Distribution and Prevalence of *Wolbachia* Infections in Native Populations of the Fire Ant *Solenopsis invicta* (Hymenoptera: Formicidae)

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ABSTRACT *Wolbachia* are endosymbiotic bacteria that commonly infect arthropods. These bacteria induce a number of phenotypes in their hosts, including cytoplasmic incompatibility, thelytokous parthenogenesis, feminization, and male killing. We surveyed native South American populations of the fire ant *Solenopsis invicta* Buren for *Wolbachia* infections by using a diagnostic polymerase chain reaction assay. In addition, we determined the fidelity of vertical transmission of the bacteria from mother to offspring in this species by assaying daughters in 24 simple-family (monogyne) colonies. Infections were common in many parts of the extensive native range of *S. invicta*. However, the proportion of individuals infected varied greatly among samples, ranging from zero in several populations from the northerly parts of the range to >90% in more southerly populations. Possible explanations for this variation in the prevalence of *Wolbachia* infections are discussed. A survey of the two social forms of *S. invicta* from four geographic areas showed that the prevalence of *Wolbachia* infections consistently was higher in the monogyne form (single queen per colony) than the sympatric polygyne form (multiple queens per colony). One likely explanation for this trend is that the selective regimes acting on *Wolbachia* in the two forms differ because of the dissimilar reproductive strategies used by each form. Finally, overall transmission efficiency was found to be very high (>99%), making it unlikely that imperfect transmission prevents the spread of the microbe to near fixation in native populations.

KEY WORDS population structure, fire ants, *Solenopsis invicta*, South America, *Wolbachia*

Wolbachia are endosymbiotic alpha-proteobacteria that infect a variety of arthropods and filarial nematodes (O'Neill et al. 1992, Werren and O'Neill 1997, Bandi et al. 1998). Recent surveys suggest that *Wolbachia* infect a substantial proportion of insect species, with estimates ranging from 17% (Werren et al. 1995, West et al. 1998, Werren and Windsor 2000) to 76% (Jeyaprakash and Hoy 2000). Extrapolation of these estimates suggests that millions of insect species currently are infected with *Wolbachia*, making these bacteria among the most widespread parasites on earth. Transmission of *Wolbachia* among hosts mainly occurs maternally through the egg cytoplasm. Consequently, *Wolbachia* have evolved several mechanisms to enhance their transmission that either increase their host's investment in daughters or decrease the reproductive success of uninfected females. These mechanisms include cytoplasmic incompatibility (CI), thelytokous parthenogenesis, feminization of genetic males, and male-killing (for recent reviews, see Werren 1997, Stouthamer et al. 1999, Stevens et al. 2001).

Numerous taxon-specific surveys for *Wolbachia* infections have been conducted, including several that specifically examined the distribution and prevalence of *Wolbachia* in ants. The first such survey reported an unusually high proportion of *Wolbachia*-infected ant species relative to the proportion of infected species found in more general surveys of randomly collected insects (Werren et al. 1995, Wenseleers et al. 1998, West et al. 1998, Werren and Windsor 2000). Subsequent surveys have shown that many ant species from a number of genera, including fire ants of the genus *Solenopsis*, are infected by *Wolbachia* (Jeyaprakash and Hoy 2000, Shoemaker et al. 2000, Keller et al. 2001, Van Borm et al. 2001, Wenseleers et al. 2002, Van Borm et al. 2003).

The presence of *Wolbachia* in fire ants was first detailed in a study by Shoemaker et al. (2000). All fire ant species within the *Solenopsis saevissima* species

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group are native to South America, but two of the species, *S. invicta* Buren and *S. richteri* Forel, were introduced into the United States early in the last century. *Solenopsis invicta,* in particular, has become widely established and is considered a significant economic and agricultural pest wherever it occurs in the United States. Shoemaker et al. (2000) found that some proportion of individuals representing all but one species of the *S. saevissima* group surveyed was infected with *Wolbachia.* Given the considerable interest in using *Wolbachia* as biological control agents (Aeschilimann 1990, Beard et al. 1992, Beard et al. 1993, Stouthamer 1993, Werren 1997, Turelli and Hoffmann 1999), the discovery of *Wolbachia* in numerous fire ant species is significant because parallel studies can be conducted on host effects and population dynamics of this microbe in naturally infected pest populations.

One noteworthy finding of Shoemaker et al. (2000) was that, although *Wolbachia* infections were common in samples of native *S. invicta,* no infections were detected in any individuals collected from populations in the introduced range in the United States (but see Jeyaprakash and Hoy 2000). One potential explanation for this result is that the colonists came from an area in South America where *Wolbachia* infections are rare or absent. However, because this previous survey included ants collected from only a relatively small part of the native range, a broader survey of *S. invicta* from additional native populations was undertaken to more accurately determine the distribution and prevalence of *Wolbachia* infections throughout the species' range. With such additional information, it becomes possible to evaluate the likelihood that the introduced populations originated from *Wolbachia*-free areas.

In the current study, we present the results of a survey for *Wolbachia* infections in *S. invicta* collected from numerous South American populations located over much of the known native range. By significantly extending the earlier survey of Shoemaker et al. (2000), this study establishes the foundation for a more comprehensive understanding of the forces influencing the geographical distribution and prevalence of *Wolbachia* in native populations of this important pest ant species.

In addition to this general geographical survey, we compared the prevalence of *Wolbachia* infections between the two social forms of *S. invicta* where they occur in sympatry. Colonies of the monogyne social form of *S. invicta* invariably contain a single reproductive queen, whereas colonies of the alternative, polygyne form contain multiple queens. Examination of the prevalence of *Wolbachia* in the two forms is of interest because the frequencies and dynamics of infections are predicted to differ between them due to form-specific differences in the selective regimes acting on *Wolbachia* (see Wenseleers et al. 1998, Shoemaker et al. 2000 for a detailed discussion of this issue). Specifically, we predict that, because of higher expressivity of CI in the monogyne form than the polygyne form, there will be a lag in the spread of *Wolbachia* through a polygyne population, resulting in consistently lower prevalence of infections than in neighboring monogyne populations. We show that infection frequencies in native *S. invicta* not only vary enormously among geographic regions but also between sympatric social forms as predicted, indicating that the factors influencing the spread and fate of *Wolbachia* in this highly social insect are likely to be numerous and complex.

Finally, we estimated the efficiency of vertical (maternal) transmission of *Wolbachia* in *S. invicta* in the wild. Monogyne fire ant colonies have a simple family structure, whereby all of the colony members are offspring of a single queen mated to a single haploid male (Ross and Fletcher 1985, Ross et al. 1993). We took advantage of the fact that all offspring in such colonies share the same maternal cytoplasm to estimate the efficiency at which *Wolbachia* infections are cotransmitted to progeny with the maternal cytoplasm. Such estimates of maternal transmission efficiency in the field, which often differ from estimates obtained in a laboratory setting (Turelli and Hoffmann 1995), have been obtained for only a few taxa (for examples, see Turelli and Hoffmann 1995, Hoffmann et al. 1998, Jiggins et al. 2002, Kittayapong et al. 2002, Wenseleers et al. 2002), yet they are essential for understanding the dynamics of *Wolbachia* infections in host populations. For instance, such transmission data are necessary for inferring whether the variable infection frequencies within many *S. invicta* populations result solely from incomplete vertical transmission, or whether other factors must be invoked, such as recent invasion by the microbe or costs to host fitness.

Materials and Methods

Individuals of *S. invicta* were collected from native populations in Argentina and Brazil in 1992 and 1998 (Table 1). Multiple workers and winged virgin queens were collected from each of 643 colonies, representing 11 geographic populations distributed over much of the known native range of *S. invicta* (Fig. 1). We previously showed that *Wolbachia* infections were equally prevalent in workers and winged queens, indicating that workers are appropriate material for detecting and estimating population infection frequencies (Shoemaker et al. 2000). Each colony was designated initially in the field as monogyne or polygyne by using well established criteria that distinguish colonies of the two social forms: worker size, mound size and distribution, numbers of sexuals, and the presence of multiple dealate queens (Greenberg et al. 1985, Ross and Fletcher 1985, Vargo and Fletcher 1989). The social form of each colony was subsequently confirmed using a diagnostic polymerase chain reaction (PCR) assay (Mescher et al. 2004). Colony social form in *S. invicta* is invariably associated with worker genotypes at the gene *Gp-9* (Ross 1997, Krieger and Ross 2002, Ross and Keller 2002), and colonies with different worker *Gp-9* genotype compositions are readily discernible using allele-specific

Table 1. *Wolbachia* infection frequencies in native *S. invicta*

Location (city, state or province, country)	Date of collection	Social form	*N*	*Wolbachia* infection frequency	95% confidence intervals
Corrientes, Corrientes, Argentina	1992	M	36	0.81	0.75–0.92
Corrientes, Corrientes, Argentina	1998	M	29	0.83	0.69–0.93
Corrientes, Corrientes, Argentina	1992	P	43	0.56	0.42–0.70
Formosa, Formosa, Argentina	1998	M	34	0.06	0.00–0.14
Formosa, Formosa, Argentina	1998	P	34	0	0.00–0.08
Roldan, Santa Fe, Argentina	1998	M	13	0.93	0.75–1.00
Rosario, Santa Fe, Argentina	1998	M	30	0.83	0.69–0.97
Arroio dos Ratos, Rio Grande do Sul, Brazil	1998	M	36	0.75	0.61–0.89
Arroio dos Ratos, Rio Grande do Sul, Brazil	1998	P	8	0.25	0.00–0.50
Rinco dos Cabrais, Rio Grande do Sul, Brazil	1998	M	81	0.11	0.05–0.19
Campo Grande, Campo Grande, Brazil	1998	M	38	0.23	0.10–0.35
Campo Grande, Campo Grande, Brazil	1998	P	5	0	0.00–0.45
Ceu Azul, Parana, Brazil	1998	M	80	0.13	0.06–0.20
Pontes E Lacerda, Mato Grosso, Brazil	1998	M	30	0	0.00–0.10
Pedra Preta, Mato Grosso, Brazil	1998	M	63	0.02	0.00–0.05
Sao Gabriel do Oeste, Mato Grosso do Sul, Brazil	1998	M	79	0	0.00–0.04

The number of individuals assayed for each sample (one per nest) is indicated by *N*. The monogyne and polygyne social forms are indicated by M and P, respectively.

PCR. Polygyne *S. invicta* colonies always contain workers with "*b*-like" alleles of *Gp-9*, whereas monogyne colonies contain only workers homozygous for the *B* alleles of this gene. Colonies of *S. invicta* seem to be mostly monogyne throughout much of the native range, with polygyny documented in only four of our 11 study populations (Mescher et al. 2004).

In addition to these worker and winged queen samples from different geographic populations, we also collected multiple winged queens from each of 24 monogyne colonies of *S. invicta* at a site near the city of Corrientes, Argentina. Winged fire ant queens are the virgin daughters of the foundress queen heading a monogyne colony. These 24 focal colonies were known from preliminary study to contain infected individuals, indicating that the foundress queen heading each colony was infected. Thus, the frequency of infection of daughter queens from these colonies could be used to estimate the efficiency of transmission of *Wolbachia* from infected queens to their female offspring.

Total genomic DNA was extracted from each ant by using the Puregene DNA isolation kit (Gentra Systems, Inc., Minneapolis, MN) (Ross and Shoemaker 1997). PCR was used to screen the DNA extracted from each ant for the presence of *Wolbachia* by using the primers *wsp*82 F (5′-GGTCCAATAAGTGATGAAGAAAC-3′) and *wsp*691R (5′-AAAAATTAAACGCTACTCCA-3′) (Zhou et al. 1998, Shoemaker et al. 2000). These *wsp* primers amplify a portion of a highly variable gene encoding the *Wolbachia* outer surface protein (Braig et al. 1998, Zhou et al. 1998). The primers EF1α-532 F (5′-AGGCAAATGTCTTATTGAAG-3′) and EF1α-610R (5′-GCGGGTGCGAAGGTAACAAC-3′) also were included in each PCR reaction to provide a positive control for template quality (Shoemaker et al. 2000). Individuals in which no bands representing the EF1α fragment occurred after three PCR experiments were not included in this study.

PCR reactions were performed in 15-μl volumes containing 12.7 μl of Platinum PCR SuperMIX (Invitrogen, Carlsbad, CA), 0.3 μl of a 25 μM solution of each *wsp* primer, 0.1 μl of a 25 μM solution of each EF1α primer, and 1.5 μl of genomic DNA. DNA amplifications were carried out in a thermal cycler (Bio-Rad, Hercules, CA) by using the following protocol: 1 min at 94°C for one cycle; 30 s at 94°C, 30 s at 60°C (-1/2°C per cycle), and 1 min at 72°C for 10 cycles (touchdown PCR); 30 s at 94°C, 30 s at 53°C, and 1 min at 72°C for 25 cycles; 5 min at 72°C for one terminal cycle. Approximately 4 μl of each PCR reaction product was electrophoresed in a 1.5% agarose gel. Gels were stained with ethidium bromide and subsequently exposed to UV light to visualize the bands. The stained gels were photographed and the images captured using a gel photodocumentation system.

A single individual per colony was PCR assayed for the presence of *Wolbachia* in the population samples. To estimate the 95% confidence intervals around the nonzero estimates of infection frequencies for each form/population, a bootstrapping procedure was used in which individuals were sampled randomly (with replacement) from the original data set for each bootstrap replicate. This procedure was repeated 1,000 times, and the 95% confidence intervals were found by eliminating the 25 lowest and 25 highest values from the ordered array of 1,000 estimates. In cases where no infected individuals were detected and sample sizes were greater than five, 95% confidence intervals were estimated from the binomial distribution (Shoemaker et al. 2000). Samples with confidence intervals that do not overlap are considered to have significantly different infection frequencies.

To estimate the efficiency of vertical (maternal) transmission of *Wolbachia*, we PCR assayed 10 to 12 winged (virgin daughter) queens from each of 24 monogyne *S. invicta* colonies for the presence of *Wolbachia*. Monogyny was confirmed in this subset of colonies by examining the genotype distributions of

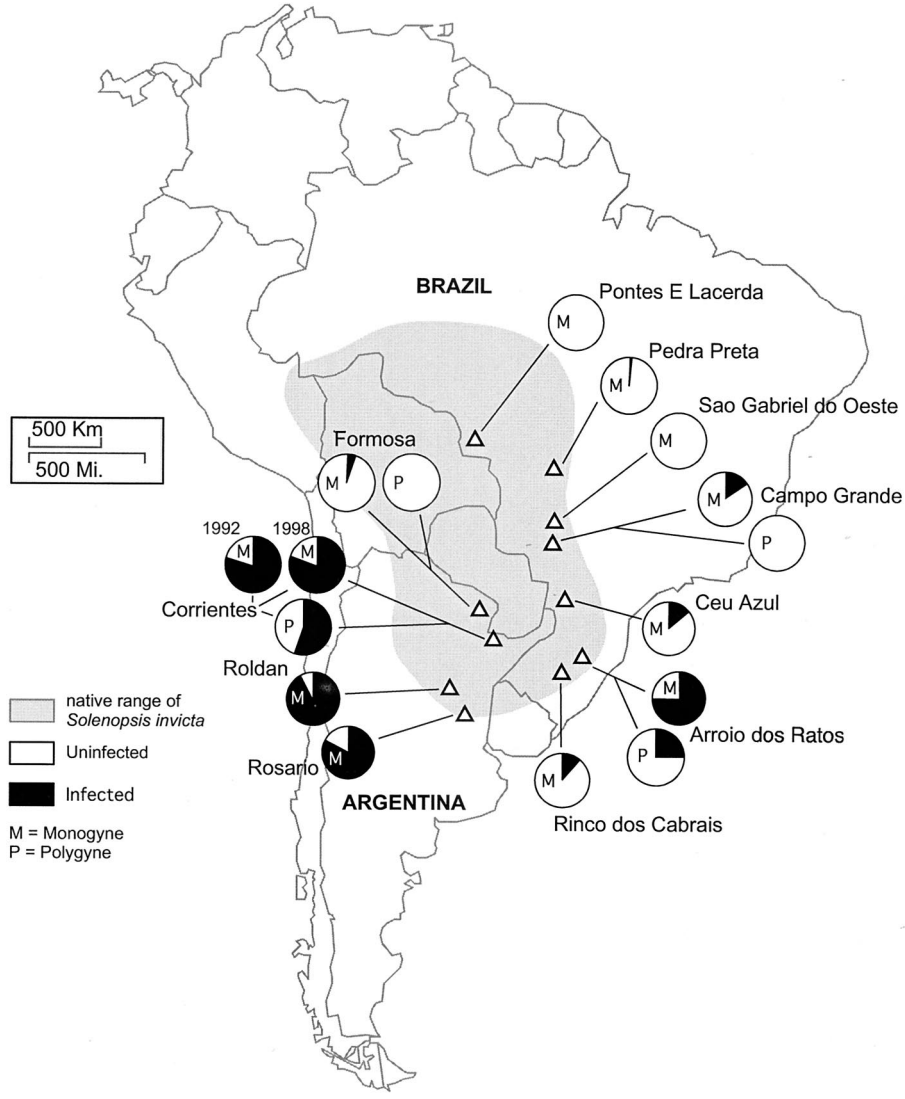

Fig. 1. *Wolbachia* infection frequencies in native *S. invicta* populations. Each pie diagram shows the proportions of infected and uninfected individuals of each social form in each geographic population. Pie diagrams representing the polygyne form contain the letter P, whereas those representing the monogyne form contain the letter M.

the winged queens at eight polymorphic allozyme loci (e.g., Ross et al. 1997); genotype distributions consistent with nestmate queens being full sisters are indicative of monogyny. Transmission fidelity within each colony was calculated by dividing the number of infected individuals by the total number of individuals assayed in that colony. We also calculated an overall estimate of transmission efficiency by dividing the total number of infected individuals in all 24 colonies by the total number of individuals assayed. The 95% confidence intervals around this estimate were calculated using the bootstrap approach described above.

Results

Geographical Distribution of *Wolbachia* in Native *S. invicta*. Results of our survey for *Wolbachia* infections in native *S. invicta* are summarized in Table 1 and Fig. 1. For eight of 643 individuals surveyed, we were unable to successful amplify the nuclear control gene EF1α. Thus, these samples are excluded from all of our analyses below and in Table 1. Infection frequencies varied dramatically among different geographic populations. *Wolbachia* was virtually or completely absent in individuals from four geographic popula-

tions, three of which are relatively close to one another in the northern half of the species' range in Brazil (Fig. 1). Variable proportions of individuals from the remaining populations harbored *Wolbachia* infections, with the infection frequencies ranging from ≈10% to >90% (Table 1; Fig. 1). The highest infection frequencies were observed in four monogyne samples from the southern part of the range: Rosario, Roldan, Arroio dos Ratos (monogyne), and Corrientes (monogyne). Infection frequencies in each of these samples were significantly higher than in all others except the Corrientes polygyne sample. *Wolbachia* infection frequencies in native *S. invicta* apparently can be relatively stable over the short term in at least some cases, given that essentially identical frequencies were found for the two samples collected from a single locality 6 yr apart (Corrientes monogyne form).

We assayed both social forms of *S. invicta* for *Wolbachia* at four different localities (Arroio dos Ratos, Corrientes, Formosa, and Campo Grande). At each locality, infection frequencies were higher in the monogyne form than the sympatric polygyne form (Table 1), and this difference was significant for two of the paired samples as indicated by nonoverlap of 95% confidence intervals as well as formal contingency tests (Arroio dos Ratos [$\chi^2 = 7.283$, df = 1, $P < 0.02$] and Corrientes [$\chi^2 = 5.433$, df = 1, $P < 0.01$]). In the other two cases, *Wolbachia* infections were detected only in ants of the monogyne form, albeit at low frequencies (Table 1).

Transmission Efficiency of *Wolbachia*. Winged daughter queens collected from 24 monogyne colonies (=simple families) were assayed for the presence of *Wolbachia* to estimate the fidelity of transmission of the parasite from mother queens to their daughters. In 22 of the families, every daughter inherited the infection from her mother, so that transmission was 100%. In the remaining two families, transmission was imperfect, with only 90 and 92% of the offspring in these two families inheriting the infection from their mother. The overall efficiency of vertical transmission of *Wolbachia* to offspring in our sample is estimated to have been >99%.

Discussion

We used a PCR assay to determine the distribution and prevalence of *Wolbachia* infections in 11 geographically widespread native populations of the fire ant *S. invicta.* In addition, we estimated the vertical transmission fidelity of *Wolbachia* in the wild by screening daughter queens from 24 monogyne colonies for the presence of these microbes. Our results reveal several patterns with important implications for the population dynamics of this parasite in native fire ant populations. The first pattern is that *Wolbachia* infection frequencies vary enormously among the different localities of *S. invicta* sampled, with some geographic populations apparently lacking infected ants and others almost fixed for the infection. In general, *Wolbachia* infections seem to be much less common in the more northerly areas of the species' range than in the more southerly areas (Fig. 1), although more extensive sampling will be required to verify this apparent pattern.

Previously, we proposed that the absence of *Wolbachia* in *S. invicta* in the United States may stem either from the absence of *Wolbachia* in the original founders or from the loss of infections in the founder population due to drift or selection in the new environment (Shoemaker et al. 2000). Our findings that *Wolbachia* infections are rare or absent throughout a large portion of the native range of *S. invicta* indicates that the absence of *Wolbachia* in the original founders is a logical scenario, especially if their source population lies in the northern part of the range (Mescher et al. 2004). However, *Wolbachia* infection data alone are not particularly informative for identifying the source population of introduced *S. invicta,* because most geographic populations are polymorphic for infections and the infection status of a few founders would be largely stochastically determined. Furthermore, we cannot rule out the possibility of secondary loss of *Wolbachia* infections in *S. invicta* founders that originated from a source population where *Wolbachia* is common.

A second important pattern in our data is that although *Wolbachia* infections were detected in most of the study populations, in no case was the frequency of *Wolbachia*-infected individuals 100%. That is, some proportion of individuals remained uninfected in every population harboring the parasite. Our finding of low-to-intermediate infection frequencies within several native *S. invicta* populations was unexpected because theoretical models generally predict that the equilibrium frequency of *Wolbachia* infections should be ≥50%, provided that the invasion threshold frequency has been achieved (Caspari and Watson 1959, Fine 1978, Turelli 1994). One factor that can prevent the bacteria from spreading to fixation within populations is inefficient transmission, which results in the production of some proportion of uninfected offspring by infected females every generation. However, given our estimate of almost perfect efficiency of vertical transmission in the wild (99.3%), it seems unlikely that the observed intermediate infection frequencies can be explained solely by this factor.

There are, however, several other potential explanations for the presence of uninfected individuals within populations harboring *Wolbachia,* as well as for the dramatic variation in infection frequencies observed among populations. Both patterns may result from differences in the length of time since *Wolbachia* invaded a population. If the initial invasion of *S. invicta* by *Wolbachia* occurred in the southern part of the species' range, then *Wolbachia* infection frequencies may be relatively high in these regions because the bacteria have had a longer period of time to spread. *Wolbachia* infections may be rare or absent in the more northerly regions of the range because the bacteria only recently invaded there (or have yet to invade) and, consequently, are still in the early stages of spreading. Alternatively, variation in infection fre-

quencies among populations may be due to the presence of different *Wolbachia* strains in different regions that vary in their host effects. Previous genetic data based on nuclear and mitochondrial DNA markers indicate that there is significant genetic differentiation among native geographic populations of *S. invicta* separated by distances of <200 km (Ross et al. 1997). Given that the species' range extends over several thousand kilometers, it is reasonable to assume that substantial genetic differentiation occurs among more distant populations and that locally adapted populations may harbor unique *Wolbachia* strains, each with specific host effects and, consequently, specific population dynamics. Finally, it is possible that one or more of the *Wolbachia* strains infecting *S. invicta* have essentially no phenotypic effects on their hosts, so that the dramatic variation in infection frequencies among populations is due largely to random drift. We will test some elements of these hypotheses by sequencing the *wsp* gene of strains from different regions, as well as by assaying northern populations with modest infection frequencies at multiple time points to learn whether infection frequencies remain constant, increase, or fluctuate stochastically over time. Our initial data from the monogyne form in Corrientes suggest that infection frequencies may remain relatively constant over short periods once they have reached high levels (Table 1).

Another significant pattern observed in our study is that *Wolbachia* infection frequencies differ in a consistent manner between sympatric populations of the two social forms of *S. invicta.* We assayed individuals of both the monogyne and polygyne forms from four different locations in South America, and in each case *Wolbachia* was more prevalent in the monogyne than in the sympatric polygyne form. Shoemaker et al. (2000) argued that such a pattern is expected if distinctive selective regimes act on *Wolbachia* in each social form, resulting in the bacteria spreading at a higher rate (and reaching a higher equilibrium frequency) in monogyne than polygyne populations. As an example of how this might occur, the expressivity of CI for a given *Wolbachia* strain is likely to be effectively complete (zero reproductive success) in the monogyne form, but the expressivity of CI in the polygyne form may be considerably lower than this. The reason has to do with the different modes of colony reproduction practiced by the two forms (Ross and Keller 1995). Uninfected queens that mate with infected males presumably produce reduced numbers of workers as a result of CI. Such queens are unlikely to succeed at founding a colony independently, as is the habit of queens of the monogyne form, because their failure to produce a sufficient worker force would doom the incipient colony (Ross et al. 1993, Ross and Shoemaker 1997). However, uninfected queens that mate with infected males may become successful reproductives in polygyne colonies, because these colonies recruit newly mated queens directly, thus bypassing the vulnerable independent-colony-founding stage characteristic of the monogyne form (polygyne colonies reproduce by means of budding or fissioning of existing colonies). Thus, the expressivity of the CI phenotype in the polygyne social form is dampened because polygyne queens with incompatible matings presumably still can produce some progeny, including uninfected males (which arise from unfertilized eggs). One predicted outcome is that there should be a lag in the spread of *Wolbachia* through a polygyne population, leading to consistently lower infection frequencies than in neighboring monogyne populations, as we observed in this study.

One caveat to the scenario described above is the assumption that the *Wolbachia* strains present in *S. invicta* induce CI. If these bacteria have some other effects on fire ants, then the rate of spread and equilibrium infection frequencies in the two forms may not differ in the manner predicted. At present, it is unknown what phenotypic effects, if any, *Wolbachia* induce in this ant. The *wsp* sequence of one of the two *Wolbachia* strains thus far found to infect *S. invicta* (*InvB*) is almost identical to a *Wolbachia* sequence from the leafcutter ant *Acromyrmex insinuator* (>99% sequence identity), and this strain seems to induce CI in the leafcutter host (Shoemaker et al. 2000, Van Borm et al. 2001). Additionally, the *wsp* sequence of the other *Wolbachia* strain found to infect native *S. invicta* (*InvA*) is almost identical (>99% sequence identity) to sequences of putative male-killing *Wolbachia* strains in the leafcutters *Acromyrmex octospinosus* and *Acromyrmex echinatior* (Shoemaker et al. 2000, Van Borm et al. 2001). It is possible that the two *Wolbachia* strains known from fire ants induce phenotypic effects similar to those reported for *Wolbachia* from leafcutter ants. However, because a given *Wolbachia* strain may induce different phenotypes in different hosts (Boyle et al. 1993, Fujii et al. 2001, Sasaki et al. 2002), direct determination of the phenotypic effects of each *Wolbachia* strain infecting native *S. invicta* will be required to achieve a more complete understanding of how these host effects influence population infection frequencies.

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