

Interspecific transmission of endosymbiotic *Spiroplasma* by mites

John Jaenike^{1,†,*}, Michal Polak^{2,†}, Anna Fiskin², Mada Helou² and Miranda Minhas¹

¹Department of Biology, University of Rochester, Rochester, NY 14627, USA

²Department of Biological Sciences, University of Cincinnati, Cincinnati, OH 45221, USA

*Author for correspondence (joja@mail.rochester.edu).

†These authors contributed equally to this work.

The occurrence of closely related strains of maternally transmitted endosymbionts in distantly related insect species indicates that these infections can colonize new host species by lateral transfer, although the mechanisms by which this occurs are unknown. We investigated whether ectoparasitic mites, which feed on insect haemolymph, can serve as interspecific vectors of *Spiroplasma poulsonii*, a male-killing endosymbiont of *Drosophila*. Using *Spiroplasma*-specific primers for PCR, we found that mites can pick up *Spiroplasma* from infected *Drosophila nebulosa* females and subsequently transfer the infection to *Drosophila willistoni*. Some of the progeny of the recipient *D. willistoni* were infected, indicating successful maternal transmission of the *Spiroplasma* within the new host species. However, the transmission rate of the infection from recipient flies to their offspring was low, perhaps due to low *Spiroplasma* density in the recipient flies.

Keywords: *Drosophila nebulosa*; *Drosophila willistoni*; host–parasite associations; *Macrocheles*; *Spiroplasma poulsonii*

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 host phylogeny (Werren *et al.* 1995; Vavre *et al.* 1999; Gasparich 2002). This pattern, as well as the occurrence of closely related endosymbionts infecting distantly related host species, indicates that these symbionts must occasionally colonize new hosts via 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; Huigens *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; Krijger & Sevenster 2001). Second, the high level of DNA sequence similarity among *Spiroplasma poulsonii* strains isolated from *Drosophila nebulosa*, *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 nebulosa 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. nebulosa* was maintained by mating infected females with males from an uninfected strain that has a normal sex ratio (14030-761.00 from the Tucson *Drosophila* Species Stock Center). *Drosophila willistoni* (strain 14030-0814.10) used in these experiments was obtained from the Tucson *Drosophila* Species Stock Center. *Macrocheles subbadius* Berlese (Macrochelidae: Mesostigmata) mites were obtained from wild *D. nigrospiracula* and cultured in the laboratory using previously published methods (Polak 1996).

(b) Infection process

Infected *D. nebulosa* 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. nebulosa* 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 via PCR using *Spiroplasma*-specific primers p18-f (5′-AGTTTATGCTGACTTGTTAATC-3′) and p18-r (5′-CTGTTGTATTACCTTGTAATGT-3′), provided by G. D. D. Hurst. The F₁ *D. nebulosa* and *D. willistoni* recipients that were positive for p18 were sequenced directly from PCR products using an internal primer, p18int131f (5′-GCAAAAACGCGAAGATGTTA-3′). To test for contamination of putatively infected *D. willistoni* with DNA from infected *D. nebulosa*, we used newly designed *D. nebulosa*-specific primers for the mtDNA *COI* gene (nebCOI fwd2: 5′-CTTATTTTACTTCTGCTAC-3′; nebCOI rev3: 5′-CTCCTGTTAATCCTCCAAC-3′). Over a range of DNA concentrations from 1.2 × 10⁻⁴ to 1.2 × 10¹ ng ml⁻¹, these primers invariably yield positive results for *D. nebulosa* but negative results for *D. willistoni*.

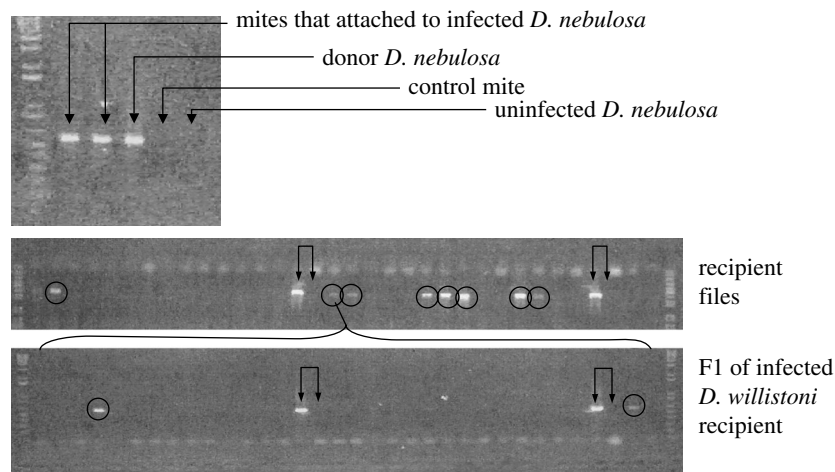


Figure 1. PCR screen for *Spiroplasma* gene p18. Top gel shows, from left to right, mites that had attached to infected *D. nebulosa* females, a donor *D. nebulosa* female from the infected strain, a control mite that had not attached to *D. nebulosa* and a female *D. nebulosa* from an uninfected strain. 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*.)

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

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*: $\chi^2=2.85$, $p=0.24$; *D. willistoni*: $\chi^2=1.24$, $p=0.54$). 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.

This work was supported by the US National Science Foundation (EF-0328363, DEB-0315521, DEB-0345990 and DEB-0542094). We thank Greg Hurst for generously providing the *Spiroplasma*-infected strain of *D. nebulosa* and the p18 primer sequences.

REFERENCES

- Anbutsu, H. & Fukatsu, T. 2003 Population dynamics of male-killing and non-male-killing *Spiroplasmas* in *Drosophila melanogaster*. *Appl. Environ. Microbiol.* **69**, 1428–1434. (doi:10.1128/AEM.69.3.1428-1434.2003)
- Bentley, J. K., Hinds, G. & Hurst, G. D. D. 2002 The male-killing *Spiroplasmas* of *Drosophila nebulosa* and *Drosophila willistoni* have identical ITS sequences. *Dros. Inf. Serv.* **85**, 63–65.
- Bourtzis, K. & Miller, T. A. (eds) 2003 *Insect symbiosis*. Boca Raton, FL: CRC Press.
- Clark, M. A., Moran, N. A., Baumann, P. & Wernegreen, J. J. 2000 Cospeciation between bacterial endosymbionts (*Buchnera*) and a recent radiation of aphids (*Uroleucon*) and pitfalls of testing for phylogenetic congruence. *Evolution* **54**, 517–525. (doi:10.1554/0014-3820(2000)054[0517:CBBEBA]2.0.CO;2)
- Gasparich, G. 2002 *Spiroplasmas*: evolution, adaptation and diversity. *Front. Biosci.* **7**, 619–640.
- Gasparich, G. E., Whitcomb, R. F., Dodge, D., French, F. E., Glass, J. & Williamson, D. L. 2004 The genus *Spiroplasma* and its non-helical descendants: phylogenetic classification, correlation with phenotype and roots of the *Mycoplasma mycoides* clade. *Int. J. Syst. Evol. Microbiol.* **54**, 893–918. (doi:10.1099/ijs.0.02688-0)
- Halliday, R. B., Walter, D. E. & Polak, M. 2005 A new species of *Gamasodes* Oudemans from Australia (Acari: Parasitidae). *Zootaxa* **1001**, 17–30.
- Heath, B. D., Butcher, R. D. J., Whitfield, G. F. & Hubbard, S. F. 1999 Horizontal transfer of *Wolbachia* between phylogenetically distant insect species by a naturally occurring mechanism. *Curr. Biol.* **9**, 313–316. (doi:10.1016/S0960-9822(99)80139-0)
- Huigens, M. E., de Almeida, R. P., Boons, P. A. H., Luck, R. F. & Stouthamer, R. 2004 Natural interspecific and intraspecific horizontal transfer of parthenogenesis-inducing *Wolbachia* in *Trichogramma* wasps. *Proc. R. Soc. B* **271**, 509–515. (doi:10.1098/rspb.2003.2640)
- Jaenike, J. & James, A. C. 1991 Aggregation and the coexistence of mycophagous *Drosophila*. *J. Anim. Ecol.* **60**, 913–928. (doi:10.2307/5421)
- Krijger, C. L. & Sevenster, J. G. 2001 Higher species diversity explained by stronger spatial aggregation across six neotropical *Drosophila* communities. *Ecol. Lett.* **4**, 106–115. (doi:10.1046/j.1461-0248.2001.00200.x)
- Montenegro, H., Solferini, V. N., Klaczko, L. B. & Hurst, G. D. D. 2005 Male-killing *Spiroplasma* naturally infecting *Drosophila melanogaster*. *Insect Mol. Biol.* **14**, 281–287. (doi:10.1111/j.1365-2583.2005.00558.x)
- Moran, N. A., Tran, P. & Gerardo, N. M. 2005 Symbiosis and insect diversification: an ancient symbiont of sap-feeding insects from the bacterial phylum Bacteroidetes. *Appl. Environ. Microbiol.* **71**, 8802–8810. (doi:10.1128/AEM.71.12.8802-8810.2005)
- Nault, L. R. 1997 Arthropod transmission of plant viruses: a new synthesis. *Ann. Entomol. Soc. Am.* **90**, 521–541.
- Polak, M. 1996 Ectoparasitic effects on host survival and reproduction: the *Drosophila-Macrocheles* association. *Ecology* **77**, 1379–1389. (doi:10.2307/2265535)
- Pool, J. E., Wong, A. & Aquadro, C. F. 2006 Finding of male-killing *Spiroplasma* infecting *Drosophila melanogaster* in Africa implies transatlantic migration of this endosymbiont. *Heredity* **97**, 27–32. (doi:10.1038/sj.hdy.6800830)
- Vavre, F., Fleury, F., Lepetit, D., Fouillet, P. & Bouletreau, M. 1999 Phylogenetic evidence for horizontal transmission of *Wolbachia* in host-parasitoid associations. *Mol. Biol. Evol.* **16**, 1711–1723.
- Werren, J. H., Zhang, W. & Guo, L. R. 1995 Evolution and phylogeny of *Wolbachia*—reproductive parasites of arthropods. *Proc. R. Soc. B* **261**, 55–63.
- Williamson, D. L. et al. 1999 *Spiroplasma poulsonii* sp. nov., a new species associated with male-lethality in *Drosophila willistoni*, a neotropical species of fruit fly. *Int. J. Syst. Bacteriol.* **49**, 611–618.