Horizontal transmission of intracellular endosymbiotic bacteria: a case between mites and fruit flies and its evolutionary implications.

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Abstract

Insect intracellular endosymbiotic bacteria of the genus *Wolbachia* are known to play an important role in the evolution of their hosts by influencing the hosts' sex ratios and creating sexual isolation within their populations. As the bacteria are confined to the egg cellular matrix and are inherited maternally, their wide distribution among arthropod taxa remains enigmatic. This ubiquitous dispersal among insects can partially be explained by the existing evidence of lateral transfers of the bacteria in host-parasite systems, usually via direct tissue contact between the agents. However, lateral transfer beyond these systems has never been reported. In the present study, we demonstrate for the first time that *Wolbachia* lateral transmission is possible between fruit flies and the non-parasitic mites *Tyrophagus noxius*. We discuss potential evolutionary implications of the reported phenomenon.

Keywords: endosymbionts; *Wolbachia*; *Tyrophagus noxius*; *Drosophila melanogaster*; lateral transfer

Introduction

Maternally inherited intracellular bacteria of the genus *Wolbachia* are widely spread among invertebrates and infect 16% to 66% of all arthropod species, including all major insect orders (Werren *et al.*, 1995; Werren, 1997; Hilgenboecker *et al.*, 2008). The bacteria are known to affect the host populations via reproductive manipulations, such as parthenogenesis, feminization, and cytoplasmic incompatibility (*e.g.*, Min and Benzer, 1997; Veneti *et al.*, 2003; Weeks *et al.*, 2003; Zchori-Fein and Perlman, 2004). Due to the last effect, individuals of the same host population cannot interbreed if infected with different *Wolbachia* strains. The resulting reproductive isolation effectively splits the population into subunits, entailing what is called *infectious speciation* (*e.g.*, Wade, 2001; Miller *et al.*, 2010). This allows a tentative presumption that *Wolbachia* may have played a crucial role in the rapid diversification of arthropods. However, as the bacteria are confined to the host eggs' cellular matrix and are usually inherited maternally (Werren, 1997), their wide distribution among insects remains largely a mystery.

Meanwhile, there is evidence that *Wolbachia* can be transmitted to a new host species via parasites. For example, such transfers have been reported between the parasitic wasps *Trichogramma kaykai* (Schilthuizen and Stouthamer, 1997; Huigens *et al.*, 2004) and *Leptopilina boulardi* (Heath *et al.*, 1999) and their respective hosts. Woodlice have also been found to acquire *Wolbachia* from conspecifics via a shared source of blood (Rigaud and Juchault, 1995). However, such cases usually imply direct tissue contact between transfer agents and are perhaps unlikely to explain the whole

network of *Wolbachia* lateral transfers that must have happened in nature, as inferred from phylogenetic studies (*e.g.*, Vavre *et al.*, 1999).

In this study, we found the mites *Tyrophagus noxius* to be infected with *Wolbachia pipientis* strain *w*Mel, the strain characteristic of the fruit flies *Drosophila melanogaster*, and experimentally demonstrate the possibility of *Wolbachia* lateral transfer between these species. The mites *Tyrophagus noxius* Zachvatkin, 1935 (*Sarcoptiformes, Acaroidea, Tyroglyphidae*) often cohabit with *Drosophila*, including cultured laboratory flies, sharing the same nutritious media but are not *Drosophila* parasites. We discuss possible evolutionary implications of this phenomenon.

Materials and Methods

Mites and flies

Mites were taken from the nutrient medium in tubes containing a reared *Drosophila* culture and identified (Figure 1) at Schmalhausen Institute of Zoology, NAS of Ukraine, Department of Acarology. Fruit flies were taken from the *Drosophila melanogaster* living laboratory culture at the Department of General and Molecular Genetics of National Taras Shevchenko University of Kyiv.

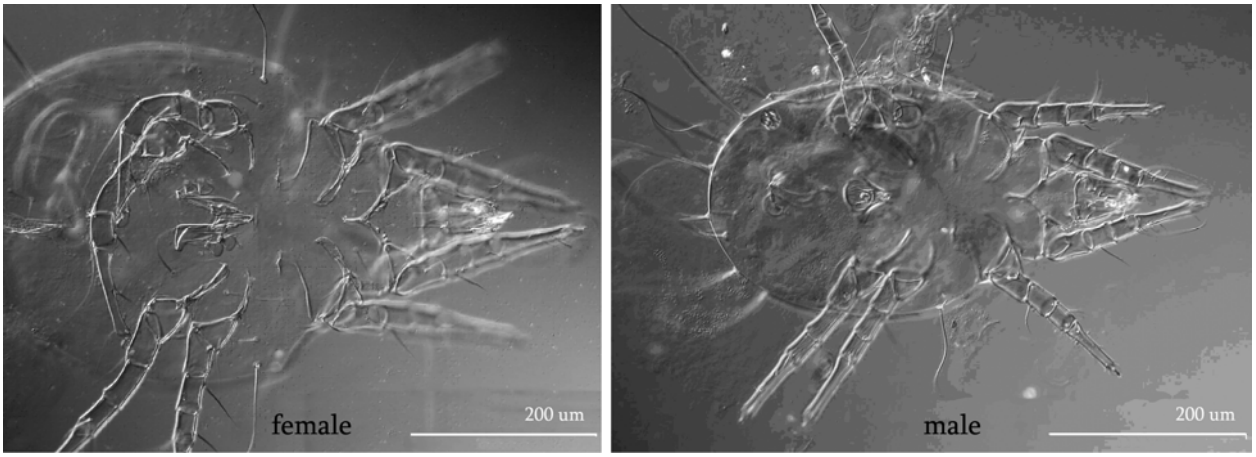

Figure 1. *Tyrophagus noxius*.

Experimental setup

First of all we ensured that the experimental mites and fruit flies were infected with *Wolbachia* by PCR with primers W-Specf and W-Specr amplifying a 438 bp fragment of the bacterial 16 rRNA gene (Werren and Windsor, 2000). Then both flies and mites were separated into two groups so that we had two infected groups of each species. After this, we separately reared two groups of flies and one group of mites on a medium containing 2 mg/ml tetacyclin hydrochloride (Morimoto *et al.*, 2006), which is an efficient antibiotic against *Wolbachia*, and ensured by PCR that these groups got rid of the bacteria. Accumulation of living material in the antibiotic-treated groups took nearly two weeks. Finally, we set up two joint cultures: 1) infected mites with uninfected flies (test culture), and 2) uninfected mites with uninfected flies (control ensuring that no bacterial revival occurs after antibiotic treatment; Figure 2). Both joint cultures were reared for 12 *Drosophila* generations. Bacterial infection status in both flies and mites was checked in both cultures by PCR three times coinciding with the *Drosophila* 2nd, 9th, and 12th generations. During the last PCR check (12th generation), we also amplified a 590-636 bp region of the *Wolbachia* surface protein Wsp gene

using the general primers *wsp81f* and *wsp691r* to identify the *Wolbachia* strain (Zhou *et al.*, 1998). The whole experiment was repeated twice. The *wsp* and *16S rDNA* amplicons from the first experiment obtained at generation 12 were sequenced.

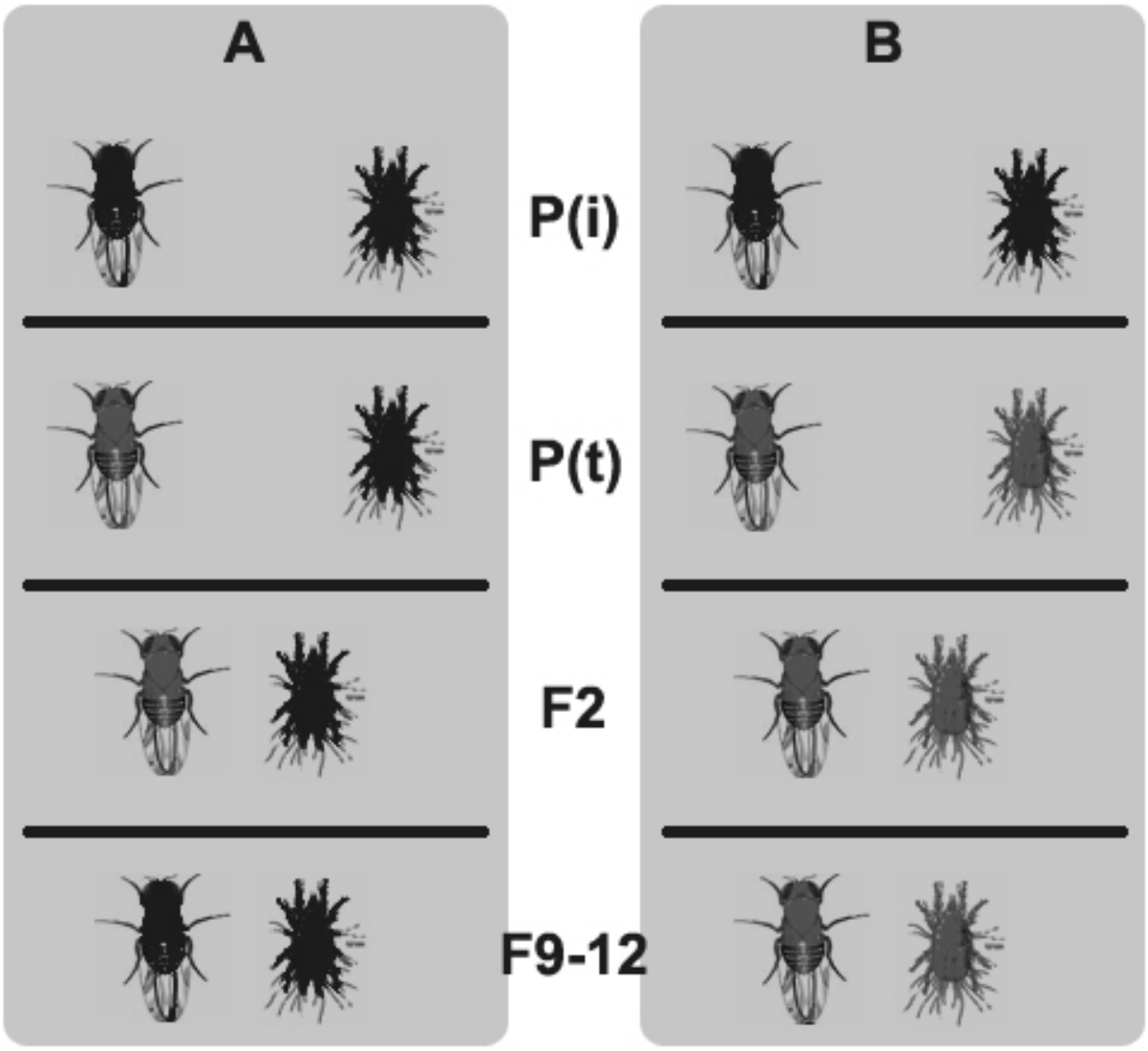

Figure 2. The experimental setup. Black flies/mites – infected, grey flies/mites – uninfected. A – experiment, B – control, P(i) – initial parental generation, P(t) – parental generation after antibiotic treatment (experimental mites were not treated). In the second generation (F2), the infection status remains the same as in P(t). Experimental flies (joint culture) get reinfected in/before the generation F9 and the infection persists through F12. No infection revival in control flies/mites.

PCR profiles

16S rRNA gene: denaturation 93°C/2 min; 30 cycles of denaturation 93°C/30 s, annealing 56°C/30 s, elongation 72°C/45 s; final elongation 72°C/8 min.

wsp gene: denaturation 94°C/2 min 50 s; 35 cycles of denaturation 94°C/30 s, annealing 55°C/40 s, elongation 72°C/50 s; final elongation 72°C/7 min.

Sequence alignment

The *wsp* and *16S rDNA* sequences were verified by BLAST (default parameters) against the NCBI's non-redundant nucleotide database. Local alignment was performed using the Vector NTI Suite 6 software (Invitrogen, USA).

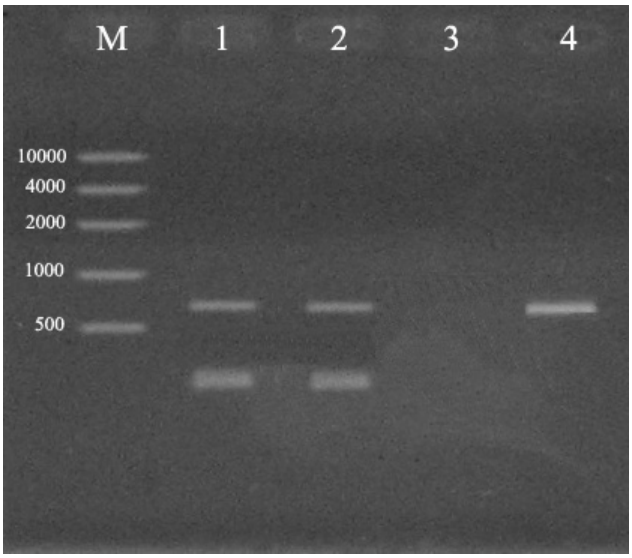

Figure 3. Infection diagnostics of *T. noxius* and *D. melanogaster* (Canton-S) after the 9th generation of cohabitation. M – molecular weight marker FastRuler High Range („Fermentas"); 1 – a fragment of *Wolbachia* sp. *wsp* gene in a *T. noxius* DNA sample; 2 – a fragment of *Wolbachia* sp. *wsp* gene in a *D. melanogaster* DNA sample (Canton-S); 3 – negative PCR control (*D. melanogaster* after tetracycline); 4 – positive PCR control for *Wolbachia* sp. (*D. melanogaster* "Uman" wild-derived lineage).

Results

In the control setups (see Materials and Methods) all three PCR checks in both experiments revealed no presence of *Wolbachia* both in mites and flies past antibiotic treatment, indicating that the treatment was reliable enough to ensure total elimination of the bacteria from tested flies and mites.

Both experiments gave identical results in the experimental setups (uninfected flies jointly cultured with infected mites). We had not detected *Wolbachia* in F2 flies, but the bacteria were present in F9 flies (Figure 3). We further tested F12 flies to check if the infection was stable, and F12 flies were also *Wolbachia*-positive. Therefore, the bacteria had been transferred from mites to flies. The presence of the bacteria in F12 flies indicates that the infection was stable and persisted into further generations.

Alignment of the mite and fly *wsp* sequences from F12 generation PCRs is shown in Figure 4 and demonstrates that both amplicons represented the same *Wolbachia* strain. BLAST alignment indicates that this strain is *w*Mel (aligned to GenBank ID: AF020064; see Zhou *et al.*, 1998), one characteristic of *Drosophila melanogaster*. Interestingly, this implies that the mites had previously (perhaps long before the experiment) acquired this bacterial strain from the flies they lived with, as we did not treat the experimental mites with an antibiotic. Therefore, our experiment just double-proofs that lateral transfer of *Wolbachia* is possible even without any invasive tissue contact between the transfer agents.

Discussion

From the evolutionary perspective, our findings indicate that either of the two players in the *D. melanogaster – T. noxius* symbiotic system has acquired the bacterium from its counterpart some time ago. Theoretically, this could occur with some "mediated" tissue contact, *e.g.*, mites eating fly eggs or vice versa from culture medium or natural nutritious media. *Wolbachia* can remain viable outside the host cell for up to one week and, more importantly, can restart their normal life cycle once back inside a cell (Rasgon *et al.*, 2006). As the mites oviposit their eggs into the medium, *Drosophila* larvae, which are not very fastidious in diet, could eat some of the eggs and thus become the vehicle of horizontal transmissions. As *T. noxius* is not a parasite, the infection gateway through fly tissue damage characteristic of host–parasite systems is unlikely in this case.

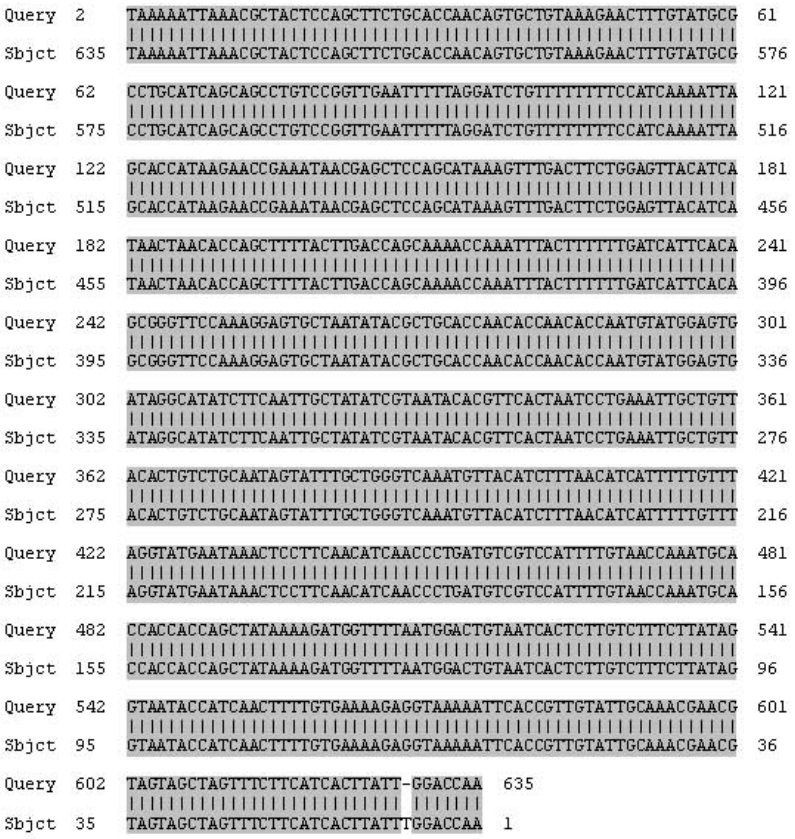

Figure 4. Alignment of *Wolbachia* sp. *wsp* gene sequences extracted from the F12 fly and mite DNA. Query – *Wolbachia* sp. *wsp* gene sequence extracted from the mite DNA; Sbjct – *Wolbachia* sp. *wsp* gene sequence extracted from the fly DNA.

Screening of the 12th generation flies suggests that the newly acquired bacterial infection gets "fixed" in ovaries and transmits further in its normal vertical way.

Although the results of this single experiment cannot be directly extrapolated to nature, such a mode of transmission could potentially explain the ubiquitous occurrence of *Wolbachia* in a wide range of hosts, because the network of species sharing common nutritious media would greatly expand the network of parasite-host and prey-predator interactions within arthropoda.

The possibility of fast *Wolbachia* transmission in nature would perhaps also mean significant effects on host evolution. One of these effects has recently been aptly designated as *infectious speciation* (Wade, 2001; Miller *et al.*, 2010). *Drosophila* flies infected with different *Wolbachia* strains within one population cannot interbreed and produce offspring because of the phenomenon called *bidirectional cytoplasmic incompatibility*, which prevents forming a normal zygote from gametes. In this way, a population infected with different bacteria strains is, in fact, reproductively separated, with the ensuing genetic drift and divergence. This bizarre way of reproductive isolation might in theory have been responsible for the rapid diversification of arthropods.

Besides, if shared laterally between many hosts, *Wolbachia* might potentially have served as a *horizontal gene transfer* (HGT) vehicle. One of the brightest examples of HGT between multicellular eukaryotes is the transfer of transposons between dipteran insects, in particular P element (a DNA transposon) between *Drosophila* species, many occurrences of which have been reported to date (reviewed in Silva and Kidwell, 2000). The most painstakingly studied case, that between *D. willistoni* and *D. melanogaster*, is known to have occurred less than a century ago. Interestingly, the rapid worldwide spread of P element in *D. melanogaster* populations, which ensued immediately upon its transfer, seems to have concurred with the spread of a particular genotype of *Wolbachia pipientis* strain *wMel*, which is now ubiquitous in *D. melanogaster* populations (Riegler *et al.*, 2005). As P element is generally harmful to fly genomes, its wide spread might have potentially benefited from its co-occurrence with *Wolbachia*, as some *Wolbachia* strains are known to give selective advantages to their host flies, particularly in antivirus (Teixeira *et al.*, 2008; Glaser and Meola, 2010) and anti-insecticide (Berticat *et al.*, 2002) defense and reproductive success (Weeks *et al.*, 2007).

HGT between multicellular eukaryotes itself probably requires bacterial and viral vectors to commute between the HGT-involved genomes. *Wolbachia* seems also to be a plausible candidate HGT vector from this perspective, as genetic exchange with its host genomes has yet been demonstrated (discussed in Introduction). A number of bacteriophages, like the phage WO (Kent and Bordenstein, 2010) known to mediate HGTs (Kent *et al.*, 2011) are encoded in the *Wolbachia* genome and might potentially serve as gene transfer vehicles between the *Wolbachia* and arthropod genomes.

This cumulative evidence, together with our results, suggests that *Wolbachia* may be a promising candidate genetic exchange driver, markedly influencing arthropod evolution. Further research is necessary to clarify if and to what extant horizontal transmission of *Wolbachia* occurs in nature, particularly in systems with no direct tissue contact between transfer agents. If proven, this phenomenon would greatly contribute to our understanding of the evolution of many invertebrate taxa and the biology of HGT between multicellular eukaryotes.

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Light-induced retinal degeneration in *Drosophila* with green fluorescent protein (GFP) attached to rhodopsin.

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Quite by serendipity, this laboratory noticed that *w*; *cn bw*; Rh1-GAL4 + UAS-Rh1GFP/TM2 *Drosophila* had degeneration of R1-6 receptors; this white-eyed stock has a transgene with GFP-labeled R1-6 rhodopsin (Rh1-GFP) driven into R1-6 by the promoter of the Rh1 gene (ninaE), so we will nickname this stock "Rh1-GFP." We utilized mostly time-tested optical ("pseudopupil") techniques summarized by Stark and Thomas (2004).

Dark-reared flies have a very obvious pseudopupil darkening, our way to visualize the amount of rhodopsin to metarhodopsin conversion (Figure 1 A *vs*. B); also R1-6's GFP was clear in the fluorescent deep pseudopupil (Figure 1 C), and R1-6's GFP-labeled rhabdomere tips were nicely imaged with optical neutralization of the cornea (Figure 1 D).

Contrast this with flies maintained 5 days in constant room light: there is a substantial decrease in the pseudopupil darkening (Figure 1 E *vs*. F); the fluorescent deep pseudopupil is hazy (Figure 1 G), and the fluorescent rhabdomere tips seen under oil immersion have missing profiles (Figure 1 H). White-eyed control *Drosophila*, without the Rh1-GFP transgene, had never shown any indications of light-induced damage in decades of research by this laboratory.

We used optical neutralization and the confocal microscope to verify the expectation that vitamin A deprived flies do not have fluorescent rhabdomere tips (Figure 1 I), while vitamin A replete flies show R1-6 GFP label (Figure 1 J). We aged vitamin A deprived flies for 8 days in constant room light, then put them in the dark on carrot juice; their GFP-labeled R1-6 rhabdomere tips look beautiful in the confocal microscope (Figure 1 K). We aged vitamin A deprived flies 5 days in constant light, then put them in the dark with carrot juice and obtained substantial recovery in the pseudopupil darkening (Figure 1 L *vs*. M); also R1-6 showed tidy GFP fluorescence in the deep pseudopupil (Figure 1 N). The work with vitamin A deprivation and replacement therapy shows that light is not damaging when Rh1-GFP is greatly reduced.

We present a light micrograph of Rh1-GFP flies maintained 23 days in room light showing cells in the process of degeneration and missing rhabdomeres (Figure 1 O). Control white-eyed flies (without Rh1-GFP), also maintained in room lighting for this same duration, did not have any signs of degeneration (not shown).

Earlier, Stark (2005) showed that white-eyed *Drosophila* with GFP driven into R1-6 had completely normal structure, rhodopsin-metarhodopsin conversions, and electrophysiology. While this may seem contradictory, GFP, in that case, was not attached to Rh1; flies were the F1 from a