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Evidence for horizontal transfer of *Wolbachia* by a *Drosophila* mite

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Abstract Mites are common ectoparasites of *Drosophila* and have been implicated in bacterial and mobile element invasion of *Drosophila* stocks. The obligate endobacterium, *Wolbachia,* has widespread effects on gene expression in their arthropod hosts and alters host reproduction to enhance its survival and propagation, often with deleterious effects in *Drosophila* hosts. To determine whether *Wolbachia* could be transferred between *Drosophila melanogaster* laboratory stocks by the mite *Tyrophagus putrescentiae*, mites were introduced to *Wolbachia*-infected *Drosophila* vials. These vials were kept adjacent to mite-free and *Wolbachia*-uninfected *Drosophila* stock vials. The *Wolbachia* infection statuses of the infected and uninfected flies were checked from generation 1 to 5. Results indicate that *Wolbachia* DNA could be amplified from mites infesting *Wolbachia*-infected fly stocks and infection in the previously uninfected stocks arose within generation 1 or 2, concomitant with invasion of mites from the *Wolbachia*-infected stock. A possible mechanism for the transfer of *Wolbachia* from flies to mites and vice versa, can be inferred from time-lapse photography of fly and mite interactions. We demonstrated that mites ingest *Drosophila* corpses, including *Wolbachia*-infected corpses, and *Drosophila* larva ingest mites, providing possible sources of *Wolbachia* infection and transfer. This research demonstrated that *T. putrescentiae* white mites can facilitate *Wolbachia* transfer between *Drosophila* stocks and that this may occur by ingestion of infected corpses. Mite-vectored *Wolbachia* transfer allows for rapid establishment of *Wolbachia* infection within a new population. This mode of *Wolbachia* introduction may be relevant in nature as well as in the laboratory, and could have a variety of biological consequences.

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Introduction

Parasitic arachnids such as ticks and mites are well known to transmit a variety of bacteria, many of them pathogenic, to and between hosts (Houck et al. 1991; Jaenike et al. 2007). The genus and species of mites present in *Drosophila* culture vary, and differ in destructiveness from those that eat the culture food to those that prey upon the flies themselves (Ashburner and Thompson 1978). Good *Drosophila* husbandry requires that fly stocks be maintained free of mites (Ashburner and Thompson 1978), yet this situation prohibits detection of rare horizontal transmission events that might be vectored by mites. Further, maintaining *Drosophila* as monocultures does not accurately mimic conditions in nature. There is experimental evidence that mites can serve as vectors of both mobile elements and bacteria. Mites are thought to be the vector that introduced *P-elements* into *Drosophila* (Houck et al. 1991). Mites have also been shown to be able to transfer *Spiroplasma* endobacteria from one species of *Drosophila* to another (Jaenike et al. 2007); *Macrocheles subbadius* mites acquired *Spiroplasma* infection after feeding on the hemolymph of infected *D. nebulosa* and were then able to transmit the infection to *D. willistoni* (Jaenike et al. 2007). This provides evidence that horizontal transmission of endobacteria via ectoparasitic mites is possible in *Drosophila*.

The endobacterial species *Wolbachia pipientis* is an obligate intracellular α-proteobacterium, a relative of the ancient α-proteobacterium that gave rise to mitochondria. *Wolbachia* mainly infect arthropod species and are thought to infect more than 20 % of all insect species (McGraw and O'Neill 2004; Tram et al. 2003; Iturbe-Ormaetxe and O'Neill 2007; Hilgenboecker et al. 2008). As obligate intracellular bacterial species, *Wolbachia* are passed vertically to the offspring through the egg cytoplasm (Serbus and Sullivan 2007). As a result, *Wolbachia* have evolved numerous means to manipulate host reproduction to enhance their vertical transmission, so to ensure propagation to the next generation. While the effect of *Wolbachia* infection ranges from parasitic to symbiotic, depending on the *Wolbachia* strain, titer, and the host organism (Serbus and Sullivan 2007), these alterations of host biology can cause deleterious effects. These effects include altering the female-male sex ratios by inducing parthenogenesis, feminization of genetic males or male killing, or by impacting offspring production and survival through cytoplasmic incompatibility, the most widely studied reproductive consequence of *Wolbachia* infection (Harris and Braig 2003; McGraw and O'Neill 2004; Iturbe-Ormaetxe and O'Neill 2007). These reproductive effects are caused by modifications to sperm and oocyte chromatin, which implies that *Wolbachia* can have widespread epigenetic effects on host gene expression (Clark et al. 2005; McGraw and O'Neill 2004).

Wolbachia are not usually considered to be susceptible to horizontal transmission—direct transfer between host individuals of the same generation. However, horizontal transfer of *Wolbachia* can be achieved experimentally through microinjection of infected egg cytoplasm between strains (Boyle et al. 1993; Schilthuizen and Stouthamer 1997; Van Meer and Stouthamer 1999; Dobson et al. 2002; Merçot and Charlat 2004; Xi and Dobson 2005). The presence of similar *Wolbachia* genomes in distantly related arthropods suggests horizontal transfer can also occur in nature (O'Neill et al. 1992; Zhou et al. 1998; Vavre et al. 1999; Schulenburg et al. 2000; Casiraghi et al. 2001; Tram et al. 2003). As *Wolbachia* typically induce reproductive isolation in their hosts, it is reasonable to postulate that these instances of horizontal transmission involve a vector (Vavre et al. 1999).

Parasites have been shown to be able to acquire *Wolbachia* from their insect hosts; *Trichogramma* and *Leptopilina* parasitic wasps can become infected with *Wolbachia* when parasitizing *Wolbachia*-infected hosts (Heath et al. 1999; Huigens et al. 2004) and in woodlice, *Wolbachia* has been transferred by contact with an infected host (Rigaud and Juchault 1995). Additionally, a monophyletic relative of *Wolbachia*, *Rickettsia,* also obligate endobacterium, can be horizontally transmitted to mammals through arachnid vectors such as ticks (Anderson and Karr 2001). Thus, it is possible that parasites such as mites might not only acquire *Wolbachia* from *Wolbachia*-infected hosts but also transmit it to other host individuals, allowing occasional horizontal transfer of *Wolbachia*.

We observed that when initially *Wolbachia*-free stocks were placed into our non mite-free room, they could become *Wolbachia*-infected with distressing frequency, often within a generation or two. Because there is abundant speculation (Jaenike et al. 2007) and empirical evidence (Rozhok et al. 2011) in the literature that mites can transmit *Wolbachia*, we tested the *Tyrophagus putrescentiae* (Astigmata: Acaridae) mites from these *Wolbachia*-infected and uninfected stocks to determine whether these mites could transmit *Wolbachia* infection between fly stocks. We found that the *Drosophila*-specific *wMel* strain of *Wolbachia* could be isolated from the mites infesting *Wolbachia*-infected *Drosophila* stocks. We then tested these mites for their ability to invade non-mite infested and non *Wolbachia*-infected *Drosophila* stocks and transmit *Wolbachia* to these flies. We found that mites were able to invade the mite-free stocks, and this was coupled with novel *Wolbachia* infection in the previously *Wolbachia*-free stocks; this infection occurred within 5 fly generations. This provides evidence that *Wolbachia* transfer between *Drosophila* laboratory populations can occur via *T. putrescentiae*. Mite-vectored *Wolbachia* transmission may also occur in natural settings and as *Wolbachia* has manifold effects on host reproduction and gene expression, mite-vectored bacterial transfer could be more biologically important that generally appreciated.

Methods

Drosophila *stock maintenance*

All *D. melanogaster* stocks were maintained at 25 ± 5 °C in 55 ml glass vials stoppered with foam bungs, all autoclaved prior to fly introduction to destroy any contaminating mites. The bungs are porous enough to allow passage of mites. The stocks were cultured on a standard cornmeal-agar-yeast-molasses medium containing methylbenzoate (0.125 %) to inhibit mold growth. All fly stocks were routinely stored on paper towel soaked with 0.1 % benzyl benzoate to inhibit mite transfer between vials. This step was omitted for the infection transfer experiment.

Mite identification and collection

Mite species was determined morphologically by H. Proctor, University of Alberta. Multiple attempts at molecular species identification by CO1 barcoding using "universal" invertebrate primers (Folmer et al. 1994) served to only detect *Drosophila* CO1 sequence, presumably from the mite guts. Live *T. putrescentiae*, were located and scraped from the food and vial surfaces of *Wolbachia*-infected and uninfected *Drosophila* stock vials using fine forceps, a fine paintbrush or a spatula. Mites were transferred to 1.5 ml microfuge

tubes for DNA extraction or to a fly-containing vial for testing of infection transfer. Mites were not sorted for sex or developmental stage.

Infection transfer experiment

Wolbachia infection transfer by mites between *Drosophila* strains was tested using uninfected *In(1)w*^{m4} (FBst0000807) and infected *O.R* strains (FBst0002376). Infection status was confirmed by PCR as described below. Mites were removed from fly stocks by rapid serial transfer to fresh vials as recommended by Ashburner and Thompson (1978). The stocks were then visually checked for mite contamination. To begin the experiment, approximately 20 mites from a mite-infested *Drosophila* stock were transferred to the *O.R* mite-free stock; no mites were added to subsequent fly generations but mites were passively transferred to new vials as the flies were transferred into new food vials each generation (Fig. 1). The *O.R* vial with mites and the *w*^{m4} vial (initially) without mites were held together with an elastic band and placed in a plastic basket in a mite-free room at 25 °C. The outsides of both vials, the basket, and the elastic were wiped with ethanol to remove any invading mites. The flies were allowed to proceed to further generations in a mite-free incubator without any other fly stocks. Each generation of flies was visually

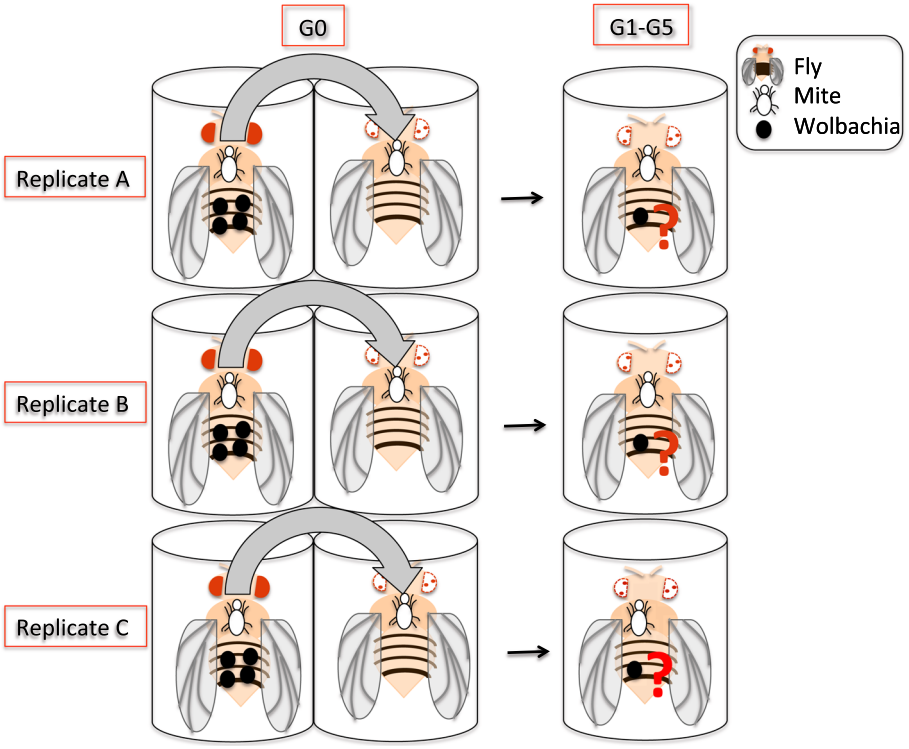

Fig. 1 Experimental design. Two mite-free fly stocks, *Wolbachia*-infected *O.R* (*red eyes*) and *Wolbachia*-uninfected *w*^{m4} (*white eyes*), were kept together in a mite-free location. Mites were introduced to the *O.R* stock and over 5 generations, the *w*^{m4} stock was examined for mite and *Wolbachia* contamination. This test of transmission was performed in triplicate, with all triplicates performed simultaneously, but physically isolated in different areas of a mite-free room. (Color figure online)

inspected to ensure that no flies had transferred between stocks, and to check if mites were present.

DNA extraction

DNA was extracted using the AquaGenomic (MultiTarget Pharmaceuticals) *Drosophila* protocol adapted from the manufacturer's tissue protocol with the following amendments; 30 µl of 1 mM Tris–HCl (pH 7.6) was added after air drying the pellet, DNA was re-suspended at 60 °C for 1 h. Extracted DNA was stored at −20 °C until further use. Five whole female flies and approximately 20 whole mites were used for DNA extraction. Any contaminating mites were removed from the flies before each DNA extraction.

Polymerase chain reaction for Wolbachia

The *Wolbachia* infection status of the starting (G0) and subsequent generations (G1, G2, G3, G4, G5) of the w^{m4} and *O.R* stock was determined by PCR. *Wolbachia* was detected by 2 rounds of amplification of 2 sections of the *Wolbachia surface protein* gene (*wsp*) using forward primer 5′-GACCCAGCAAATACTATTGCAGACA-3′, and reverse primer 5′-AG CGGGTTCCAAAGGAGTGC-3′, with an annealing temperature of 53 °C, producing a 150 bp amplicon (Jaenike et al. 2010), and the forward primer 5′-TGGTCCAATA AGTGATGAAGAAACTAGCTA-3′ with the reverse primer 5′-AAAATTAAAGTAC TCCAGCTTCTGCAC-3′ with an annealing temperature of 61.6 °C amplifies a 548 bp fragment, which with the flanking primers, generates a 605 bp amplicon (Braig et al. 1998). A portion of the *Drosophila garnet* gene was amplified as a positive control for DNA integrity using the forward primer 5′-CTCTTTGAGTTTGGGAAATGC-3′ and the reverse primer 5′-TACAAATGCTGGGCTACGAC-3′ with an annealing temperature of 58 °C generating a 501 bp amplicon.

All amplifications used GoTaq Green Master Mix (Promega), and used 95 °C for 5 min for denaturation, 35 cycles at 30 s at 95 °C, 30 s at 57 °C along a gradient of ±5 °C to satisfy the annealing temperatures of the primers, and 72 °C for 40 s, followed by a final elongation (72 °C for 5 min). The second round of amplification of *Wolbachia* DNA from mites used 5 µl of the first round PCR product for each sample, with the same primer sets as the first round; the negative control used water in place of DNA. The PCR products were electrophoresed on 1.2 % agarose gel with 1× sodium borate buffer stained with SYBR Safe DNA gel stain (Cedarlane). Sequencing was performed by Genome Québec at McGill University. Sequence alignments and analyses were performed using SeaView, Codon-Code Aligner, and MEGA.

Drosophila/*mite video preparation*

Day old *Drosophila* and mite carcasses, killed by freezing overnight, live mites and live *Drosophila* larva were placed, in various combinations, in the well of a 9.5 cm^2 culture plate with 2 ml 2 % agar on the bottom. Sequential still images were obtained using a EZ4D dissecting scope (Leica) and integrated camera programmed with LAS EZ through Macro Express to take photographs every 30 s, illuminated by an L2 fiber-optic illuminator (Leica). For photographs taken under red light, the illuminator lenses were masked with 3 M lithography tape and otherwise performed identically. The images were rendered and

edited using Photoshop CS4 (Windows) at 10 frames/s, therefore every second of video represents 300 s of real time.

Results and discussion

The goal of this study was to determine whether *T. putrescentiae* mites could transmit *Wolbachia* between *D. melanogaster* individuals.

Wolbachia *DNA is present in mites*

If mites can transmit *Wolbachia*, one would expect mites present in a *Wolbachia*-infected *Drosophila* stock to, themselves, contain *Wolbachia*. Mites were collected from a *Wolbachia*-infected *Drosophila* stock, their DNA extracted and *Wolbachia* DNA amplified. Two *Wolbachia surface protein* (*wsp*) fragments of approximately 550 and 150 bp were amplified from a sample of 15–20 whole mites, indicating the presence of *Wolbachia* DNA within the mites (Fig. 2). Sequence analysis of the 150 bp product showed that it aligned to bases 223–369 of the *wsp* gene obtained from *Wolbachia* strain typically found in *Drosophila* (Saridaki et al. 2011) (Fig. 3). Evidence of a *Drosophila*-specific strain of *Wolbachia* in mites is completely consistent with the results of Rozhok et al. (2011) who also found that 635 bp of the *wMel wsp* gene could be isolated from mites living with *Wolbachia*-infected *Drosophila*. Mites have been implicated in other horizontal transfer events, the most well known being the presumed transfer of *P-elements* from *D. willistoni* to *D. melanogaster* (Houck et al. 1991) and the evidence for their role in this transfer was, similarly, the presence of *Drosophila* DNA in mites. However, to extend this analysis, we initiated a more rigorous test of the ability of mites to transfer *Wolbachia* between *Drosophila* strains.

Evidence of Wolbachia *transfer*

Amplification of the *wMel* strain of *Wolbachia* from mites could represent genuine infection of mites with this strain of *Wolbachia*, or alternatively, simply *Wolbachia*-infected

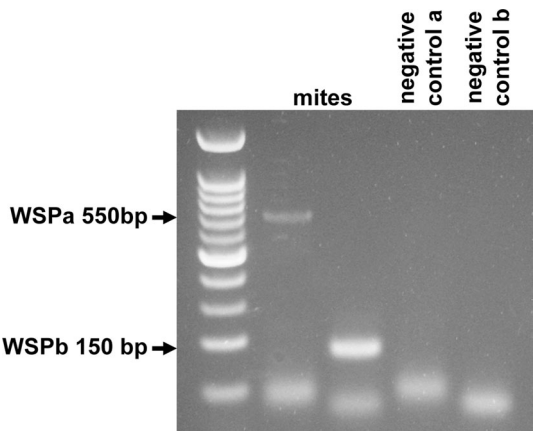

Fig. 2 *Wolbachia* DNA isolated from mites. *Wolbachia* DNA was amplified from mites by two sets of primers specific for *Wolbachia surface protein* (to produce the 550 bp *wsp* amplicon fragment a and 150 bp *wsp* amplicon fragment b). Negative controls for each of the two amplifications are shown to the right (negative control fragment a and fragment b)

```
sel=0       205                                                                272
Wolbachia   TAAAGATGTAACATTTGACCCAGCAAATACTATTGCAGACAGTGTAACAGCAATTTCAGGATTAGTGA
Consensus60 ------------------CCCCGCAAATACTATTGCAGACAGTGTAACAGCAATTTCAGGATTAGTGA

sel=0       273                                                                340
Wolbachia   A-CGTGTATTACGATATAGCAATTGAAGATATGCCTATCACTCCATAC-ATTGGTGTTGGTGTTGGTG
Consensus60 AACGTGTATTACGATATAGCAATTGAAGATATGCCTATCACTCCATACCATTGGTGTTGGTGTTGGTG

sel=0       341                                                                408
Wolbachia   CAGCGTATATTAGCACTCCTTTGGAACCCGCTGTGAATGATCAAAAAAGTAAATTTGGTTTTGCTGGT
Consensus60 CAGCGTATATTAGCACTCCTTTGGAACCC----------------------------------------
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Fig. 3 The consensus sequence of *Wolbachia* DNA amplified from mites. The consensus sequence of *Wolbachia* DNA amplified from mites, aligned from bases 223 to 369 of the GenBank sequence for *wMel Wolbachia surface protein (wsp)*

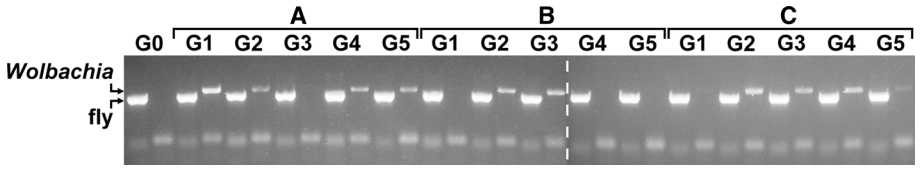

Fig. 4 Changes in *Wolbachia* infection status of previously uninfected and mite-free w^{m4} stocks. The initial uninfected status is indicated by no amplification of *Wolbachia surface protein* in the G0 flies. In subsequent generations, in replicates A, B, and C, *Wolbachia* DNA is amplified as indicated by a *wsp* amplicon. In all cases, confirmation of DNA integrity was confirmed by amplification of the same sample with fly-specific primers ("fly")

Drosophila tissue associated with the mite bodies, either externally or in their gut. To determine if the mite-associated *Wolbachia* were viable and capable of being transmitted, mite infested and *Wolbachia*-infected *Drosophila* stock vials were placed in close proximity to uninfected *Drosophila* stock vials and the previously uninfected stocks monitored for new *Wolbachia* infection occurring concomitantly with mite invasion.

Approximately 15–25 live mites from mite-infested *Wolbachia*-infected fly stocks were collected and transferred to three vials of mite-free but *Wolbachia*-infected *O. R* stocks (Fig. 1). Each of these vials were kept adjacent to a culture vial containing a mite-free and *Wolbachia*-uninfected *In(1)*w^{m4}. This experimental design, rather than the transfer of mites directly to a vial of uninfested *Wolbachia*-free flies, is somewhat analogous to conditions in nature where mites would have to travel along the substrate to encounter flies. These experimental conditions also mimic the conditions that might be occurring in a mite-infested laboratory. Unlike many acarid mites, *T. putrescentiae* does not have a phoretic nymphal stage (Colloff 2009) and previous observations in our lab had indicated that mites had no difficulty walking along surfaces and invading stoppered vials, and could do so in a matter of hours to days. The flies from each previously mite-free and *Wolbachia*-uninfected w^{m4} stock were monitored visually for mite invasion and for *Wolbachia* using PCR for *wsp,* from the original to the fifth generation, to determine if the mites were able to transfer *Wolbachia*.

Visual inspection revealed that mites invaded the previously mite-free w^{m4} vials by day 7, successfully established themselves in the new vials and established breeding populations that persisted through generations 1–5. PCR of 5 whole flies per vial from the w^{m4} stock vials showed no evidence of *Wolbachia* infection in the first, G0, generation (Fig. 4). However, in replicate A, evidence of *Wolbachia* infection was first seen in the next generation, G1. Evidence of infection was evident in all subsequent generations with the exception of

generation 3 (Fig. 4). In replicate B, evidence of *Wolbachia* infection was present within generations 2 and 3 (Fig. 4). In replicate C, *Wolbachia* DNA was stably detectable from generations 2 to 5 (Fig. 4). In no case was there evidence of contamination of the w^{m4} vials by the *O.R* flies, which would have been evident by finding red-eyed flies in the w^{m4} vials. Thus, although initially being uninfected with *Wolbachia*, as determined by amplification of a portion of *wsp*, all three *In(1)*w^{m4} stocks appeared infected within 1–2 generations. These results are consistent with a study by Kozeretska and colleagues who in a preliminary report (Bilousov et al. 2011) and subsequent more detailed report (Rozhok et al. 2011) documented transfer of *Wolbachia* infection by *Tyrophagus noxius* mites. In a similar experimental design, but one in which mites from *Wolbachia*-infected stocks were transferred directly into vials with uninfected flies, they observed stable transfer of *Wolbachia* by generation 9, but not by generation 2. Despite more direct access to the flies in this experimental design, it appears to have taken longer for *Wolbachia* to be transferred. This may reflect the number of *Wolbachia* required to produce an amplicon, as only single round PCR amplification was used in these experiments so that low titer infections in earlier generations might have been missed. Thus, the results presented here and those of Bilousov et al. (2011) and Rozhok et al. (2011) demonstrate that *Wolbachia* can be transmitted to new hosts by mites.

In at least one of the three replicates (C), the infection appeared to be stable, however, in replicates A and B, *Wolbachia* infection appeared rapidly but did not persist throughout the experiment. Similar sporadic maintenance of transferred *Wolbachia* was also found in horizontal transmission in parasitic wasps (Heath et al. 1999; Rigaud et al. 2001; Huigens et al. 2004), presumably due to the failure to efficiently colonize the germ line. The apparent transient infection could represent an infection that failed to establish in the fly germ line, a complex process involving interactions between bacterial and host proteins (Serbus and Sullivan 2007). Alternatively, the number of *Wolbachia* cells may have been too low for successful amplification in the intervening generations. The intermittent and low abundance of *Wolbachia* DNA suggests that the number of cells transferred is low and that they may not necessarily establish immediately in the new host. Additional mite colonization events and/or infection from potentially *Wolbachia*-infected progeny of the originally invading mites are also a possible source of infection of the *Drosophila* stocks. Similarly, it is not clear whether *Wolbachia* establish in the germ line of the mites and are transmitted transovarially or whether continued exposure to *Wolbachia*-infected *Drosophila* is necessary for *Wolbachia* to be present in mites. Regardless of the ease of establishing stable vertically-transmitted infections, in all replicates, mites appeared able to transfer *Wolbachia* to previously uninfected *Drosophila* hosts. Further, this transfer occurred within a few generations and may have generated *Wolbachia* infections that were stable over generations.

Possible mechanism of Wolbachia *transfer*

Tyrophagus putrescentiae mites are a common pest of stored products and of *Drosophila* cultures but are not known to prey upon living flies, so it was not entirely clear how they could obtain *Wolbachia* from flies and how other flies could obtain it from them. However, these mites are known to feed upon dead and decaying bodies (Braig and Perotti 2009) so one possible mechanism for the *Wolbachia* transfer is through mutual consumption of corpses, possibly compounded by cannibalism by *Drosophila* larvae. To see if mites were eating *Drosophila* corpses, and if *Drosophila* larvae would eat mite-infested *Drosophila* corpses, we used time-lapse photography to capture the behavior of mites in the presence of dead flies, and *Drosophila* larvae in the presence of dead mite-infested adult fly corpses

(Fig. 5 and Supplemental Videos S1 and S2). These images show evidence of mites consuming fly tissue, including ovaries that typically contain a high titer of *Wolbachia* (Dobson et al. 1999). Similarly, imaging of second and third instar larva revealed ready ingestion of dead adult flies. Immature eggs within dead female carcass seemed especially attractive. Additionally, third instar larva were imaged ingesting dead mites.

The ingestion of carcasses or eggs could be a possible source of *Wolbachia* introduction, complementing the usual vertical transmission of these bacteria by inheritance in the egg cytoplasm. As *Wolbachia*-infected mites contaminate a fly stock, the fly larvae can eat the mites, either dead or alive as they scavenge the corpses of dead adults. The larvae could then become infected with *Wolbachia*. New mites may be able to acquire this infection by feeding on the corpses and *Wolbachia*-rich eggs of the adult flies. This scenario relies on *Wolbachia* surviving, if not necessarily replicating, in extracellular conditions and being able to invade new cells. Rasgon et al. (2006) have demonstrated that *Wolbachia* are viable for at least a week outside of cells and that these cells were able to establish subsequent infection. An alternative mode of transmission may be direct transfer or injection of *Wolbachia*-infected host cells by mites during the feeding process, as was proposed for *P-element* transfer by *Proctolaelaps regalis* mites (Houck et al. 1991).

Endobacteria such as *Wolbachia* can provide invaluable insight into the acquisition and evolution of eukaryotic organelles. Because *Wolbachia* has, for the most part, not yet evolved a symbiotic relationship with the host, it is profoundly valuable in examining the initial molecular negotiations between an endobacterium and its host eukaryotic cell (Clark et al. 2005; Iturbe-Ormaetxe and O'Neill 2007). These complex networks of interactions between the endobacterium and host cell have optimized vertical transmission through host eggs, however, in this work we show that mites can mediate horizontal transmission of *Wolbachia*. Traditionally, *Drosophila* mites have been primarily studied in the context of the threat they poses to delicate fly stocks. However, this work shows that mites may pose a greater threat to *Drosophila* research than previously appreciated. Horizontal transmission of *Wolbachia* by mites can be problematic for *Drosophila* research. *Wolbachia* is capable of inducing a variety of reproductive effects in the fly and has widespread effects on gene expression, including epigenetic effects (Clark et al. 2006; Xi et al. 2008; Zheng et al. 2011). So both the presence of *Wolbachia* and its capacity for rare horizontal transmission must be taken into account in laboratory experiments. In nature, rare instances of horizontal transmission vectored by mites could also occur, as mites are present on

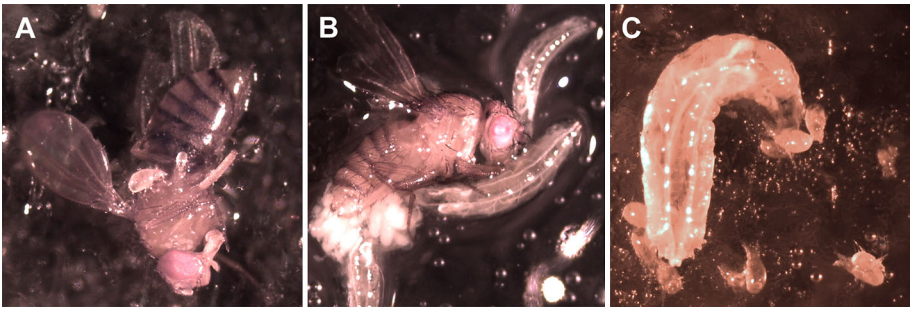

Fig. 5 Still images obtained from time-lapse photography of fly and mite interactions, suggesting a potential mechanism for *Wolbachia* acquisition and transfer. **a** Mites are observed ingesting fly carcasses. **b** Second and third instar *Drosophila melanogaster* larva were observed ingesting fly carcasses including hemolymph and immature eggs. **c** Third instar larva (observed under *red light*) ingesting dead mites. (Color figure online)

Drosophila individuals in the wild, and *Drosophila* and mite population densities can be high at feeding and breeding sites. Horizontal transmission of endobacteria, although probably rare in nature, may be evolutionarily significant and the role of mites in mediating this transfer warrants further investigation.

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Conflict of interest The authors declare that they have no conflict of interest.

Ethical standard All applicable international, national, and/or institutional guidelines for the care and use of animals were followed and all procedures performed in studies involving animals were in accordance with the ethical standards of the institution at which the studies were conducted.

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