**Wolbachia superinfection in an Ecuadorian sample of the sand-flea Tunga penetrans**

(Preliminary note)

**Andrea LUCHETTI, Barbara MANTOVANI, Massimo TRENTINI**

Dipartimento di Biologia Evoluzionistica Sperimentale, Università di Bologna, Italy

**Abstract**

Wolbachia spp. are intracellular endosymbionts that cause reproductive alterations in their hosts. We here demonstrate the co-existence (superinfection) of both the arthropod- and the filarial-infecting strain in a sample of Tunga penetrans (L.) from Ecuador.

**Key words:** superinfection, Tunga penetrans, Tunga trimamillata, Wolbachia, Wolbachia 16S rDNA.

Obligate intracellular bacteria of the genus Wolbachia infect a wide variety of arthropods (insects, mites and isopods) and filarial nematodes. Wolbachia spp. are maternally inherited and can cause a number of reproductive alterations (phenotypes) in their hosts. These range from cytoplasmic incompatibility to sex-ratio distortion; the former determines the inviability of the offspring derived from crosses either between infected males and uninfected females, or between individuals infected by different Wolbachia strains; the latter produces a female-biased sex-ratio (Stouthamer et al., 1999). Owing to the peculiar effects on its hosts, Wolbachia spp. have been recently involved in the control of arthropods of economic and public health interest (Beard et al., 1998; Rasgon et al., 2003).

From a phylogenetical point of view, six supergroups (A-F) have been so far detected: two of these (C and D) comprise Wolbachia strains from nematodes, the others embody strains pertaining to arthropods (Lo et al., 2002).

In the tropical sand-flea Tunga penetrans (L., 1758) a filarial-related Wolbachia strain was identified (Fischer et al., 2002; Heukelbach et al., 2004). Through PCR amplification of the 16S rDNA gene and cell division protein gene ftsZ, we recently demonstrated the presence of an arthropod-infecting Wolbachia strain in T. trimamillata Pampiglione, Trentini, Fioravanti, Onore and Rivasi, 2002 and the complete absence of this bacterium in Ecuadorian populations of T. penetrans (Luchetti et al., 2004).

The widening of our studies to other South American and African populations, led to the analyses of five T. penetrans specimens from Olmedo, Ecuador: these were neosomic females extracted from swine. DNA isolation and Wolbachia 16S rDNA amplification were performed as described in Luchetti et al (2004). PCR approach revealed the presence of a band of the expected size (1.0 kbp) in all analysed samples, thus indicating a 100% prevalence of Wolbachia infection.

The sequencing of the first 524 bp of one amplicon (TpOLMw1) was performed following standard procedures (Luchetti et al., 2004) and then the obtained electropherogram was checked for the presence of nucleotide polymorphisms, as described in Feliciello et al. (2005). The final determined sequence has been submitted to Genbank as a “consensus sequence”, with IUBMB single letter code for multiple bases in the same position (A.N.: DQ015672).

BLAST search in public databases confirmed that we are dealing with a Wolbachia 16S rDNA sequence. This datum represents the first invention of Wolbachia in Ecuadorian T. penetrans and contrasts with a previous survey where no infection was reported (Luchetti et al., 2004); however, it is in agreement with other literature data indicating a 100% prevalence of Wolbachia in T. penetrans (Fischer et al., 2002; Heukelbach et al., 2004). In our previous work, both general (O’Neill et al., 1992) and specific (Fischer et al., 2002) primers were used to evidence the presence of the endosymbiont, but no amplicons were ever obtained. Given the protocol followed, we think that previously analysed populations were uninfected. Obviously the possibility that they were infected with a very low prevalence in the population and/or with a low bacterial density in the organism cannot be at present ruled out.

The careful check of the electropherogram of presently analysed sequence revealed the presence of two bases in 15 sites out of 524 bp (table 1). The 60% of these variable sites showed contemporaneously the nucleotides diagnostic for the filarial-related strain from T. penetrans (A.N.: AY150558) and the arthropod-related strain from T. trimamillata (A.N.: AY350621 and AY350622) (table 1). This situation may be explained only considering the possibility that different strains of Wolbachia are present in this sample of T. penetrans. Furthermore, considering the other variable sites, it could be assumed the co-presence of more than two Wolbachia strains. These results support a superinfection of presently analysed sample and help to explain the divergence of detected Wolbachia strains in T. penetrans and T. trimamillata (Fischer et al., 2002; Luchetti et al., 2004). In fact, even if it is well known that Wolbachia phylogeny is usually incongruent with that of its hosts (Stouthamer et al., 1999; Heath et al., 1999; Vavr et al., 1999), the presence of highly differentiated Wolbachia strains in Tunga species appears quite peculiar.
Table 1. Nucleotide variable positions, with single letter IUMB code (R = G or A; Y = C or T; W = A or T), of the *Wolbachia* 16S rDNA. Asterisks mark diagnostic sites for filarial- and arthropod-infecting strain.

<table>
<thead>
<tr>
<th></th>
<th>37</th>
<th>46</th>
<th>61</th>
<th>70</th>
<th>116</th>
<th>122</th>
<th>123</th>
<th>126</th>
<th>128</th>
<th>164</th>
<th>199</th>
<th>254</th>
<th>298</th>
<th>409</th>
<th>426</th>
</tr>
</thead>
<tbody>
<tr>
<td>R</td>
<td>R</td>
<td>Y</td>
<td>R</td>
<td>R</td>
<td>W</td>
<td>R</td>
<td>Y</td>
<td>R</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>R</td>
<td>R</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The condition of superinfection has been already demonstrated in several arthropods (Jeyaprakash and Hoy, 2000) and it represents an interesting aspect for evolutionary models dealing with *Wolbachia* dynamics. While endosymbiotic theory predicts a general trend toward clonality, *Wolbachia* constitutes an exception in which there is selection to maintain diversity with superinfection as a stage in which a novel *Wolbachia* variant will co-exist with the original infection type within a host (Dobson, 2004). This dynamics may explain the observation that *Wolbachia* phenotype can change frequently (Stouthamer et al., 1999; Jiggins et al., 2002), given that under this assumption additional pathways for the evolution of novel phenotypes are allowed.

Acknowledgements

This work was supported by 60% funds of the University of Bologna. We wish to thank Giovanni Onore for sample collecting in Ecuador.

References


Corresponding author: Andrea Luchetti, Dipartimento di Biologia Evoluzionistica Sperimentale, Alma Mater Studiorum Università di Bologna, via Selmi 3, 40126 Bologna, Italy (andluch@alma.unibo.it).

Received April 7, 2005. Accepted May 18, 2005.