for definitive confirmation (R. Comont, Personal Communication). No other *Hesperomyces* species use *H. axyridis* as a host. Social contact between adult hosts as they attempt to mate at feeding sites or as they establish overwintering aggregations permits horizontal transmission of *H. virescens* (Welch et al. 2001; Riddick and Schaefer 2005). Transfer of *H. virescens* from *H. axyridis* to related coccinellids occupying the same habitat may be rare, but could represent an avenue for spread of this parasite to new hosts (Riddick and Cottrell 2010).

Although Laboulbeniales are widely regarded as causing minimal effects on hosts aggregation of *H. virescens* thalli around the legs, mouthparts, and antennae of *H. axyridis* adults can inhibit the detection of predators, food, and mates (Nalepa and Weir 2007).

Furthermore, infected *H. axyridis* females mate less frequently than uninfected conspecific females (Nalepa and Weir 2007) and *H. virescens* can potentially reduce the winter survival of *H. axyridis* in the USA (Riddick and Cottrell 2010).

Microsporidia: Nosematidae

There is a scarcity of studies on interactions between microsporidia and coccinellid as hosts. Although microsporidia attack several coccinellid species and are relatively common pathogens of predacious coccinellids, little information on host specificity and routes of transmission appears in the literature. Five species of *Nosema* infect the adult stage of different Coccinellidae in the field. The prevalence of *Nosema*-infected coccinellids rarely exceeds 10–15% (Lipa et al. 1975; Bjørnson 2008). Microsporidia cause chronic disease, with sublethal effects such as reduced fecundity and increased developmental time that lower host fitness. As such, microsporidia may be a group of natural enemies of potential importance in regulating populations of some coccinellids.

Transmission routes for *Nosema*-infection of coccinellids include horizontal transmission, involving the release of spores that invade (via ingestion) a susceptible host. Vertical transmission occurs when infection transfers from mother to offspring.

Microsporidia infection of *H. axyridis*

There is very little information available on microsporidia attacking *H. axyridis* in its native range. Kuznetsov (1997) states that unidentified microsporidia attack *H. axyridis* in Primorsky Territory in eastern Russia but does not present further data. To our knowledge, there are no records of microsporidia from *H. axyridis* in the introduced range, although histological studies of British populations of *H. axyridis* suggest possible infection in *H. axyridis* but this needs confirmation (G. Stentiford, Personal Communication). However, Saito and Bjørnson (2006) succeeded in transmitting *Tubulinosema hippodamiae* from *H. convergens* to *H. axyridis* larvae in laboratory experiments. While the microsporidium had no effect on larval mortality, it did prolong developmental time of its host. All larvae of *H. axyridis* were successfully infected, but they produced fewer spores per individual compared to other susceptible coccinellid species in

the experiment. This may indicate that *H. axyridis* is a less suitable host for microsporidia infection although it is clearly within the physiological host range of the *Tubulinosema* species originating from a heterologous host. Since *H. axyridis* is an intraguild predator of eggs of other coccinellid species (Cottrell 2004), larvae of *H. axyridis* are likely to encounter microsporidia infected prey when feeding on eggs or older life stages of coccinellid species within the host range of *Nosema* and *Tubulinosema* species. Future studies should evaluate whether *H. axyridis* is also within the ecological host range of microsporidia species that infect coccinellids sharing habitats in both its native and its introduced range.

The information available on interactions of fungal pathogens with populations of *H. axyridis* is limited for both the native and the introduced range of this invasive species. This also applies to Coccinellidae in general, especially when considering microsporidia infections. Laboratory studies have shown that *H. axyridis* is less susceptible to infection by the hypocrealean fungus *B. bassiana* compared to native coccinellid species (Cottrell and Shapiro-Ilan 2008; Roy et al. 2008). While it is possible to transmit *Tubulinosema* from *H. convergens* to *H. axyridis*, infection of the latter species limits the reproductive success of the microsporidium. An evaluation of the apparent robustness of *H. axyridis* to infection by fungal pathogens compared to other coccinellid species is premature due to the few data available. It may be that *H. axyridis* has an inherent low susceptibility to infection by hypocrealean fungi or microsporidia as compared with most other coccinellid species, in its native and introduced range.

Septate eugregarines (Apicomplexa: Eugregarinorida: Septatorina)

Unicellular eugregarine protozoa are principally parasites of annelids and arthropods, although some are commensals or even mutualists, inhabiting alimentary canals, coeloemic spaces and reproductive vesicles. Septate eugregarines infect the digestive tract of coccinellids in terrestrial habitats and seem more common in warm climates. Coccinellids are primarily infected by several species of the genus *Gregarina*, although recent work suggests that this is

a polyphyletic group that eventually will be separated further (Clopton 2009).

Ceryngier et al. (2011) lists 12 eugregarine species found in coccinellids from most continents. Ten species belong to *Gregarina* and some of the species have been recorded from several coccinellid species. However, this is not necessarily an implication of a wide host range, as cross-infectivity studies with species of eugregarines in other insect taxa have shown morphologically indistinguishable species to be adapted strictly to the homologous host (Clopton and Gold 1996).

Kuznetsov (1997) reported of *H. axyridis* being infected with eugregarines but did not provide further details. So far, no records exist of eugregarines in *H. axyridis* in the introduced range, but this may reflect a lack of studies focusing at detecting pathogenic microorganisms in coccinellids. Microscopic examination of digestive tracts of large numbers of Danish *H. axyridis* and indigenous coccinellid species, respectively, is ongoing and has not yet confirmed the presence of this group of potential natural enemies (Steenberg and Harding, unpublished data).

If *H. axyridis* has significant habitat overlap with confirmed coccinellids hosts of *Gregarina* spp., direct transmission via ingestion of oocysts might occur in the introduced range, provided the eugregarine species in question is not species-specific. Whilst infection with these protozoans results in destruction of intestinal cells (Ceryngier and Hodek 1996), in general they are considered to be weak pathogens that have limited negative effects on their hosts. However, at least one report describes that gametocyst blockage of the intestinal tract in heavily infected individuals may reduce longevity (Laudého et al. 1969). As such, whilst infection with eugregarines alone may exert little effect on coccinellid population density, high densities of trophozoites and/or gametocysts in the midgut would add stress to a host infected with other pathogens.

Bacteria

There are very few empirical studies of bacterial pathogens in coccinellids (Ceryngier et al. 2011). A few studies have considered the use of *Bacillus thuringiensis*, or more specifically the crystalline delta-endotoxin, as an insecticide for the control of phytophagous Epilachninae, considered crop pests (Song et al. 2008) with harmful effects noted for

Henosepilachna vigintioctomaculata (larvae only), *E. varivestis* (adults) and sublethal effects for *H. vigintioctopunctata* (adults). However, the information available on effects of entomopathogenic bacteria on coccinellids is scant.

In contrast, many studies have investigated maternally-inherited bacterial endosymbionts such as *Wolbachia*, *Spiroplasma*, *Cardinium*, *Rickettsia*, and Flavobacteria, which are believed to infect at least one-third of all arthropod species (Weinert et al. 2007; Werren et al. 2008; Duron et al. 2008; Hilgenboecker et al. 2008). These bacteria impose a wide spectrum of effects on the reproductive biology of their hosts: from killing infected male eggs or larvae early in development (“male-killing”), or inducing cytoplasmic incompatibility or parthenogenesis, to more obviously beneficial (mutualistic) effects such as host protection from natural enemies such as parasitoids, viruses, pathogenic bacteria and fungi (reviewed in Brownlie and Johnson 2009; Zindell et al. 2011).

Endosymbionts are known from 14 coccinellid host species, and are suspected to occur in many others (Hurst et al. 1992b; Majerus and Hurst 1997; Weinert et al. 2007). All five of the bacterial groups mentioned above have been reported in coccinellids, and are generally regarded as male-killers because of the highly female-biased sex ratios observed in some populations. Although male-killing has been directly confirmed several times by breeding experiments, it has not been confirmed for every known host-endosymbiont combination. It should not therefore be assumed that all coccinellid endosymbionts are male-killers. Indeed, observation of infected male coccinellids suggests that male-killing is imperfect, and/or that hosts have evolved resistance (Weinert et al. 2007).

The interaction between male-killing bacteria and their coccinellid hosts is intriguing. Although the killing of males may seem to be a negative consequence of infection, infected female ladybirds benefit from the death of their brothers by freeing up of resources (resource reallocation), reduced kin competition and avoidance of inbreeding (e.g. Majerus and Hurst 1997; Roy et al. 2007). There is therefore an indirect fitness advantage to females from male-killer infection. Negative fitness effects, beyond the loss of males, have also been documented and include decreased oviposition rates, lower overall fecundity,

higher infertility levels, or shorter adult life-span (Hurst et al. 1992a, 1994, 1999; Majerus et al. 2000; ElNagdy et al. 2011), as well as reduced genetic variation in the host’s mitochondrial and nuclear genomes, which could reduce their adaptive potential (e.g. Randerson et al. 2000; Telschow et al. 2006; Engelstadter and Hurst 2007).

Investigating the role of endosymbionts in invasion success of their hosts is important because of the implications for host fitness and adaptation, and for biological control. Endosymbionts could be useful in biological control of arthropod pests either by introducing endosymbionts that have a negative effect on their host, or by disrupting the relationship between beneficial endosymbiont and host (e.g. Shoemaker et al. 2000). However the relationship between these bacteria and their hosts is complex, and variable between different host-bacteria combinations. The complex biology of the host-endosymbiont relationship needs to be fully understood before such methods can be implemented. Theoretically, female-biased sex ratio distortion, combined with enhanced female fitness, could facilitate host invasion and male-killers should promote dispersal in their hosts to facilitate their own spread (Bonte et al. 2008). In spite of this important debate, few studies have so far investigated endosymbionts in the context of biological control or invasions (but see e.g. Shoemaker et al. 2000; Zindell et al. 2011). However, a recent study provided elegant demonstration that *Rickettsia* spp. nr. *bellii* infection of sweet potato whitefly, *Bemisia tabaci* Gennadius (Hemiptera: Aleyrodidae) has facilitated the spread of this highly invasive species throughout the USA, by enhancing host fitness and increasing the proportion of females (Himler et al. 2011). Further investigation is needed to fully understand whether endosymbionts are directly relevant to the enemy release or EICA hypotheses, and indeed whether release from endosymbionts is most likely to facilitate or inhibit host invasion (Zindell et al. 2011).

Female-biased sex ratios were first documented for *H. axyridis* in the 1970s (Matsuka et al. 1975). *Spiroplasma* is widespread in the native range, occurring at least in Japan, Russia, Mongolia, China and South Korea (Majerus et al. 1998; Majerus 2006). Although the overall prevalence of *Spiroplasma* seems to be low, it can be staggeringly high in some populations, for example 49% in one Japanese

Table 1 ERH mechanisms in relation to parasites and pathogens and evidence for relevance to the establishment and spread of *H. axyridis*. Further details of mechanisms provided by Roy et al. 2011

Mechanism	Refuting evidence	Supporting evidence			Comments	Reference
		None	Tentative	Strong		
Escaping natural enemies from native range			×		More diverse range of natural enemies in native range particularly parasitoids. However, a systematic survey of natural enemies in the native versus invaded range has not been undertaken.	(Ceryngier et al. 2011; Osawa 1992)
Low probability of parasite arrival because of small founding population of <i>H. axyridis</i>		×			It is likely that there are many source populations of <i>H. axyridis</i> . Additionally <i>H. axyridis</i> was released in large numbers (founding population) throughout the invaded range.	(Brown et al. 2011)
Invading life-history stage (egg, larva, pupa, adult) of <i>H. axyridis</i> uninfected by hitch-hiking parasites	×				The interactions between <i>H. virescens</i> and <i>H. axyridis</i> provide an interesting scenario. It is likely that infection of <i>H. axyridis</i> in the invaded range is from exposure to the fungus in USA and that the movement of <i>H. axyridis</i> from one invaded region to another could be a global transmission route for this hitch-hiking fungus.	(Riddick and Schaefer 2005)
Infected <i>H. axyridis</i> do not survive founding stage of invasion		×			Male-killers are present in <i>H. axyridis</i> within the invaded range and as such these can be considered hitch-hiking parasites. There is also tentative evidence of microsporidia infection in British populations of <i>H. axyridis</i> but this requires confirmation. It is possible that some parasites and pathogens were removed through screening in the biological control facilities or that infected <i>H. axyridis</i> did not survive the founding stage of invasion.	(Brown et al. 2008a, b; Lombaert et al. 2010)
Density-dependent parasites unable to persist at low densities of <i>H. axyridis</i>	×				<i>H. axyridis</i> spreads rapidly and becomes highly abundant within the introduced range, therefore density-dependent parasites should be able to persist within <i>H. axyridis</i> in the invaded range.	(Brown et al. 2011)
Environmental conditions in invaded range unsuitable for parasite survival	×				<i>H. axyridis</i> is now established globally and, therefore, is present across a wide climatic range. It is highly unlikely that environmental conditions would be adversely affecting potential parasite/pathogen–host interactions.	(Brown et al. 2011)

Table 1 continued

Mechanism	Refuting evidence	Supporting evidence			Comments	Reference
		None	Tentative	Strong		
Parasites in native range not adapted to <i>H. axyridis</i>				×	<i>Harmonia axyridis</i> is extremely well-defended and appears to be a sub-optimal host for a range of parasites/pathogens in the introduced range. Many studies have indicated higher susceptibility of native coccinellids to parasites and pathogens in comparison to <i>H. axyridis</i> . However, it is possible that <i>H. axyridis</i> is also more resilient to parasites and pathogens within the native range compared to the coccinellid community. Indeed <i>H. axyridis</i> in Japan are a marginal host to <i>D. coccinellae</i> .	(Berkvens et al. 2010; Burgio et al. 2008; Cottrell and Shapiro-Ilan 2003; Koyama and Majerus 2008; Roy et al. 2008; Saito and Bjørnson 2006; Shapiro-Ilan and Cottrell 2005; Sloggett et al. 2011)
Environmental conditions in invaded range affect interactions between <i>H. axyridis</i> and native parasites/pathogens		×			It is possible that the phenology of the host is asynchronous with parasites/pathogens in the invaded range.	
Reallocation of resources from defence to population growth (EICA)			×		<i>H. axyridis</i> could potentially divert resources from chemical and physical defence to population growth over time and this is worthy of research attention. However, there is no evidence of EICA currently.	(Sloggett et al. 2011)

population of *H. axyridis* and 99.98% in another (Majerus et al. 1998, 1999). Nakamura et al. (2005) have confirmed the male-killing phenotype and high transmission efficiency in Japanese *Spiroplasma*-infected *H. axyridis*. *Wolbachia* and *Rickettsia* have also been detected in the native range, with *Wolbachia* reaching quite high frequencies (A. Aebi, R. Zindel, C. Thomas and L. Lawson Handley, unpublished). Surveys for sex ratio distortion and male-killers in the invaded range of *H. axyridis* have yielded interesting results. There is so far no evidence for sex ratio distortion of *H. axyridis* in North America (Heimpel and Lundgren 2000), or the majority of rest of the invaded range, however some UK populations do have female-biased sex ratios (L. Lawson Handley, unpublished). *Wolbachia*, *Spiroplasma*, *Rickettsia* and *Flavobacteria* have been detected at low frequencies in the invaded range, and unexpectedly, *Wolbachia* was found at quite high frequency in European biocontrol populations (A. Aebi, R. Zindel, C. Thomas and L. Lawson Handley, unpublished). It is currently unknown whether these bacteria were transmitted vertically with the initial invasive population, or whether they have been subsequently acquired by horizontal transfer (e.g. through shared parasitoids). It is therefore impossible to ascertain, at present, the role of endosymbionts in invasion success of *H. axyridis*. However, research is ongoing into the transmission dynamics and impact of these bacteria on *H. axyridis* fitness and genetic diversity.

Conclusions and future directions

Roy et al. (2011) outlined the mechanisms behind the ERH including the various ways in which natural enemies are lost through invasion (Torchin et al. 2003). Despite the range of studies on natural enemies of coccinellids, there is still a paucity of information with respect to the relevance of these enemy release mechanisms and EICA to *H. axyridis* (Table 1). There is no information on whether or not *H. axyridis* has carried hitch-hiking parasites into the introduced range, although it is likely that we will find intracellular parasites in invasive alien *H. axyridis*. The focus of research has been on assessing interactions between *H. axyridis* and parasites native to the invaded range, however there has been little consideration given to their capacity to

regulate *H. axyridis* populations. Indeed there is no evidence that undisputedly attributes the regulation of coccinellid populations to parasites and pathogens (Ceryngier and Hodek 1996). Construction of life history tables to assess the role of parasites and pathogens in the population dynamics of coccinellids, particularly *H. axyridis* both within its native and introduced range, should be a priority for future study.

It is apparent that the prevalence of natural enemies is temporally and spatially variable. Additionally there is considerable variation in the susceptibility of different host species. In many of the studies, covering taxonomically diverse range of parasitic natural enemies, *H. axyridis* is an inferior host. *H. axyridis* is a well-defended coccinellid (Sloggett et al. 2011) and this almost certainly contributes to low prevalence of parasitisation. The behavioural responses (such as detection and avoidance) of native coccinellids compared to *H. axyridis* to parasites and pathogens could be enlightening (Ormond et al. 2011). However, studies that comprehensively assess the parasites of *H. axyridis* in its native and introduced range should be considered a priority. Such work is critical if we are to further our understanding of the complexities and relevance of the ERH to *H. axyridis*. It will also be essential to examine and compare life history traits of *H. axyridis* in both the native and introduced ranges. It is plausible that *H. axyridis* could benefit from both enemy release and EICA within the introduced range but these mechanisms are likely to be coupled with a number of other factors that contribute to the success of this invader.

Acknowledgments HER is funded through the Centre for Ecology & Hydrology (Natural Environmental Research Council (NERC)). HER is also funded by the Joint Nature Conservation Committee (JNCC) and receives funding from the Department for Environment, Food and Rural Affairs (Defra) for work on the GB Non-Native Species Information Portal. ER is funded by Microsoft Research. Mention of a commercial or proprietary product does not constitute an endorsement of the product by any of the universities or by the United States Department of Agriculture.

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Author Biographies

Helen E. Roy is an ecologist leading zoological research in the Biological Records Centre at the NERC Centre for Ecology & Hydrology (UK). The focus of her research is insect community interactions with particular emphasis on the effects of environmental change. She is convenor of the IOBC/WPRS Working Group “Benefits and Risks of Exotic Biological Control Agents” which was the impetus for this special issue of BioControl. She is currently leading the development of the

GB Non-Native Species Information Portal. She is an associate editor of BioControl.

Emma Rhule is a PhD candidate at the University of Cambridge. Her research interest focuses on host-parasite interactions, particularly that of the ectoparasitic mite, *Coccipolipus hippodamiae* and Coccinellids. In addition to studying the relationship between the mite and its hosts, she is interested in studying the genetic structure of the parasite population that may have been caused by variation in host use and geographic location.

Susanne Harding is an insect ecologist performing applied entomological research at the Department of Agriculture and Ecology at the Faculty of Life Sciences, University of Copenhagen. The core area of her research is regulation of insect populations, focusing on insect–host plant interactions, insect–natural enemy interactions, impact of climate change on insect population dynamics, integrated and biological control, and invasive arthropods.

Lori-Jayne Lawson Handley is a lecturer at the University of Hull. Her research interests centre on molecular ecology and she has published widely in this field. She has a particular interest in using molecular techniques to increase understanding of invasive alien species and has been effective at applying the latest tools to key questions in relation to routes of invasion.

Remy L. Poland is a secondary school teacher (biology) at Clifton College. She has extensive research expertise in intra-guild interactions. Her research on the harlequin ladybird and natural enemy interactions is ongoing. She co-leads the UK Ladybird Survey.

Eric W. Riddick is a research entomologist with the Agricultural Research Service, United States Department Agriculture. He is interested in behavioural, chemical, and nutritional ecology of natural enemies. Recent research has examined parasite–host interactions between the parasitic fungus *Hesperomyces virescens*, the parasitic mite *Coccipolipus hippodamiae*, and the ladybird *Harmonia axyridis* in North America.

Tove Steenberg is an insect pathologist at the University of Aarhus. Her main research interest is the ecology of entomopathogenic fungi and their use in biological control. Currently her studies include extensive field sampling to demonstrate how various natural enemies interact with the harlequin ladybird *Harmonia axyridis* following its arrival to Denmark.