Insect Molecular Biology (1998) 7(4), 393–396

SHORT NOTE

Phylogenetic analysis of parthenogenesis-inducing *Wolbachia* in the genus *Aphytis* (Hymenoptera: Aphelinidae)

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Abstract

Parthenogenesis-inducing intracellular bacteria of the genus *Wolbachia* are found in a variety of parasitoid wasp genera. The presence of *Wolbachia* in the uniparental *Aphytis* species *A. lingnanensis* Compere, *A. diaspidis* (Howard), *A. chilensis* Howard, and *A. chrysomphali* (Mercet) was tested using primers specific for the *ftsZ* gene. The symbiont was detected in all of these species. *Wolbachia ftsZ* genes that were sequenced from the four hosts show a high degree of similarity. Both the PCR with specific primers for group 'A' and phylogenetic analysis place these *Wolbachia* in group 'A'. The fact that the tested *Aphytis* species belong to different phylogenetic groups and harbour what seem to be almost identical *Wolbachia*, lends credence to the horizontal transmission hypothesis.

Keywords: *ftsZ* gene, symbionts, parasitic wasps, thelytoky.

Introduction

Wolbachia are intracellular bacteria belonging to the alpha subdivision of the purple bacteria (Woese, 1987). This symbiont is known to cause reproductive and sex-ratio disorders in many insects and other arthropods (for review, Werren, 1997). The bacteria are located in the reproductive tissues of their hosts and are transferred from the female to her offspring through the egg cytoplasm. *Wolbachia* has been found in over 10% of the insects surveyed (Werren *et al.*, 1995).

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To date, three major phenomena are known to be associated with the presence of *Wolbachia*: (1) Cytoplasmic incompatibility which occurs between infected and uninfected strains. This phenomenon has been described in many insect species and several mites (O'Neill & Karr, 1990; Breeuwer & Werren, 1990). (2) Feminization diversion of genetic males into phenotypic females, which has been described in several Isopod species (Rigaud *et al.*, 1991). (3) Parthenogenetic production of female offspring without fertilization by males, which has been found only in species of parasitic Hymenoptera (Stouthamer *et al.*, 1990; Zchori-Fein *et al.*, 1995; Pijls *et al.*, 1996).

Since it is not yet possible to rear *Wolbachia* in artificial media, their identification and systematic characterization is performed using molecular phylogenetic methods. Until recently, sequences of the 16S rDNA gene were used for phylogenetic analysis (O'Neill *et al.*, 1992; Stouthamer *et al.*, 1993). Since comparisons of *Wolbachia* phylogeny from some infected species using the 16S rDNA gene failed to show any significant differences among these species, it was necessary to look for a less conserved gene. The *ftsZ* cell cycle gene was found to be more variable among species, and is expected to reveal greater differences (Werren *et al.*, 1995).

Phylogenetic analysis of *Wolbachia* has revealed that the bacteria can be divided into two different groups. Group 'A' members show fewer differences among the infected species in comparison with group 'B' (Werren *et al.*, 1995). Although it is possible to divide the symbionts into distinct groups, no connection has been found between the *Wolbachia* strain and the reproductive disorder it causes. Based on the two genes, the phylogenetic studies have also shown that very different host species harbour identical *Wolbachia*. These findings lead to the hypothesis of horizontal transfer of the symbiont among its hosts (O'Neill *et al.*, 1992; Werren *et al.*, 1995; Moran & Bauman, 1994).

Support for this hypothesis has come from different phylogenetic methods and from the fact that some hosts having a parasite–host relationship harbour identical *Wolbachia* (Werren *et al.*, 1995).

Aphytis (Hymenoptera: Aphelinidae) are minute wasps which develop exclusively as primary ectoparasites of armoured scale insects (Homoptera: Diaspididae), and are the most important natural enemies of these serious pests (Rosen & DeBach, 1979). Based on morphological and biological characters, the genus is divided into six different groups: vittatus, chilensis, proclia, mytilaspidis, lingnanensis and chrysomphali (Rosen & DeBach, 1976). In the genus *Aphytis* one quarter of the species whose sexuality is known exhibit thelytokous parthenogenesis in which unfertilized eggs develop into females. In these species the frequency of males in the population is 1–5% (DeBach, 1969). *Wolbachia* infections have previously been studied in two *Aphytis* species: *A. lingnanensis* Compere which has both a biparental and a uniparental line, and *A. diaspidis* (Howard) which is strictly uniparental. Using molecular methods, *Wolbachia* were detected in both of the uniparental lines, and their presence was confirmed in uniparental *A. lingnanensis* by electron microscopy and antibiotic treatment (Zchori-Fein *et al.*, 1994, 1995).

The aim of this study was to identify the parthenogenesis-inducing symbionts in uniparental species representing the major *Aphytis* groups, and to establish their phylogenetic relationships in order to learn about horizontal transmission of *Wolbachia* within this important group.

Results and Discussion

Detection of Wolbachia *in various* Aphytis *species*

The uniparental *Aphytis* species, *A. lingnanansis* (UP), *A. diaspidis*, *A. chilensis* and *A. chrysomphali*, showed a band size of about 900 bp in PCR with *Wolbachia ftsZ* gene general primers (Fig. 1). The two biparental species (*A. lingnanensis* BP line and *A. paramaculicornis*) did not show any band, indicating the positive results are from the *Wolbachia* gene (Fig. 1). Using primers for 18S rDNA gene, the DNA was confirmed as amplifiable (data not shown).

Parthenogenesis-inducing *Wolbachia* are widespread within the Chalcidoidea, and their presence has been verified by molecular methods in more than fifteen species (Stouthamer, 1997). Our results show correlation between the presence of *Wolbachia* and thelytokous reproduction in species of the genus *Aphytis*.

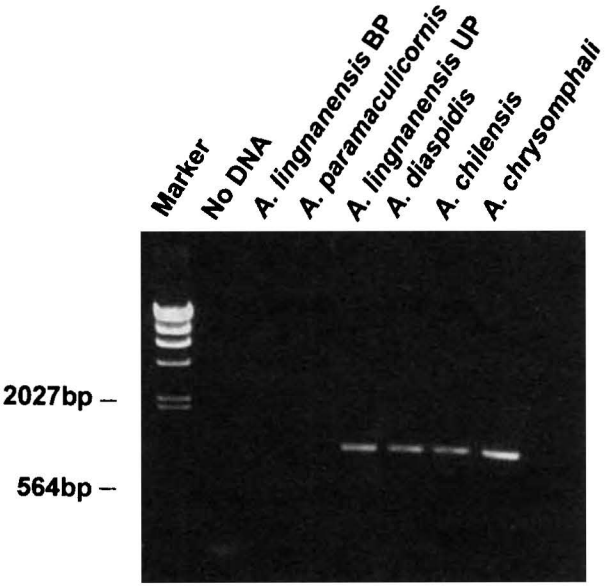

Figure 1. PCR analysis of *Wolbachia* infection in *Aphytis* species. DNA from uniparental and biparental *Aphytis* species was amplified using PCR with *ftsZ* general primers. PCR products were electrophorated on 1% agarose gel and stained with ethidium bromide. A = *Aphytis*, BP = biparental, UP = uniparental.

Phylogeny of Wolbachia *from uniparental* Aphytis *species*

All the tested species also gave a band of the right size in PCR with *Wolbachia* 'A' group specific primer (data not shown). Each of the four *Wolbachia* sequences (897 bp long) differ from the other three by one base pair. The phylogenetic analysis of the sequencing results could not detect any differences among the *Wolbachia* found in the various *Aphytis* species, and placed them all in the same branch of the 'A' group (Fig. 2).

Both the results of PCR with 'A' group specific primers and the sequence-based phylogenetic analysis show that *Wolbachia* from *Aphytis* belong to the 'A' group, together with the parthenogenesis-inducing *Wolbachia* found in *Muscidifurax uniraptor*. Based on the 16S rDNA, a previous phylogenetic analysis placed *Wolbachia* from *A. lingnanensis* and *A. diaspidis* at the same position (Zchori-Fein *et al.*, 1995). According to the phylogenetic tree constructed by Werren *et al.* (1995) on the basis of *ftsZ* gene, *Wolbachia* from *A. yanonensis* DeBach & Rosen belong to the Adm subgroup within the 'A' group. Our analysis places the *Wolbachia* from all tested *Aphytis* in that group. The Adm subgroup is a heterogenic group of hosts that carry very similar *Wolbachia*. This high degree of similarity has led researchers to suggest recent horizontal transmission (O'Neill *et al.*, 1992; Moran & Bauman, 1994; Werren *et al.*, 1995). The present study lends further support to this hypothesis.

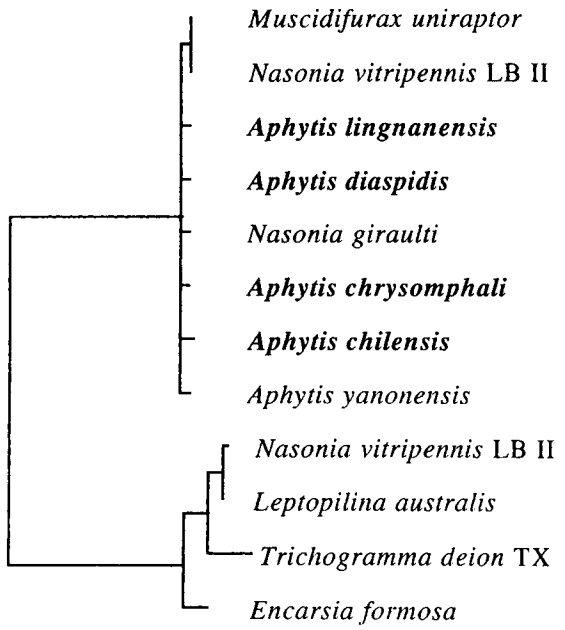

Figure 2. A phylogenetic tree based on *ftsZ* gene sequences from different *Wolbachia* groups. The tree was constructed using the branch-and-bound method of PAUP. *E. coli* is used as an outgroup (100 bootstrap). Insect names indicate *Wolbachia* origin. Bolds indicate species examined in this paper.

The *Aphytis* species chosen for this study represent four (chilensis, proclia, lingnanensis and chrysomphali) out of the six phylogenetically distinct *Aphytis* groups and are estimated to have separated tens of millions years ago (Rosen & DeBach, 1979). The calculated divergence rate of 0–2.5 million years within the Adm group (Werren *et al.*, 1995), combined with the phylogeny of the species *Aphytis*, make it less likely to assume that a common ancestor of *Aphytis* was infected with *Wolbachia*, and the infection remained through the speciation process.

Smith *et al.* (1992) suggest three lines of supporting evidence for horizontal transmission of genes: (1) Unexpected position of the organism in a phylogenetic tree. This evidence is reinforced if more than one phylogenetic method gives the same topology. (2) If there are other sequences that give the same phylogeny. (3) When the life history of the organisms involved includes contact relationships. In addition, the more organisms tested that give the same phylogeny, the stronger the evidence. There is some evidence for horizontal transmission of genes from prokaryotes to eukaryotes (Smith *et al.*, 1992), and evidence for the transmission of P element among *Drosophila* species through the mite *Proctolaelaps regalis* DeLeon (Houck *et al.*, 1991). Though *Wolbachia* is an entire organism which is probably horizontally transmitted among arthropods, the criteria for gene transmission can be applied: (1) When *Aphytis* phylogeny (Fig. 3) is compared with the phylogeny of *Wolbachia* found in that group, it can be seen that virtually identical Wolbachiae are found in *Aphytis* species belonging to distant phylogenetic groups. (2) The sequences of two different *Wolbachia* genes (16S rDNA and *ftsZ*) from *Aphytis* give the same phylogenetic tree (Zchori-Fein *et al.*, 1995; this paper). (3) Because they are gregarious, contact relationships among different *Aphytis* species are possible and it often happens that larvae parasitizing the same host cannabilize each other (Ben-Shalom, 1996). For example, there is evidence that *A. diaspidis*, *A. chrysomphaly* and *A. chilensis* are natural parasites of the oleander scale *Aspidiotus nerii* in the Middle East (Rosen & DeBach, 1979). Such contact, however, does not necessarily result in transmission of the symbionts, since *A. paramaculicornis* also parasitizes the oleander scale but does not carry *Wolbachia*. In light of the above, it is reasonable to assume that *Wolbachia* was horizontally transmitted among *Aphytis* species.

Experimental procedures

Detection of Wolbachia *in various* Aphytis *species*

Aphytis *species*. Four uniparental (UP) *Aphytis* species were chosen, each representing a distinct morphologic group (Fig. 3, after Rosen & DeBach, 1976), from the primitive to the advanced: Chilensis group: *A. chilensis* Howard, field collected on *Aspidiotus nerii* (Bouché) on citrus from Spain. Proclia group: *A. diaspidis* (Howard), which was field collected in Rehovot from *Diaspis echinocacti* (Bouché), and subsequently reared on that host in the laboratory. Lingnanensis group: *A. lingnanensis* Compere (UP); this uniparental line was

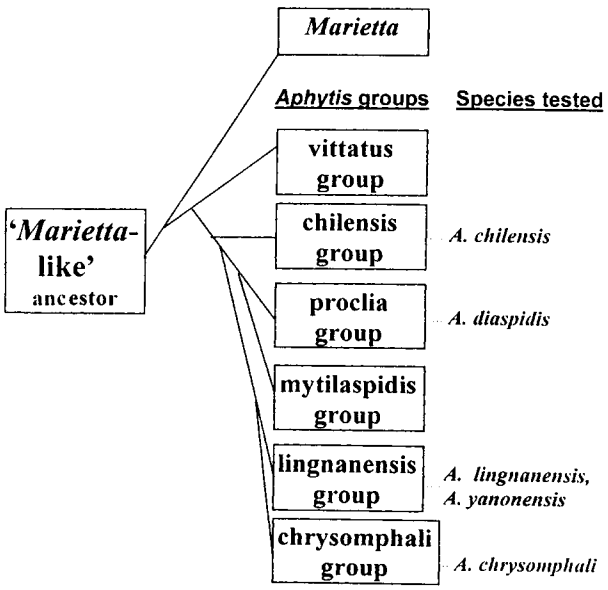

Figure 3. A phylogenetic tree of *Aphtyis* species, based on morphological characters (after Rosen & DeBach, 1976).

originally collected in 1987 from *Aonidiella* sp. in the Philippines, and subsequently maintained in the laboratory in Israel on *A. nerii*. Chrysomphali group: *A. chrysomphali* (Mercet), field collected on *Aonidiella aurantii* (Maskell) on citrus from Spain. Two biparental (BP) lines served as controls: Lingnanensis group: *A. lingnanensis* (BP), a parasite of *A. aurantii*. This line was imported to Riverside, California, from China in 1947, and from there to Israel in 1988, where it was subsequently maintained in the laboratory on *A. nerii*. Proclia group: *A. paramaculicornis* DeBach & Rosen was field collected on *Parlatoria oleae* (Colvèe) from Texas.

DNA extraction and amplification. Total DNA was extracted from the wasps using Chelex 100 (Bio-Rad) solution (4% Chelex, 100 μg/ml proteinase K) for grinding, followed by holding the tubes for 35 min at 56°C. They were vortexed, boiled for 15 min, and placed for 3 min in a centrifuge at 14,000 rpm. The reactions were performed according to Werren *et al*. (1995), with *ftsZ* general primers for detection of *Wolbachia*.

Phylogeny of Wolbachia *from uniparental* Aphytis *species*

PCR, cloning and sequencing. The reactions were performed according to Werren *et al*. (1995), and 'A' specific *ftsZ* primer was used to identify the group to which the detected *Wolbachia* belongs. PCR products were cloned using a TA cloning kit (Invitrogen). Plasmid mini-preparation was performed using CTAB after Del-Sol *et al*. (1989). Three positive clones were sequenced using a 373A automatic sequencer (Applied Biosystems).

Phylogenetic analysis. The four *ftsZ* sequences (access codes: Y13281, Y13280, Y13666, Y14954), and *Wolbachia ftsZ* sequence from *A. yanonensis* (Werren *et al*., 1995), were introduced into the GCG and aligned against other *ftsZ* genes from both 'A' group and 'B' group found in other species within the Hymenoptera. In order to determine the systematic position of the endosymbionts of *Aphytis* in comparison with *Wolbachia* from other Hymenoptera, evolutionary tree analyses were performed. The tree was constructed by the branch-and-bound method of the PAUP (phylogenetic analysis using parsimony) 3.1 for Macintosh computer program, with *Escherichia coli* as an outgroup (100 bootstrap), consensus tree constructed from all most parsimonious trees by the strict method (Swofford, 1993).

Acknowledgements

We thank J. H. Werren for a visit to his laboratory, for primers, use of facilities and advice. Thanks are extended to F. Gracia-Mari and M. Rose for supplying *Aphytis* species. This research was supported by grant US-2062-91 from BARD, the United States–Israel Binational Agricultural Research and Development Fund.

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