Symbiont infection affects aphid defensive behaviours

Emilie Dion, Sarah Erika Polin, Jean-Christophe Simon and Yannick Outreman*

1 UMR 1099 INRA-Agrocampus Ouest-Université Rennes 1 ‘Biologie des Organismes et des Populations appliquée à la Protection des Plantes’, 65 rue de Saint-Brieuc CS 84215, 35042, Rennes Cedex, and BP 35327, 35653 Le Rheu Cedex, France.

*Author for correspondence (yannick.outreman@rennes.inra.fr).

Aphids harbour both an obligate bacterial symbiont, Buchnera aphidicola, and a wide range of facultative ones. Facultative symbionts can modify morphological, developmental and physiological host traits that favour their spread within aphid populations. We experimentally investigated the idea that symbionts may also modify aphid behavioural traits to enhance their transmission. Aphids exhibit many behavioural defences against enemies. Despite their benefits, these behaviours have some associated costs leading to reduction in aphid reproduction. Some aphid individuals harbour a facultative symbiont Hamiltonella defensa that provides protection against parasitoids. By analysing aphid behaviours in the presence of parasitoids, we showed that aphids infected with H. defensa exhibited reduced aggressiveness and escape reactions compared with uninfected aphids. The aphid and the symbiont have both benefited from these behavioural changes: both partners reduced the fitness decrements associated with the behavioural defences. Such symbiont-induced changes of behavioural defences may have consequences for coevolutionary processes between host organisms and their enemies.

Keywords: Acrithosiphon pisum; defensive behaviours; symbiont infection; Hamiltonella defensa; symbiont-mediated phenotype.

1. INTRODUCTION

An extraordinary number of arthropods harbour various types of vertically transmitted symbionts. Among insects, symbionts of aphids are perhaps the best studied [1]. In addition to their essential nutrient-providing symbiont Buchnera aphidicola, many aphid species also carry one or a few facultative bacterial symbionts. These symbionts may exert diverse effects on their host, such as plant adaptation, heat tolerance or parasite resistance (reviewed in [1]). Most of these effects are beneficial for the host, thus favouring the spread and the persistence of symbionts within aphid populations. In various mutualistic associations, such evidence of symbiont effects concerns morphological, developmental and physiological host traits but rarely behavioural ones [2].

Recent studies on facultative symbionts concern Hamiltonella defensa, a γ-proteobacterium that provides parasitoid protection to the pea aphid, Acrithosiphon pisum [3]. Pea aphids also present a large repertoire of defensive strategies, including morphological, social, chemical and behavioural defences [4]. Most prevalent, however, are defensive behaviours like aggressiveness towards the enemy, or escape reactions [5]. These behaviours are beneficial, because they reduce an aphid’s risk of being attacked. However, this benefit is balanced by costs, such as loss of feeding opportunities or lower survival rates, leading to fitness decrements (e.g. [6,7]). This balance between costs and benefits associated with defensive behaviours induces variation for these traits in aphid populations [8].

Here, we investigated the possible influence of H. defensa on the propensity of aphids to defend themselves against enemies. Because aphids harbouring this symbiont are protected against parasitoids, we hypothesize that behavioural defence pay-offs should be reduced: defensive responses against parasitoids would induce useless fitness decrements in both partners. By contrast, defensive behaviours may be the unique way to avoid parasitism in aphids uninfected with H. defensa. Consequently, a reduction in defensive behaviours may be expected in aphids infected with H. defensa. To test this possible contribution of symbiosis to defensive behavioural variation in insect populations, we compared the aggressiveness and escape responses of aphids infected or not infected with H. defensa.

2. MATERIAL AND METHODS

(a) Insects

Pea aphids were collected in 2008 and 2010 around Rennes (Western France) in several alfalfa (Medicago sativa) fields since populations specialized on this plant harbour Hamiltonella defensa at a high rate [9]. The genotypes and the symbiotic status of the sampled aphids were determined (see [10] and [9] for details on genotyping and symbiont detection, respectively). From these analyses, we selected 10 aphid genotypes infected or not infected with H. defensa (table 1). Some genotypes were represented by lineages infected or not infected with H. defensa and were considered to have identical genetic backgrounds (i.e. 14 highly polymorphic markers were used for genotyping, a number sufficient to identify clonal copies from distinct genotypes), and just differing by their symbiotic status. The Aphidius ervi parasitoids originated from parasitized aphids collected in alfalfa fields around Rennes in 2008. Parasitoids were reared on a culture of A. pisum feeding on broad bean (Vicia faba). Insects were maintained at 20°C under a long-day regime (16 L: 8 D).

For the experiments, only second and third-instar larvae of A. pisum were used. To obtain parasitoid females, parasitized aphids were collected from cultures and placed individually in gelatin capsules. Newly emerged females were enclosed in plastic tubes containing moistened cotton, droplets of honey and one male for mating, and were used once for experiment the day after.

(b) Experimental set-up

The experiment aimed to measure the influence of the symbiotic status of aphids on their defensive behaviours. Two experimental series were implemented with exactly the same design. In the first series, we compared the defensive behaviours of three aphid genotypes harbouring H. defensa with three genotypes free of facultative symbionts. In the second series, we more precisely analysed the influence of H. defensa on defensive behaviours of aphids with identical genetic backgrounds, by considering four genotypes that each presented naturally-co-occurring lineages infected or not infected with H. defensa (table 1).

The procedure consisted of introducing one parasitoid female into a cage (20 × 25 × 30 cm) containing one broad bean leaf (10 cm height) infested with 10 genetically and symbiotically identical aphids. Observation began the day after the wasp landed on the leaf and started searching for aphids and ended when it left the leaf. The insect behaviours were recorded using the event-recorder ‘The Observer’ (Noldus Information Technology, Wageningen,
with H. defensa uninfected individuals (figure 1). Aggressiveness was significantly higher in colonies of H. defensa-infected genotypes as compared to uninfected individuals. The rate of rejections owing to aphid aggressive behaviours (i.e. quick motions or piercing of legs and/or body repelling the wasp to undertake a stabbing behaviour) or the parasitoid’s decision (without aphid aggressive behaviour). As parasitoid attack could elicit the dropping behaviour, the occurrence of this escape reaction was also recorded. Once the parasitoid left the leaf, all aphids were transferred onto a broad bean plant and followed over four weeks in order to measure the parasitism rate (i.e. proportion of aphids parasitized). This procedure was repeated 148 times (table 1).

### 3. RESULTS

#### (a) Aphid defensive behaviours

When faced with parasitoids, the behavioural responses of aphids strongly depended on their symbiotic status. In both experimental series, A. pisum individuals infected with H. defensa were less defensive than uninfected individuals. The Hamiltonella-free individuals dropped off the plant more frequently than the Hamiltonella-infected aphids when confronted with parasitoids (figure 1a). Once encountered by the enemy, the Hamiltonella-free aphids were more aggressive than the Hamiltonella-infected ones. Consequently, the rate of rejections owing to aphid aggressiveness was significantly higher in colonies of uninfected individuals (figure 1b). Aphids infected with H. defensa were more prone to parasitoids and, consequently, ovipositor insertions occurred more frequently in colonies of H. defensa-infected hosts (figure 1c).

#### (b) Parasitism rate

In the first experimental series, parasitism rates did not differ significantly between H. defensa-infected and uninfected aphids. This lack of significance was mainly owing to high parasitism variance among H. defensa-infected genotypes: while two H. defensa-infected genotypes were strongly resistant (about

<table>
<thead>
<tr>
<th>experimental series</th>
<th>aphid genotypes</th>
<th>H. defensa infection status</th>
<th>number of replicates</th>
</tr>
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<tbody>
<tr>
<td>series 1 distinct genetic background</td>
<td>G1</td>
<td>infected</td>
<td>11</td>
</tr>
<tr>
<td>G2</td>
<td>infected</td>
<td>11</td>
<td></td>
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<tr>
<td>G3</td>
<td>infected</td>
<td>11</td>
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<tr>
<td>G4</td>
<td>uninfected</td>
<td>10</td>
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<tr>
<td>G5</td>
<td>uninfected</td>
<td>11</td>
<td></td>
</tr>
<tr>
<td>G6</td>
<td>uninfected</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>series 2 pairs of lineages with identical genetic background</td>
<td>G7</td>
<td>infected</td>
<td>11</td>
</tr>
<tr>
<td>G8</td>
<td>uninfected</td>
<td>10</td>
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</tr>
<tr>
<td>G9</td>
<td>infected</td>
<td>11</td>
<td></td>
</tr>
<tr>
<td>G10</td>
<td>uninfected</td>
<td>10</td>
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The Netherlands). When the parasitoid stopped its searching activity after both locating and contacting with an aphid, the event was regarded as an attack by parasitoid. The outcome of an attack was either the aphid acceptance for oviposition or its rejection. Piercing the aphid skin with the ovipositor was counted as an ovipositor insertion. Conversely, any attack by a parasitoid that did not lead to a piercing of the aphid skin with the ovipositor was counted as an ovipositor insertion. The outcome of an attack was regarded as an attack by parasitoid. The occurrence of this escape reaction was also recorded. Once the parasitoid left the leaf, all aphids were transferred onto a broad bean plant and followed over four weeks in order to measure the parasitism rate (i.e. proportion of aphids parasitized). This procedure was repeated 148 times (table 1).

(c) Statistical analyses

The dependent variables analysed were the total number of aphid dropping behaviours, the rate of host rejections owing to aphid aggressiveness, the total number of ovipositor insertions and the parasitism rate. The analyses consisted of testing the effect of the symbiotic status of aphids on each dependent variable. In both experiments, several genotypes were tested and genotype was considered as a random independent variable in our statistical modelling. Generalized linear mixed models (GLMMs) were fitted using the lme4 package [11] in R v. 2.8.1 [12] and by assuming either a Poisson or Binomial error according to the dependent variable and a log- and logit-link function, respectively.

### Figure 1. Effects of infection with H. defensa on (a) the number of A. pisum individuals that dropped off the plant during colony exploitation by an A. ervi female; (b) the rate of A. ervi host rejections owing to the aggressiveness of the attacked aphid; and (c) the number of A. ervi ovipositor insertions during colony exploitation. Black bars, aphids harboring H. defensa; open bars, aphids free of facultative symbionts. See §2 for description of experimental series 1 (distinct genetic background) and experimental series 2 (identical genetic background). The mean and the standard errors are shown. Statistical significance was evaluated by the GLMM (**p < 0.001, *p < 0.01, †p < 0.05 and n.s. p > 0.05).
10% of parasitism rate), one genotype presented an intermediate level of symbiont-mediated resistance (about 40% of parasitism rate). In the second experimental series, the rate of parasitism was lowest in colonies of *H. defensa*-infected aphids (figure 2).

4. DISCUSSION

Our results indicate that *A. pismum* individuals infected with *H. defensa* dropped off the plant less frequently than uninfected individuals and exhibited a reduced aggressiveness towards the enemy. These results were observed irrespective of aphid genetic backgrounds, supporting the influence of *H. defensa* on aphid behavioural defences. Consequently, we experimentally demonstrated that the presence of defensive symbionts can produce high behavioural variation in their host populations.

We also showed that parasitoids do not benefit from the reduced defensive behaviours in *Hamiltonella*-infected aphids: even if the infected aphids are exposed to parasitoid oviposition more frequently, the symbiont-mediated resistance maintained low parasitism rates. The *H. defensa* infection enables aphids to save costs associated with the defensive behaviours while protecting the aphid against parasitoids. The factor responsible for this symbiont-mediated behavioural variation is obviously unknown. It may be parsimonious to assume that the reduction of defensive behaviours in infected aphids is an adaptive behavioural response of aphids rather than a consequence of behavioural manipulation by the bacterial symbiont. In this evolutionary scenario, uninfected individuals have been selected for efficient behavioural defences, while the selection has been attenuated in infected aphids. We must also consider that the aphid behavioural changes can be a pathological consequence of symbiont infection and are coincidentally beneficial for both partners. Whatever the underlying mechanism of this symbiont-mediated phenotype is, it minimizes the costs associated with defensive behaviours and so favours both host and symbiont.

At first sight, the vertically transmitted symbiont *H. defensa* increases host fitness by conferring resistance to parasitoids and reducing costly defensive behaviours. These beneficial effects on the host increase the spread and the persistence of the facultative symbiont within aphid populations. However, these benefits are strongly dependent on the ecological context. While *H. defensa* confers specific protection against parasitoids, behavioural defences protect aphids against a wider range of natural enemies [5]. The reduction of behavioural defences in infected aphids would then increase their susceptibility against predators and predation may lead to lower prevalence of symbionts in host populations. The interplay between predation and parasitism pressures would certainly have a considerable impact on the persistence of symbionts in natural populations.

5. CONCLUSION

Our experiments identified symbiosis as an important factor generating behavioural variation in host populations. As insect symbionts are extremely common, we suggest that symbiont-induced changes in insect behaviours may have played a key role in the evolution of ecological interactions in many species. Symbionts may promote evolution in insect enemies by changing host defensive strategies like conferring resistance and affecting defensive behaviour expressions.

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