

On the taxonomic status of the intracellular bacterium *Wolbachia pipientis*: should this species name include the intracellular bacteria of filarial nematodes?

The recent article by Lo *et al.* (2007) aiming to recognize the *Wolbachia* endobacteria of arthropods and filarial nematodes as *Wolbachia pipientis*, while admirable for attempting to codify the nomenclature in this field of research, may not address the stark differences of the endobacteria found in nematodes compared with the endobacteria found in arthropods.

Lo *et al.* (2007) propose that all endobacteria of arthropods and nematodes commonly called *Wolbachia* be formally declared as *Wolbachia pipientis*. Part of their reasoning is based on the difficulty of assigning the strains to monophyletic species due to the lack of an appropriate outgroup and the fact that to name arthropod strains after the species they infect would be unwieldy because of the huge number of arthropod species infected with these endobacteria (Lo *et al.*, 2007). This was the consensus reached at the 2004 international conference on *W. pipientis* (Heron Island, Australia, August 2004). However, in 2005, the genome of *Wolbachia* from the filarial nematode *Brugia malayi* (*wBm*) was published (Foster *et al.*, 2005) and compared with the previously published genome of the *Wolbachia* from *Drosophila melanogaster* (*wMel*) (Wu *et al.*, 2004). Analysis of the genome and the biology of the *Wolbachia* in nematodes argues against including these endobacteria with those from arthropods as *W. pipientis*.

The *wBm* genome is ~200 kb smaller than that of *wMel*, resulting in fewer predicted proteins (Foster *et al.*, 2005). In addition to the difficulty in making phylogenetic

trees without an appropriate outgroup, there is a high degree of recombination in arthropod strains, characteristic of horizontally transmitted bacteria, requiring multilocus strain typing for reliable identification (Baldo *et al.*, 2006). However, while *wMel* has the genes necessary for recombination, prophage sequences that could facilitate recombination and promote exchange of genes or gene fragments and a high proportion of repetitive DNA elements (14.2%) (Wu *et al.*, 2004), *wBm* has no active system for DNA recombination and no prophages and a much smaller proportion of repetitive elements (5.4%). As a result, *wBm* shows no or little recombination (Jiggins, 2002). Despite the difficulties in making phylogenetic trees for *Wolbachia*, all trees consistently group the varied nematode *Wolbachia* together into supergroups C, D and F (Casiraghi *et al.*, 2005), with the phylogeny of the endobacteria in supergroups C and D being concordant with the phylogeny of their hosts (Casiraghi *et al.*, 2001, 2004; Fenn *et al.*, 2006).

In addition to molecular evidence for nematode *Wolbachia* being different from the endobacteria in arthropods, evidence can be found by looking at the biology of these organisms. The first is that all adult worms in species infected with *Wolbachia* have the endobacteria. Besides a block in embryonic and larval development, adult worms die when the endobacteria are depleted with antibiotics (Taylor *et al.*, 2005), i.e. they are mutualists rather than parasites. In contrast, not all members of an arthropod population are infected with *Wolbachia*, and the majority of these infections can be cured with antibiotics without adverse effects on the host. Second, horizontal transfer of *Wolbachia* in arthropods has been described in nature (Turelli & Hoffmann, 1991; Vavre *et al.*, 1999), and stable infections of the

endobacteria from one species to another have been performed in the lab using micro-injection or natural mechanisms (Heath *et al.*, 1999; Riegler *et al.*, 2004). In contrast, micro-injection of *Wolbachia* from the nematode *Litomosoides sigmodontis* into the *Wolbachia*-free nematode *Acanthocheilonema viteae* resulted in apparent replication of the endobacteria, but none were detected in the larvae released from these injected worms (Hartmann *et al.*, 2003). Unfortunately, the endobacteria were not localized in the worms to see where they had established themselves – in cells or only in the body cavity. Attempts to infect *Caenorhabditis elegans* or insect cell lines with nematode *Wolbachia* that are then able to replicate have been unsuccessful (B. Slatko, unpublished results). This probably reflects the fact that the *Wolbachia* in nematodes are mutualists, and their hosts have become dependent on their endosymbionts for embryonic and larval development as well as adult survival (Pfarr & Hoerauf, 2006).

Furthermore, the significant phylogenetic distance of the hosts should also be considered. Arthropods and nematodes are in different animal phyla and are estimated to be separated by nearly a billion years of evolution (Wang *et al.*, 1999).

In summary, we have attempted to define several points that argue for a different species name for the *Wolbachia* in nematodes. Until the endobacteria in the genus *Wolbachia* are formally characterized, typed and named according to the *Bacteriological Code* (Lapage *et al.*, 1992), we believe that there is enough evidence to support naming, for purposes of discussion, the *Wolbachia* of supergroups C and D after a key host species in each supergroup, e.g. '*Wolbachia volvulus*' (*Onchocerca volvulus*) and '*Wolbachia malayi*' (*Brugia malayi*),

The names used in this manuscript are used within the context of general discussion as covered by Rule 28a of the 1990 revision of the *Bacteriological Code* and are not being formally proposed. As such, they do not meet any of the criteria required for valid publication of a name.

respectively. The endobacteria of *Mansonella* spp., in *Wolbachia* supergroup F, which have both nematode and arthropod hosts, should remain *W. pipientis* until more study clarifies their phylogeny. By assigning different names to *Wolbachia* of the C and D supergroups, their unique biology and phylogeny will be better highlighted and the taxonomy of *Wolbachia* will be more in line with the biology of these fascinating bacteria.

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