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CHAPTER 3.1.21

# The Genus *Wolbachia*

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## Introduction

Numerous invertebrate species form long lasting symbioses with bacteria (Buchner, 1949; Buchner, 1965). One of the most common of these bacterial symbionts is *Wolbachia pipientis*, which has been estimated to infect anywhere from 15–75% of all insect species (Werren et al., 1995a; West et al., 1998; Jeyaprakash and Hoy, 2000; Werren and Windsor, 2000) as well as many species of arachnids, terrestrial crustaceans and filarial nematodes (O'Neill et al., 1997a; Bandi et al., 1998). In most arthropod associations, *Wolbachia* act as reproductive parasites manipulating the reproduction of their hosts to enhance their own vertical transmission. There appears to be little direct fitness cost to the infected host besides the costs arising from the reproductive manipulations. However instances have been reported where *Wolbachia* can be either deleterious (Min and Benzer, 1997; Bouchon et al., 1998) or beneficial (Girin and Boultreau, 1995; Stolk and Stouthamer, 1995; Wade and Chang, 1995; Vavre et al., 1999b; Dedeine et al., 2001) to their hosts.

*Wolbachia* were first described as intracellular *Rickettsia*-like organisms (RLOs), infecting the gonad cells of the mosquito, *Culex pipiens* (Hertig and Wolbach, 1924), and were later named "*Wolbachia pipientis*" (Hertig, 1936). It was not until the work of Yen and Barr (Yen and Barr, 1971; Yen and Barr, 1973) that *Wolbachia* were implicated in causing crossing incompatibilities between different mosquito populations (Laven, 1951; Ghelelovitch, 1952). When polymerase chain reaction (PCR) diagnostics for *Wolbachia* became available, it became clear that this agent was both extremely widespread and also responsible for a range of different reproductive phenotypes in the different hosts it infected (O'Neill et al., 1992; Rousset et al., 1992; Stouthamer et al., 1993). The most common of these are cytoplasmic incompatibility, inducing parthenogenesis, overriding host sex-determination, and male-killing (O'Neill et al., 1997a). As of the time of this writing, more than 450 different *Wolbachia* strains with unique gene sequences, different phenotypes, and infecting different hosts have been deposited in GenBank and the *Wolbachia* host database (http://www.wolbachia.sols. uq.edu.au).

## Phylogeny

*Wolbachia pipientis* (Hertig, 1936) is the only species of the genus *Wolbachia*, family Anaplasmataceae, order Rickettsiales, class α-proteobacteria (Dumler et al., 2001). Two other species, *Wolbachia persica* (Suitor and Weiss, 1961) and *Wolbachia melophagi* (Nöller, 1917; Philip, 1956), originally in the same genus have since been removed (Dumler et al., 2001). *Wolbachia*'s closest relatives in the Anaplasmataceae are the genera *Anaplasma*, *Ehrlichia* and *Neorickettsia* (Fig. 1). They are all obligate intracellular bacteria that reside in vacuoles of eukaryotic cells (Dumler et al., 2001).

On the basis of the phylogeny of the 16S *rRNA* gene, the genus *Wolbachia* as currently defined is monophyletic (O'Neill et al., 1992; Bandi et al., 1998; Lo et al., 2002). *Wolbachia* have been divided into six supergroups A–F on the basis of 16S *rRNA* and *ftsZ* gene sequences; A, B, E and F are associated with arthropods (Vandekerckhove et al., 1999; Lo et al., 2002), and C and D are associated with filarial nematodes (Bandi et al., 1998; Lo et al., 2002). The phylogenetic relationship between these supergroups is currently not well resolved (Lo et al., 2002; Fig. 2).

The validity of the A and B supergroups that infect insects has been confirmed by phylogenetic analysis of the heat shock operon *groE* (Masui et al., 1997), of the spacer 2 region between the 23S and the 5S *rRNA* coding genes (Van Meer et al., 1999) and of the surface protein gene *wsp* (Zhou et al., 1998; Fig. 3). There is evidence that homologous recombination between different *Wolbachia* strains occurs (Jiggins et al., 2001; Werren and Bartos, 2001) and thereby has the potential to confound the interpretation of phylogenetic data based on the sequences of single genes. The lack of congruency between phylogenetic trees of *Wolbachia*

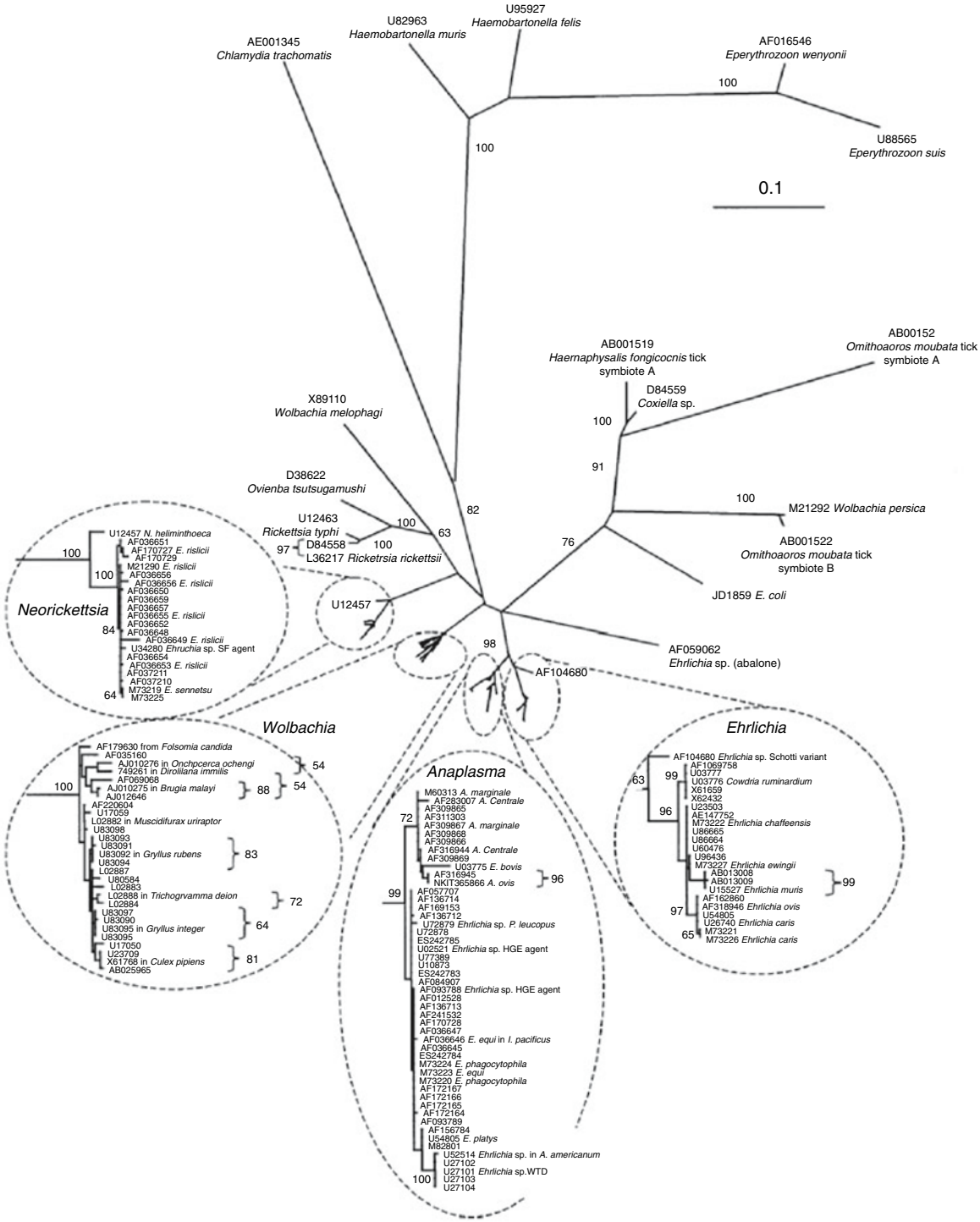


Fig. 1. Phylogenetic tree inferred from the small subunit (16S) rRNA gene sequences of *Ehrlichia*, *Anaplasma*, *Neorickettsia* and *Wolbachia* species, including 455 sites after removal of sites containing a gap in any sequence. The sequence from *Chlamydia trachomatis* (accession no AE001345 [www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=nucleotide=search=AE001345]) was used as an outgroup. Numbers above internal nodes indicate the percentage of 1000 bootstrap replicates that supported the branch. All bootstrap values are included for clades that were consistently observed using the phylogenetic methods applied (maximum parsimony, minimum evolution, maximum likelihood and majority-rule bootstrap analysis of neighbor-joining trees). The maximum-likelihood tree is shown. Bars, estimated number of substitutions per site; the scales for the figure and insets are the same. From Dumler et al. (2001).

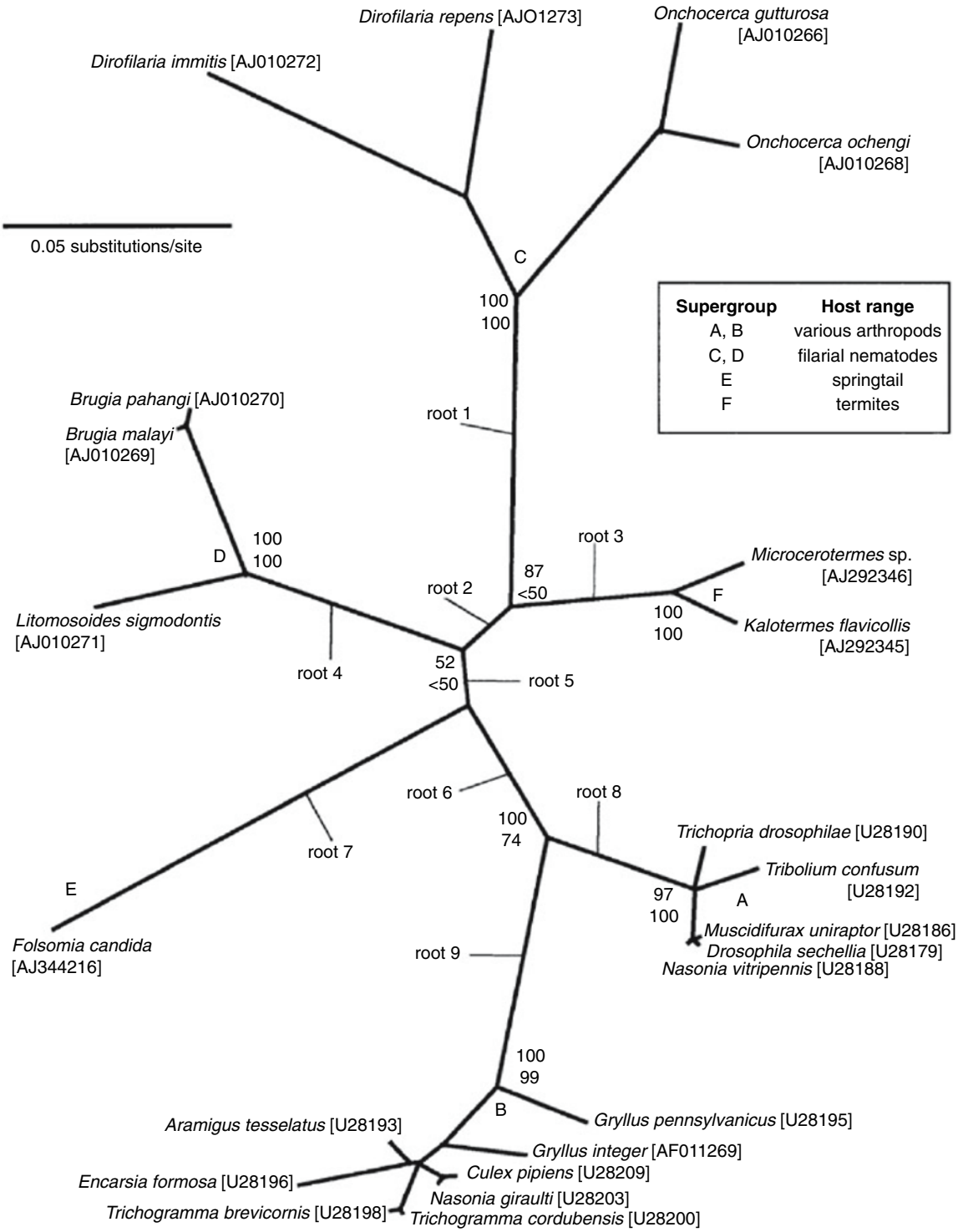


Fig. 2. Unrooted phylogenetic tree of *Wolbachia* endosymbionts of arthropods and filarial nematodes based on *ftsZ*, estimated using Bayesian inference of phylogeny. Posterior probabilities supporting nodes of interest are shown above bootstrap values from a maximum parsimony analysis. Names represent host species. Roots 1-9 indicate positions where the *ftsZ* gene of the outgroup *Anaplasma marginale* was constrained during likelihood estimations to examine the most appropriate root placement. Accession numbers are shown adjacent to each taxon. Each supergroup is labelled with one of the letters A-F. From Lo et al. (2002).

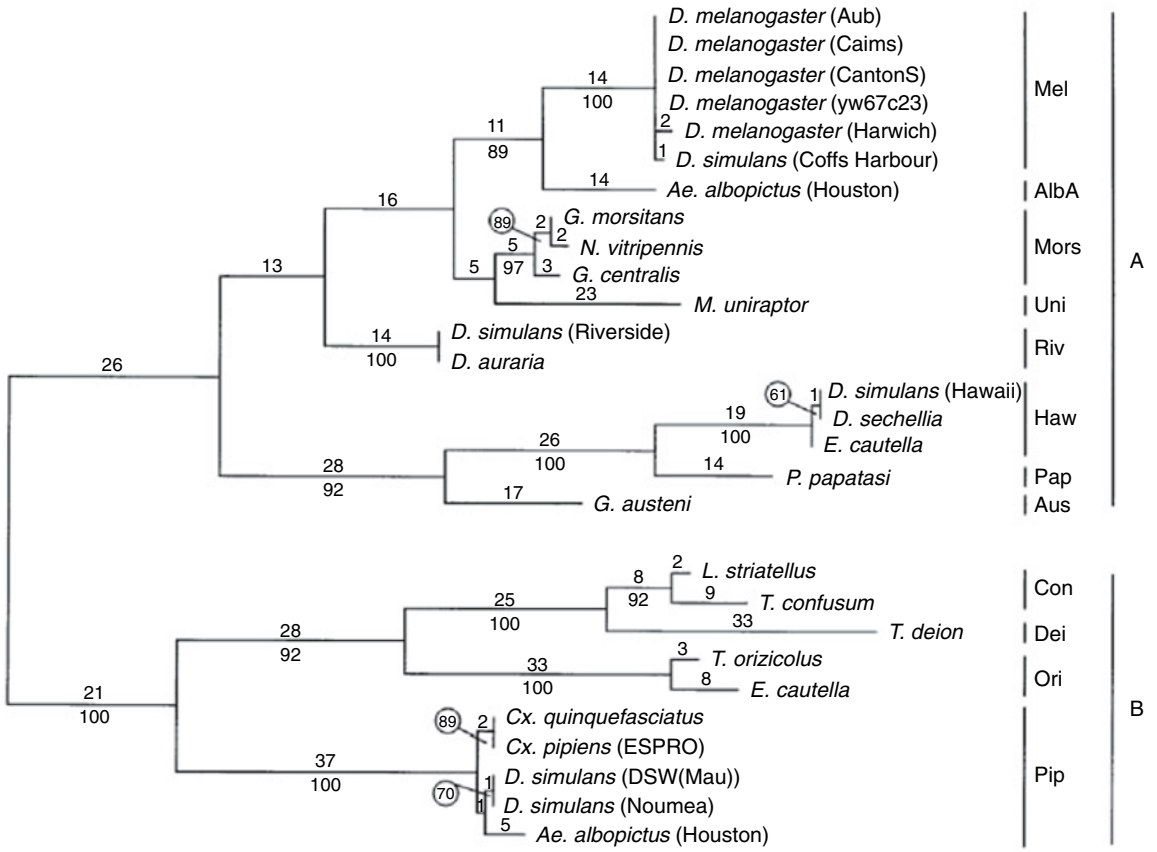


Fig. 3. One of four most parsimonious trees generated from a branch-and-bound search of aligned *wsp* sequences (tree length = 472; CI = 0.64). Tree shown is midpoint rooted. Branch lengths, as determined from the Phylogenetic Analysis using Parsimony (PAUP) table of linkages, are labelled above branches and bootstrap values (500 replicates) are labelled below branches. Bootstrap values less than 50 are not shown. Taxa are labelled as the host from which the *Wolbachia* strain was isolated. From Zhou et al. (1998).

strains and their hosts indicates that horizontal transfer of *Wolbachia* to new host species has occurred on multiple occasions during evolutionary history (O'Neill et al., 1992; Werren et al., 1995b; Vavre et al., 1999a).

## Taxonomy

The species name *Wolbachia pipientis* (Hertig, 1936) was originally assigned to the infection in a particular host, the common house mosquito *Culex pipiens*. The name has since been extended to highly similar bacteria in arthropods and filarial nematodes. In most associations, the literature only refers to the genus name "*Wolbachia*." With the rapid discovery of numerous *Wolbachia* strains, a uniform nomenclature system became necessary. The current system was based on an abbreviation style *w*Host (Rousset and de Stordeur, 1994), as proposed in Zhou et al. (1998). This system is now widely accepted and has been further specified by Charlat et al. (2002a). Separate names should be assigned to strains differing in any of the following three traits: *Wolbachia* gene sequences, phenotypic effects on hosts, or the host species infected. Strain names should then consist of a *w* followed by two or three letters to refer to the *Wolbachia* strain and a subscript referring to the host species (e.g., $w\mathrm{No}_{D.sim}$ for the *Wolbachia* infection of *Drosophila simulans* originally identified from Noumea or $w\mathrm{Cer1}_{R.cer}$ for one of the *Wolbachia* infections of *R. cerasi*). Most *Wolbachia* strains of the A and B supergroup have now been assigned strain names on the basis of the sequence of the surface protein gene *wsp* (Zhou et al., 1998). It should be noted that while strain designation in itself makes no assumptions about relatedness and as such can be based upon any consistent genetic or ecological feature, rigorous phylogenetic analysis might require the use of multiple gene sequences to account for potential recombination between strains. The discovery in recent years of a large number of diverse *Wolbachia* strains suggests that the taxonomy of the

*Wolbachia pipientis* group as a whole will soon need to be re-examined with the possibility that the current species might end up being split into a number of new species, potentially based on supergroup assignments.

## Habitat

*Wolbachia* are obligate intracellular bacteria of invertebrates, with the majority of the currently described hosts living in terrestrial habitats. *Wolbachia* have an impressive host range. Infections have been detected in all major orders of insects, arachnids (such as spiders and mites), terrestrial crustacean species, and filarial nematodes. This extreme diversity of hosts makes *Wolbachia* one of the most ubiquitous intracellular symbionts yet described. Within the host cell, *Wolbachia* is always seen within a vacuole, presumably of host origin (Fig. 4). The nature of this compartment and the extent it is modified by *Wolbachia* have yet to be determined. *Wolbachia* are inherited vertically by transovarial transmission through the cytoplasm of host eggs. As a result *Wolbachia* always infects the female germline of its host. *Wolbachia* is usually lost from the cytoplasm of sperm cells during spermatogenesis (Clark et al., 2002; Veneti et al., 2003) and as such is only maternally inherited. In addition to the germline, a range of other somatic tissues is known to be infected. The extent and diversity of somatic tissues infected vary with host and *Wolbachia* strain (Dobson et al., 1999).

## Isolation

*Wolbachia*'s entire life cycle is dependent on the cytoplasmic environment of the host. The bacteria cannot yet be cultivated on cell-free media and can only be maintained in individual hosts or cell lines (O'Neill et al., 1997b; Dobson et al., 2002; Noda et al., 2002). *Wolbachia* infections have been established in a variety of insect cell lines including those originating from *Aedes albopictus* (O'Neill et al., 1997b; Dobson et al., 2002; Noda et al., 2002), *Drosophila melanogaster*, *Spodoptera frugiperda* (Dobson et al., 2002), and *Heliothis zea* (Noda et al., 2002). *Wolbachia* can also be maintained in a mammalian cell line originating from mouse connective tissue (Noda et al., 2002). *Wolbachia* strains can be artificially transferred between host species by embryonic microinjections (Nigro, 1991; Boyle et al., 1993; Braig et al., 1994) or inoculation with crushed tissues from pupae (Williams et al., 1993), ovaries, and fat- and nervous tissues (Bouchon et al., 1998). Transinfection experiments have enabled the comparison of different *Wolbachia*-host interactions and provided a means to determine what aspects of the association are regulated by either host or symbiont (Clancy and Hoffmann, 1997; Poinsot et al., 1998; McGraw et al., 2001; Riegler et al., 2004). Transinfection experiments have also been used to segregate multiple infections from a single host (Charlat et al., 2002b; Riegler et al., 2004) or establish multiple infections within a single host (Rousset et al., 1999).

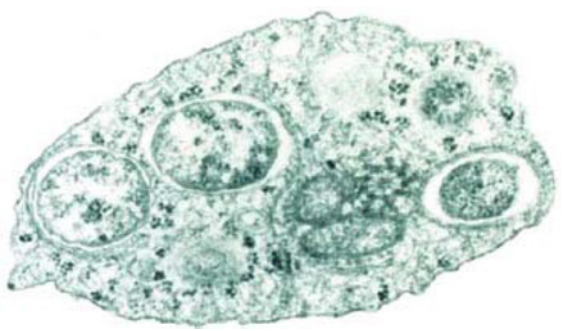

Fig. 4. Transmission electron micrograph of *Wolbachia* (arrow) within a developing spermatid of the moth *Ephestia cautella*. Courtesy of Scott O'Neill.

## Identification

*Wolbachia* are coccoid or bacilliform in morphology, 0.8–1.5 μm long (Hertig, 1936). These Gram-negative bacteria have two cell membranes and are enclosed within a vacuole (Fig. 4). Several techniques have been utilized to visualize *Wolbachia* within host tissue. *Wolbachia* can be readily stained by Giemsa (Hertig, 1936) or general DNA-binding fluorochromes such as DAPI (4′,6-diamino-2-phenylindole dihydrochloride; O'Neill and Karr, 1990; Bressac and Rousset, 1993). Monoclonal and polyclonal antibodies have been developed for the outer membrane proteins of several species of the order Rickettsiales (Ohashi et al., 1998) and specifically also for *Wolbachia* (Kose and Karr, 1995; Dobson et al., 1999; Masui et al., 2001). *Wolbachia* have also been successfully detected using in situ hybridization techniques with *Wolbachia* specific DNA probes (Heddi et al., 1999). Several polymorphic genes of *Wolbachia* have been isolated and characterized for a wide range of strains. *Wolbachia* specific PCR primers have been designed and are commonly used to identify *Wolbachia* infections in total host genomic DNA extracts (Table 1). The best primers to detect a wide range of different *Wolbachia* strains are those in the 16S primer-set, which can be used for strains from all supergroups described so far (O'Neill et al., 1992; Bandi et al., 1998; Vandekerckhove et al.,

Table 1. List of diagnostic *Wolbachia* primers.[a]

| Gene | | Primer 5′-3′ | Super-group | References |
|---|---|---|---|---|
| 16S | 16S*f* | TTGTAGCCTGCTATGGTATAACT | A, B | O'Neill et al., 1992 |
| | 16S*r* | GAATAGGTATGATTTTCATGT | A, B | |
| | *FILf* | TATATAGCTTGCTATAGTGTA | C | Sironi et al., 1995 |
| | *FILr* | TCGAACAGGCATAATTTCCA | C | |
| | *Bsymbf* | ACGAGTTATAGTATAACT | D | Taylor et al., 1999b |
| | *Bsymbr* | CCTTCGAATAGGAATAAT | D | |
| *ftsZ* | *ftsZunif* | GGYAARGGTGCRGCAGAAGA | A–F | Lo et al., 2002 |
| | *ftsZunir* | ATCRATRCCAGTTGCAAG | A–F | |
| | *ftsZf1* | GTTGTCGCAAATACCGATGC | A, B | Werren et al., 1995 |
| | *ftsZr1* | CTTAAGTAAGCTGGTATATC | A, B | |
| *wsp* | *81F* | TGGTCCAATAAGTGATGAAGAAAC | A, B | Zhou et al., 1998 |
| | *691R* | AAAAATTAAACGCTACTCCA | A, B | |
| | *WSPintF* | TAGYTACTACATTCGCTTGCA | C, D | Bazzocchi et al., 2000 |
| | *WSPintR* | CCAAYAGTGCYATAAAGAAC | C, D | |

[a]Primers are for the 16S *rRNA* gene, the cell cycle gene *ftsZ* and the outer surface protein gene *wsp*, used for the detection of the six different *Wolbachia* supergroups A–F.

1999). However the polymorphism in the 16S gene is low, which makes the detection of multiple infections in individuals and the characterization of single strains difficult. For the latter, primers designed to amplify the cell cycle gene *ftsZ* (Holden et al., 1993; Werren et al., 1995b; Bandi et al., 1998; Lo et al., 2002) and the outer surface protein gene *wsp* (Braig et al., 1998; Zhou et al., 1998) are more suitable.

## Preservation

Because of their intracellular biology, *Wolbachia* cannot easily be preserved and are best maintained in cultures of their hosts. Some of *Wolbachia*'s hosts undergo diapause phases to survive extreme conditions. Under such circumstances *Wolbachia* are usually retained in most associations. However loss of infections during diapause has been observed in a few cases (Perrot-Minnot et al., 1996). *Wolbachia* can also be kept in cell lines (O'Neill et al., 1997b; Dobson et al., 1999; Noda et al., 2002), where they can be stored at –80°C (O'Neill et al., 1997b). Treatment with antibiotics, particularly with tetracycline or rifampicin, in combination with high temperature is commonly used for removing *Wolbachia* infections (Dobson and Rattanadechakul, 2001a; Fenollar et al., 2003b; Volkmann et al., 2003). Rearing insects under nutritional stress has been reported to reduce the efficiency of maternal transmission (Sinkins et al., 1995; Clancy and Hoffmann, 1998).

## Genomics

*Wolbachia* strains have small streamlined genomes comprised of a single circular chromosome. No plasmids are known to occur in *Wolbachia*. The genome size among the members of different *Wolbachia* supergroups varies considerably. Strains of the nematode-associated C and D supergroup have a chromosome size of 0.9–1.1 Mb, whereas members of the insect-associated A supergroup have chromosome sizes of 1.3–1.6 Mb (Sun et al., 2001) and 1.8 for B supergroup (Fenollar et al., 2003a). The reduced size in nematode *Wolbachia* correlates with their observed obligate mutualism and concordant evolution with their hosts (Bandi et al., 1998; Casiraghi et al., 2001).

The genome of the *Wolbachia* strain *w*Mel$_{D.\,mel}$ that naturally infects *Drosophila melanogaster* has recently been sequenced (Wu et al., 2004). This has revealed a striking number of repetitive elements within the *Wolbachia* genome. Over 14% of the chromosome is comprized of repeat sequences, many of them transposable elements. This high level of repetitive DNA is unique for a streamlined intracellular genome. Associated with this high level of repetitive DNA is the common occurrence of translocations and inversions between *Wolbachia* strains (Sun et al., 2003; Wu et al., 2004). Initial data indicate that the presence of repetitive DNA on the *Wolbachia* chromosome is an extremely sensitive marker for discriminating between different *Wolbachia* strains (M. Riegler, personal communication). The genome sequence of *Wolbachia* has also revealed a striking number of genes encoding ankyrin repeat domains. These domains, which mediate protein-protein interactions, are found in over 23 predicted genes of unknown function within the *w*Mel$_{D.mel}$ genome. It is currently hypothesized that they may play a major role in the interaction of *Wolbachia* with its various eukaryotic hosts (Wu et al., 2004).

## Ecology

The majority of *Wolbachia* infections in arthropods are physiologically benign. Two strains have been reported as virulent so far, the *popcorn* infection in *D. melanogaster*, *w*MelPop$_I$ (Min and Benzer, 1997), and *w*Vul in *Porcellio dilatus* (Bouchon et al., 1998). Some *Wolbachia* associations have evolved obligate mutualisms with their hosts, as is seen in C and D supergroup infections of filarial nematodes (Bandi et al., 2001). In some insect associations, *Wolbachia* have positive effects on fertility (Girin and Boultreau, 1995; Vavre et al., 1999b) and sperm competition (Wade and Chang, 1995) and are essential for oogenesis (Dedeine et al., 2001). *Wolbachia* can also counteract the deleterious effects of certain mutations in *D. melanogaster* by rescuing oogenesis defects (Starr and Cline, 2002). In most associations, *Wolbachia* manipulate host reproduction, thereby favoring their own dispersal into host populations, a characteristic that leads to the most general definition of *Wolbachia* as reproductive parasites. These reproductive manipulations include cytoplasmic incompatibility, thelytokous parthenogenesis, male-killing and feminization (O'Neill et al., 1997a; Stouthamer et al., 1999).

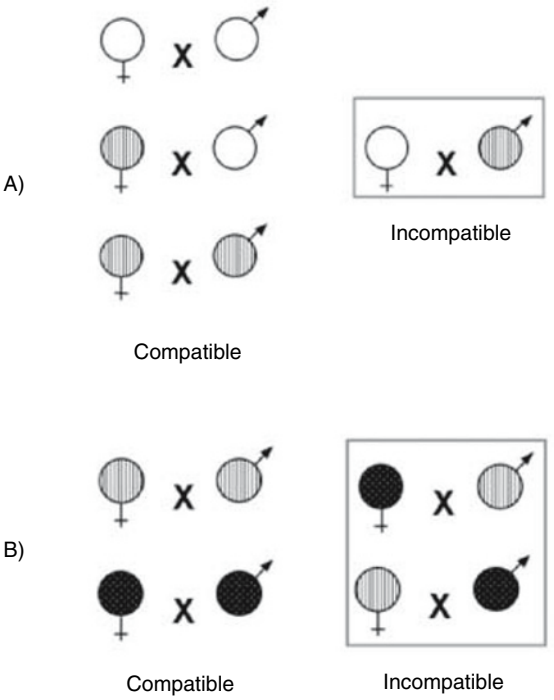


Fig. 5. Schematic illustration of cytoplasmic incompatibility. A) Unidirectional incompatibility is expressed when *Wolbachia* infected males (shaded) mate with uninfected females. These matings produce few viable offspring. All other crosses are compatible. B) Bidirectional incompatibility occurs when insects carrying different *Wolbachia* strains mate. In this case only crosses between individuals infected by the same *Wolbachia* strain are compatible.

### Cytoplasmic Incompatibility

Cytoplasmic incompatibility (CI) is the most commonly described phenotype associated with *Wolbachia* infection and has been observed in many insect, mite and crustacean species (Hoffmann and Turelli, 1997). CI arises when infected males mate with females that are either uninfected or infected with a different strain of *Wolbachia* (Fig. 5). The result is a failure of the male pronucleus to successfully complete karyogamy during fertilization of the female eggs. This leads to embryonic lethality in diploid species (O'Neill and Karr, 1990) and either to production of males (Breeuwer and Werren, 1993) or embryonic lethality in haplodiploids (Vavre et al., 2000). Crosses between infected females and uninfected males or between individuals infected with the same strain of *Wolbachia* are fully compatible. The molecular basis of CI is not yet known. Cytological analyses suggest that *Wolbachia* influences proteins involved in host cell cycle regulation (Tram and Sullivan, 2002). *Wolbachia* delay the entry of male pronuclei into the first mitotic divisions either by a direct inhibition of the enzymatic machinery that drives cells into mitosis or indirectly through activation of cell cycle checkpoints (Tram et al., 2003).

A two-component model has been suggested to explain CI (Werren, 1997; Poinsot et al., 2003). *Wolbachia* modify the sperm of infected males during spermatogenesis (modification, or "mod function"), leading to embryonic death unless a related *Wolbachia* is present in the egg and restores viability (rescue, or "resc function"). *Wolbachia* strains can be classified according to their mod and resc capability. For example, a mod+ resc+ strain can induce CI as well as rescue the modification of the same strain, whereas a mod– resc+ strain is incapable of modifying sperm but can rescue sperm modified by closely related *Wolbachia* strains. The mod/resc model also explains other CI relationships such as bidirectional incompatibility between individuals infected with a different *Wolbachia* variant (Fig. 5) or unidirectional incompatibility between individuals infected with a variable number of different *Wolbachia* strains. In the latter case, multiple infections express unidirectional CI when males carry an additional CI inducing strain that is not present in the infected females (Merot et al., 1995; Rousset and Solignac, 1995; Perrot-Minnot et al., 1996; Dobson et al., 2001b; Riegler and Stauffer, 2002).

## Thelytokous Parthenogenesis

Hymenoptera and some species of other insect orders possess a haplo-diploid sex determination system (Normark, 2003). Haploid embryos generally develop into males and diploid embryos develop into females. Females can control the laying of unfertilized and fertilized eggs, which then develop into males and females, respectively. Thus sexual haplodiploids are able to produce male offspring parthenogenetically and female offspring sexually (i.e., reproduce by arrhenotoky). Some *Wolbachia* strains are known to interfere with this system during the stage of chromosome segregation and enable females to produce parthenogenic female offspring (i.e., reproduce by thelytoky). In *Wolbachia* infected eggs, two haploid nuclei fuse at the end of the first mitotic division or during interphase before the second division (Gottlieb et al., 2002). Embryos thus develop into homozygous diploid females (Stouthamer and Kazmer, 1994). Thelytokous parthenogenesis induced by *Wolbachia* has been discovered in a variety of parasitoid species (Stouthamer, 1997). Parthenogenesis in the collembolan *Folsomia* has also been assumed to be induced by a *Wolbachia* strain (Vandekerckhove et al., 1999).

## Feminization

*Wolbachia* infections in some terrestrial crustaceans are known to cause feminization (Rigaud, 1997; Bouchon et al., 1998). A similar phenotype has also been detected in a butterfly species (Kageyama et al., 1998; Kageyama et al., 2002; Kageyama et al., 2003). Both groups, crustaceans and butterflies, have unique ZZ or WZ sex chromosome sets with males being the homogamous and females the heterogamous gender (Rigaud, 1997; Kageyama et al., 2002). Feminizing *Wolbachia* strains induce genetic males to develop into functional phenotypic females, thereby providing an opportunity for the symbiont to be maternally transmitted to the next generation. In the isopod *Armadillidium vulgare*, *Wolbachia* prevents the formation of the androgenic gland and also changes the reaction of the host to androgenic hormone activity (Rigaud, 1997).

## Male Killing

*Wolbachia* strains are known that exclusively kill male embryos in some host species. These "male-killing *Wolbachia*" have been found in Coleoptera (Majerus et al., 2000), Lepidoptera (Jiggins et al., 1998) and Diptera (Hurst et al., 2000). In infected populations, male-killing bacteria cause a female biased sex ratio and are thereby theorized to allow a more beneficial resource allocation that increases the reproductive fitness of female hosts and the bacteria they transmit (Hurst et al., 1997). A stable infection equilibrium balanced by resistance factors of the hosts is necessary to prevent the eradication of the infected populations (Randerson et al., 2000).

The type and level of expression of the various *Wolbachia*-induced phenotypes are determined by a mixture of strain and host genotypes. The most intriguing observations so far are switches from one phenotype to another. A switch from CI to a male-killing phenotype was documented after the transfer of *Wolbachia* between two moth species (Sasaki et al., 2002). Another switch in phenotype originally reported as a change from feminizing in one butterfly host species to male-killing in another butterfly species (Fujii et al., 2001) has since been resolved as overlapping feminizing and male-killing phenotypes in the original host (Kageyama and Traut, 2004). It has also been shown that the strength of CI in *Drosophila melanogaster* is dependent on male age (Reynolds and Hoffmann, 2002) and host genotype (McGraw et al., 2001; Reynolds et al., 2003). In many transfer experiments the attenuation or exacerbation of phenotypic effects suggests a strong involvement of host factors (McGraw et al., 2002; Riegler et al., 2004).

The strategy of being a reproductive parasite is not restricted to *Wolbachia*. Other microorganisms are also known to induce biased reproductive phenotypes in arthropods and crustaceans. Feminizing microsporidia are known in amphipod crustaceans (Bulnheim and Vavra, 1968), and male killers are known from the γ-proteobacteria as well as *Spiroplasma* and Bacteroidetes in beetles (Hurst et al., 1997), flies (Williamson et al., 1999), and wasps (Werren et al., 1986). Recently, *Cardinium hertigii*, a member of the Bacteroidetes group, has been shown to cause feminization (Weeks et al., 2001), parthenogenesis (Zchori-Fein et al., 2001; Zchori-Fein et al., 2004) and CI (Hunter et al., 2003) in its arthropod hosts.

The variety of *Wolbachia*-induced reproductive manipulations suggests that these infections impact the population genetics of their hosts. Most apparent is an indirect impact on other cytoplasmic factors, such as the mitochondrial genome. *Wolbachia* infections that cause unidirectional CI and spread into uninfected populations over generations also favor the spread of the infected mitochondrial haplotype and can cause a replacement of original mitochondrial haplotypes (Turelli and Hoffmann, 1991; Ballard et al., 1996). A linkage of *Wolbachia* strains with mitochondrial haplotypes has been shown in the case of CI-inducing strains (Turelli et al.,

1992), as well as in *Wolbachia* associations with other phenotypes such as feminization (Grandjean et al., 1993) and male killing (Jiggins, 2003).

However, CI does not lead to a reduction of nuclear gene flow between populations as long as it is unidirectional (Caspari and Watson, 1959). A few exceptions are seen in island situations (Telschow et al., 2002a). Alternatively, bidirectional incompatibility if complete can inhibit genetic exchange between host populations. In this context the reproductive isolation promoted by *Wolbachia* infections has been seen as a factor promoting speciation in infected hosts (Werren, 1998; Bordenstein et al., 2001; Telschow et al., 2002b).

The strict vertical inheritance of *Wolbachia* has been questioned after the finding of dissimilarities between phylogenetic trees of *Wolbachia* strains and their hosts (O'Neill et al., 1992; Werren et al., 1995b; West et al., 1998; Vavre et al., 1999a). Although efficient horizontal transfer between infected and uninfected individuals of the same parasitoid species has been observed (Huigens et al., 2000) and horizontal transfer of the *Wolbachia* infection of a fly species to a parasitoid wasp described (Heath et al., 1999), it is likely that the limiting factor for efficient horizontal transfer between species is the establishment of the infections in the germline and in the populations of the new host species (Heath et al., 1999; Riegler et al., 2004).

## Disease

Despite the fact that *Wolbachia* can reach quite high densities in infected hosts, they do not appear to induce an innate immune response from their hosts (Bourtzis et al., 2000). Moreover most infections do not appear to reduce physiological host fitness appreciably (Hoffmann et al., 1990; Hoffmann et al., 1994; Hoffmann et al., 1998; Giordano et al., 1995; Turelli and Hoffmann, 1995; Bourtzis et al., 1996; Clancy and Hoffmann, 1997; Poinsot and Merot, 1997). Few *Wolbachia* infections cause disease. The best documented is the *popcorn* infection *w*MelPop$_{D.mel}$, which presumably by overreplicating drastically reduces the lifespan of the host *Drosophila melanogaster* (Min and Benzer, 1997). This strain shows a similar virulence phenotype when transferred into the related host species *D. simulans* (McGraw et al., 2002), suggesting that the virulence determinants are encoded by the genome of this particular *Wolbachia* strain. Another virulent *Wolbachia* strain has been described from isopods. Massive symbiont proliferation, followed by necrosis of the nervous tissues, was observed after the artificial transfer of a *Wolbachia* strain naturally infecting *Armadillidium* to *Porcellio dilatatus* (Bouchon et al., 1998).

In recent years *Wolbachia* has been implicated in the inflammatory pathogenesis of human filariasis. It is hypothesized that bacterial toxins released from the *Wolbachia* that infect the filarial nematode induce an inflammatory response in the mammalian host (Taylor and Hoerauf, 1999a; Taylor, 2003). This response has been thought to be largely mediated by lipopolysaccharide (LPS) released from *Wolbachia*. Curiously the genome sequence of the *Drosophila* infecting strain *w*Mel$_{D.mel}$ shows that this *Wolbachia* strain, like many intracellular symbionts, does not contain an intact pathway for LPS biosynthesis (Wu et al., 2004), suggesting either that nematode *Wolbachia* have gained this capability or that some other mechanism may be responsible for the observed pathogenesis.

## Applications

The potential applied use of *Wolbachia*-mediated incompatibilities to control insect pests and associated diseases was suggested even before it was understood that *Wolbachia* was the etiological agent responsible for cytoplasmic incompatibility (Laven, 1967; Yen and Barr, 1971). Since then, *Wolbachia* has been proposed as a method to directly suppress pest populations, to modify the ability of insects to transmit disease agents, to enhance the mass production of beneficial insects used for biological control, and in recent years, as a new target for the control of filariasis.

### Host Population Suppression

The application of CI as a means to suppress pest insect populations has been considered since its discovery (Laven, 1967; Boller et al., 1976; Brower, 1980). Analogous to the Sterile Insect Technique (SIT; Krafsur, 1998), an inundating release of incompatible males in natural populations should decrease or inhibit successful fertilization of wild females and thereby suppress wild populations. Field experiments with the mosquito *Culex pipiens* have shown promising results (Laven, 1967). However, this technique has a few drawbacks such as immigration of fertilized females (Curtis et al., 1982) and the risk of releasing compatible females along with males. An isolated population, combined with a reliable sexing technique, which guarantees the release of only males is essential for the success of population suppression. A combination of CI and SIT, whereby insects could be irradiated at lower doses and then sterilized in a conventional SIT program, has been suggested. *Wolbachia*

would induce the crossing sterility and the irradiation would prevent the release of fertile infected females (Arunachalam and Curtis, 1985; Shahid and Curtis, 1987). The use of cytoplasmic incompatibility, or the Incompatible Insect Technique (IIT; Blümel and Russ, 1989), has been suggested for a range of pests of agricultural and medical importance (Laven, 1967; Boller et al., 1976; Brower, 1980).

### Modulating Insect-Transmitted Disease

In addition to direct population suppression, *Wolbachia*-based strategies could be used to interfere with the ability of insect populations to transmit pathogens, either through modifying their population age structure or by spreading genes into populations that block transmission of pathogens. In the first instance it has been proposed that overreplicating virulent *Wolbachia* strains (Min and Benzer, 1997; McGraw et al., 2002) could be used to skew population age-structure towards younger individuals and in so doing reduce the ability of the insect population to transmit disease agents such as dengue virus (Sinkins and O'Neill, 2000; Brownstein et al., 2003; Rasgon et al., 2003).

Alternatively *Wolbachia* could be used to drive refractoriness genes located either in themselves or in other maternally inherited factors such as mitochondria, viruses or inherited nutritive symbionts through host populations by a hitch-hiking effect (Beard et al., 1993; Curtis and Sinkins, 1998; Turelli and Hoffmann, 1999; Sinkins and O'Neill, 2000). The drive of nuclear genes into host populations using *Wolbachia* is less feasible, as there is no linkage between infections and the host nuclear genome. However, transformation of organisms with constructs that contain the genes involved in the induction of *Wolbachia* phenotypes together with the desired genes has been suggested (Curtis and Sinkins, 1998; Turelli and Hoffmann, 1999; Sinkins and O'Neill, 2000).

### *Wolbachia* as a Target for Filariasis Control

Recent work suggests that *Wolbachia* endosymbionts of filarial nematodes are a major source of the inflammatory response observed in humans suffering from lymphatic filariasis (Taylor et al., 2000) and onchocerciasis (Saint Andre et al., 2002). Antibiotic therapy trials have led to long-term reductions of *Wolbachia* and interruption of nematode embryogenesis (Hoerauf et al., 2002). Similar antibiotic therapy strategies are currently being tried for other filarial diseases and should open new ways of controlling human filarial infection and disease (Taylor and Hoerauf, 2001).

### *Wolbachia* as a Fitness Enhancer in the Rearing of Beneficials

Associations have been reported where *Wolbachia* clearly have favorable effects on their hosts. *Wolbachia* is known to increase fitness parameters in hymenopteran parasitoids (Girin and Boultreau, 1995; Stolk and Stouthamer, 1995; Vavre et al., 1999b). Similarly, male-killing bacteria are seen in the context of a better resource allocation in lady bird beetles (Hurst et al., 1997). Both groups of insects are commercially used in biological control. Hence, *Wolbachia*'s potential of favoring the reproduction of their hosts could become an important trait for the rearing of beneficials. This aspect has been discussed in the comparison of sexually and parthenogenetically reproducing *Trichogramma* lines (Stouthamer, 1993; Silva et al., 2000; Tagami et al., 2001; Tagami et al., 2002). *Wolbachia* infected thelytokous lines seem to perform better than uninfected arrhenotokous counterparts (Silva et al., 2000).

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