

No evidence for *Wolbachia*-induced parthenogenesis in the social Hymenoptera

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Abstract

In some parasitoid wasps, infection by the micro-organism *Wolbachia* leads to asexual reproduction. Within the Hymenoptera, the limits of distribution of parthenogenesis inducing *Wolbachia* have not yet been established. To address this issue, we screened all known thelytokous social hymenopteran species using a PCR assay. None was infected, and therefore we conclude that worker thelytoky evolves independently of *Wolbachia* in ants and bees. This supports the previously proposed hypothesis that a sex determining system based on heterozygosity may form a proximate limitation to *Wolbachia*-induced parthenogenesis.

Introduction

The typical pattern in hymenopteran species is that females are produced from fertilized eggs but that males arise parthenogenetically from unfertilized eggs (arrhenotoky). In some species, however, unfertilized eggs can yield diploid female offspring through the process of thelytokous parthenogenesis. Indeed, it is puzzling why such asexual mutants have not outreproduced their sexual counterparts more often (Williams, 1975; Maynard Smith, 1978; Bell, 1982). A recent theory poses that asexual reproduction should be opposed by the male sex, as males can gain fitness benefits through sexual reproduction only (Hurst & Peck, 1996; Hurst, 1997). Perhaps not surprisingly then, maternally derived plasmagenes have been discovered that adopt the opposite strategy: induction of asexual reproduction, hence escaping the two-fold cost of sex (Hurst, 1993). Most notably, in some parasitoid wasps infection by the micro-organism *Wolbachia* leads to asexual reproduction and all-female broods (reviewed in Stouthamer, 1997). Being maternally transmitted, these microbes make their host produce asexually broods of mostly female offspring. This phenomenon is reversible upon treatment by antibiotics or excessive heat (Stouthamer *et al.*, 1990).

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In addition, manipulative strategies have evolved several times and are not limited to parthenogenesis induction. Effects of sex ratio distortion also occur through feminization of genetic males (Rigaud, 1997) or killing of male offspring (Hurst *et al.*, 1999), and in most insect species the bacteria spread by sterilizing competing uninfected females (reviewed by O'Neill *et al.*, 1997; Werren, 1997). The limits of distribution of each of these phenotypes have not yet been established. Parthenogenesis induction has been found to be the most common phenotype in chalcidoid and cynipoid parasitoid wasps (Stouthamer, 1997), but its exact distribution within the Hymenoptera is unknown. As a first systematic survey of an aculeate group, we investigated whether *Wolbachia* is involved in parthenogenesis induction in any of the known thelytokous social hymenopteran species (six ant species and the cape bee, Table 1) using a PCR-based assay. Of the handful of species where thelytoky has been documented, only *P. pungens* and *C. biroi* show obligatory thelytoky (Itow *et al.*, 1984; Tsuji & Yamauchi, 1995); in the other species thelytoky is thought to occur only in orphaned colonies.

In striking contrast to the apparent rarity of thelytoky in the social Hymenoptera, *Wolbachia* seems to be extremely common in ants, with at least 50% of the species being infected (Wenseleers *et al.*, 1998). Indeed, several authors have suggested that *Wolbachia* might have some importance in explaining the reproductive patterns found in social insects (Crozier & Pamilo, 1993,

Table 1 Collection localities, number of colonies and type of material screened for *Wolbachia* infection.

Species	Thelytoky reported by	Collection localities and number of colonies screened	No. of workers per colony screened
APIDAE			
<i>Apis mellifera capensis</i>	see refs in Rothenbuhler <i>et al.</i> (1968) and Page & Erickson (1988)	6 colonies from Grahamstown (S. Africa)	10
FORMICIDAE			
Ponerinae			
<i>Platythyrea punctata</i>	Heinze & Hölldobler (1995); Schilder <i>et al.</i> , 1999	2 colonies from Puerto Rico (USA), 2 colonies from Barbados, 1 colony from Central Florida (USA)	3
Cerapachyinae			
<i>Cerapachys biroi</i>	Tsuji & Yamauchi (1995)	4 colonies from Okinawa (Japan)	3
Myrmicinae			
<i>Messor capitatus</i>	Grasso <i>et al.</i> (1998)	1 colony from Berceto (Italy) 1 colony from Rome (Italy) 2 colonies from Brescia (Italy)	10
<i>Pristomyrmex pungens</i>	Itow <i>et al.</i> (1984); Tsuji (1988)	3 colonies from Nagoya (Japan)	5
Formicinae			
<i>Cataglyphis cursor</i>	T.W., unpublished data	1 colony each from St. Gilles, Beaucaire, Tarascon, Aix-en-Provence, Apt, Luberon and Bagnols-sur-Cèze (France) and Peguerinos and Gredos (Spain)	6
<i>Cataglyphis piliscapus</i>	see refs. in Lenoir <i>et al.</i> (1988)	1 colony each from Girona (Spain) and Banyuls-sur-Mer, Leucate, Narbonne and Montpellier (France)	6

1996; Bourke & Franks, 1995; Heinze & Tsuji, 1995; Schilder *et al.*, 1999). The present study assesses the likelihood of a role in parthenogenesis induction.

Materials and methods

Sampling and DNA extraction

A total of approximately 250 workers belonging to seven thelytokous species were collected from various localities (Table 1) and stored in ethanol 99%. DNA was extracted using a proteinase K digest: individual insects were incubated at 55 °C for 5 h in a solution consisting of 90 µL 1× PCR buffer, 0.6 µL 10 mg mL⁻¹ proteinase K (Sigma), 5 µL of 10% Tween 20 (Merck) and 5 µL of 10% NP 40 (Calbiochem). Extraction volume was increased five-fold for cape bee samples. The samples were subsequently incubated at 95 °C for 20 min to inactivate the proteinase K, centrifuged and stored at -20 °C prior to use.

PCR amplification

Based on Genbank deposited *Wolbachia* 16S rDNA sequences, a generic primer pair was developed that targets a 1000-bp stretch of the SSU rDNA of all known *Wolbachia* strains (including the recently discovered C and D strains found in nematodes, Bandi *et al.*, 1998) but no other related bacteria: WOLB16SF1 5'-AGT CCT GGC

TAA CTC CGT GCC A-3' and WOLB16SR1 5'-TCA CCC CAG TCA CTG ATC CCA C-3'. Empirical results have shown that this primer pair is highly specific for *Wolbachia*, as it successfully amplifies the *Wolbachia* from various insect groups (including those found in ants, Wenseleers *et al.*, 1998), but fails to amplify other members of the alpha proteobacteria. Comparison with the approximately 23 000 previously published SSU rDNA sequences using ProbeMatch further confirmed specificity (Maidak *et al.*, 1999). In addition, we devised an insect-specific primer pair amplifying a 555-bp stretch of the nuclear 18S rDNA of the host: 18SF1 5'-TTG GAG GGC AAG TCT GGT GC-3' – 18SR1 5'-ACT TCG GCG GAT CGC TAG CT-3'. This gene was used to ascertain the quality of the DNA extractions.

PCR amplifications were carried out in a 15-µL reaction mixture consisting of 0.5 µM of each primer (0.25 µM for the 18S rDNA primers), 0.2 mM of each dNTP, 1.5 mM MgCl₂, 1.5 µL template, 0.3 U of *Taq* polymerase (Ampli-Taq, Perkin Elmer Cetus), 1 µL of the crude DNA extract and 1× enzyme buffer supplied by the manufacturer. Each reaction mixture was overlaid with about 20 µL of mineral oil. PCR was performed on a Biometra TGradient 96 Thermoblock with initial denaturation at 94 °C for 3 min, followed by 30 cycles consisting of 94 °C for 30 s, 63 °C for 1 min and 72 °C for 2 min, and a final 10' terminal extension step at 72 °C. Using these PCR conditions, the host DNA detection threshold corresponded to the detection limit of *Wolbachia*. Samples for

which the control PCR failed were discarded. *Gnamptogenys menadensis* (A strain infected, collected from Karaenta, Sulawesi) and *Gnamptogenys moelleri* (uninfected, collected from Mexico) were included as positive and negative controls in every amplification in order to discriminate between an experimental failure in PCR amplification and lack of *Wolbachia* infection.

Results

All material from the thelytokous species was examined for *Wolbachia* infection using a PCR assay (for details see Table 1), but none turned out to be infected. The correct amplification of the positive control indicates true lack of infection and not a failure in the set-up of the PCR reactions. For all samples, the 18S rDNA host gene was successfully amplified, eliminating the possibility of either failed DNA extractions or presence of PCR inhibiting substances. Also, since for each species several colonies and several individuals per colony were analysed, absence of an amplification product is unlikely to have been the result of infection polymorphism.

Discussion

This study clearly shows that it is highly unlikely that *Wolbachia* induces parthenogenetic reproduction in social insects. Foremost, none of the thelytokous social Hymenoptera is infected by the micro-organism. In addition, *Wolbachia* occurs abundantly in ants (Wenseleers *et al.*, 1998), while thelytoky has been documented in just a handful of species (Bourke, 1988; Choe, 1988; Heinze & Tsuji, 1995).

Why does *Wolbachia* not induce parthenogenesis in the social Hymenoptera? From the perspective of *Wolbachia*, such a strategy would yield a direct transmission advantage and unlike alternative strategies like male killing (Hurst *et al.*, 1999) or cytoplasmic incompatibility (O'Neill *et al.*, 1997) would not require relatedness to be selected for (Frank, 1997). This study sheds light on this question in that proximate constraints probably matter. *Wolbachia*-induced parthenogenesis generally relies on a cytogenetic mechanism of gamete duplication, yielding completely homozygous diploid offspring (in *Trichogramma*: Stouthamer & Kazmer, 1994; in *Diplolepis*: Stille & Dävring, 1980; Stille, 1985; Plantard *et al.*, 1998; in *Encarsia formosa*: mentioned without reference in Quicke, 1997; all allozyme loci screened so far are also homozygous, e.g. De Oliveira & De Lima, 1997; in *Muscidifurax uniraptor*: Legner, 1985). Only in the chalcidoid and cynipoid wasps, where the sex determining system is independent of heterozygosity, does such a system result in the production of female offspring (the exact nature of the sex determining system of chalcidoids and cynipoids is still controversial, see Dobson & Tanouye, 1998). In the social Hymenoptera, where offspring sex is determined

by heterozygosity at a single sex locus (Beye *et al.*, 1996; reviewed by Cook, 1993; Cook & Crozier, 1995; Crozier & Pamilo, 1996), this would end in exclusive production of sterile diploid male offspring. This limitation was mentioned previously by Stouthamer & Kazmer (1994), Hurst & Peck (1996) and Werren (1997), an hypothesis that is corroborated by our results. Our data therefore highlight the role of proximate constraints in evolution. *Wolbachia* might also have evolved other mechanisms to induce parthenogenesis that would not reduce heterozygosity (e.g. automictic parthenogenesis with suppression of recombination as in the cape bee, Verma & Ruttner, 1983; Moritz & Haberl, 1994). But in social insects this is probably not what has happened. What phenotype *Wolbachia* causes in social insects remains unknown, but parthenogenesis induction is no longer among the possibilities.

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