Interspecific transmission of endosymbiotic Spiroplasma by mites

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1. INTRODUCTION

Innumerable species of insects are infected with maternally transmitted bacterial endosymbionts, whose effects range from parasitic to mutualistic (Bourtzis & Miller 2003). Some mutualistic symbionts, such as Buchnera and Candidatus Sulcia muelleri, are vertically transmitted with effectively perfect fidelity over extended evolutionary periods, resulting in congruent phylogenies of hosts and their symbionts (Clark et al. 2000; Moran et al. 2005). In contrast, many commensal and parasitic endosymbionts, such as Wolbachia (Phylum Proteobacteria) and Spiroplasma (Phylum Firmicutes), show little congruence with many commensal and parasitic endosymbionts, such as lateral transfer. However, the mechanisms by which such lateral transmission occurs are unknown. To date, the only experimental studies to demonstrate interspecific transmission of endosymbionts have focused on parasitic wasps that can pick up Wolbachia infections either from their insect host or from other parasitic wasp species sharing the same host (Heath et al. 1999; Hugens et al. 2004).

Here, we ask whether a generalist ectoparasitic mite can serve as a vector to transmit Spiroplasma from one Drosophila species to another. There are several reasons to think that this may be possible. First, many species of Drosophila are infected with ectoparasitic mites in the wild (Polak 1996; Halliday et al. 2005), and interspecific aggregation of Drosophila around breeding sites may provide an arena for movement of mites from one Drosophila species to another (Jaenike & James 1991; Kriger & Sevenster 2001). Second, the high level of DNA sequence similarity among Spiroplasma poulsonii strains isolated from Drosophila nebula, Drosophila willistoni and Drosophila melanogaster (Bentley et al. 2002; Montenegro et al. 2005; Pool et al. 2006) suggests that S. poulsonii has undergone lateral transfer in the recent evolutionary past. Furthermore, S. poulsonii belongs to the citri–poulsonii clade of Spiroplasma, which has a broad host range, including flies, honeybees, leafhoppers and ticks, the latter belonging to the same order (Acari) as mites (Gasparich et al. 2004). Finally, ectoparasitic mites ingest the haemolymph of infected insects and thus may act as ‘dirty needles’ to transmit haemolymph-dwelling microbes, such as Spiroplasma, from one host to another, in much the same way that aphids and certain other insects can transmit viruses from one plant to another (Nault 1997). Here, we show that mites are capable of effecting such interspecific transmission.

2. MATERIAL AND METHODS

(a) Drosophila and mites

Drosophila nebula infected with male-killing S. poulsonii (Williamson et al. 1999) were provided by G. D. D. Hurst who collected the flies in Guadeloupe. This strain of D. nebula was maintained by mating infected females with males from an uninfected strain that has a normal sex ratio (140±30:76±1.00 from the Tucson Drosophila Species Stock Center). Drosophila willistoni (strain 140±30:081±10) used in these experiments was obtained from the Tucson Drosophila Species Stock Center. Macrocheles subbadius Berlese (Macrocheles: Mesostigmata) mites were obtained from wild D. nigrospiracula and cultured in the laboratory using previously published methods (Polak 1996).

(b) Infection process

Infected D. nebula females (donors) were placed individually with two mites in pipette tips to facilitate host–parasite contact. After 24 h, mites were detached from the flies and transferred to a pipette tip containing an uninfected female fly (recipient) of either D. nebula or D. willistoni. Recipient flies that had an attached mite were maintained for 3 days at 24°C, mated with conspecific males and allowed to oviposit for 6 days. These recipient flies were noted to have mite-induced scars, confirming that the mites had breached the host’s integument.

(c) Infection assay

Spiroplasma infection in donors, mites, recipients and the offspring of recipients was assessed using PCR using Spiroplasma-specific primers p18-f (5′-AGTTATATGAGTTATAATGT-3′) and p18-r (5′-CTGTTGAATTACCTTGTAATGT-3′), provided by G. D. D. Hurst. The F1, D. nebula and D. willistoni recipients that were positive for p18 were sequenced directly from PCR products using an internal primer, p18int131f (5′-CGAAAAACGCCGAGA TGTTA-3′). To test for contamination of putatively infected D. willistoni with DNA from infected D. nebula, we used newly designed D. nebula-specific primers for the mtDNA COI gene (nbcCOIfwd: 5′-CTTATTTTACTTCTGCTAC-3′; nbcCOIrev3: 5′-CTTCTGTTAATCCCTCCAC-3′). Over a range of DNA concentrations from 1.2×10−4 to 1.2×10−1 ng ml−1, these primers invariably yield positive results for D. nebula but negative results for D. willistoni.
mites that attached to infected D. nebulosa

donor D. nebulosa

control mite

uninfected D. nebulosa

recipient files

F1 of infected D. willistoni recipient

Figure 1. PCR screen for Spiroplasma gene p18. Top gel shows, from left to right, mites that had attached to infected D. nebulosa females, a donor D. nebulosa female from the infected strain, a control mite that had not attached to D. nebulosa and a female D. nebulosa from an uninfected strain. Middle panel shows representative recipient flies (D. willistoni and D. nebulosa). Bottom panel shows representative offspring of a recipient D. willistoni female that had been scored positive for infection. In the bottom two panels, circled individuals indicate recipient flies and F1 progeny scored positive for Spiroplasma infection, and arrows denote adjacent positive (left: Spiroplasma-infected D. nebulosa) and negative (right: uninfected D. nebulosa) controls.

Table 1. Offspring sex ratios and Spiroplasma infection. (Data shown for infected recipient flies whose offspring were either positive or negative for Spiroplasma infection. Also shown are the offspring sex ratios for control flies that had not been exposed to Spiroplasma.)

<table>
<thead>
<tr>
<th>recipient species</th>
<th>parental female type (n)</th>
<th>offspring sex ratio</th>
<th>proportion female offspring (total number of offspring)</th>
</tr>
</thead>
<tbody>
<tr>
<td>D. nebulosa</td>
<td>infected recipient (1)</td>
<td>≥1 positive</td>
<td>0.52 (93)</td>
</tr>
<tr>
<td></td>
<td>infected recipient (6)</td>
<td>all negative</td>
<td>0.54 (512)</td>
</tr>
<tr>
<td></td>
<td>uninfected control (7)</td>
<td>—</td>
<td>0.496 (1436)</td>
</tr>
<tr>
<td>D. willistoni</td>
<td>infected recipient (1)</td>
<td>≥1 positive</td>
<td>0.58 (113)</td>
</tr>
<tr>
<td></td>
<td>infected recipient (2)</td>
<td>all negative</td>
<td>0.55 (138)</td>
</tr>
<tr>
<td></td>
<td>uninfected control (7)</td>
<td>—</td>
<td>0.530 (747)</td>
</tr>
</tbody>
</table>

3. RESULTS

Out of 17 mites that had attached to infected D. nebulosa females, 14 (82%) were positive for Spiroplasma infection. Among recipient flies, 28% of D. nebulosa (n=98) and 21% of D. willistoni (n=19) were positive for Spiroplasma, indicating that mites can transmit the infection from infected to uninfected flies and that transmission can occur both within and between Drosophila species. Among the progeny of infected recipient flies, 0.3% of D. nebulosa (n=306) and 3.6% of D. willistoni (n=162) were infected (figure 1).

The p18 gene fragments that were amplified from the Spiroplasma-infected strain of D. nebulosa, the mites that attached to these flies, and F1 progeny of infected D. nebulosa and D. willistoni recipients were either identical or differed at several polymorphic sites out of 559 bp (GenBank accession numbers DQ885999–DQ886015 and DQ886017). The Spiroplasma in two of the infected offspring of a single infected D. willistoni recipient differed from each other at 18 out of 375 sites sequenced (GenBank accession numbers DQ886004 and DQ886005). All of these sites are apparently polymorphic (represented by double peaks in electropherograms) in the Spiroplasma-infected D. nebulosa strain used in this study, suggesting the existence of a double infection in D. nebulosa and segregation of Spiroplasma strains among the F1 of infected recipient flies. However, because these sequences were obtained directly from PCR products rather than clones, we cannot rule out sequencing ambiguities as the cause of this apparent polymorphism and segregation. The D. nebulosa-specific COI primers failed to amplify any sequences from the 10 Spiroplasma-positive offspring produced by infected D. willistoni recipients, indicating that the presence of Spiroplasma in these flies was not due to contamination with DNA from infected D. nebulosa.

Although infected recipient females produced a slightly greater proportion of female offspring than did uninfected females, offspring sex ratios did not differ significantly between infected recipient D. willistoni and D. nebulosa that produced one or more infected offspring, infected recipients that produced only uninfected offspring and uninfected control flies (table 1; D. nebulosa: χ²=2.85, p=0.24; D. willistoni: χ²=1.24, p=0.54). None of the infected recipient flies produced strongly female-biased offspring sex ratios.

4. DISCUSSION

Our results show that mites can act as vectors to bring about interspecific transmission of endosymbionts. Given the abundance of ectoparasitic mites in natural communities, we suspect that generalist mites...
could be important vectors of symbionts that occur within the haemolymph of insects.

Significant sex ratio distortion was not evident in the offspring of Spiroplasma-infected recipient flies. It is possible that the S. poulsonii from D. nebulosa is poorly adapted to D. willistoni. However, conspecific D. nebulosa recipients also manifested low rates of maternal transmission and male killing, suggesting that the infection process itself, rather than maladaptation to a new host species, is responsible for the lack of sex ratio distortion.

The low levels of transmission and male killing may result from low intra-host densities of Spiroplasma (Anbutsu & Fukatsu 2003). Mite-vectored Spiroplasma would initially occur on the cuticle or in the haemolymph of recipient flies, but must then enter the oocytes for maternal transmission and subsequent male killing to occur. Three findings support this interpretation. First, on average, only a small fraction of offspring of infected recipient females were infected, suggesting a low mean density of infection. Second, there was considerable variation among infected recipient females in the fidelity of Spiroplasma transmission. For D. willistoni, 10 out of 99 offspring from one infected recipient female were infected, whereas 0 out of 63 offspring were infected from three other infected recipient females. Such variation is consistent with random variation around a low mean density of infection. Finally, we found evidence suggesting that Spiroplasma strains segregated in these offspring of infected recipients. It is therefore likely that Spiroplasma go through population bottlenecks in undergoing transmission from donor fly to mite to recipient fly to the offspring of these recipients. The attainment of higher within-host Spiroplasma densities may depend on incubation period and environmental conditions. Regardless of post-infection dynamics, our experiments identify an ecologically plausible mechanism by which these endosymbionts may be transmitted among species in the wild.

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