SHORT COMMUNICATION

Specialisation of bacterial endosymbionts that protect aphids from parasitoids

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Abstract. 1. Infection by the bacterial endosymbiont Hamiltonella defensa is capable of protecting the pea aphid from parasitism by Aphidius ervi and the black bean aphid from parasitism by Lysiphlebus fabarum. Here we investigate protection of a third aphid species, the cowpea aphid, Aphis craccivora, from four parasitoid species: Binodoxys communis, B. koreanus, Lysiphlebus orientalis, and Aphidius colemani.

2. We compared parasitism of A. craccivora lines that were either infected with, or cured of H. defensa separately for the four parasitoid species. Infection by H. defensa almost completely eliminated parasitism of A. craccivora by B. communis and B. koreanus, but had no effect on parasitism by L. orientalis and A. colemani.

3. This indicates at least genus-level specificity of protective effects by H. defensa and we discuss implications of our findings on the known world-wide distribution of this host/symbiont interaction.

Key words. Aphidius, Aphis craccivora, Binodoxys, defensive symbiont, Hamiltonella defensa, Lysiphlebus.

Introduction

In protection mutualisms, members of one species protect members of another species from one or more enemy species, and receive a benefit in return – often food and/or a suitable domicile (Bronstein, 2009). In one class of protection mutualisms, aphids harbour endosymbiotic bacteria that confer resistance against parasitoids or pathogens (Oliver et al., 2014). In the best-studied example of this interaction, the pea aphid, Acyrthosiphon pismum (Harris), often harbours Hamiltonella defensa Moran, a facultative bacterial symbiont that can confer resistance to the parasitoid Aphidius ervi Haliday (Oliver et al., 2014). Bacteriophage-encoded toxins are suspected to play a role in this interaction as a virus called A. pismum secondary endosymbiont (APSE) must infect H. defensa for the pea aphids to receive protection (Moran et al., 2005; Oliver et al., 2009).

H. defensa also protects the black bean aphid, Aphis fabae Scopoli, from parasitism by Lysiphlebus fabarum (Marshall) (Schmid et al., 2012). To date, however, the specificity of H. defensa protection against various parasitoid species has not been addressed. This latter point is particularly important given that many aphids are routinely attacked by many parasitoid species (Stary, 1988; Hågvar & Hofsvang, 1991). Here, we document H. defensa-mediated protection of the cowpea aphid, Aphis craccivora Koch, from parasitism and compare the effectiveness of this protection across four parasitoid species in three genera.

Our experiments were motivated by evidence of poor parasitism success of the parasitoid Binodoxys communis (Gahan) in A. craccivora that tested positive for H. defensa (Desneux et al., 2009). This host exhibited much lower suitability than other aphids did that did not test positive for H. defensa. While these results suggested a protective role of H. defensa, they were only correlative. Our goal here was to confirm these results experimentally and to determine whether H. defensa in A. craccivora confers resistance to other parasitoid species.

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Materials and methods

Insect cultures

_Aphis craccivora_ were collected from alfalfa, _Medicago sativa_ L., in Lexington, Kentucky, USA in 2009, and cultured on fava bean, _Vicia faba_ L., in 8.5 cm pots within plant growth chambers at 18°C and an LD 16:8 h photoperiod. Isofemale lines were initiated from single female aphids on individual _V. faba_ plants. Ten lines from the original population of _A. craccivora_ tested positive for infection with only _H. defensa_ using denaturing gradient gel electrophoresis analysis of polymerase chain reaction (PCR)-amplified fragments of 16S rRNA and confirmed with diagnostic PCR (Russell _et al._, 2013). _Hamiltonella defensa_-free _A. craccivora_ lines were generated by a selective curing technique in which aphids were fed an artificial diet that was treated with equal parts of the antibiotics gentamycin, defatoxiamine, and ampicillin for 3 days, after which the aphids were placed on _V. faba_ plants (Dykstra _et al._, 2014). One of these lines (AC1) was used for these experiments. Clearing of _H. defensa_ from offspring of aphids from this line was confirmed by PCR and an _H. defensa_-free line was maintained for at least 10 generations before resistance studies. Both cured (‘Ham−’) and uncured (‘Ham+’) lines were tested using diagnostic PCR for _H. defensa_ before assays (Brady & White, 2013).

Four species of parasitoid hymenopterans within the braconid aphidine tribe Aphidiini were used: _Binodoxys communis_, _B. koreanus_ Stary (subtribe Trioxina), _Aphidius colemani_ Viereck, and _Lysiphlebus orientalis_ Starý, and Rakhshani (both subtribe Aphidiina) (Shi & Chen, 2005). The two _Binodoxys_ species and _L. orientalis_ are native to Eurasia and were introduced into a quarantine laboratory in the U.S.A. as prospective biological control agents against the Asian soybean aphid, _Aphis glycines_ (Ragsdale _et al._, 2011). _Aphidius colemani_ is a commercially available aphid parasitoid known to attack _A. craccivora_ (Rakhshani _et al._, 2005) that we purchased for this study. All parasitoids were reared within growth chambers on a colony of _A. glycines_ stemming from a single collection in 2003 on soybean plants at 25°C, 65% RH, and a photoperiod of LD 16:8 h. (Wyckhuys _et al._, 2008). _Aphis glycines_ is not known to harbour _H. defensa_ and diagnostic PCR assays had previously confirmed the absence of _H. defensa_ in our colony (N. Desneux and G. E. Heimpel, unpublished). _Aphis glycines_ harbours bacteria in the genus _Arsenophonus_, but these do not appear to protect against parasitoids (Wulff _et al._, 2013).

Bioassays and analyses

Fifty _A. craccivora_, 10 of each developmental stage (first, second, third, and fourth instar nymphs, and adults) that were all either uncured (Ham+) or cured (Ham−) were exposed to individual female parasitoids on potted _V. faba_ plants covered by cylindrical Plexiglas cages (diameter 11 cm, height 21 cm) as in Desneux _et al._ (2009). As adult aphids can reproduce, this design allows for some reproduction during the assays. Although _A. craccivora_ were reared at 18°C, parasitoid assays were run at 25°C. Diagnostic PCR was used before each experimental sequence to ensure that Ham+ aphids tested positive for _H. defensa_ and that Ham− aphids tested negative. We introduced single, unmated, honey-fed females that had eclosed within 24 h into the arenas; only apparently healthy parasitoids were used (i.e. active and without wing or body damage). The two _Binodoxys_ species and _A. colemani_ are sexual species and thus uncured females produce only male offspring whereas _L. orientalis_ is thelytokous (Stary _et al._, 2011; Petrovic _et al._, 2013) and thus unmated females produce daughters. Parasitoids were removed from the arenas 24 h after introduction and any parasitised aphids (‘mummies’ – aphid remains with discoloured cuticles containing developing parasitoids) were collected 10 days later. All mummies from a given arena were placed into a single plastic petri dish (10 cm diameter) and held in a plant growth chamber at 25°C and LD 16:8 h photoperiod for emergence. The numbers of mummies and the emergence rate from the mummy to the adult stage were scored from each arena. The numbers of mummies were compared between the Ham+ and Ham− treatments separately for each of the four parasitoid species using Wilcoxon rank sum tests with normal approximation, and the emergence rates for the four parasitoid species exposed to Ham− _A. craccivora_ were compared using ANOVA on square root arcsin-transformed values weighted for mummy numbers. We also compared the emergence rates between Ham+ and Ham− aphids for parasitoids that produced mummies on both aphid treatments using _t_-tests on square root arcsin-transformed values weighted for mummy numbers. Sample sizes ranged between six and nine female parasitoids per aphid treatment per parasitoid species for a total of 58 assays.

Results and discussion

The presence of _H. defensa_ caused complete and significant suppression of parasitism by _B. communis_ (χ² = 10.636; _P_ = 0.0011) and near-complete and significant suppression of _B. koreanus_ parasitism (χ² = 10.425; _P_ = 0.0012). In contrast, rates of successful parasitism, as measured by mummy production, were not significantly affected by _H. defensa_ infection in either _A. colemani_ (χ² = 0.02; _P_ = 0.9614) or _L. orientalis_ (χ² = 10.332; _P_ = 0.5644) (Fig. 1). Average emergence rates from the mummy stage of the four parasitoid species that were cured of _H. defensa_ did not differ significantly (_F_3,20 = 2.32; _P_ = 0.11; _B. communis_ mean ± SEM: 0.62 ± 0.13, _B. koreanus_: 0.80 ± 0.08, _A. colemani_: 0.82 ± 0.04, _L. orientalis_: 0.72 ± 0.04). There was also no significant difference between the average emergence rates of Ham+ and Ham− aphids attacked by _A. colemani_ or _L. orientalis_ (_A. colemani_ mean ± SEM: Ham+: 0.76 ± 0.03, Ham−: 0.82 ± 0.04, _t_12 = 1.46, _P_ = 0.17; _L. orientalis_: Ham+: 0.66 ± 0.09, Ham−: 0.61 ± 0.11, _t_16 = 1.27, _P_ = 0.23). Thus, our results demonstrate that _H. defensa_ protects _A. craccivora_ from parasitism by two species of _Binodoxys_ parasitoids, but not from parasitism by _A. colemani_ or _L. orientalis_.

The mechanisms causing these differences may involve countermeasures on the part of the parasitoids. Two studies have shown that parasitoids can evolve improved capability against symbiiont-defended aphids (Dion _et al._, 2011; Rouchet & Vorburger, 2014). In the latter case, the scope of counter
Defensive symbiosis was quite narrow, and parasitoids showed improved performance only against the symbiont strains with which they had an evolutionary history. In our study, most of the parasitoids were imported species previously unexposed to U.S. populations of *A. craccivora*. However, some of these parasitoid populations may have encountered similar strains of *H. defensa* in the past, either within other infected populations of the cosmopolitan *A. craccivora* (Brady et al., 2014), or in other aphid species that share related strains of *H. defensa* (Russell et al., 2003). Behavioural resistance (i.e. avoidance of *H. defensa*-harbouring aphids) is also possible, and previous assays showed that *B. communis* accepted *H. defensa*-harbouring *A. craccivora* at a slightly lower rate than most other aphids, although acceptance was still relatively high (Desneux et al., 2009). Alternatively, phylogenetic differences among the parasitoids might explain their differential ability to parasitise defended *A. craccivora*. Our studies utilised a single aphid clone infected with a single strain of *H. defensa*. It is possible that different *H. defensa* strains and their associated APSE phages may confer protection against particular natural enemies. However, all *H. defensa* strains characterised in North American *A. craccivora* carry APSE-4, which encodes a shiga-like toxin, and are identical across multiple bacterial and virus sequence typing loci (Degnan & Moran, 2008; Dykstra et al., 2014). Limited strain diversity in introduced populations may reduce the range of enemies protected by symbionts and present opportunities for more effective biological control.

The specificity of defensive symbionts has implications for expected infection rates of host aphids. Infection rates of *A. craccivora* by *H. defensa* are relatively low world-wide. In a survey of 44 *A. craccivora* populations from 18 countries across six continents, six populations showed evidence of *H. defensa* infections (Brady et al., 2014) and in these cases infection rates were at intermediate levels. Additionally, laboratory studies showed fitness costs of infection by *H. defensa* as well as an imperfect (but high) vertical transmission rate (Dykstra et al., 2014). Thus, despite clear fitness benefits in the presence of susceptible parasitoids, *H. defensa* would be expected to be lost from populations of *A. craccivora* in the absence of such parasitoids. A defensive symbiont that protects against a broad range of parasitoid species would provide a greater benefit and probably be retained in more populations.

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References


**Fig. 1.** The numbers of parasitised (= ‘mummified’) aphids produced by individual females of *Binodoxys communis* (a), *B. koreanus* (b), *Aphidius colemani* (c), and *Lysiphlebus orientalis* (d). Shown are means ± SEM.


