

# Social parasitism in fire ants (*Solenopsis* spp.): a potential mechanism for interspecies transfer of *Wolbachia*

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## Abstract

One possible mechanism for interspecific transfer of *Wolbachia* is through the intimate contact between parasites and their hosts. We surveyed 10 species of fly parasitoids (*Pseudacteon* spp.) and one inquiline social parasite, *Solenopsis daguerrei*, for the presence and sequence identity (*wsp* gene) of *Wolbachia*. Two *Wolbachia* variants infecting *S. daguerrei* were identical to known variants infecting the two common ant host species, *Solenopsis invicta* and *Solenopsis richteri*, suggesting possible transfers of *Wolbachia* between this parasite and their hosts have occurred. Our data also revealed an unexpectedly high diversity of *Wolbachia* variants within *S. daguerrei*: up to eight variants were found within each individual, which, to our knowledge, is the highest reported number of *Wolbachia* variants infecting a single individual of any host species.

**Keywords:** horizontal transmission, *Pseudacteon*, social parasitism, *Solenopsis* ants, *Wolbachia*, *wsp*

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## Introduction

*Wolbachia* are a genus of intracellular bacteria infecting numerous arthropods and filarial nematodes. In arthropods, these bacteria often manipulate the reproduction of their hosts to increase their own transmission by distorting the host-sex ratio or by inducing cytoplasmic incompatibility (for recent reviews see Werren 1997; Stouthamer *et al.* 1999). Recent surveys have suggested that *Wolbachia* infect a substantial proportion of insect species, with estimates ranging from 17% (Werren *et al.* 1995a; West *et al.* 1998; Werren & Windsor 2000) to 76% (Jeyaparakash & Hoy 2000). Extrapolation of these estimates indicates that *Wolbachia* are certainly one of the most widespread parasites on earth.

Although *Wolbachia* predominantly are maternally transmitted, it is clear from phylogenetic and experimental data as well as their widespread distribution among numerous taxa that they are also transmitted horizontally among arthropod species (O'Neill *et al.* 1992; Werren *et al.* 1995b; Schilthuisen & Stouthamer 1997; Heath *et al.* 1999; Van Meer *et al.* 1999; Vavre *et al.* 1999; Huigens *et al.* 2000; Noda *et al.* 2001; Huigens *et al.* 2004). While the mechanisms of

such interspecific horizontal transfer of *Wolbachia* are poorly understood, one commonly suggested mechanism is that they are transferred between insect hosts and their associated parasitoids. Although the findings of several studies support this mechanism of transfer (Schilthuisen & Stouthamer 1997; Van Meer *et al.* 1999; Vavre *et al.* 1999; Noda *et al.* 2001), the findings of several other studies do not (Schilthuisen & Stouthamer 1998; West *et al.* 1998; Shoemaker *et al.* 2002). These conflicting results underscore the need for additional studies examining the relative importance of parasitoids in transferring *Wolbachia* as well as attempting to identify other possible mechanisms of horizontal transmission of *Wolbachia*.

Ants potentially represent an appealing insect group for studying horizontal transmission of *Wolbachia* for three main reasons. First, recent studies have revealed a high incidence of *Wolbachia* infections in many ant species from a number of genera (Wenseleers *et al.* 1998; Jeyaparakash & Hoy 2000; Shoemaker *et al.* 2000; Shoemaker *et al.* 2003a; Tsutsui *et al.* 2003; Van Borm *et al.* 2003). Second, many ant species are major predators of a diversity of invertebrate species (Hölldobler & Wilson 1990) from which they potentially may acquire *Wolbachia*. Third, many ant species commonly share natural enemies, including parasitoids and other parasites (Schmid-Hempel 1998), that could serve as vectors for *Wolbachia* transfer.

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In this study, we surveyed for the presence and identity of *Wolbachia* infections in parasites that attack the two fire ant species, *Solenopsis invicta* and *Solenopsis richteri*. These parasites comprise 10 fly parasitoid species of the genus *Pseudacteon* (Diptera: Phoridae) and the inquiline ant social parasite *Solenopsis daguei* (Hymenoptera: Formicidae). We hypothesized that if one or more of these parasite species has played a role in *Wolbachia* transfer, then one might expect that both the parasite and the host ant species harbour identical or nearly identical *Wolbachia* variants. To test this hypothesis, we sequenced a portion of the *Wolbachia* genome (*wsp* gene) from individuals representing each *Wolbachia*-infected parasite species, and subsequently compared these sequence data to existing sequences representing the same gene region of *Wolbachia* infecting *S. invicta* and *S. richteri*.

## Materials and methods

### Samples and molecular analyses

*Pseudacteon* flies were collected from the surface of mounds of various *Solenopsis* species, including *Solenopsis invicta*. Individuals of the inquiline social parasite *Solenopsis daguei* were collected from within three nests of *S. invicta* (see Table 1). Total DNA was extracted from each individual using the Puregene DNA Isolation Kit (Gentra Systems). A portion of the *Wolbachia* genome (*wsp*) was amplified by

polymerase chain reaction (PCR) using the primers *wsp81-F* and *wsp691-R* (Zhou *et al.* 1998). These primers amplify a 575–625 bp portion of a highly variable gene encoding a bacterial surface protein, *wsp* (Zhou *et al.* 1998). PCR-amplified products were then purified and sequenced. For a detailed description of PCR reaction mixes, thermal cycling profiles, and sequencing reaction protocol, see Shoemaker *et al.* (2003b). In cases where our sequencing results revealed the presence of more than one *Wolbachia* variant, as evident in multiple peaks or frameshifts in electropherogram profiles, the *wsp* PCR amplicons were cloned into a vector (TOPO TA Cloning Kit, Invitrogen Corp.). Colonies were screened for the presence of the desired *wsp*-PCR insert using the primers previously discussed. PCR-amplified products from nine to 21 colonies representing each individual were purified and sequenced as previously described.

### Phylogenetic analyses

Only nonredundant *Wolbachia* (*wsp*) sequences from each species were used for tree-based phylogenetic methods. We also included in our analyses a subset of published sequences from other insects obtained from GenBank, including a representative of each unique *wsp* sequence found previously in *S. invicta* and *S. richteri* (see Fig. 1). For all analyses, the third hypervariable region of *wsp* was removed (Zhou *et al.* 1998; Shoemaker *et al.* 2002). The

**Table 1** Collecting locality and *Wolbachia* infection status of each parasite species attacking fire ants (*Solenopsis* spp.).

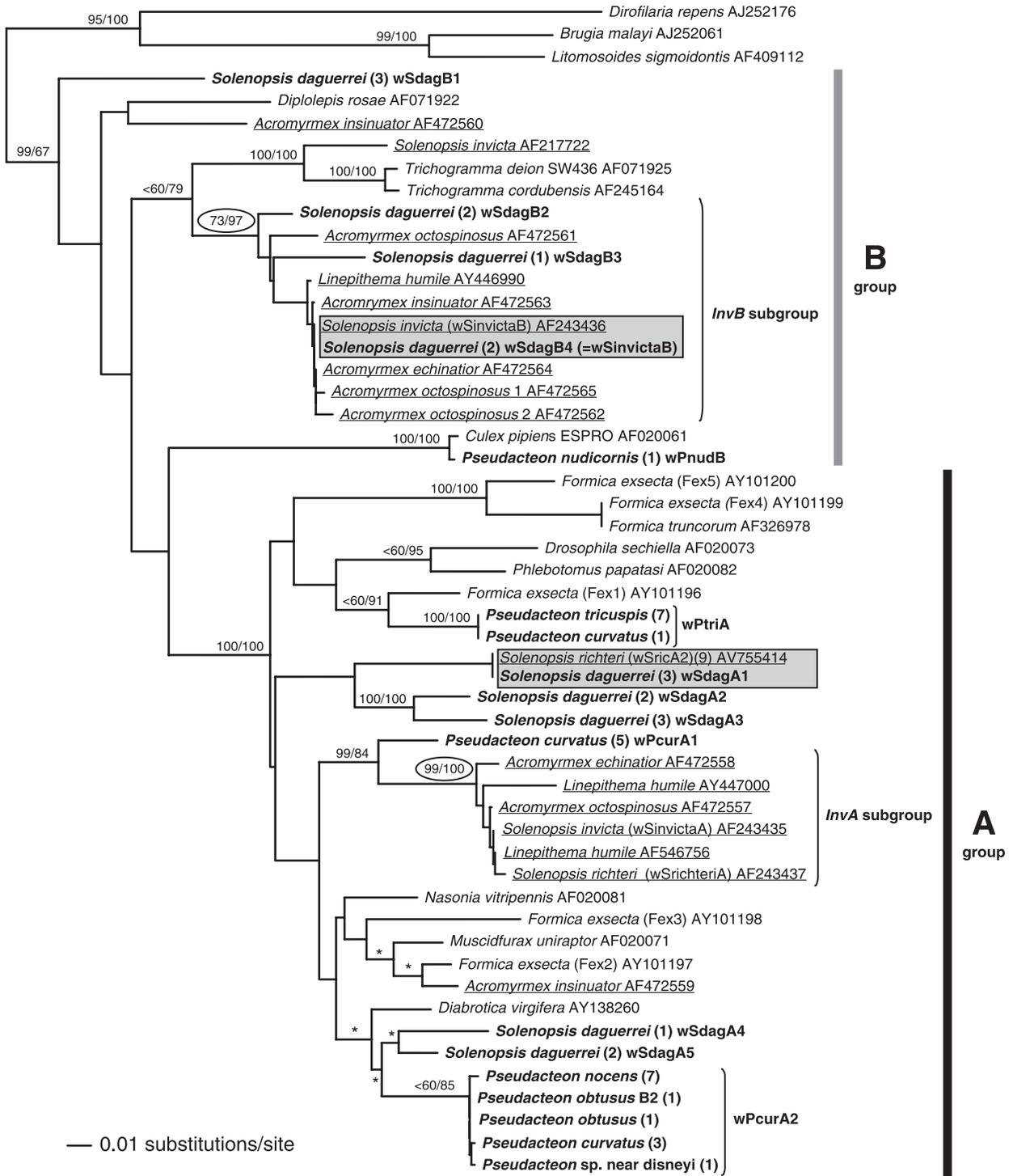
Species	Population	N <sup>A</sup>	n <sub>inf</sub> <sup>B</sup>	n <sub>wsp</sub> <sup>C</sup>	<i>Wolbachia</i> ( <i>wsp</i> ) Strains
<b><i>Pseudacteon</i> spp. (parasitoid flies)</b>					
<i>Pseudacteon borgmeieri</i>	Collected from the surface of	1	0	0	—
<i>Pseudacteon cultellatus</i>	nests in the field in Argentina	2	0	0	—
<i>Pseudacteon curvatus</i>	(Buenos Aires, Corrientes, Formosa, and Tucuman),	5	5	14 (3)	wPcurA1 (2) <sup>D</sup> ; wPcurA1 + wPcurA2 + wPtriA (1)
<i>Pseudacteon litoralis</i>	Bolivia (Tarija), or from field	4	0	0	—
<i>Pseudacteon</i> sp. near <i>disneyi</i>	collected nests (Corrientes, Formosa, and Buenos Aires)	1	1	1	wPcurA2 (1)
<i>Pseudacteon nocens</i>	that were returned to the	3	3	3	wPcurA2 (3)
<i>Pseudacteon nudicornis</i>	laboratory.	4	1	1	wPnudB (1)
<i>Pseudacteon obtusus</i>		3	1	1	wPcurA2 (1)
<i>Pseudacteon obtusus</i> (B)		2	1	9 (1)	wPcurA2 (1)
<i>Pseudacteon tricuspis</i>		5	5	17 (4)	wPtriA (4)
<b><i>Solenopsis daguei</i></b> (inquiline social parasite)	Resistencia, Argentina	1	1	15	wSdagA1 + wSdagA3 + wSdagA5 + wSdagB1
	Guaira, Brazil	1	1	21	wSdagA1 + wSdagA2 + wSdagA3 + wSdagA4 + wSdagB1 + wSdagB2 + wSdagB3 + wSdagB4 (= wSinvictaB)
	Planalto, Brazil	1	1	21	wSdagA1 + wSdagA3 + wSdagA5 + wSdagB1 + wSdagB2 + wSdagB4 (= wSinvictaB)

<sup>A</sup>N equals number of individuals surveyed for *Wolbachia* infection.

<sup>B</sup>n<sub>inf</sub> equals the number of individuals infected with *Wolbachia*.

<sup>C</sup>n<sub>wsp</sub> equals the total number of *wsp* sequences (number of individuals in parentheses).

<sup>D</sup>values in parentheses represent the number of individuals with a particular *Wolbachia* strain or combination of strains.



**Fig. 1** Neighbour-joining (NJ) tree of *wsp* sequences from *Solenopsis daguerrei* and various *Wolbachia*-infected *Pseudacteon* spp. (this study, in bold) as well as representative *wsp* sequences from other insects including the New World ants obtained from GenBank. Numbers along branches represent posterior probability values from Bayesian analyses followed by bootstrap support values from NJ analyses. Only values greater than 60% for both analyses are shown. Asterisks (\*) indicate additional nodes with bootstrap and posterior probability values greater than 60%. The values at the nodes of the two clades containing the two subgroups of *Wolbachia* previously discussed to be specific to the New World ants (*InvA* and *InvB*, indicated by brackets) are encircled. All *wsp* sequences obtained from the New World ants are underlined. The vertical black and grey bars represent the A and B groups of *Wolbachia*, respectively. The two instances where *S. daguerrei* and one of its ant hosts harbour identical *wsp* sequences (presumed cases of horizontal transfer) are indicated by shaded boxes (see text).

program MODELTEST (Posada & Crandall 1998) was used to find the best-fit model of sequence evolution and genetic distance values, which were then calculated between pairs of sequences using this model (GTR +  $\Gamma$ , with  $\Gamma = 0.51$ ). The resulting distance data matrix was used to infer relationships among the *wsp* sequences using neighbour-joining (NJ) methods as implemented in the program PAUP\* version 4.0b10 (Swofford 1999) while Bayesian methods were implemented using MRBAYES version 2.0 (Huelsenbeck & Ronquist 2001). For NJ analyses, ties were broken randomly and the support for particular nodes within the NJ tree was assessed using bootstrap analyses performed with 50 000 data resamplings. Bayesian analyses were used to generate posterior probability distribution values for clades (Huelsenbeck & Ronquist 2001; Ronquist & Huelsenbeck 2003). No a priori assumptions about the tree topology were made and all metropolis coupled Markov chain Monte Carlo (MCMC) searches were provided with a uniform prior based upon the values obtained from MODELTEST. We performed four independent searches, each of which was started from a random tree, run with four chains and for 4 million generations with every 100th tree sampled to obtain 40 001 sample points. Posterior probabilities (or the percentage of samples that recover any particular clade) were calculated using the trees visited by the Markov chains after the burn-in samples (i.e. the number of generations required for samples to reach stationarity as determined by visual inspection of the log-likelihood values) were discarded.

## Results

### *Wolbachia* variants in *Pseudacteon* flies

Our survey revealed that individuals representing seven of the 10 *Pseudacteon* species were infected with *Wolbachia* (Table 1). Out of 46 *wsp* sequences from these seven species, only four unique sequences were detected indicating that different *Pseudacteon* species harbour identical *Wolbachia* variants [deposited in European Molecular Biology Laboratory (EMBL) and GenBank data libraries under Accession nos AY878108–AY878111]. Multiple infections were detected only in *Pseudacteon curvatus*. Crucially, none of the 46 *wsp* sequences from the infected *Pseudacteon* flies was identical or even closely related to the *wsp* sequences obtained from infected *Solenopsis invicta* and *Solenopsis richteri*.

### *Wolbachia* variants in *Solenopsis daguerrei*

All three *Solenopsis daguerrei* individuals surveyed were *Wolbachia*-infected and our preliminary sequence analyses revealed that each individual contained more than one *wsp* variant. Subsequent analyses of 57 *wsp* sequences revealed nine unique sequences, presumably representing nine dif-

ferent *Wolbachia* variants (deposited in EMBL and GenBank data libraries under Accession nos AY878099–AY878107; Table 1). The percentage of *wsp* nucleotide sequence dissimilarity among these nine variants ranged from 3.8 to 21.8%. The number of different *Wolbachia* variants found within each *S. daguerrei* individual ranged from four to eight. Furthermore, six of these nine variants were common to more than one individual. Two of the *wsp* sequences found in *S. daguerrei* were identical to the *wsp* sequences obtained from their ant hosts *S. invicta* and *S. richteri*: wSdagA1 is identical to wSricA2 obtained from nine individuals of *S. richteri*, and wSdagB4 to wSinictaB commonly found in *S. invicta* (Shoemaker *et al.* 2000, 2003b).

### Phylogenetic analyses

The neighbour-joining (NJ) and Bayesian analyses recovered trees virtually identical in topology (Fig. 1). Consistent with earlier studies, the sequences representing the A and B groups of *Wolbachia* cluster together. Further, our results are also consistent with those of Van Borm *et al.* (2003) showing that the *wsp* sequences from free-living species of the New World ants fall into one of two well-supported clades (i.e. subgroups *InvA* and *InvB*) at the exclusion of *wsp* sequences from all other insects, with two exceptions: *S. invicta* from the United States (Jeyaprakash & Hoy 2000) and *S. richteri* from Arroio dos Ratos, Brazil (D.D.S., unpublished).

None of the four unique *wsp* sequences obtained from *Wolbachia*-infected *Pseudacteon* flies falls within either well-supported subgroup containing the New World ant *Wolbachia* variants, although one variant (wPcurA1) is a sister group to the variants within the *InvA* subgroup (Fig. 1). The nine *wsp* sequences we obtained from the social parasite *S. daguerrei* are distributed across the A and B *Wolbachia* groups. Furthermore, only three of the nine *wsp* sequences fall within the subgroup *InvB*, and none falls within subgroup *InvA*.

## Discussion

Numerous lines of evidence indicate that *Wolbachia* are transmitted horizontally between arthropod species. Although the mechanisms of such interspecies transmission in nature are poorly understood, one suggested mechanism is that *Wolbachia* are transferred between hosts and their parasitoids (O'Neill *et al.* 1992; Werren *et al.* 1995b; Schilthuisen & Stouthamer 1997; Heath *et al.* 1999; Van Meer *et al.* 1999; Vavre *et al.* 1999; Huigens *et al.* 2000; Noda *et al.* 2001; Huigens *et al.* 2004). In this study, we found no evidence for horizontal transmission of *Wolbachia* between the two fire ant species *Solenopsis invicta* and *Solenopsis richteri* and their associated parasitoid flies of the genus *Pseudacteon*. Indeed, although many *Pseudacteon* species are *Wolbachia*-infected (at least seven out of 10 species), our analyses

revealed that none of the *Wolbachia* variants infecting these flies is identical or even closely related to *Wolbachia* variants known to infect *S. invicta* and *S. richteri*. Thus, despite the intimate interactions among them, these fly parasitoids do not appear to be candidates for transferring *Wolbachia* infections among *Solenopsis* ant species, lending further support to the idea that host–parasitoid associations are certainly not the only mechanism of horizontal transmission of *Wolbachia* (Schilthuizen & Stouthamer 1998; West *et al.* 1998; Shoemaker *et al.* 2002).

In contrast, our results do suggest horizontal transmission between the inquiline social parasite *Solenopsis daguerrei* and its hosts. Indeed, two of the nine *Wolbachia* variants infecting *S. daguerrei* are identical to the variants present in *S. invicta* and *S. richteri*. The possibility that the sharing of identical *Wolbachia* variants is because of the contamination of host tissues resulting from the intimate association between the ant host and its social parasite [a possibility recently raised by Hughes *et al.* (2004) for certain host–parasitoid systems] can be ruled out because all *S. daguerrei* individuals harbouring *Wolbachia* variants identical to *S. richteri* (wSricA2) were collected from nests of another species, *S. invicta*. The alternative hypothesis that the sharing of *Wolbachia* variants between *S. daguerrei* and its hosts as a result of shared ancestry also seems very unlikely. First, *S. daguerrei* is not closely related to *S. invicta* and *S. richteri* within the *Solenopsis saevissima* species-group (Pitts 2002), and more important, several species more closely related to *S. daguerrei* are not infected with *Wolbachia* (Shoemaker *et al.* 2000). Also, our phylogenetic analyses show that the shared *Wolbachia* variant infecting *S. daguerrei* and *S. richteri* (wSdagA1) is unique to these two species and differs from all other *Wolbachia* variants infecting the New World ants (including other *Solenopsis* spp.), ruling out the possibility that this variant has been coinherited in *S. daguerrei* and *S. richteri* from a common ancestor. Thus, the most parsimonious explanation for the sharing of identical *Wolbachia* variants between *S. daguerrei* and the host ants *S. invicta* and *S. richteri* is that it results from horizontal transmission of *Wolbachia*. Given the intimate interactions between inquiline social parasites and their hosts (i.e. feeding by trophallaxis, caring of eggs by workers; Hölldobler & Wilson 1990), one can easily envision sufficient opportunities for *Wolbachia* to be transferred from the host to the social parasite and, possibly, from the social parasite to the host.

Another intriguing finding of our study is the high number of *Wolbachia* variants that each individual of *S. daguerrei* harbours compared with individuals of free-living fire ant species, which rarely harbour more than one *Wolbachia* variant (Shoemaker *et al.* 2000, 2003b). Indeed, we found eight *Wolbachia* variants within a single individual of *S. daguerrei*, which to our knowledge, is the highest number of coinfecting variants reported in a single host individual of any species. Furthermore, in contrast to most

of the *Wolbachia* variants infecting the New World ants, phylogenetic analyses revealed that many *Wolbachia* variants in *S. daguerrei* do not belong to the *InvA* and *InvB* subgroups but rather are quite divergent and scattered across the *Wolbachia* A and B groups (Fig. 1). The coexistence of multiple and unrelated *Wolbachia* infections suggests that the biology of *S. daguerrei* allows individuals to frequently acquire new *Wolbachia* variants by horizontal transmission, and that the new *Wolbachia* can persist within host populations. While multiple *Wolbachia* variants may also be the result of recombination, which has been demonstrated to occur in *Wolbachia* (Jiggins *et al.* 2001; Werren & Bartos 2001; Reuter & Keller 2003), our analyses of the nine *wsp* sequences from *S. daguerrei* did not reveal any obvious patterns consistent with recombination. In any case, regardless of the roles of recombination and horizontal transmission in the origin of multiple *Wolbachia* infections in *S. daguerrei*, an interesting question that remains is why do individuals of this parasitic ant species harbour such a diversity of *Wolbachia* variants in contrast to their free-living ant host species. One speculative hypothesis is that the lifestyle of ants (free-living vs. parasite) may influence the spread and the persistence of *Wolbachia* within host populations.

In conclusion, our data suggest that social parasitism in ants may represent an additional mechanism of interspecies transfer of *Wolbachia*. Further, because social parasitism is relatively common in ants, which are widespread and play key roles in many ecosystems, this mechanism of *Wolbachia* transmission may be significant in arthropod communities. Nonetheless, because social parasites are often rather host-specific and generally parasitize only closely related ant species or groups of ant species (Hölldobler & Wilson 1990), the mechanisms leading to the occurrence of similar or identical *Wolbachia* strains infecting the New World ants (*InvA* and *InvB*) from divergent genera remain to be explained.

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