Taxonomic status of the intracellular bacterium *Wolbachia pipientis*

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*Wolbachia pipientis* is a maternally inherited, intracellular bacterium found in more than 20% of all insects, as well as numerous other arthropods and filarial nematodes. It has been the subject of a growing number of studies in recent decades, because of the remarkable effects it has on its arthropod hosts, its potential as a tool for biological control of arthropods of agricultural and medical importance and its use as a target for treatment of filariasis. *W. pipientis* was originally discovered in the cells of the mosquito *Culex pipiens* (Hertig & Wolbach, 1924) and was formally described in 1936 (Hertig, 1936). In recent decades, the use of PCR and sequencing techniques has revealed a large number of bacteria with close phylogenetic affinity to *W. pipientis*. Although they form a clear monophyletic cluster with *W. pipientis*, many of these undescribed strains show significant differences in the 16S rRNA gene when compared to the type strain (i.e. more than 3%). Due to some uncertainty about whether such bacteria might represent different species, most researchers in the field now commonly refer to *W. pipientis* simply as *Wolbachia*. In this note, we briefly review higher-level phylogenetic and recombination studies of *W. pipientis* and propose that all the intracellular symbionts known to cluster closely with the type strain of *W. pipientis*, including those in the currently recognized supergroups (A–H), are officially given this name.

Introduction

*Wolbachia pipientis* (class Alphaproteobacteria, order Rickettsiales) and its taxonomically undescribed close relatives are intracellular, maternally transmitted symbiotic bacteria found in more than 20% of all insect species, numerous other arthropods and filarial nematodes (Bandi et al., 1998; Jeyaprakash & Hoy, 2000; Plantard et al., 1999; Taylor & Hoerauf, 1999; Wenseleers et al., 1998; Werren et al., 1995; Werren & Windsor, 2000). They are well known as manipulators of arthropod host reproduction, causing cytoplasmic incompatibility, feminization, parthenogenesis and male-killing (Stouthamer et al., 1999; Werren, 1997), and have, more recently, been shown to act as obligate mutualists in filarial nematodes (Bandi et al., 2001; Taylor & Hoerauf, 1999).

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Molecular phylogenetic and recombination analyses of *W. pipientis*

No *W. pipientis* strains have yet been isolated in pure culture, largely because of their fastidious requirements. Traditional methods for bacterial species and strain determination, such as those involving DNA–DNA hybridization and biochemical parameters, have therefore not been feasible. Early phylogenetic analysis of 16S rRNA gene sequences assigned *W. pipientis* clearly to the Alphaproteobacteria, revealing a sister-group relationship with the genera *Ehrlichia*, *Anaplasma* and *Neorickettsia* (O’Neill et al., 1992). This study showed that *W. pipientis sensu stricto* formed a monophyletic clade with other insect-associated microorganisms and suggested ‘the classification of these bacteria as members of the same species’ (O’Neill et al., 1992).

Further 16S rRNA-based studies of intracellular bacteria from various arthropods and filarial nematodes showed that they formed a monophyletic group with *W. pipientis* (Rousset et al., 1992; Sironi et al., 1995; Stouthamer et al., 1993) (see also Dumler et al., 2001). A study of several different *W. pipientis* arthropod strains based on the more variable gene *ftsZ* showed that two divergent clades exist (A, B) (Warren et al., 1995). The type strain (from *C. pipiens*) was found within clade B. A subsequent analysis of intracellular bacteria from filarial nematodes found that they formed two additional clades (C, D) (Bandi et al., 1998). These clades have since been termed ‘supergroups’; a schematic summary of currently recognized supergroups is shown in Fig. 1. A number of other supergroups have been proposed more recently, including supergroups E (Collembola) (Czarnetzki & Tebbe, 2004; Vandekerckhove et al., 1999), F (arthropods and filarial nematodes) (Campbell et al., 1992; Casiraghi et al., 2005; Lo et al., 2002; Rason & Scott, 2004), G (spiders) (Rowley et al., 2004) and H (termites) (Bordenstein & Rosengaus, 2005). A number of divergent lineages, including those from various flea species and the filarial nematode *Dirofilaria repens*, have yet to be designated supergroups (Casiraghi et al., 2005; Gorham et al., 2003).

Relationships among the various supergroups are not well understood, primarily because of the absence of a suitable outgroup for rooting the inferred trees (Lo et al., 2002). The closest known relatives of *W. pipientis* are members of a clade including the genera *Anaplasma* and *Ehrlichia*, which have corrected 16S rRNA gene sequence identities of ~90% with *W. pipientis*. The last common ancestor of *W. pipientis* and these outgroups is likely to have existed several hundred million years ago, which has led to the erosion of phylogenetic signal in many sequence characters. In a recent study (Casiraghi et al., 2005), three protein-coding genes were combined and analysed, including their homologues in *Anaplasma* and *Ehrlichia* as outgroups. Using a statistical test that distinguishes between competing hypotheses (Shimodaira & Hasegawa, 1999), the scores of a number of different trees with differing outgroup positions were compared. It was found that no outgroup placement was significantly more likely than another. Without a reliable root for the tree, it is not possible to assign supergroups confidently to different, monophyletic species. A more prudent approach is to consider all strains as members of *W. pipientis*.

A number of recent studies have shown that exchange of genetic information has occurred among supergroups. Evidence has been found for recombination of genes within and between supergroups A and B (Baldo et al., 2005, 2006a; J. C. Dunning Hotopp and J. H. Werren, unpublished data). Exchange of genes/gene fragments within bacteriophages that infect different *W. pipientis* supergroups has also been demonstrated (Bordenstein & Wernegreen, 2004; Masui et al., 2000). Genome sequencing studies show that *W. pipientis* has the genetic machinery for gene transfer (Wu et al., 2004) and a number of arthropod strains have been shown to be infected by both A and B representatives (Warren et al., 1995; Warren & Windsor, 2000), which would provide a means of gene flow. It has been clearly demonstrated that recombination in the *wsp* gene encoding an outer-surface protein is widespread among supergroup A and B bacteria (Baldo et al., 2005; Jiggins et al., 2001; Warren & Bartos, 2001). This gene should therefore be avoided when trying to understand relationships among *W. pipientis* strains. Due to the level of recombination in *W. pipientis* and close relatives, reliable strain identification requires a multilocus strain typing (MLST) approach (Maiden et al., 1998). A comprehensive MLST system has been developed for *W. pipientis* using five standard housekeeping genes (Baldo et al., 2006b; Paraskevopoulos et al.,

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**Fig. 1.** Schematic diagram of *W. pipientis* phylogeny based on various phylogenetic studies of the genes *ftsZ*, *groEL*, *gltA* and *dnaA* (see text). Letters represent supergroups that have been confirmed on the basis of these four genes. The position of supergroup G is tentative since it was estimated using the *wsp* and 16S rRNA genes. Host species are indicated next to each clade or lineage. Two lineages, from *Dipetalonema gracile* and *Ctenocaphalides felis*, have not yet been classified into supergroups. Bar, 0.1 substitutions per site (an approximation based on a concatenated gene analysis of these four genes).


