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Influence of the secondary endosymbiont *Serratia symbiotica* on the resistance to the parasitism in the aphid *Acyrtosiphon pisum*

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Abstract

Aphids have an obligate association with the primary symbiont *Buchnera aphidicola* and is known to affect aphid fitness. Aphids commonly harbour other facultative bacterial endosymbionts and may benefit from their presence through increased resistance to parasitoids^[1]. Present results suggest that the ability of hosts to defend against natural enemies depend not only on the presence of symbionts but also on the host genotype. Aphids represent a complex micro-environment for the parasitoid larvae and both the aphid physiology and endosymbiont influence their survival until mummification.

Keywords: *Serratia symbiotica*, *Acyrtosiphon pisum*, *Aphidius ervi*, resistance, facultative endosymbionts, parasitism, oviposition.

1. Introduction

In any given ecosystem, the multi-trophic interactions between the different trophic levels, e.g. between plants, herbivorous insects and their natural enemies (predators, parasitoids and pathogens) result from a long co-evolution.

Phloem-feeding insects (aphids, whiteflies, psyllids, scales and mealybugs) harbour bacterial endosymbionts with both obligate and facultative relationships. Most of the aphid species possess the obligate primary bacterial endosymbiont, *Buchnera aphidicola*, which provides the aphid with essential amino acids lacking from their phloem sap diet^[2]. Many species of aphid can also harbour facultative secondary endosymbiotic bacteria among which three species are frequently cited: *Serratia symbiotica*, *Hamiltonella defensa*, and *Regiella insecticola* have been discovered^[3]. The significance of these bacteria remained elusive until recent studies on the secondary symbionts of the pea aphid *Acyrtosiphon pisum* revealed several effects on aphid fitness and phenotypes. They are not essential for survival, they play an important role in the ecology and evolution of aphids by affecting important traits such as insecticide resistance, natural enemy resistance, thermal resistance, viral transmission, reproduction and development^[4, 5, 6].

Compared with uninfected aphids, infected aphids were more probably to be attacked by parasitoids but less probably to support parasitoid development. This interchange between a symbiotic bacteria and a host provides a tool for the endurance and dispersion of symbiotic bacteria^[7]. There is an enormous clonal variation for susceptibility to parasitoids in natural populations of aphids^[8, 9]. Some of this variation is explained by genetic differences among aphid clones^[8, 9], but most of the variation is owing to endosymbiotic bacteria that some clones possess^[7]. Some studies have also revealed that the prevalence of facultative bacteria such as *S. symbiotica*, *H. defensa* and *R. insecticola* varies considerably across pea aphid host plant races and geographic areas^[10, 11, 12] possibly influenced by the selective advantage of carrying different symbionts in different habitats^[13, 14].

A big difference was also observed between *A. pisum* clones considering their resistance to the natural ennemi *A. ervi* development following oviposition^[7]. However, information on the possible role of *Serratia symbiotica* regarding the resistance to parasitoids remains quite poor. In consequence, in order test if *S. symbiotica* increases resistance of *A. pisum* (Homoptera: Aphididae) to parasitoids, we used two genotypes of *A. pisum* Madison and Tucson, each genotype represented by two lines of aphids (symbiotic and cured).

We recorded the mummification rate of aphids parasitized by the parasitoid *A. ervi* (Hymenoptera: Braconidae). We thus expect that the ability of hosts to defend against natural enemies depends not only on the presence of symbiont but also on the host genotype.

2. Materials and Methods

(a) Study organisms

All the experiments were performed with the Tucson and Madison infected and uninfected subclones of *A. pisum* (Hemiptera: Aphididae) provided by Dr. Nancy Moran (University of Texas). The Tucson and Madison pea aphid were originally collected from *Vicia faba* and *Medicago lupulina* in Tucson and Madison respectively in Arizona (1999) and were naturally infected by *S. symbiotica*.

The Tucson uninfected sub-colonies were established in 2000 and 2006 through curing of *S. symbiotica* with heat-shock. The 5AR aphid sub-clones were established from the naturally uninfected 5A clone collected in Madison by microinjection of body fluids containing symbionts from the Tucson.

The infection status of subclones was verified using diagnostic PCRs with primers 16SA1 (5'-AGAGTTTGATCMTGGCTCAG-3') and PASS cmp (5'-GCAATGCTTATTAACACAT-3')^[15].

As parasitoid, we used *Aphidius ervi* (Hymenoptera: Braconidae), a species that is commonly used in the biocontrol of pest aphids and capable of parasitizing *Myzus persicae* as well as *A. pisum*^[16]. *A. ervi* wasps were reared on a non-resistant *M. persicae* colonies synchronized L3 and maintained on a broad bean plant (*Vicia faba*) in order to assess preference and to evaluate the ability of *A. ervi* females to discriminate between the two genotypes infected and uninfected aphids with *S. symbiotica* during the experiments.

Colonies of *A. ervi*, and *A. pisum* (infected and uninfected) were maintained on bean plant while *M. persicae* was reared on sweet pepper plants at 19.5 ± 0.6 °C, 40-50% RH, under a 16L: 8 D photoperiod.

On the Tucson and Madison pea aphid strains, we compared the rates of successful parasitism of each aposymbiotic or symbiotic lines using the uninfected line of the same genotype as control. In each experiment, 30 pea aphids were placed on a potted *V. faba* plant in a cup cage 20-24h before *A. ervi* introduction. To measure susceptibility to parasitoids, we exposed groups of aphid for the two genotypes to parasitoids for a fixed period of time and determined the proportion of individuals that were successfully parasitized as recommended by^[17].

Before the start of the experiment, we reconfirmed the infection status of aphids by diagnostic PCR. *A. ervi* individuals used for parasitism assays were previously isolated at the mummy stage, fed and mated under direct observation after emergence. Wasps used in the experiments were all aged 24 h post-emergence and were assumed to be mated. Females of *A. ervi* were given oviposition experience by exposing them to five uninfected third instar aphids *M. persicae* in a Petri dish (diameter 5 cm) just before the experiment. Any wasp that did not oviposit in an aphid within 5 min after introduction in the Petri dish was excluded from the experiment. Females with oviposition experience were then individually assigned at random to either treatment (inoculated with SS) or control (uninfected) arenas for the two genotypes of aphids. The

wasps were removed from the cup cages after 6 h. The cages were incubated at 19.5 ± 0.6 °C, 40-50% RH, under a 16L: 8D photoperiod and examined after 10 days and the number of mummies were counted to determine susceptibility to parasitism.

Secondary symbionts association with resistance were measured by rate of mummification, calculated as the fraction of aphids that formed the characteristic yellow hardened mummy from the total number of aphids that were transferred to the cup cages in the beginning of the experiment.

(b) Statistics

All statistical analyses were carried out in R.2.12.0 (R development Core Team 2010, <http://www.r-project.org>). The proportion of aphids exposed to *A. ervi* that were mummified was analysed using a generalized linear model.

3. Results and Discussion

Exposing symbiotic and cured aphids to the parasitoid showed clearly that *S. symbiotica* increases resistance to parasitic wasps: the mummification rate is higher in cured aphid than in symbiotic aphids and depends on the aphid genotype (figure 1). *A. pisum* infected with the *S. symbiotica* showed a reduction in mummy formation when compared with uninfected controls (ANOVA, $F_{1:116} = 258.13$, $P < 0.001$) for the two genotypes. Moreover, the mummification rate depends on the aphid genotype: so, green aphids collected from Tucson are mummified at a higher rate than red aphids collected from Madison (ANOVA, $F_{1:116} = 122.55$, $P < 0.001$).

These results are in agreement with previous results showing that the infection with *S. symbiotica* confer resistance of *A. pisum* to the parasitoid *A. ervi*. At this point, it seems that resistance is linked to the development process of the parasitoid larva inside its host more than to acceptance level and behavioural rejection^[7].

Oppositely, other studies did not showed any significant effect of *S. symbiotica* on resistance against *A. ervi*^[18, 19]. It is also possible that parasitoid larvae develop successfully despite possible decrease in overall survival, indeed reduced emergence could be observed even after successful mummification^[18]. In earlier studies it has been stated that *H. defensa* and *S. symbiotica* co-infection increase the resistance against *A. ervi*.^[20]

Color variation within populations of the pea aphid influences relative susceptibility to predators and parasites. The high number of mummies observed with green aphids (Tucson) compared with the low number of mummies observed with red aphids (Madison) may be due to the aphid body color. The ecological studies showed that parasitoid wasps preferentially attack green aphids^[21].

In a recent study, it has been found that infection with a facultative endosymbiont of the genus *Rickettsiella* changes the insects body color from red to green in natural populations^[22]. In our case the induced green color of aphid may induce the parasitism risk by the parasitoid, but the resistance of aphids against the parasitoid may be incomplete. Parasitoid larvae is able to develop successfully despite possible decrease in overall survival of the parasitoid larvae, indeed reduced emergence could be observed even after successful mummification^[18].

Present results suggest that the ability of insect hosts to defend themselves against natural enemies not only depends on the presence of symbiont but also on the host genotype and the aphid body color. Aphids represent a complex microenvironment for the parasitoid larvae: both aphid physiology and endosymbiont influence their survival until mummification.

For the future, it will be important to investigate parasitoid behavior facing a symbiotic aphid strain and particularly to see if symbionts could influence aphids cuticular hydrocarbon variation. In fact, cuticular hydrocarbons, are reported to serve as chemical cues in the recognition process during inter-individual interactions between host and parasitoid^[23].

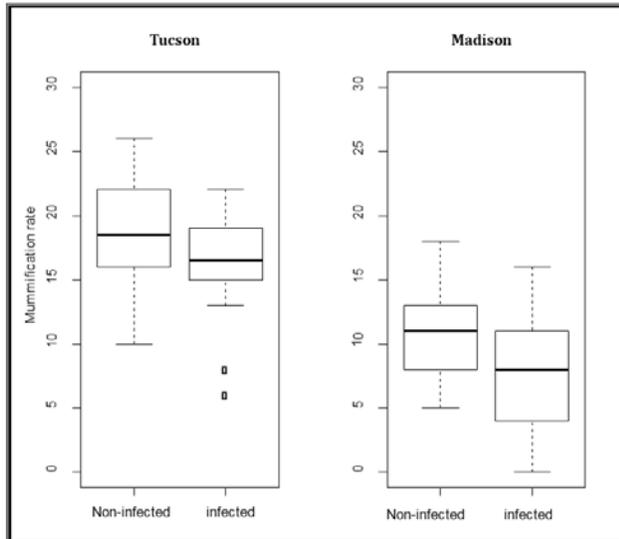


Fig 1: Mummification 10 days after parasitization for aphids from Madison and Tucson infected and non-infected with *S. symbiotica*

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