Insect. Soc. 53 (2006) 269–273
0020-1812/06/030269-5
DOI 10.1007/s00040-006-0868-7

Insectes Sociaux

Research article

Wolbachia in the invasive European paper wasp *Polistes dominulus*

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Received 28 October 2005; revised 25 January 2006; accepted 17 February 2006.
Published Online First 30 June 2006

Abstract. The European paper wasp *Polistes dominulus* has been expanding its North American range since its introduction in the 1970s. We screened *P. dominulus* from Italy and the northeastern U.S. for the presence of the intracellular reproductive symbiont *Wolbachia.* Infection rates among females varied from 16 % to 87 % among U.S. sites and from 33 % to 71 % in Italy. We also found infected haploid and diploid males, indicating that this is not a male-killing *Wolbachia* infection. Our data show that infected individuals from New York, Massachusetts, and Italy carry the same *Wolbachia* strain, and that some mtDNA haplotypes include both infected and uninfected individuals. We discuss possible implications of *Wolbachia* infection in this invasive social hymenopteran.

Keywords: Polistes dominulus, Wolbachia, infection prevalence, invasion biology.

Introduction

Polistes wasps are considered a model system for behavioral and ecological studies of social insects (Reeve, 1991). The European paper wasp *P. dominulus* – the most studied member of the genus – is an invasive species that was first found in Massachusetts in the 1970s (Eickwort, 1978), continues to spread through North America (Cervo et al., 2000), and may be displacing native congenerics (Gamboa et al., 2002, 2004; Liebert et al., in press). Like other *Polistes* wasps, *P. dominulus* is an important predator in terrestrial communities, but it is more of a generalist than many of its congenerics (Cervo et al., 2000; Pickett and Wenzel, 2000). Thus, a change in *Polistes* species composition may have broad impacts on the structure of local insect communities.

The invasiveness of *P. dominulus* may be partially due to to its high productivity (Pickett and Wenzel, 2000). Though decreased genetic variability may enhance invasiveness in social insects (Tsutsui et al., 2000), this is unlikely to account for the success of *P. dominulus*, which has probably been introduced to the U.S. multiple times from yet-unidentified source populations, minimizing bottleneck effects (Johnson and Starks, 2004). *P. dominulus* would also incur a high cost in genetically depauperate populations due to the production of diploid males and triploid females (Liebert et al., 2004, 2005), phenomena that provide evidence of complementary sex determination, or CSD (Beye et al., 2003). We here report another aspect of *P. dominulus* biology – *Wolbachia* infection – which may affect its success as an invader.

Wolbachia are obligate, maternally transmitted endosymbionts of arthropod and nematode reproductive tissues. Two major strain groups, designated A and B, are common in insects (Werren et al., 1995), and *Wolbachia* infections in both strain groups may alter host reproduction to enhance production of infected females. Infection phenotypes may include feminization (F), parthenogenesis induction (PI), male-killing (MK), and cytoplasmic incompatibility (CI) (O'Neill et al., 1997). While all of these mechanisms aid the spread of *Wolbachia* in populations, they do not all affect host population growth in the same way. In particular, because F, PI, and MK infections increase the overall production of females in a population, they can enhance mean colony productivity, and thus the potential rate of spread by infected populations. In contrast, CI infections lead to reproductive failures when infected males mate with uninfected females, potentially reducing mean colony productivity. However, all of these *Wolbachia* phenotypes increase the variance in productivity among females, which can cause different lineages to contribute unequally to a growing population. Thus the presence and influence of *Wolbachia* may also have implications for the interpretation of many behavioral and demographic studies of this species.

We obtained *P. dominulus* females from native populations in Italy and introduced populations in Massachusetts, New York, Connecticut and Michigan, USA. We screened

these wasps for *Wolbachia* to estimate infection prevalence at each site, sequenced a *Wolbachia* gene from a subset of infected wasps to determine the number of strains present, and sequenced an mtDNA gene from a subset of infected and uninfected wasps to explore possible infection patterns. We also screened males from several sites to determine whether adult males could survive *Wolbachia* infection.

Materials and methods

Collection

Female wasps from New York and Connecticut were netted in flight in the spring of 2005, and processed fresh or after brief ethanol storage. Females from Massachusetts, Michigan, and Italy were collected from nests for other population studies between 2001 and 2004, and stored in ethanol at –20 °C or below until use; to avoid pseudoreplication, only one female per nest was used. Males were collected from nests in Michigan and Italy in 2003 and Massachusetts in 2004. Only females were used to estimate *Wolbachia* prevalence, since we had no *a priori* knowledge of whether sex-ratio effects would bias prevalence estimates that were based on both sexes. Males were screened to determine only whether they could survive infection; because males are seasonal and thus more difficult to obtain, multiple nestmates were used when available.

DNA isolation

For microsatellite genotyping, DNA was isolated from insect legs following the methods of Johnson and Starks (2004) and Liebert et al. (2004, 2005). For *Wolbachia* screening, we isolated DNA from insect abdomens using Gentra Puregene™ reagents according to manufacturer's directions. For fresh or briefly ethanol-stored specimens, we used only dissected abdominal tissue, including eggs or gonads but avoiding the gut and sting apparatus. For longer-preserved specimens, whole abdomens were used. Specimens from Italy were inspected before preparation to be sure that no Strepsiptera were visible (Hughes et al., 2003).

Wolbachia screening and strain-group typing

We performed PCR-based *Wolbachia* screens on *P. dominulus* females collected from five sites in the U.S. (n = 101) and four sites in Italy (n = 39). We also screened males from Italy (n = 3) and the U.S. (n = 37) to determine whether infected males could survive to adulthood. All specimens were screened for *Wolbachia* with 16s rDNA W-Spec primers (Werren and Windsor, 2000) and the *Wolbachia* surface protein gene (*wsp*) primers 81f and 691r (Braig et al., 1998). After initial screening, we amplified DNA from a subsample of 21 infected wasps with primers specific for the A group (16s rDNA A) and B group (*ftsZ* B) of *Wolbachia* (Werren et al., 1995). For insect mtDNA amplification, we used the cytochrome oxidase I barcoding primers LCO 1490 and HCO 2198 (Hebert et al., 2002).

Each PCR reaction contained 5.65 µl water, 1.0 µl 10X reaction buffer, 0.5 µl 50 mM $MgCl_2$, 1.0 µl 2 mM dNTPs, 0.4 µl each of 10 µM forward and reverse primers, 0.05 µl *Taq* polymerase, and 1.0 µl of specimen DNA solution. Amplifications were performed with pre-dwell at 94º for 2 min, 35 to 38 cycles of 94º for 30–60 s, 55º for 50–60 s, and 72º for 1 min 30 s, and a post-dwell of 72º for 10 min. PCR products were electrophoresed and visualized on 1.5 % agarose-ethidium bromide gels, using a DNA ladder to estimate fragment size. When either or both of the *Wolbachia* primer sets yielded a band of the expected size, the wasp was scored as infected. When neither *Wolbachia*-specific band was present but the COI primers amplified a fragment of expected size, the wasp was scored as uninfected. If no bands were obtained with any primers, the DNA isolation was considered unsuccessful, and the wasp was not included in the study (nor in reported specimen counts). For PCR controls, we used DNA from *Wolbachia*-infected and uninfected *Drosophila spp.* and *Nasonia vitripennis* maintained in cultures at the University of Rochester.

Sequencing

We sequenced a 512 bp *wsp* fragment from infected wasps from Italy (n = 6), Massachusetts (n = 6), and New York (n = 2). A 625 bp COI mtDNA fragment was also sequenced from these wasps and from uninfected wasps from Italy (n = 6), Massachusetts (n = 5) and New York (n = 1). Because expected fragments were short, we used only the forward primers for sequencing with the BigDye® Terminator Cycle Sequencing Kit (Applied Biosystems, Inc.). Excess dNTPs and primers were removed using ExoSAP-IT® (USB Corp.). Final sequencing steps were performed at the University of Rochester Functional Genomics Center core facility. Sequences were viewed and aligned using Sequencher 4.2.2 (GeneCodes Corp.)

Determination of male ploidy

Diploid males were identified via the presence of at least one heterozygous genotype among the six microsatellite loci Pdom1CAG, Pdom25AAG, Pdom121AAG, Pdom122AAT, Pdom127bAAT, and Pdom139AAC (Henshaw, 2000). Males were presumed haploid if they possessed only one allele at each of the six loci. The probability that a diploid male was homozygous at all six loci was less than 0.0005 as calculated from population allele frequencies (Liebert et al. 2005).

Genotyping was performed as described in Johnson and Starks (2004) and Liebert et al. (2004, 2005) in the I.S.I.R.F. genetics laboratory at Tufts University, Medford, MA.

Results

Infection status – females

Forty-nine of 101 females (49 %) collected in the U.S. and 23 of 39 females (59 %) collected in Italy were infected with *Wolbachia.* Local prevalences ranged from 16 % to 87 % in the U.S. and from 33 % to 71 % in Italy (Table 1). The difference in prevalence among U.S. sites was significant (χ^2 = 27.2, 4 df, $p < 0.001$), but overall prevalence was similar between the U.S. and Italy (χ^2 = 1.23, 1 df, p = 0.27). Sample sizes were too small to test for differences among Italian sites.

Infection status – males

Two of three haploid males from Italy, 11 of 23 haploid males from Massachusetts, and the only Michigan haploid male were infected with *Wolbachia*, as were five of 12 diploid males and one male of undetermined ploidy from Massachusetts. These 40 males came from only 11 nests (three from Italy, one from Michigan, and seven from Massachusetts), and nine of these nests produced infected males, so

Table 1. *Wolbachia* prevalence among *P. dominulus* females collected in the U.S. and Italy.

Site	Collection dates	N	Number infected	Prevalence
Andover, MA	Aug 2001	23	6	0.26
Carlisle, MA	Aug 2001	17	10	0.59
Rochester, NY	Mar–May 2005	19	3	0.16
Enfield, CT	Jun 2005	19	10	0.53
Rochester, MI	Sep–Nov 2003	23	20	0.87
Total USA	**–**	**101**	**49**	**0.49**
Florence, Italy	Summer 2003	24	17	0.71
Monte Nerone, Italy	Aug 2004	5	2	0.40
Uccellina, Italy	Jun 2004	4	2	0.50
Venturina, Italy	Aug 2004	6	2	0.33
Total Italy	**–**	**39**	**23**	**0.59**
Overall	**–**	**140**	**72**	**0.51**

independent replicates by site and infection status were too small to test for differences in infection prevalence between males and females.

Strain group

Primers specific for *Wolbachia* strain group A failed to amplify fragments from any of the 21 infected wasps tested, while primers specific for group B amplified fragments from 20 of these 21 wasps.

Wolbachia sequences

A 512-bp *wsp* fragment (GenBank DQ172917) was identical in all 14 individuals tested. Its closest matches were with *Wolbachia* strains found in the mosquito *Coquillettidia richardii* (Ricci et al., 2002), the fig wasp *Blastophaga nipponica* (Shoemaker et al., 2002), the green rice leafhopper *Nephotettix malayanus* (Kittayapong et al., 2003), and the crambid moths *Ostrinia scapulalis and O. furnacalis* (Kageyama et al., 2002, 2003). The *wsp* sequence from *C. richardii* differed from that of *P. dominulus* by 1 bp. Sequences from the other four insects did not differ from that of *P. dominulus*, but in each of these cases the overlap between the published *wsp* sequence and the sequence we obtained from *P. dominulus* was incomplete (Table 2).

mtDNA haplotypes

Six haplotypes of a 625 bp fragment of the COI mtDNA gene were present among 14 *Wolbachia*-infected and 12 *Wolbachia*-uninfected females (GenBank DQ172911-172916). Each of these haplotypes differed from its most closely related haplotype(s) by a single base. Five haplotypes contained at least one infected wasp. The two most common haplotypes (>2 individuals each) contained both infected and uninfected wasps. One haplotype included uninfected wasps from Italy and New York and infected and uninfected wasps from Massachusetts. Sequence data showed no evidence of the presence of other arthropod DNA. Figure 1 shows infection status superimposed on a network of host haplotypes.

Discussion

Our results show that (a) *Wolbachia* occurs in both males and females of *P. dominulus*, (b) infection prevalence varies among local U.S. populations, but is similar overall between Italy and the northeastern U.S., (c) infected individuals from Italy and the northeastern U.S. carry the same B-group *Wolbachia* strain, (d) the infection is associated with multiple

Table 2. The five closest matches between *P. dominulus Wolbachia wsp* (GenBank DQ172917) and previously published *wsp* sequences from other *Wolbachia* strains.

GenBank accession number	Host species	Host order and family	Length	Overlap with *P. dominulus wsp* (512 bp)	# bp differences	Bit score	E-value	Reference
AJ311039	*Coquillettidia richardii*	Diptera: Culicidae	549	512 bp	1	941	0.0	(Ricci et al., 2002)
AF521155	*Blastophaga nipponica*	Hymenoptera: Agaonidae	555	503 bp	0	929	0.0	(Shoemaker et al., 2002)
AF481176	*Nephotettix malayanus*	Homoptera: Cicadellidae	555	503 bp	0	929	0.0	(Kittayapong et al., 2003)
AB077201	*Ostrinia scapulalis*	Lepidoptera: Crambidae	555	503 bp	0	929	0.0	(Kageyama et al., 2003).
AB056664	*Ostrinia furnacalis*	Lepidoptera: Crambidae	555	503 bp	0	929	0.0	(Kageyama et al., 2002)

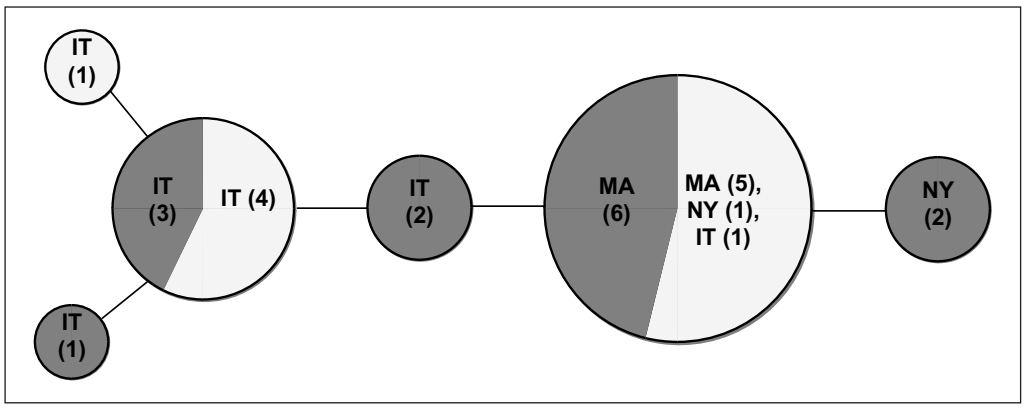

Figure 1. Unrooted mtDNA haplotype network for 26 wasps from Massachusetts (MA), New York (NY), and Italy (IT). Each circle represents one haplotype with an area proportional to haplotype frequency; shaded areas show proportion of wasps infected. Sample sizes are in parentheses. Each haplotype differs from its adjacent haplotype(s) by only 1 bp.

mitochondrial haplotypes, and (e) infected and uninfected individuals can share the same mitochondrial haplotype.

Wolbachia strains are highly variable among host taxa. Almost every insect host species surveyed harbors a *Wolbachia* strain with a unique *wsp* sequence (Zhou et al., 1998), and strains with highly similar *wsp* sequences often infect distantly related hosts (Werren et al., 1995). The five known strains that are most similar to the *P. dominulus* strain are found in a mosquito, a fig wasp, a leafhopper, and two crambid moths. The mosquito, *Coquillettidia richardii*, is found in Italy (Ricci et al., 2002), but soft-bodied terrestrial larvae and nymphs are the preferred prey of *P. dominulus* (Nannoni et al., 2001). The other hosts are Asian species that *P. dominulus* is unlikely to encounter in either the U.S. or Italy. Also, currently available evidence suggests that there have been multiple introductions of *P. dominulus* into the northeastern U.S. from source populations that have not yet been identified and are not necessarily located in Italy (Johnson and Starks, 2004; Liebert et al., in press). Therefore, the association of only one *Wolbachia* strain with this generalist predator on two continents suggests a single established infection rather than repeated, transient exposures via prey or other infected insects. Since unambiguous single COI sequences were obtained from each individual, it is also unlikely that the infection is harbored by arthropod endoparasites (e.g. Strepsiptera) rather than by the wasps themselves.

If all infected individuals of *P. dominulus* are direct descendants of one originally infected female, then this infection is old enough for at least five mutations to have accumulated within a 625 bp sequence of the COI gene in the host mtDNA. The alternative possibility is that there has been horizontal transmission of the infection within *P. dominulus*. However, such horizontal transmission will often result in the colonization of cytoplasmic lineages carrying distantly related mitochondrial haplotypes. The infected *P. dominulus* haplotypes that we identified are very closely related, a pattern more consistent with an established, vertically-transmitted infection (Dyer and Jaenike, 2004).

The presence of both infected and uninfected individuals with the same haplotype indicates that the infection is imperfectly transmitted or can be lost during host life. The occurrence of both infected and uninfected individuals across sites and haplotypes suggests that the infection is being maintained at intermediate (and possibly equilibrium) frequencies (Dyer and Jaenike, 2004).

The presence of infected haploid and diploid males rules out the MK, F, and PI phenotypes, unless penetrance is low. Moreover, the F and PI phenotypes are both unlikely in hymenopterans with CSD (Werren, 1997). Our data do not show whether this infection causes CI or other reproductive effects, but if this infection has persisted as suggested above, *P. dominulus* is probably not in the process of losing a phenotypically "dead" infection. If such a loss were going on, then one would expect the infection to be associated with only a random subset of mitochondrial haplotypes, a pattern we do not see. Furthermore, if infection status influences female reproductive success, there may be a history of mitochondrial sweeps originating in one or a few matrilines (Baudry et al., 2003). If such a sweep has occurred, mtDNA haplotype frequencies in this species may not exhibit neutral dynamics, with implications for their use in invasion tracking (Hurst and Jiggins, 2005).

Previous studies of *P. dominulus* have emphasized early reproduction and high colony productivity as keys to its invasiveness (Pickett and Wenzel, 2000; Gamboa et al., 2002; Gamboa et al., 2004; Liebert et al., in press). Through various mechanisms, *Wolbachia* infections can have a substantial effect on the productivity of individuals and populations. Consequently, heterogeneity among populations in infection prevalence may contribute to differential rates of expansion and colonization by populations of an invasive species. Some invasive social insects lose *Wolbachia* infections in new habitats, presumably because of either drift or novel selection pressures (Shoemaker et al., 2000; Tsutsui et al., 2003; Reuter et al., 2005). However, *P. dominulus* populations in the U.S. may be less affected by drift if multiple introductions have increased effective founder-population size. Also, the cited studies of *Wolbachia* in invasive ants have focused on longer-established populations which have had more time to adapt to local conditions. The more recent expansion of *P. dominulus* provides a rare opportunity to study geographic and temporal variation in infection prevalence during an invasion, and to compare *Wolbachia* dynamics between native and invaded ranges. By investigating possible reproductive effects of *Wolbachia* in *P. dominulus*, and by monitoring changes in infection prevalence and host density as the inva-

sion proceeds, it may be possible to infer whether a "selfish" endoparasite can influence spread of a host population. *P. dominulus* may thus become an attractive subject for *Wolbachia* research in the context of invasion biology.

Acknowledgements

Funds for this work were provided by the U.S. National Science Foundation (EF-0328363 and DEB-0315521). We also thank Stefano Turillazzi, George Gamboa, R. Ray Choudhury, and Jo Kozaczka for providing wasp specimens, Miranda Minhas for technical assistance, and four anonymous reviewers for helpful comments on earlier versions of this manuscript.

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