



Potential role of the sexually transmitted mite *Coccipolipus hippodamiae* in controlling populations of the invasive ladybird *Harmonia axyridis*

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ABSTRACT

The enemy release hypothesis is often invoked to explain the invasion success of species occurring outside their native range. The natural or artificial introduction of natural enemies into populations of invasive species can therefore be an effective control method. The ladybird *Harmonia axyridis* (Pallas) (Coleoptera: Coccinellidae) is native to Asia, but in recent decades has been introduced into Europe and North America, where it has rapidly spread and had a detrimental impact on native species. A promising candidate for the biological control of this species is the sexually transmitted mite *Coccipolipus hippodamiae* (McDaniel & Morrill) (Acarina: Podapolipidae). This mite occurs naturally in some European coccinellid populations, where it can reach a high prevalence and induce host sterility. We report that colonies consisting of reproducing adult mites and their eggs can be established on *H. axyridis* both through artificial transfer from, and sexual contact with, infected individuals of another species. These infected *H. axyridis* can then infect further conspecifics during copulation and infected females become sterile. Additionally, we report natural *C. hippodamiae* infections in recently established populations of *H. axyridis* in Poland. However, the prevalence of the mites in the field is currently low, and further work is required to determine whether the mites will reach the high prevalence required to effectively control *H. axyridis* populations. Overall, our results demonstrate that *C. hippodamiae* has the potential to reduce the population size of *H. axyridis* following either natural or artificial transfer from other species.

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1. Introduction

Harmonia axyridis (Pallas) (Coleoptera: Coccinellidae) has been released widely in Europe and North America during the last few decades as a biological control agent of aphids and coccids (Brown et al., 2008a). It is now an invasive alien species in many countries where it has become established outside initial release sites (Majerus and Roy, 2005). Its voracity causes it to outcompete native ladybirds and other aphidophages (Brown and Miller, 1998; Colunga-Garcia and Gage, 1998). In addition, *H. axyridis* is a polyphagous predator and, as well as eating target and non-target aphid species, it will also attack non-pest insects, including lepidopteran eggs and larvae (Koch et al., 2003) and other aphidophages, including the immature stages of other coccinellids (Pell et al., 2008; Ware and Majerus, 2008; Ware et al., 2008).

Whilst not intentionally introduced into Britain, *H. axyridis* arrived here in 2004 from continental Europe, most likely via multiple routes (Brown et al., 2008b; Majerus et al., 2006). Large numbers of species, including non-target aphids and coccids, other aphidophages and coccidophages, and the predators, parasites and

pathogens that attack them may be negatively affected by *H. axyridis*. Negative effects on humans have also been reported, including the aggregation of beetles inside people's homes during the winter months, occasional allergic reactions to bites (Yarborough et al., 1999; Ray and Pence, 2004; Davis et al., 2006; Goetz, 2007), and damage to fruit and wine production (Ejbich, 2003).

Despite its pest status in North America, little has been done with regards to finding ways to control *H. axyridis*. Chemical insecticides are considered undesirable due to the potentially harmful effects on people and pets when used inside buildings. In addition, due to the lack of specificity of such chemicals, they are likely to negatively affect those native ladybird species which also overwinter inside buildings. The repellent properties of some volatile chemicals, such as camphor and menthol, may have some potential when applied to a building's exterior (Riddick et al., 2000).

In its native Asia, *H. axyridis* is not considered a pest. This may be due to the natural population control exerted by sympatric predators, parasites and pathogens. The enemy release hypothesis suggests that one of the reasons that some alien species become invasive is that when they colonize new regions, they escape the natural enemies found in their native range (Torchin et al., 2003; Colautti et al., 2004; Carroll et al., 2005). Native ladybirds inhabiting the introduced range of *H. axyridis* are attacked by a wide range

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of natural enemies, including parasitoid wasps and flies, fungal pathogens, sexually transmitted mites and male-killing bacteria (Majerus, 1994; Majerus et al., 2000). However, these enemies will only have co-evolved adaptations to host species with which they have been sympatric for a large part of their evolutionary history, and not necessarily with *H. axyridis*.

Studies of the effects of several enemies of British ladybirds, including the parasitoid wasp *Dinocampus coccinellae* (Schrank) (Hymenoptera: Braconidae) and the pathogenic fungus *Beauveria bassiana* (Balsamo) (Ascomycota: Hypocreales), demonstrate the reduced susceptibility of *H. axyridis* compared to native ladybirds (Koyama and Majerus, 2008; Roy et al., 2008). Whilst native natural enemies may adapt to *H. axyridis* as a novel host, there is a risk that, in the time that elapses before they adapt sufficiently, *H. axyridis* may have already caused considerable damage to elements of native biodiversity. The sexually transmitted mite *Coccipolipus hippodamiae* (McDaniel & Morrill) (Acarina: Podoplipidae), a known parasite of some European coccinellids, may offer some hope.

Coccipolipus hippodamiae is an ectoparasitic mite that lives on the underside of the elytra of coccinellids (Majerus, 1994) and is transmitted sexually (Knell and Webberley, 2004). Adult female mites attach to the host's elytron and feed on its haemolymph. The females lay eggs, which hatch into motile larvae, and these migrate between hosts during copulation. Once on a novel host, larvae embed their mouthparts and metamorphose into adults. Thereafter, adult mites are entirely sedentary. Establishment and subsequent maintenance of this sexually transmitted infection within a host population is dependent on two key factors—high levels of promiscuity to permit horizontal transmission between host individuals, and overlapping generations to facilitate transmission between successive generations. *Harmonia axyridis* in Britain is highly promiscuous, (Majerus and Roy, 2005), long-lived, and undergoes at least two overlapping generations each year (Brown et al., 2008b). Therefore, it is possible that *C. hippodamiae* may be able to spread through populations of *H. axyridis*.

C. hippodamiae has previously been identified on four species of coccinellid collected in the wild: *A. bipunctata*, *Oenopia conglobata* (Linnaeus), *Calvia quatuordecimguttata* (Linnaeus) and *Harmonia quadripunctata* (Pontoppidan) in continental Europe (Webberley et al., 2004). Infection of European coccinellids by *C. hippodamiae* causes female hosts to become infertile. This has been well studied in *A. bipunctata*, in which complete sterility was induced within approximately three weeks of infection (Hurst et al., 1995; Webberley et al., 2004). Hurst et al. (1995) speculate that sterility results from mite infection interfering with the production of the egg chorion, since eggs laid by infected females were observed to shrivel and desiccate within 24 h of oviposition.

The host range of *Coccipolipus* species is somewhat limited and there is evidence that it is not possible to infect all coccinellid species. For example, attempts to artificially infect *Exochomus quadripustulatus* (Linnaeus), which co-exists in locations inhabited by infected *A. bipunctata*, failed (Webberley et al., 2004). Epidemiological factors may also prevent the mites from spreading through populations of many species, and this may explain why *C. hippodamiae* is absent from the majority of British coccinellids. This is thought to be due to the paucity of mating between overwintered populations and the new generation, which presents a barrier to transmission (Hurst et al., 1995).

The fact that *C. hippodamiae* has been recovered from *H. quadripunctata* makes it reasonable to suggest that its congener, *H. axyridis*, may also be a suitable host. If similar effects on female fertility are found in *H. axyridis* as have been documented in *A. bipunctata*, it seems that infection with *C. hippodamiae* may represent a promising avenue for controlling invasive *H. axyridis* populations. Here, we test whether *C. hippodamiae* can successfully establish reproducing colonies on *H. axyridis* following artificial transfer or sexual

contact, and if the infection causes sterility. Additionally, Polish populations of *H. axyridis* were surveyed for the presence of *C. hippodamiae*. Polish populations of *A. bipunctata* are known to be infected at a relatively high prevalence (Webberley et al., 2004) and thus we also tested whether *C. hippodamiae* moved between species in the wild.

2. Materials and methods

All experiments were conducted on *A. bipunctata* alongside *H. axyridis*, in order to allow comparison of data with a known natural host of *C. hippodamiae*.

2.1. Can *C. hippodamiae* successfully establish on *H. axyridis* following artificial transfer?

Wild samples of *A. bipunctata* (from deciduous trees in Poland), *H. quadripunctata* (from coniferous trees in France) and *H. axyridis* (from England, sent into the Harlequin Ladybird Survey) were collected in 2007. All ladybirds were scored for the presence or absence of *C. hippodamiae* using the procedure described in Webberley et al. (2004). Briefly, ladybirds were anaesthetized under CO₂, fixed on their backs on a bed of Blu-tac[®], and the elytra exposed using entomological pins under a dissecting microscope. Each individual elytron was checked for mite infection, recording the presence of reproducing females and any immature stages (eggs, larvae).

Mite-infected stocks of *A. bipunctata* and *H. quadripunctata* provided a source of mites for transfer to uninfected individuals. Host ladybirds were anesthetized and fixed as described above. Artificial transfer followed the protocol of Hurst et al. (1995). Either six mite eggs or four mite larvae were transferred from the elytron of an infected *A. bipunctata* to that of an uninfected ladybird on the tip of a fine entomological pin. Fourteen days post-manipulation, and every 4 days thereafter, the recipient ladybirds were scored for mite presence, and numbers of adults, eggs and larvae noted. Sixty *H. axyridis* females and 20 female *A. bipunctata* were artificially infected. A successfully established colony was defined as one in which transferred eggs or larvae completed development resulting in a colony containing at least one sexually reproducing adult female with eggs present. This is because the motile larviform female is the infective stage of the life cycle. Fisher's Exact test was used to test whether the ratio of infected:uninfected ladybirds was different in the different treatments.

2.2. Can *C. hippodamiae* be transferred to *H. axyridis* during sexual contact?

The rate at which mites were transmitted to *H. axyridis* following sexual contact with infected individuals of another species was measured. An infected ladybird, either *A. bipunctata* ($N = 17$: 8 females, 9 males) or *H. quadripunctata* ($N = 15$: 7 females, 8 males), was placed in a 9 cm Petri dish with an uninfected *H. axyridis* of the opposite sex. Infected *A. bipunctata* ($N = 15$: 7 females, 8 males), were paired with uninfected *A. bipunctata* of the opposite sex to provide an expected rate of transmission in a known natural host. Couples were maintained for 8–12 days, during which pairs were fed on a daily diet of excess pea aphids, *Acyrtosiphon pisum* (Harris) (Homoptera: Aphididae) and dishes changed daily. After this time, they were separated and mite presence scored. Subsequently, mite scores were recorded every 4 days. Any mating encounters observed during this time were recorded.

Next, sexual transmission between copulating *H. axyridis* was monitored. *Harmonia axyridis* that had become mite-infected following pairing with infected *H. quadripunctata* were used. An

infected *H. axyridis* ($N = 9$: 6 females, 3 males) was placed in a 9 cm Petri dish with an uninfected *H. axyridis* of the opposite sex. Couples were maintained for 10–12 days and any matings observed were noted. Again, dishes were changed daily and pairs fed a diet of excess aphids. The infection status of each donor *H. axyridis* was scored every 4 days during this period. Couples were then separated and scored. Subsequently, mite scores were recorded every 4 days for both donor and recipient ladybirds. As with the artificial transfer experiments, Fisher's Exact test was used to test whether the ratio of infected:uninfected ladybirds was different in the different treatments.

2.3. Does infection by *C. hippodamiae* cause sterility in *H. axyridis*?

A stock of uninfected female *H. axyridis* ($N = 10$) were maintained in culture alongside experimental stocks, as a control against the negative effects of mite infection. Manipulated (ladybirds to which eggs or larvae were artificially transferred) and control ladybirds were fed daily on excess pea aphids. Once a fortnight, uninfected male ladybirds were introduced to the Petri dish containing manipulated females for a period of 24 h. This facilitated mating and ensured that the failure of eggs to hatch was due to mite presence as opposed to sperm depletion. Egg clutches were collected every other day. The numbers of eggs laid and the subsequent hatch rates were recorded. Petri dishes were changed three times a week. The median egg hatch rates of infected and uninfected individuals were compared using an exact Wilcoxon Rank Sum Test.

2.4. Is *C. hippodamiae* present in Polish *H. axyridis* populations?

Samples of *H. axyridis* were collected from sites around Toruń and Bydgoszcz, Poland, by eye and by beating trees. The distance between the built up areas of these two cities is approximately 30 km. These were scored, as in Webberley et al. (2004) for the presence or absence of *C. hippodamiae*. Those individuals hosting mite populations were preserved in 100% ethanol.

3. Results

3.1. *C. hippodamiae* can successfully establish on *H. axyridis* following artificial transfer

Fourteen days after artificial transfer of eggs or larvae, 38% ($N = 60$) *H. axyridis* females and 50% ($N = 20$) *A. bipunctata* females hosted mites (Table 1). There was no significant difference in the proportion of the two species successfully infected (Fisher's Exact

Table 1
The number of individuals in which *C. hippodamiae* established successfully reproducing populations after sexual contact or artificial transfer.

	Infected	Not infected
<i>Artificial transfer</i>		
<i>H. axyridis</i> female	23	37
<i>A. bipunctata</i> female	10	10
<i>Sexual contact—heterospecific</i>		
<i>A. bipunctata</i> ♂ → <i>H. axyridis</i> ♀	4	5
<i>A. bipunctata</i> ♀ → <i>H. axyridis</i> ♂	3	5
<i>H. quadripunctata</i> ♂ → <i>H. axyridis</i> ♀	8	0
<i>H. quadripunctata</i> ♀ → <i>H. axyridis</i> ♂	5	2
<i>Sexual contact—conspecific</i>		
<i>A. bipunctata</i> ♂ → <i>A. bipunctata</i> ♀	4	1
<i>A. bipunctata</i> ♀ → <i>A. bipunctata</i> ♂	3	4 ^a
<i>H. axyridis</i> ♂ → <i>H. axyridis</i> ♀	3	0
<i>H. axyridis</i> ♀ → <i>H. axyridis</i> ♂	3	3

^a Two males died before the first check and one escaped.

test, $p = 0.435$). Once established, mite populations developed at similar rates on *A. bipunctata* and *H. axyridis*; at 18 days post-manipulation 52% ($N = 23$) infected *H. axyridis* and 80% ($N = 10$) infected *A. bipunctata* hosted adult females and immatures as opposed to just adults or immatures (Fisher's Exact test: $p = 0.245$).

3.2. *C. hippodamiae* can be transferred to *H. axyridis* during sexual contact

Harmonia axyridis can also become infected when it mates with other species of ladybird. In pairings with infected *A. bipunctata*, 44% ($N = 9$) female and 38% ($N = 8$) male *H. axyridis* became infected. When paired with infected *H. quadripunctata*, 100% ($N = 8$) female and 71% ($N = 7$) male *H. axyridis* became infected (Table 1). The rate of mite transmission to *H. axyridis* was higher from the congeneric donor, *H. quadripunctata* (87% ($N = 15$)) compared to transfer from *A. bipunctata* (41% ($N = 17$)) (Fisher's Exact test, $p = 0.0118$). The rate at which *A. bipunctata* transmitted mites to *H. axyridis* is similar to the rate of transmission between conspecifics (Table 1; Fisher's Exact test, $p = 0.462$). There was no significant difference in the degree of establishment whether mites were transferred artificially (50% ($N = 20$)) or via sexual contact (58% ($N = 12$)) between individuals of *A. bipunctata* (Fisher's Exact test, $p = 0.726$).

The mite can also be transmitted sexually between infected and uninfected *H. axyridis*. In 10 pairings between infected and uninfected individuals, 43% ($N = 7$) male and none of the female ($N = 3$) *H. axyridis* became infected. At least two matings were observed per couple. There was no significant difference in the conspecific infection rate between pairs of *H. axyridis* (67% ($N = 9$)) and pairs of *A. bipunctata* (58% ($N = 12$)) (Fisher's Exact test, $p = 1.00$).

3.3. Infection by *C. hippodamiae* causes sterility in *H. axyridis*

The egg hatch rates from infected female *H. axyridis* after artificial transfer was initially high (>70%), followed by a steep decline at approximately day 19 (Fig. 1). After this point there is almost complete sterility. In contrast, data from uninfected control females of the same age, collected over the same time period, show that hatch rates were consistently high (Fig. 1).

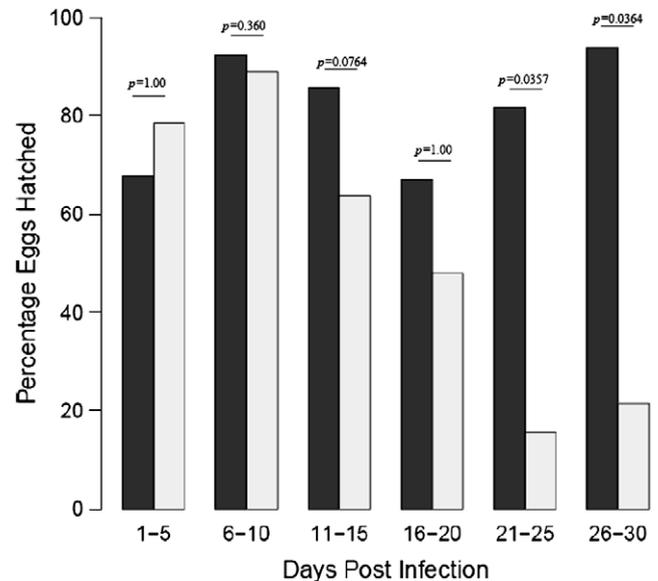


Fig. 1. Egg hatch percentages from artificially infected and control females over the 30 day period post-manipulation. Egg hatch rates were compared using the Wilcoxon Rank Sum Test.

3.4. *C. hippodamiae* is present in Polish *H. axyridis* populations

Five hundred and seventeen *H. axyridis* individuals were collected in Torun and 22 individuals collected in Bydgoszcz between 22/05/2009 and 11/06/2009, of which, 3.71% were infected ($N = 539$; number infected = 20; 95% confidence intervals: 0.0228–0.0567). Of the 20 infected beetles, 11 carried only immature stages or non-reproducing adults, eight harbored adults and eggs, and all three stages were found on one beetle.

4. Discussion

We have demonstrated that the mite *C. hippodamiae* can be successfully transferred to *H. axyridis* both by artificial transfer and sexual contact from *A. bipunctata* and *H. quadripunctata*. Once on *H. axyridis*, the mite successfully undergoes metamorphosis, resulting in reproducing adult females. Within two to 3 weeks of infecting a female, the mite causes a reduction in egg hatch rate and ultimately complete sterility. The long-term effects on males are not yet known. *Coccipolipus hippodamiae* was also shown to successfully transfer between copulating *H. axyridis*, which is essential in order for it to establish as a sexually transmitted disease within wild populations.

The mites appear remarkably well adapted to their new host. Whether artificially or sexually transferred, there was no significant difference in the time taken for the mites to successfully establish and reproduce on *H. axyridis* than on *A. bipunctata*. Additionally, when infected and uninfected beetles are confined together in a Petri dish, there are high rates of transmission between species. This may also occur in natural populations, as Webberley et al. (2004) reported transmission between species in locations where they overwinter in close proximity.

The discovery of naturally occurring *C. hippodamiae* infections in Polish populations of *H. axyridis* is particularly significant. That the mite has naturally made the jump across the species barrier proves that the abilities of *C. hippodamiae* to transmit and reproduce on *H. axyridis* are not just phenomena restricted to the laboratory setting. Furthermore, as there is natural transmission of the mites from native species to *H. axyridis*, this may naturally reduce the size of *H. axyridis* populations without the need to artificially release parasites.

Coccipolipus hippodamiae currently occurs at a low prevalence in Polish populations, and is unlikely, at present, to significantly impact the population size of *H. axyridis*. It is unclear whether this is a consequence of the recent arrival of the mites in this population, and therefore starting the season from a very low prevalence, or whether they will never reach a high prevalence. It is also unknown whether the mite persists in *H. axyridis* populations throughout the year, or if it relies on recurrent transfer from a reservoir of mites in *A. bipunctata* populations. The mating rate, the dynamics of mite transmission, the effect of mite infection on host survival and the degree of overlap between generations will determine whether *C. hippodamiae* ultimately reaches a high prevalence and has a significant impact on *H. axyridis* populations.

These results suggest it should be possible to artificially introduce *C. hippodamiae* into British *H. axyridis* populations, where the mite is not present in populations of any of the native species. From then on, high promiscuity and overlapping generations mean that it is possible that the mite will spread to significant prevalence levels. A question that must now be addressed is whether an artificial introduction should actually be implemented. Infected European migrants of species native to the UK sporadically introduce *C. hippodamiae* into British ladybird populations (Majerus, 1994), yet the mite does not persist in these populations. The introduction of *C. hippodamiae* into *H. axyridis* populations would act as a more permanent reservoir for infection through interspecific mating.

However, although mating does occur between species (Pascoe et al., 2007), it is quite rare—during the 2009 field season in Poland only one of 99 (*H. axyridis* $N = 52$; *A. bipunctata* $N = 46$; *H. axyridis*–*A. bipunctata* $N = 1$) observed mating pairs involved different species (E. Rhule, unpublished data). Therefore, ongoing transmission from *H. axyridis* to native species is unlikely to directly lead to a high prevalence of infection in native species, and instead the main threat would be if a host shift from *H. axyridis* led to the infection circulating within populations of native species. Webberley et al. (2004) surveyed 19 European species of coccinellid that were collected at sites where at least one other coccinellid species was found to be infected with *C. hippodamiae*. Of these 19, only four were found to successfully host the mite. Of the remaining 15 species, 11 are native to the UK. This may indicate that British populations of these species would also be immune to infection, although this is something that needs to be investigated as part of a wider community impact assessment. While the factors that have kept Britain free of this mite so far, notably the lack of overlapping generations (Majerus, 1994), should prevail, it is possible that *H. axyridis* could provide a new link that introduces the mite into susceptible British species.

By definition, risk assessments must look to the future. With progressive global warming, there is a possibility that British coccinellids will become fully bivoltine, and mites introduced into native British species through interspecific matings with *H. axyridis* could permanently establish. However, as the mite is already present in Northern European populations of species commonly found in Britain, it is likely that the mite would colonize Britain anyway.

Our results suggest that *C. hippodamiae* has great potential to control the spread and increase of the invasive alien *H. axyridis*. In many populations this may occur naturally without the need for artificial releases. However, if migrants do not naturally introduce the mite into British populations of *H. axyridis*, artificial releases could be considered. Our current knowledge of the mite's ecology suggests that this is unlikely to have negative side effects on other coccinellids. However, given the harmful effects that introducing the *H. axyridis* into the UK has had, much more research would be needed before releasing another species outside of its native range.

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