Update TRENDS in Ecology and Evolution Vol.19 No.4 April 2004

Research Focus

Are filarial nematode *Wolbachia* obligate mutualist symbionts?

Katelyn Fenn and Mark Blaxter

School of Biological Sciences, University of Edinburgh, Ashworth Laboratories, King's Buildings, Edinburgh, UK, EH9 3JT

The intracellular symbiotic bacteria of filarial nematodes have inspired new ideas for the control of disease using antibacterial drugs. For effective, long-term control, this requires that the bacteria are essential to their nematode hosts. Two recent studies offer conflicting evidence: long, close coevolution between most filarial nematodes and their symbionts contrasts with many species having naturally lost them. An attempt to transfer symbionts to an uninfected host found that the bacteria did not thrive, suggesting they are adapted to one host.

Much of the biotic world lives in symbioses, ranging from parasitic to mutualistic, and from essential collaborations to temporary associations (Box 1) [1]. Symbioses between eubacteria and metazoans are key components of many ecosystems and are also of importance to medical science, because filarial nematodes, causative agents of river blindness and elephantiasis, harbour intracellular bacterial symbionts [2]. These symbionts are very closely related to *Wolbachia pipientis*, an alphaproteobacterial symbiont of arthropods, and are usually described as filarial nematode *Wolbachia* in the absence of a formal species name. Filarial nematode *Wolbachia* display features that are suggestive of a close, possibly essential, mutualistic relationship (Box 1) and this finding is part of the argument for using antibacterial drugs, such as tetracycline, to cure filarial disease [3,4]. If the filarial nematodes rely on the bacteria, mass antibacterial treatment will aid in eradicating infection and disease.

Box 1. Symbiosis and *Wolbachia*

Symbiosis

'Symbiosis' simply means 'living together', and is usually reserved for very close associations between different organisms. Symbiotic relationships can be classified based on whether they are essential to either partner. In cases where the benefits to host and symbiont differ (enslavement and parasitism) there is usually a fitness cost for the nonessential partner (Table I).

Wolbachia in filarial nematodes

Filarial nematodes parasitize a wide range of mammal hosts. They are vectored by a diverse set of arthropod intermediate hosts, including mosquitoes, blackflies, mites and tabanid flies (Figure I). Many species of filarial nematode also carry a symbiotic partner, *Wolbachia*, which are intracellular alphaproteobacteria related to the pathogens *Rickettsia* and *Erlichia*. *Wolbachia* are found in most tissues of the nematodes, especially the hypodermal cords, where they pack the cytoplasm (Figure I). They are transmitted from mother to offspring through the oocytes. Their position in Table I remains unclear.

Wolbachia in arthropods

Wolbachia bacteria have been detected in many arthropod species. They cause several reproductive modifications that promote their spread through infected host populations, including induction of parthenogenesis (especially in haplodiploid species, such as wasps) and generation of cytoplasmic incompatibility between infected and noninfected hosts. Arthropod *Wolbachia* fall into the 'parasite' class in Table I.

Table 1. Classification of symbiotic relationships

		Is the symbiosis essential to the larger ('host') partner?	
		No	**Yes**
Is the symbiosis essential to the smaller partner?	**No**	Commensalism	Enslavement
	Yes	Parasitism	Obligate mutualistic

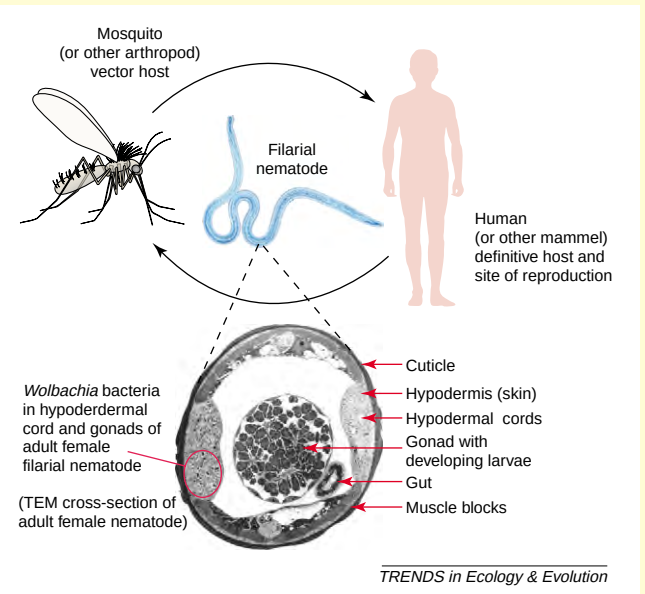

Figure I.

Corresponding author: Katelyn Fenn (k.fenn@ed.ac.uk).

However, if the bacteria are not essential, and impose some fitness cost (i.e. are 'parasitic'; Box 1), then treatment might cure the filarial nematodes of their infection, resulting in fitter, more aggressive parasites. Recent papers by Casiraghi *et al.* [5] and Hartmann *et al.* [6] address this key issue.

Wolbachia are best known as intracellular symbionts of arthropods [7,8]. They were first recognized as non-nuclear genetic elements controlling mating types [9], and are present in many arthropod taxa [10]. Arthropod *Wolbachia* are maternally transmitted within a species, but the bacterial phylogeny suggests frequent horizontal transfer between unrelated hosts [8]. There appears to be no host-taxonomic barrier to *Wolbachia*, and artificial transfer from infected to uninfected hosts has been readily achieved [7,11]. Arthropod *Wolbachia* split into two major clades ('A' and 'B'; additional 'minor' clades have also been recognized [10]) that arose ~25 million years ago. Importantly, within a given species, individuals can be either infected or not, and treatment with antibacterials, such as tetracycline, cures most arthropods of infection [7,11]. The arthropod–*Wolbachia* relationship is thus classed as parasitic (Box 1).

Patterns of symbiosis in filarial nematodes

The filarial nematode–*Wolbachia* symbiosis has a very different pattern of phylogeny and response to drug treatment, suggestive of a mutualistic relationship [5,12,13]. Tetracycline treatment of infected animals results in blocking of nematode development, stunting of growth and elimination of fecundity [4,14,15]. Naturally *Wolbachia*-free filarial nematodes are not affected [16] (Figure 1a). Tetracycline treatment has already proved effective in humans: patients carrying *Onchocerca volvulus* have undetectable patent infections up to a year after treatment [4].

The presence of *Wolbachia* is fixed within a filarial nematode species [12], in contrast to that observed in most arthropod species. Casiraghi *et al.* [5] used mitochondrial 12S rRNA and cytochrome oxidase I [13] sequences to show close coevolution of filarial nematodes with their intracellular symbionts (Figure 1b). *Onchocerca* species, including the causative agent of human river blindness, are the sister group to *Dirofilaria*, parasites of dogs and cats. The other major human parasites, *Brugia* and *Wuchereria*, are very closely related, but distinct from the *Onchocerca*–*Dirofilaria* clade. A dataset of ribosomal 16S sequences was used to derive a bacterial phylogeny.

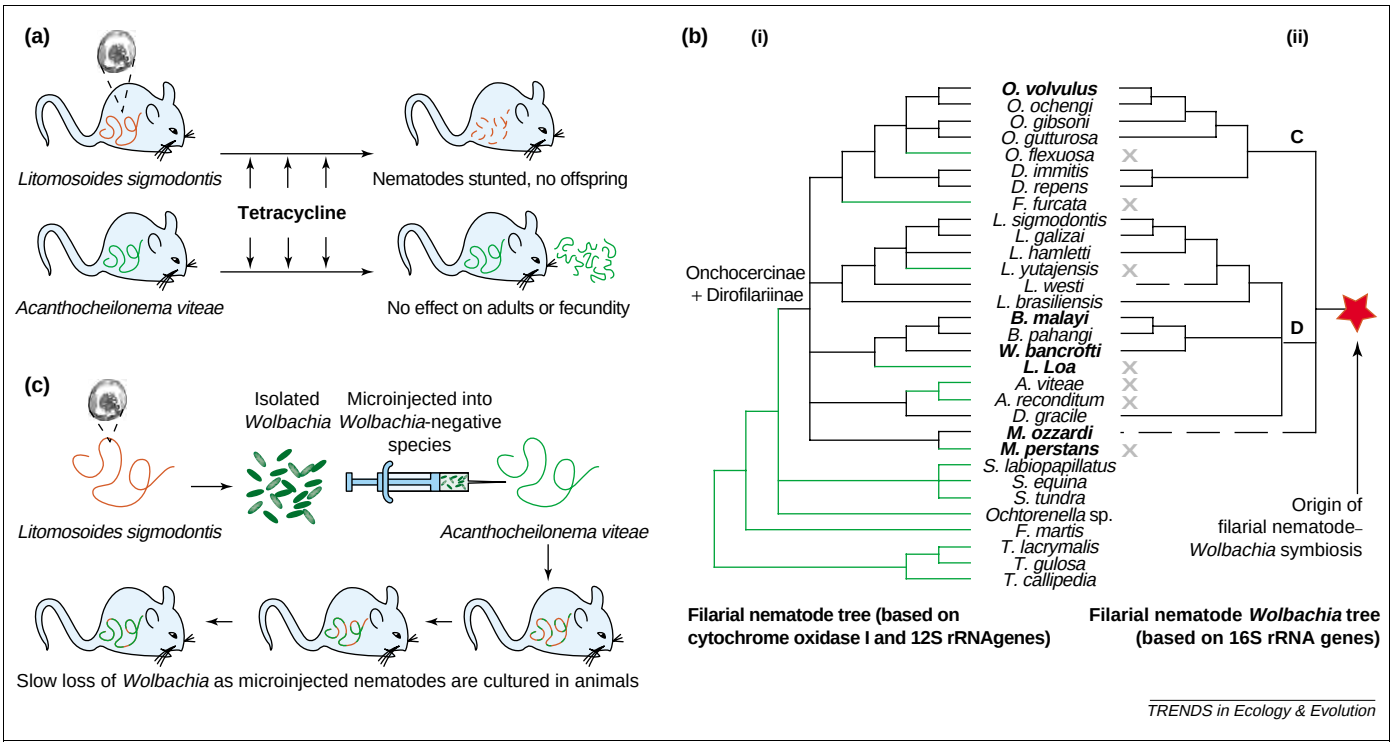

Figure 1. Biological evidence for a mutualistic symbiosis in the filarial nematode–*Wolbachia* association. **(a)** Tetracycline treatment of mammal hosts carrying filarial nematodes has different outcomes, depending on the presence of intracellular symbionts in the nematodes [16,25]. In *Litomosoides sigmodontis*, which carry intracellular symbionts (red nematodes), tetracycline results in stunting of filarial nematode growth, and inhibition of reproduction. In some cases, the nematodes are killed, but this might be a synergistic effect of the antibacterial and the immune response. In filarial nematodes that do not have intracellular symbionts, such as *Acanthocheilonema viteae* (green nematodes), antibacterial treatment has no effect on the outcome of an infection [16]. **(b)** The filarial nematode and filarial nematode *Wolbachia* trees are in the main congruent (a split in the filarial nematode lineage, to the right, is matched by a split in the filarial nematode *Wolbachia* lineage, to the left). This pattern suggests a single, ancient ((50 million years ago) origin of filarial nematode–*Wolbachia* association (red star) and an essential, possibly mutualistic, symbiosis since then. However, at least six independent losses [5,13,19–21] of intracellular symbionts have occurred since the association first arose (green branches in filarial nematode tree on right; green X on left panel), which indicates that the filarial nematodes might not be completely reliant on their intracellular bacterial partners in a long-term phylogenetic sense. Human parasitic filarial nematodes are indicated by bold lettering. The clades of filarial nematode *Wolbachia* ('C' and 'D') are indicated in (bi). Dashed lines in (bii) indicate the assumed placements of the symbionts of *Litomosoides westii* and *Mansonella ozzardi* in the absence of 16S sequence data. (Genus names not mentioned above: *Brugia* (*malayi* and *pahangi*); *Dipetalonema gracile*; *Dirofilaria* (*immitis* and *repens*); *Filaria martis*; *Foleyella furcata*; *Loa loa*; *Mansonella* (*ozzardi* and *perstans*); *Setaria* (*labiopapillatus*, *equine* and *tundra*); *Thelazia* (*lacrymalis*, *gulosa* and *callipedia*). Redrawn from data from [5]. **(c)** Filarial nematode *Wolbachia* can be transferred from a naturally infected species, such as *L. sigmodontis* (red nematodes) to a naturally uninfected one, *A. viteae* (green nematodes). However, the level of bacteria in the artificially infected filarial nematodes diminishes the longer that they are cultured in animals (mixed red–green nematodes), suggesting that the new association is not stable.

Filarial nematode *Wolbachia* are divided into two clades, 'C' and 'D' (Figure 1b), which are more diverse than are the arthropod *Wolbachia* (an inferred divergence of ~50 million years ago) [12]. All the filarial nematodes have arthropod intermediate vector hosts, often also infected with arthropod-type *Wolbachia* and, thus, the nematodes could have caught their infections from their vectors. The depth of the filarial nematode *Wolbachia* branches is significantly greater than that of the arthropod parasites [13], and it is perhaps more likely that the arthropod reproductive parasites arose from a filarial nematode intracellular symbiont.

Importantly, the bacterial phylogeny splits at the same time as the filarial nematode phylogeny (Figure 1b). Closely related bacteria are found in closely related filarial nematode hosts (e.g. clade C bacteria are found exclusively in filarial nematodes from the *Onchocerca–Dirofilaria* clade; Figure 1b). There appears to have been a long and stable relationship between the filarial nematodes and their intracellular symbionts, in contrast to the more promiscuous pattern observed in the arthropod–*Wolbachia* system. Casiraghi *et al.* also surveyed some more obscure parasites, and were able to define a natural group, 'Onchocercinae plus Dirofilariinae', which includes all the *Wolbachia*-carrying filarial nematodes [13], placing the origin of the symbiosis in the last common ancestor of this group. A wide range of nonfilarial nematodes is also *Wolbachia* free [17]. Fascinatingly, some of the species within the *Wolbachia*-infected clade lack bacteria. *Onchocerca flexuosa* has no bacteria [18], whereas all other *Onchocerca* species do. Similarly, *Litomosoides yutajensis* lacks bacteria [5], whereas *L. sigmodontis* has them. The human parasite *Loa loa* lacks bacteria [19–21]. Casiraghi *et al.* identify a total of six, independent events of intracellular symbiont loss when mapped across the filarial nematode tree (Figure 1b) [5]. This is not a pattern that would be expected if the symbiosis were essential to the filarial nematode partner.

The benefits of symbiosis

Wolbachia have not been cultured outside their host cells and, thus, for them, symbiosis is (probably) essential. The detrimental effects of tetracycline treatment on infected filarial nematodes (Figure 1a) could be due to either the disruption of an essential relationship, or to necrosis owing to dead bacteria [16]. A mutualist hypothesis (Box 1) implies that filarial nematode *Wolbachia* benefit their hosts. But what might these benefits be?

To address the role(s) of filarial nematode *Wolbachia,* a model system is needed where parallel experiments on infected and noninfected filarial nematodes of the same species can be done. Recent studies that suggest a role for filarial nematode *Wolbachia* in immunotolerance in the mammalian host of the filarial nematodes [22] are compromised by between-species comparisons. No naturally infected species is known to have uninfected members, but there are several filarial nematode species that do not harbour *Wolbachia* [12,19–21]. Hartmann *et al.* [6] now report the transfer of bacteria from *L. sigmodontis* through microinjection into the naturally *Wolbachia*-free *Acanthocheilonema viteae* (Figure 1c). Microinjected *A. viteae* were implanted into a mammalian host and maintenance and multiplication of *Wolbachia* was verified by PCR over many weeks. Thus, an uninfected host is nontoxic to the *Wolbachia* and appears to support some *Wolbachia* growth. However, there was no evidence of transmission of *Wolbachia* vertically to offspring, and levels of infection declined [6]. Perhaps directed microinjection into *A. viteae* ovaries would enable *Wolbachia* to invade the germ line. Definitive analysis of the effects of *Wolbachia* on the development of the parasite and the pathology of disease require a stable *Wolbachia*-containing *A. viteae* line. Other *Wolbachia*-free nematodes could also be used [5], but, as yet, none of these are maintained in laboratory animals.

Where next for filarial nematode *Wolbachia*?

Thus, a preliminary answer to the question of mutualism has been gained: 'nearly but not quite essential'. Many questions remain. Why would *Wolbachia* be essential in some species but be easily lost in others? How do filarial nematode *Wolbachia* ensure transmission? Arthropod *Wolbachia* use an array of mechanisms to manipulate their hosts [7]: is a similar variety of mechanisms active in filarial nematode *Wolbachia* (however, no evidence for reproductive manipulation has been found thus far)? Are the associations very species specific? Will type D *Wolbachia* survive and thrive in a type C host? Can a *Wolbachia*-negative strain of a currently infected species be established by antibiotic treatment of stages that are unlikely to be killed by necrotic reactions (such as microfilaria)? Can *Wolbachia* be transferred to a free-living nematode, such as *Caenorhabditis elegans*, to facilitate study? Can the *Wolbachia* be transferred to an arthropod or an arthropod cell line (no nematode cell lines are available)? Are the nematode *Wolbachia* competent to manipulate these hosts?

The genomes of several *Wolbachia*, including filarial nematode (*Brugia malayi* 'D' and *Onchocerca volvulus* 'C') and arthropod (*Drosophila melanogaster* 'A') strains, are currently being sequenced [23]. Comparison of these genomes with those of related intracellular pathogens (*Rickettsia* [24] and *Erlichia* species) might identify genes involved in mutualism or parasitism, and perhaps even genes involved in causing disease. The filarial nematode–*Wolbachia* relationship must be a balance between costs and benefits, and emphasizing the costs could identify a route to treatment. However, until the basis of the interaction is known, it is difficult to foresee the epidemiological effect that the use of antibacterials will have on filarial nematodes.

References

1 Margulis, L. and Fester, R. eds (1991) *Symbiosis as a Source of Evolutionary Innovation*, The MIT Press
2 Sironi, M. *et al.* (1995) Molecular evidence for a close relative of the arthropod endosymbiont *Wolbachia* in a filarial worm. *Mol. Biochem. Parasitol.* 74, 223–227
3 Hoerauf, A. *et al.* (2000) Endosymbiotic bacteria in worms as targets for a novel chemotherapy in filariasis. *Lancet* 355, 1242–1243
4 Hoerauf, A. *et al.* (2001) Depletion of *Wolbachia* endobacteria in *Onchocerca volvulus* by doxycycline and microfilaridermia after ivermectin treatment. *Lancet* 357, 1415–1416

5 Casiraghi, M. *et al*. Mapping the presence of *Wolbachia pipientis* on the phylogeny of filarial nematodes: evidence for symbiont loss through evolution. *Int. J. Parasitol*. (in press)
6 Hartmann, N. *et al.* (2003) Trans-species transfer of *Wolbachia*: microinjection of *Wolbachia* from *Litomosoides sigmodontis* into *Acanthocheilonema viteae*. *Parasitology* 126, 503–511
7 Werren, J.H. (1997) Biology of *Wolbachia*. *Annu. Rev. Entomol.* 42, 587–609
8 Werren, J.H. *et al.* (1995) Evolution and phylogeny of *Wolbachia*: reproductive parasites of arthropods. *Proc. R. Soc. Lond. Ser. B* 261, 55–63
9 Waddington, C.H. (1956) *Principles of Embryology*, Allen and Unwin
10 Rigaud, T. (1999) Further *Wolbachia* endosymbiont diversity: a tree hiding in the forest. *Trends Ecol. Evol.* 14, 212–213
11 Weeks, A.R. *et al.* (2002) Wolbachia dynamics and host effects: what has (and has not) been demonstrated? *Trends Ecol. Evol.* 17, 257–262
12 Bandi, C. *et al.* (1998) Phylogeny of *Wolbachia* bacteria in filarial nematodes. *Proc. R. Soc. Lond. Ser. B* 265, 2407–2413
13 Casiraghi, M. *et al.* (2001) A phylogenetic analysis of filarial nematodes: comparison with the phylogeny of *Wolbachia* endosymbionts. *Parasitology* 122, 93–103
14 Chirgwin, S.R. *et al.* (2003) *Brugia pahangi* and *Wolbachia*: the kinetics of bacteria elimination, worm viability, and host responses following tetracycline treatment. *Exp. Parasitol.* 103, 16–26
15 Casiraghi, M. *et al.* (2002) Tetracycline treatment and sex-ratio distortion: a role for *Wolbachia* in the moulting of filarial nematodes? *Int. J. Parasitol.* 32, 1457–1468
16 Hoerauf, A. *et al.* (1999) Tetracycline therapy targets intracellular bacteria in the filarial nematode *Litomosoides sigmodontis* and results in filarial infertility. *J. Clin. Invest.* 103, 11–18
17 Bordenstein, S.R. *et al.* (2003) Absence of *Wolbachia* in nonfilariid nematodes. *J. Nematol.*
18 Brattig, N.W. *et al.* (2001) Neutrophil accumulation around *Onchocerca* worms and chemotaxis of neutrophils are dependent on *Wolbachia* endobacteria. *Microbes Infect.* 3, 439–446
19 McGarry, H.F. *et al.* (2003) Evidence against *Wolbachia* symbiosis in *Loa loa*. *Filaria J.* 2, 9
20 Grobusch, M.P. *et al.* (2003) No evidence of *Wolbachia* endosymbiosis with *Loa loa* and *Mansonella perstans*. *Parasitol. Res.* 90, 405–408
21 Buttner, D.W. *et al.* (2003) Obligatory symbiotic *Wolbachia* endobacteria are absent from *Loa loa*. *Filaria J.* 2, 10
22 Saint Andre, A. *et al.* (2002) The role of endosymbiotic *Wolbachia* bacteria in the pathogenesis of river blindness. *Science* 295, 1892–1895
23 Slatko, B.E. *et al.* (1999) The *Wolbachia* genome consortium. *Microb. Comp. Genomics* 4, 161–165
24 Andersson, S.G. *et al.* (1998) The genome sequence of *Rickettsia prowazekii* and the origin of mitochondria. *Nature* 396, 133–140
25 Bandi, C. *et al.* (1999) Effects of tetracycline on the filarial worms *Brugia pahangi* and *Dirofilaria immitis* and their bacterial endosymbionts *Wolbachia*. *Int. J. Parasitol.* 29, 357–364

0169-5347/$ - see front matter
doi:10.1016/j.tree.2004.01.002

Allelic histories: positive selection on a HIV-resistance allele

Michael P.H. Stumpf[1] and Hilde M. Wilkinson-Herbots[2]

[1]Department of Biological Sciences, Wolfson Building, Imperial College London, London, UK, SW7 2AZ
[2]Department of Statistical Science, University College London, London, UK, WC1E 6BT

The *CCR5-Δ32* allele crucially determines the course of HIV infection and appears to be highly protective against the disease. Population genetic studies suggest that the allele has been under positive selection in Europe in the past. In a recent paper, Alison Galvani and Montgomery Slatkin collate the available evidence and use a mathematical model to strongly suggest that smallpox could have exerted sufficient selection pressure to explain the distribution of the allele across Europe. This is a beautiful example of the power of mathematical models in evolutionary genetics.

The G protein-coupled chemokine receptor CCR5 is of crucial importance during HIV infection and the *CCR5-Δ32* deletion allele seems to confer protection against infection with HIV [1]; complete protection has been reported for homozygotes, whereas, whilst heterozygotes can be infected, the onset of AIDS is delayed by two to four years. The geographical distribution of this allele is intriguing and the population frequency shows a clear decline from ~16% in Finland to 4% in the south of Europe (Sardinia). Population frequencies in Africa and Asia are negligible but are predicted to increase if the HIV pandemic continues [2]. There has been considerable interest in the population genetics of the *CCR5-Δ32* allele, its history and geographical distribution. Recurrent questions, which have now been re-examined by Galvani and Slatkin [3], are has selection has given rise to its relatively high frequency and what was or is the source of the selection pressure?

The plague and *CCR5-Δ32*: cause or coincidence?

Previous studies of the age of the *CCR5-Δ32* allele yielded estimates ranging from 700 years before present (ybp) [4] to 1700 ybp [5] (with associated confidence intervals of 275–1875 and 375–3675 ybp, respectively). The first of these point estimates brings the age of the allele into close proximity with the historical plague epidemics [4]. It has, thus, been argued that the selection pressure on the *CCR5-Δ32* allele stems from *Yersinia pestis*, the bacterium that causes bubonic plague. In spite of the lack of a detailed molecular mechanism for why the two very different agents, HIV and *Y. pestis*, should share a dependence on the same receptor, CCR5, it is, at least in theory, possible that they do.

Corresponding author: Michael P.H. Stumpf (m.stumpf@imperial.ac.uk).