Parasitoids as vectors of facultative bacterial endosymbionts in aphids

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Heritable bacterial endosymbionts play an important role in aphid ecology. Sequence-based evidence suggests that facultative symbionts such as Hamiltonella defensa or Regiella insecticola also undergo horizontal transmission. Other than through male-to-female transfer during the sexual generation in autumn, the routes by which this occurs remain largely unknown. Here, we tested if parasitoids or ectoparasitic mites can act as vectors for horizontal transfer of facultative symbionts. Using symbiont-specific primers for diagnostic PCR, we demonstrate for the first time, to our knowledge, that parasitoids can indeed transfer H. defensa and R. insecticola by sequentially stabbing infected and uninfected individuals of their host, Aphis fabae, establishing new, heritable infections. Thus, a natural route of horizontal symbiont transmission is also available during the many clonal generations of the aphid life cycle. No transmissions by ectoparasitic mites were observed, nor did parasitoids that emerged from symbiont-infected aphids transfer any symbionts in our experiments.

Keywords: Aphis fabae; bacterial endosymbiont; horizontal transmission; maternal transmission; parasitoid; vector

1. INTRODUCTION

Heritable bacterial endosymbionts are common in insects and mediate ecologically important traits of their hosts [1,2]. Aphids harbour an obligate intracellular endosymbiont, Buchnera aphidicola, which serves a nutritional function and is required for aphid survival [3]. Perfect phylogenetic congruence between aphids and their Buchnera indicates long-term vertical and no horizontal transmission of this symbiont [4]. In addition to Buchnera, aphids may possess several facultative endosymbionts that are not indispensable but beneficial by providing protection against parasitoids, pathogens or thermal stress [1,2]. They exhibit virtually perfect maternal transmission [5]; yet sequence-based evidence indicates that horizontal transmission occurs as well [6]. In contrast to Buchnera, facultative symbionts are also found extracellularly in the aphids’ haemolymph, which may facilitate horizontal transmission. Indeed, it is possible to transfer them experimentally between lineages of the same or even different species by microinjection of symbiont-containing haemolymph [7] or by feeding aphids with an artificial diet containing symbiotic bacteria [5].

However, it remains unclear how relevant these findings are for the natural situation. In the whitefly Bemisia tabaci, another phloem-feeding insect, plant-mediated oral transmission of the endosymbiont Rickettisia has been documented recently [8]. In aphids, the only natural mechanism of horizontal transfer known so far is by sex: during mating, infected males may transmit facultative symbionts to uninfected females through their ejaculate [9]. Because aphids reproduce by cyclical parthenogenesis, this route is available only once per year, during the single sexual generation of the annual cycle in autumn. It would be important to establish if there are natural routes of horizontal transmission that are also available during the many clonal generations throughout the growth season.

Potential candidates are natural enemies that come in contact with haemolymph of multiple individuals and may thus act as natural vectors. For example, Jaenike et al. [10] found that ectoparasitic mites can transfer endosymbiotic Spiroplasma between Drosophila species. This finding is important because aphids are part of complex communities in which species are strongly linked through a multitude of natural enemies. Transmission by some of these enemies would provide facultative endosymbionts with substantial mobility within their communities and could help explain their broad occurrence. We investigated this possibility in aphids using ectoparasitic mites and parasitoid wasps as candidate vectors, and we were able to show for the first time, to our knowledge, that parasitoids are indeed able to transfer bacterial endosymbionts horizontally.

2. MATERIAL AND METHODS

(a) Aphids, parasitoids and mites

We worked with five different clones of the black bean aphid, Aphis fabae, all collected in Switzerland in 2006 and maintained in the laboratory on seedlings of broad bean (Vicia faba) under conditions ensuring continuous parthenogenetic reproduction (20 °C, 16 h photoperiod). These clones were genetically distinct; each possessed a different multilocus genotype over eight microsatellite loci. Three clones were naturally uninfected with any known facultative endosymbiont of aphids and served as recipients (nos 256, 401 and 405). One clone (no. 402) was naturally infected with Hamiltonella defensa, an endosymbiont known to increase aphid resistance to parasitoids [11], and one clone (no. 259) was naturally infected with Regiella insecticola, an endosymbiont known to protect aphids against fungal pathogens [12]. These two clones served as donors.

As parasitoids we used a laboratory stock culture of the commercially available biocontrol agent Aphidius colemani, originally obtained from Andermatt Biocontrol AG (Grossdietwil, Switzerland), as well as six thelytokous (asexual) isofemale lines of Lysiphlebus fabarum, the most important parasitoid of A. fabae in the field. These lines were also collected in 2006 from different sites in Switzerland, Germany, Italy and France, and they have been maintained on A. fabae in the laboratory since then.

Larvae of an undetermined species of ectoparasitic mite from the family Trombidiidae were obtained in June and July 2010 from infested colonies of the aphid Brachycaudus cardui growing on a stand of Senecio encelioides near Zurich, Switzerland. We used the mites in experiments immediately after collection. Note that by using field-collected mites, we could not exclude that they had already sucked on symbiont-infected aphids prior to collection. Nevertheless, any transmission of symbionts by these mites to aphids in the laboratory would support their role as vectors.

(b) General experimental procedures

The basic protocol for testing the horizontal transmission of bacterial endosymbionts consisted of allowing potential vectors to first attack aphids of a donor clone and then a sub-adult aphid of a recipient

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clone. If the recipient survived the attack, it was reared to adulthood and allowed to produce offspring until it ceased to reproduce or died. The last few offspring of each recipient were reared to adulthood and then collected into 1.5 ml Eppendorf tubes for DNA extraction and detection of endosymbionts by PCR. We tested the offspring rather than the recipients themselves because our prime interest was in transmissions resulting in new, heritable infections. The last born offspring were tested because new infections require several days of ‘incubation’ before they are transmitted maternally [13]. The DNA of individual aphids was prepared with Chelex to test for the presence of either donor clone as well as 10 mites straight from the field for mummies. We reared parasitoids (only *L. fabarum*) on both donor clones but did not give them any opportunity to stab infected aphids by isolating mummies prior to emergence. We then caged these wasps in groups of five on plants containing a colony of approximately 50 third and fourth instar nymphs of a recipient clone. Wasps were left in the cages until they died, which—at this ratio of parasitoids to hosts—should have resulted in multiple attacks per recipient. Ten days after adding the wasps, we collected those aphids that had resisted parasitism and were still alive and reared them individually to produce offspring for diagnostic PCR. We also tested nine wasps emerging from each of the donor clones for the presence of symbionts by diagnostic PCR.

3. RESULTS

The sample sizes and outcomes of all the three experiments are summarized in table 1. Of 89 recipient aphids that were attacked by mites that had previously fed on symbiont-infected donors, 75 survived and reproduced. We tested between one and five (mean \( \approx 4.45 \)) of their latest born offspring but did not find a single individual testing positive for symbionts. Neither did diagnostic PCR detect the presence of *H. defensa* or *R. insecticola* in any of the mites that were tested after feeding on a donor clone or directly after collection.

Of 154 recipients that were stabbed by a parasitoid that had attacked donor aphids before (parasitoid experiment 1), only 58 survived and reproduced. All others died before reproduction, mostly because they were parasitized successfully. We used between one and five (mean \( \approx 4.35 \)) of the survivors’ latest born offspring for diagnostic PCR and found 23 individuals from five recipient mothers to be positive (table 1). Thus, we observed five cases of horizontal transmission via parasitoid wasps that led to new heritable infections with facultative endosymbionts. Two cases involved the transmission of *R. insecticola* to recipient clone 256, once by *A. colemani* and once by *L. fabarum*, three cases involved the transmission of *H. defensa* to recipient clone 405 by *A. colemani*.

| Table 1. Summary of results from the three experiments testing for horizontal transmission of aphid endosymbionts by animal vectors. (No. of recipients is the number of uninfected aphids that were exposed to a potential vector; no. of recipients lost is the number of these aphids that died or were killed by parasitoids before leaving any offspring; no. of surviving recipients is the number of recipients that survived the vectors’ attack and reproduced; and no. of infected recipients is the number of these survivors whose offspring tested positive for endosymbionts in diagnostic PCRs, demonstrating that they acquired the symbiont from the vector and passed it on to their offspring.) |
|---|---|---|---|---|
| mite experiment | no. of recipients | no. of recipients lost | no. of surviving recipients | no. of infected recipients |
| no. of recipients | 8 | 2 | 18 | 7 |
| no. of surviving recipients | 1 | 2 | 4 | 1 |
| no. of infected recipients | 0 | 0 | 0 | 0 |
| transmission rate (95% CI) | 0 (0.00-0.04) | 0 (0.00-0.04) |
| parasitoid experiment 1 | no. of recipients | no. of recipients lost | no. of surviving recipients | no. of infected recipients |
| no. of recipients | 27 | 26 | 24 | 27 |
| no. of surviving recipients | 15 | 14 | 6 | 9 |
| no. of infected recipients | 5 | 5 | 3 | 3 |
| transmission rate (95% CI) | 0.033 (0.013-0.073) | 0.086 (0.037-0.186) |
| parasitoid experiment 2 | no. of recipients | no. of recipients lost | no. of surviving recipients | no. of infected recipients |
| no. of recipients | 14 | 14 | 39 | 15 |
| no. of surviving recipients | 9 | 9 | 13 | 4 |
| no. of infected recipients | 5 | 5 | 13 | 4 |
| transmission rate (95% CI) | 0 (0.03-0.033) | 0 (0.05-0.053) |

- Wilson score 95% binomial CI [15], calculated using the ProCIs package in R v. 2.12.1 [16].
In parasitoid experiment 2, we obtained 113 recipients that resisted parasitism by wasps that had developed in one of the donor clones. Of those, 44 died soon after isolating them on individual plants and 69 survived and reproduced. We tested between one and five of their last born offspring (mean = 4.75) but did not detect any infections with facultative symbionts (table 1). Diagnostic PCRs on wasps were all negative, too.

4. DISCUSSION

Here, we demonstrate that parasitoids can transfer endosymbionts of aphids between clones by sequentially stabbing infected and uninfected aphids—a previously undescribed route of horizontal transmission. The wasp’s ovipositor appears to act as a ‘dirty needle’ that can inoculate previously uninfected aphids. If the recipient aphid resists the parasitoid and survives the attack, this can result in a new, heritable infection. Considering the fact that many aphid parasitoids use multiple hosts, it is likely that they can transfer endosymbionts not just within but also between aphid species.

That some maternally transmitted endosymbionts of plant sap-sucking insects also exhibit horizontal transmission is indicated by phylogenetic evidence [6]. Identifying the mechanisms has important implications for understanding the ecology and evolution of these insects. Combined with the recent discovery of transfer via plants in whiteflies [8], the novel route we describe here in aphids suggests that facultative symbionts enjoy considerable mobility in phloem-feeding insects, mediated by trophic links in the complex food webs of which they are a part. In the case of symbionts like H. defensa, which increases host resistance to parasitoids, such transfers may also modify the strength of trophic links. The estimated transmission rates via parasitoids were low but not negligible (3.3% over all recipients; 8.6% if only survivors are considered; table 1), and that two different endosymbionts could be vectorised by both parasitoid species tested suggests that this mechanism is sufficiently general to be of broad relevance.

We neither observed any transmissions via wasps that developed in symbiont-infected aphids, nor did we observe any transmissions via ectoparasitic mites. However, rare events remain easily undetected with a limited sample size, and we cannot exclude low transmission rates via these routes (see confidence intervals in table 1). At least for mites it is known that they can act as vectors of endosymbiotic Spiroplasma in Drosophila [10].

As a final remark, we point out that in the case of H. defensa, which provides aphids with protection against parasitoids, the wasps are spreading a symbiont that is detrimental to their own fitness. They may thus be under selection to minimize such transfers, which could explain the low rates of transmission observed.

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