



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

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