Distribution and prevalence of *Wolbachia* in introduced populations of the fire ant *Solenopsis invicta*

A. M. Bouwma†, M. E. Ahrens‡, C. J. DeHeer§ and D. DeWayne Shoemaker†
†Department of Entomology, University Wisconsin-Madison, Madison, USA; ‡Department of Entomology, North Carolina State University, Raleigh, USA

Abstract

*Wolbachia* are intracellular bacteria that induce phenotypic effects in many arthropod hosts to enhance their own transmission within host populations. *Wolbachia* commonly infect the Red Imported Fire Ant, *Solenopsis invicta*, in native South American populations. A previous study failed to detect *Wolbachia* in fire ants from the introduced range in the USA. We conducted an extensive study of individuals collected from 1157 nests from 10 widespread geographical populations in the USA. *Wolbachia* were detected in ants from two nearby populations in southern Mississippi, with different variants (*wsp* gene sequences) infecting ants from colonies of the multiple-queen (polygyne) vs. single-queen (monogynie) social forms. The parsimonious explanation for the presence of *Wolbachia* in introduced *S. invicta* is that there have been one or more recent introductions of *Wolbachia*-infected fire ants into the southern USA.

Keywords: Fire ants, *Solenopsis invicta*, Wolbachia.

Introduction

*Wolbachia* are intracellular bacteria found in a wide range of arthropods and filarial nematodes (Bandi *et al*., 1998; O’Neill *et al*., 1992). These bacteria are primarily maternally transmitted, and many variants enhance their transmission within host populations by a variety of mechanisms, termed phenotypic effects, that either distort the sex ratio of their host’s offspring in favour of females (parthenogenesis, feminization of males, male-killing), or decrease the reproductive success of uninfected female hosts (cytoplasmic incompatibility) (Werren, 1997). The strong manipulation of host reproduction by *Wolbachia* enables this microbe to spread even if it induces a physiological cost that reduces fitness in its hosts (Turelli, 1994).

*Wolbachia* infections are common in ants (Van Borm *et al*., 2001; Wenseleers *et al*., 1998; Jeyaprakash & Hoy, 2000; Shoemaker *et al*., 2000). Further, *Wolbachia* variants found in New World ants are strikingly similar to each other (based on the highly variable *Wolbachia* outer surface protein gene *wsp*) yet different from all currently known *Wolbachia* variants in other insect groups suggesting that these *Wolbachia* variants are ant specialists (Tsutsui *et al*., 2003). Three New World *Wolbachia* host ants, the Argentine ant *Linepithema humile* (Reuter *et al*., 2005; Tsutsui *et al*., 2003), and the Red and Black Imported Fire Ants, *Solenopsis invicta* and *S. richteri* (Shoemaker *et al*., 2003, 2000), have been introduced independently into North America where they have become significant economic pests. Recent studies by Tsutsui *et al*. (2003) and Reuter *et al*. (2005) suggest that *Wolbachia* infections may have been lost during the colonisation of new habitats by Argentine ants since *Wolbachia* infections are common in the native range of this species, but rare or absent among introduced individuals. Similarly, Shoemaker *et al.* (2003, 2000) found that *Wolbachia* infections are common in numerous native populations of the two fire ant species *S. invicta* and *S. richteri*, but apparently absent in introduced populations in the USA (however, see Jeyaprakash & Hoy, 2000). While the phenotypic and fitness effects induced by the *Wolbachia* in these three ant species are as yet unknown, these studies raise the possibility that the absence of this microbe contributes to the success of these ants in their introduced range (Shoemaker *et al*., 2000; Tsutsui *et al*., 2003).

While *Wolbachia* infections are common in native *S. invicta*, *Wolbachia* prevalence varies considerably among different geographical populations (Shoemaker *et al*., 2003), suggesting that similar geographical variation in *Wolbachia* prevalence occurs among introduced populations. If so, the previously reported absence of *Wolbachia* infections among *S. invicta* in the USA may be due to inadequate geographical sampling. Indeed, a recent study by Jeyaprakash & Hoy (2000), using a highly sensitive long PCR assay, found *Wolbachia* in a single *S. invicta* individual sampled...
from a laboratory colony (presumably from Florida, USA). For the present study we conducted an extensive survey for Wolbachia in introduced S. invicta, using both the long PCR protocol of Jeyaprakash & Hoy (2000) and a standard PCR assay (Shoemaker et al., 2003; Zhou et al., 1998). In our study, the different PCR assays produced identical results, and while individuals from most populations were uninfected, multiple individuals from two populations in Mississippi, USA did harbour Wolbachia infections. Surprisingly, sequence analyses revealed that two Wolbachia variants occur in the USA, both of which are identical to variants previously described in native S. invicta (Shoemaker et al., 2000), yet differ from the variant found in S. invicta by Jeyaprakash & Hoy (2000).

Results

We did not detect Wolbachia in any individuals from eight of the 10 S. invicta populations studied. However, 11 individuals from two nearby populations in southern Mississippi (Hurley and Pascagoula) tested positive for Wolbachia (Fig. 1, Table 1). In samples collected in 1996, ants from eight of 61 colonies (13%) of the polygyne social form from Hurley, Mississippi harboured Wolbachia, whereas individuals from all 59 monogyne colonies from this same site were uninfected. (Monogyne colonies possess a single egg-laying queen, whereas polygyne colonies possess several to hundreds [Glancey et al., 1973]; there is limited gene flow between these two social forms [Ross & Shoemaker, 1993, 1997; Shoemaker & Ross, 1996]). In samples collected from Hurley, southern Mississippi in 2003, we did not find any infected ants from 76 polygyne colonies, however Wolbachia were detected in one individual from one of the 82 monogyne colonies (1.2%) at this site. In the same year, Wolbachia-infected individuals from two of 41 monogyne colonies (4.9%) in Pascagoula, Mississippi were observed, while a single individual from a polygyne colony was uninfected (Fig. 1, Table 1).

Sequence analyses of the Wolbachia variants (wsp; Wolbachia surface protein) from the 11 infected individuals (colonies) yielded one major result: all infected individuals

Figure 1. Wolbachia infection prevalence in introduced populations of Solenopsis invicta in the USA. All populations are uninfected unless indicated otherwise.

© 2006 The Royal Entomological Society, Insect Molecular Biology, 15. 89–93
from polygyne colonies (1996 Hurley, \( n = 8 \)) had an identical Wolbachia variant, as did all of the Wolbachia-infected individuals from monogyne nests (2003 Hurley, \( n = 1 \)) and 2003 Pascagoula, \( n = 2 \). The variants infecting individuals of each social form, however, were quite different from each other. Specifically, the Wolbachia variant in infected ants from monogyne colonies was identical to a variant known to infect native S. invicta (\( wSinvictaA \) [AF243435]) and belongs to the InvA subgroup of fire ant Wolbachia (Wolbachia supergroup A) whereas the variant in ants from polygyne colonies is identical to another variant known to infect native S. invicta (\( wSinvictaB \) [AF243436]) but belongs to the divergent InvB subgroup (Wolbachia supergroup B) (Fig. 1).

### Discussion

Our study revealed that two of 10 sampled populations of S. invicta in the USA harbour one of two Wolbachia variants at low prevalence (1–13%). The only previous evidence for Wolbachia in introduced S. invicta comes from a study by Jeyaprakash & Hoy (2000) in which the authors reported a single Wolbachia-infected individual from a laboratory colony presumably collected in Gainesville, Florida, USA. These authors demonstrated that a long PCR protocol was more sensitive at detecting Wolbachia in a diversity of hosts. We used this same protocol in our study of Wolbachia infections in 116 colonies in Gainesville, Florida USA (as well as a subset of colonies from other locations) in order to see if any infections escaped detection simply due to lack of sensitivity of our standard PCR assay. However, the long PCR assay produced identical results to our standard assay in all cases, and both assays failed to detect Wolbachia within individuals from any of these colonies. Thus, the Wolbachia variant found by Jeyaprakash & Hoy (2000) presumably was rare enough to escape detection or has recently been lost from natural S. invicta populations in Gainesville, Florida.

The low prevalence of Wolbachia infections is of particular interest since some theoretical models predict that such low frequencies likely are unstable (Turelli, 1994), suggesting that these Wolbachia infections are either in the process of sweeping through fire ant populations in the southern USA or are on the verge of being lost. (While male-killing Wolbachia variants may persist at a low equilibrium prevalence, the Wolbachia variants in fire ants likely do not induce this phenotype since males are commonly infected [Shoemaker et al., 2000]). Indeed, for one of the sites where we have data for two collections from different years (i.e. the polygyne population from Hurley, Mississippi), Wolbachia infection prevalence was significantly lower in 2003 than in 1996 (non-overlap of 95% confidence intervals). Nonetheless, we cannot rule out that the observed difference in Wolbachia prevalence is due to microgeographical variation rather than temporal variation in prevalence since the collection locations in Hurley, Mississippi were not the same in 1996 and 2003 (samples came from neighbouring pastures), and polygyne S. invicta consistently exhibit high levels of mtDNA genetic structure even at small spatial scales (Ross & Shoemaker, 1997).

We see three possible scenarios that could explain the restricted distribution of Wolbachia in S. invicta to individuals from Pascagoula and Hurley, Mississippi: (1) the Wolbachia variants we found in Hurley and Pascagoula were present in some proportion of the original monogyne founders introduced into the USA in the late 1930s or polygyne founders in the early 1970s that subsequently became established, but the infection has failed to spread throughout the USA; (2) a more recent introduction of Wolbachia-infected queens of S. invicta into the southern USA has occurred subsequent to the original invasion of ants representing each social form; or (3) Wolbachia-infections have been recently horizontally transmitted into introduced S. invicta from another host in the USA.

We consider the second scenario the most likely explanation for the distribution of Wolbachia infections in introduced S. invicta for several reasons. Firstly, if a proportion of the original monogyne foundresses introduced into Mobile, Alabama in the 1930s, or polygyne foundresses in the 1970s, were infected with Wolbachia, one might predict that Wolbachia infections would have spread throughout the USA along with the rapid spread of the ants shortly after their initial invasion. However, we failed to detect Wolbachia

### Table 1. Prevalence of Wolbachia infections in introduced populations of the fire ant Solenopsis invicta in the USA

<table>
<thead>
<tr>
<th>Location</th>
<th>Social form</th>
<th>Year</th>
<th>N</th>
<th>Prevalence of Wolbachia infections</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Austin, TX</td>
<td>M</td>
<td>1996</td>
<td>59</td>
<td>0.05</td>
<td>0.05</td>
</tr>
<tr>
<td></td>
<td>P</td>
<td>1996</td>
<td>59</td>
<td>0.05</td>
<td>0.05</td>
</tr>
<tr>
<td>DeQuincy, LA</td>
<td>M</td>
<td>1996</td>
<td>43</td>
<td>0.07</td>
<td>0.07</td>
</tr>
<tr>
<td></td>
<td>P</td>
<td>1996</td>
<td>25</td>
<td>0.12</td>
<td>0.12</td>
</tr>
<tr>
<td>Hammond, LA</td>
<td>M</td>
<td>1996</td>
<td>64</td>
<td>0.05</td>
<td>0.05</td>
</tr>
<tr>
<td></td>
<td>P</td>
<td>1996</td>
<td>55</td>
<td>0.05</td>
<td>0.05</td>
</tr>
<tr>
<td></td>
<td>M</td>
<td>2003</td>
<td>62</td>
<td>0.05</td>
<td>0.05</td>
</tr>
<tr>
<td></td>
<td>P</td>
<td>2003</td>
<td>44</td>
<td>0.07</td>
<td>0.07</td>
</tr>
<tr>
<td>Hurley, MS</td>
<td>M</td>
<td>1996</td>
<td>59</td>
<td>0.05</td>
<td>0.05</td>
</tr>
<tr>
<td></td>
<td>P</td>
<td>1996</td>
<td>61</td>
<td>0.13</td>
<td>0.05, 0.22</td>
</tr>
<tr>
<td></td>
<td>M</td>
<td>2003</td>
<td>82</td>
<td>0.01</td>
<td>0.04</td>
</tr>
<tr>
<td></td>
<td>P</td>
<td>2003</td>
<td>76</td>
<td>0.04</td>
<td>0.04</td>
</tr>
<tr>
<td>Pascagoula, MS</td>
<td>M</td>
<td>2003</td>
<td>41</td>
<td>0.05</td>
<td>0.02, 0.08</td>
</tr>
<tr>
<td></td>
<td>P</td>
<td>2003</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Vaughan, MS</td>
<td>M</td>
<td>2003</td>
<td>64</td>
<td>0.05</td>
<td>0.05</td>
</tr>
<tr>
<td></td>
<td>P</td>
<td>2003</td>
<td>55</td>
<td>0.05</td>
<td>0.05</td>
</tr>
<tr>
<td>Demopolis, AL</td>
<td>M</td>
<td>2003</td>
<td>3</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>P</td>
<td>2003</td>
<td>63</td>
<td>0.05</td>
<td>0.05</td>
</tr>
<tr>
<td>Mt. Vernon, AL</td>
<td>M</td>
<td>2003</td>
<td>59</td>
<td>0.05</td>
<td>0.05</td>
</tr>
<tr>
<td></td>
<td>P</td>
<td>2003</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Monroe, GA</td>
<td>M</td>
<td>1995</td>
<td>34</td>
<td>0.09</td>
<td>0.09</td>
</tr>
<tr>
<td></td>
<td>P</td>
<td>1995</td>
<td>31</td>
<td>0.09</td>
<td>0.09</td>
</tr>
<tr>
<td>Gainesville, FL</td>
<td>M</td>
<td>1996</td>
<td>60</td>
<td>0.05</td>
<td>0.05</td>
</tr>
<tr>
<td></td>
<td>P</td>
<td>1996</td>
<td>56</td>
<td>0.05</td>
<td>0.05</td>
</tr>
</tbody>
</table>

© 2006 The Royal Entomological Society, *Insect Molecular Biology, 15*, 89–93
infections in ants from a total of 837 nests collected outside of two nearby populations in Mississippi. In addition, Wolbachia infections were present in individuals from locations that are within 65 km of a likely port of entry (Mobile, Alabama), consistent with a recent introduction. The finding of two very different Wolbachia variants that are each associated with different social forms in the USA suggests that there likely have been two separate, recent introductions of Wolbachia-infected ants involving each of the two social forms. However, because both of these Wolbachia variants are found in sympatric native populations of each social form of S. invicta (Ahrens & Shoemaker, 2005), we cannot rule out completely the possibility of a single introduction involving queens of both social forms and both Wolbachia variants.

We consider the third scenario of horizontal transmission from another host the least likely. While the Wolbachia variant found by Jeyaprakash & Hoy (2000) has never been found in another ant and probably came from a non-ant host (Tsutsui et al., 2003), the two different Wolbachia variants we found in introduced S. invicta are known to infect S. invicta from South America (Shoemaker et al., 2003). Further, these two variants are associated with the same mtDNA haplotypes in the USA as they are in South America (D. Shoemaker, unpublished data), and have never been found in another insect. Thus, the parsimonious explanation is that these Wolbachia variants in introduced S. invicta are the result of one or more recent introductions of Wolbachia-infected S. invicta into the USA.

The two infected USA populations of S. invicta may serve as model systems for studying the dynamics and phenotypic effects of Wolbachia infections in naturally infected host populations, an opportunity rarely afforded to researchers (Hoshizaki & Shimada, 1995; Turelli & Hoffmann, 1991). Our study is an important first step toward collecting the necessary baseline geographical and temporal data on Wolbachia prevalence in introduced populations of fire ants that will allow us to determine both the fate of these infections and their impact on populations in the introduced range of S. invicta.

Experimental procedures

Collection of ants

Over three separate collecting trips in the spring of 1995, 1996 and 2003, we collected ants representing different life stages and castes from 1157 colonies at a total of 10 locations distributed throughout the range of introduced S. invicta in the southern USA (Fig. 1, Table 1). For each site, we included only nests located within 40 km of all other nests sampled at that site. We placed all collected ants immediately into liquid nitrogen in the field and subsequently stored them at −80 °C pending DNA analyses. We also made an effort to collect colonies representing both social forms of S. invicta from each site. We approximated social form in the field using cues from nest density, worker size, and nest brood composition (Ross & Shoemaker, 1997), and then subsequently confirmed it in the laboratory using one of two methods described below.

Screening for Wolbachia

We isolated total genomic DNA from individual ants and bulk samples (see below) using the Puregene DNA isolation kit (Shoemaker et al., 2000) (Gentra Systems Minneapolis, Minnesota). Shoemaker et al. (2003) showed that Wolbachia infections in S. invicta are transmitted to the offspring with nearly 100% fidelity, which means that screening one individual is a reliable method for determining whether individuals within a monogyne colony are infected. However, because the offspring within polygyne colonies are produced by multiple, unrelated queens, this procedure measures the proportion of infected polygyne queens in the population rather than the proportion of Wolbachia-infected polygyne colonies. We screened total genomic DNA from each ant for the presence of Wolbachia using PCR with the primers Wsp81F and Wsp691R (Zhou et al., 1998). Details of the PCRs, PCR profiles, and electrophoresis of products are described in Shoemaker et al. (2003, 2000). For all of the colonies collected from Florida (n = 116) and subset of the other colonies (n = 298), we also screened for the wsp gene and the presence of Wolbachia using the primers Wsp-Forward and Wsp-Reverse following the long PCR protocol detailed in Jeyaprakash & Hoy (2000).

Determination of social form

For all samples collected in 1995 and 1996, we confirmed polygyny by finding two or more wingless (reproductive) queens in a nest, by detecting multiple families represented among eight or more nestmate offspring females assayed at six polymorphic allozyme loci, and/or by detecting the presence of the b allele of the gene Gp-9, which occurs only in the polygyne social form (Krieger & Ross, 2002; Ross, 1997). We confirmed monogyny by detecting a single family represented among eight or more nestmate females assayed at the six allozyme loci and by failing to find the Gp-9a allele among these females (DeHeer & Tschinkel, 1998). For the colonies we collected in 2003, we determined social form using a 2-stage, allele-specific PCR assay at the gene Gp-9 designed to amplify ‘b’-like alleles only. (see Krieger & Ross, 2002; Ross et al., 2003). For those colonies that were negative for the stage-2 PCR, we performed bulk extractions of an additional five individuals and repeated the 2-step PCR assay to confirm monogyny.

Sequencing of Wolbachia strains

We sequenced a portion of the wsp gene from all Wolbachia-infected individuals in order to identify the Wolbachia variants infecting these ants. We PCR-amplified Wolbachia DNA using the primers Wsp81F and Wsp691R. PCR reaction components, thermal cycling conditions, and sequencing methods were identical to those described in Ahrens & Shoemaker (2005).

Acknowledgements

We wish to thank Franck Dedeine and two anonymous reviewers for their comments on an earlier version of the manuscript. This study was supported by grants from the College of Agriculture and Life Sciences at the University of Wisconsin and the United States Department of Agriculture USDA 2003-35302-13497 NRICGP to DDS.
References


