Applied and Environmental Microbiology

# Recombination in *Wolbachia* Endosymbionts of Filarial Nematodes?

**Jeremy Foster, Barton Slatko, Claudio Bandi and Sanjay Kumar**

***Appl. Environ. Microbiol.*** **2011, 77(5):1921. DOI: 10.1128/AEM.02380-10.**

Applied and Environmental Microbiology, Mar. 2011, p. 1921–1922 Vol. 77, No. 5
0099-2240/11/$12.00 doi:10.1128/AEM.02380-10


# Letters to the Editor

## Recombination in *Wolbachia* Endosymbionts of Filarial Nematodes?†

We read with interest the recent paper by Ros and colleagues (8) reporting recombination in the citrate synthase gene (*gltA*) from *Wolbachia* endosymbionts of filarial nematodes that belong to *Wolbachia* supergroups C and D. We were intrigued by this observation, since unlike the mostly parasitic relationship between *Wolbachia* endosymbionts and arthropod hosts, where coinfection with two or more *Wolbachia* strains and genetic exchange via recombination are common (1, 9), neither phenomenon had been detected previously for *Wolbachia* strains which mutualistically colonize nematodes (2, 5, 6). Furthermore, the nematode hosts of the *Wolbachia* strains implicated in the recombination event infect different mammalian hosts and are transmitted by distinct insect vectors. Since the phylogeny of filarial nematodes appears highly congruent with that of their *Wolbachia* endosymbionts, indicative of long, stable coevolution (3), and horizontal transfers between nematode hosts are not known, it is difficult to understand how such recombination might have occurred.

We suspected that the apparent recombination was due to a hybrid sequence previously deposited in GenBank and used in the analysis. The software used to identify recombination would not be able to discriminate such hybrids. The *gltA* sequence in question (GenBank accession no. AJ609644) from the *Wolbachia* endosymbiont of *Wuchereria bancrofti* (supergroup D) was obtained as part of an earlier study involving amplification of *Wolbachia* genes from 40 invertebrate hosts, including *Brugia malayi* (also supergroup D *Wolbachia*) and *Onchocerca gibsoni* (supergroup C) (4). In this study, each *gltA* amplicon was sequenced bidirectionally with the primers used for PCR. We believe a tracking issue during amplification, sequencing, and assembly of forward and reverse reads of the *gltA* fragment from the *Wolbachia* endosymbiont of *W. bancrofti* resulted in the accidental generation of a hybrid sequence. Ros et al. subsequently observed that the 5′ end of the *gltA* gene fragment (~210 bp) amplified from *W. bancrofti* is almost identical to the corresponding fragment from *O. gibsoni*, while the 3′ end (~625 bp) is a close match to the sequence from *B. malayi*, giving the appearance of recombination between *Wolbachia* supergroups C and D (8).

The recent draft genome sequence of *W. bancrofti* and its endosymbiont (http://www.broadinstitute.org/annotation/genome/filarial_worms/MultiHome.html) indicates no such recombination in the *gltA* genes of *Wolbachia* from filarial nematodes. Figure 1 presents an alignment of the *Wolbachia gltA* fragments depicted by Ros et al. (8) but is extended to include the new *gltA* sequence from *W. bancrofti*. At the 5′ end of the alignment, where 33 nucleotides of the earlier *W. bancrofti* sequence (GenBank accession no. AJ609644) had an identical match to *O. gibsoni* rather than *B. malayi* (8), we find that 31 of 32 positions covered by the new *W. bancrofti gltA* fragment exactly match the *Wolbachia* sequence from *B. malayi* and not that from *O. gibsoni.* The 3′ ends of both sequences from *W. bancrofti* are almost identical to that from *B. malayi* (not shown). While we cannot formally rule out the possibility that different isolates of *W. bancrofti* might contain *Wolbachia* strains with different *gltA* sequences, all available evidence argues against recombination in this gene. This is consistent with earlier studies that failed to detect recombination in filarial nematode *Wolbachia* strains (2, 5, 6) and the notion that recombination does not occur in mutualistic *Wolbachia* strains that show no horizontal transmission between worm hosts (6).

```
                                                  1111111111111111222
                                                124467888889112444445566789990 00
                                               16570800125790583023575835502585 78
AJ609639            C  Onchocerca gibsoni      .g.............................. .
AJ609644            D  Wuchereria bancrofti    ACTTACGGGAGGATCTTGTCATGCCTAGGGAATG
Supercontig.410     D  Wuchereria bancrofti    --ccg.aaagcagctccactggaatccaaaggaa
AJ609643            D  Brugia malayi           gaccgtaaagcagctccactggaatccaaaggaa
```

FIG. 1. *Wolbachia gltA* sequence alignment presented as by Ros et al. (8) using GenBank accession numbers (where available), supergroups (boldface), and host species as names and preserving the numbering (read vertically by column) used by Ros et al. (8). Only polymorphic sites are shown. Differences from the reference *W. bancrofti Wolbachia* sequence are in lowercase, with identities as dots. The new sequence from the *Wolbachia* endosymbiont of *W. bancrofti* was obtained from the Broad Institute (annotated as *Wolbachia-Wuchereria bancrofti* supercontig 410 with coordinates 337 to 936 on the plus strand). Sequences were aligned using the TCoffee server via www.tcoffee.org using default parameters for regular TCoffee (7).

**REFERENCES**

1. **Baldo, L., S. Bordenstein, J. J. Wernegreen, and J. H. Werren.** 2006. Widespread recombination throughout *Wolbachia* genomes. Mol. Biol. Evol. **23:** 437–449.
2. **Bordenstein, S. R., et al.** 2009. Parasitism and mutualism in *Wolbachia*: what the phylogenomic trees can and cannot say. Mol. Biol. Evol. **26:**231–241.
3. **Casiraghi, M., T. J. Anderson, C. Bandi, C. Bazzocchi, and C. Genchi.** 2001. A phylogenetic analysis of filarial nematodes: comparison with the phylogeny of *Wolbachia* endosymbionts. Parasitology **122**(Pt. 1)**:**93–103.
4. **Casiraghi, M., et al.** 2005. Phylogeny of *Wolbachia* pipientis based on *gltA*, *groEL* and *ftsZ* gene sequences: clustering of arthropod and nematode symbionts in the F supergroup, and evidence for further diversity in the *Wolbachia* tree. Microbiology **151:**4015–4022.
5. **Casiraghi, M., J. H. Werren, C. Bazzocchi, A. Biserni, and C. Bandi.** 2003. *dnaA* gene sequences from *Wolbachia pipientis* support subdivision into supergroups and provide no evidence for recombination in the lineages infecting nematodes. Parassitologia **45:**13–18.
6. **Jiggins, F. M.** 2002. The rate of recombination in *Wolbachia* bacteria. Mol. Biol. Evol. **19:**1640–1643.
7. **Poirot, O., E. O'Toole, and C. Notredame.** 2003. Tcoffee@igs: a Web server for computing, evaluating and combining multiple sequence alignments. Nucleic Acids Res. **31:**3503–3506.
8. **Ros, V. I., V. M. Fleming, E. J. Feil, and J. A. Breeuwer.** 2009. How diverse is the genus *Wolbachia*? Multiple-gene sequencing reveals a putatively new *Wolbachia* supergroup recovered from spider mites (Acari: Tetranychidae). Appl. Environ. Microbiol. **75:**1036–1043.
9. **Werren, J. H., D. Windsor, and L. Guo.** 1995. Distribution of *Wolbachia* among neotropical arthropods. Proc. R. Soc. Lond. B. **262:**197–204.



**Jeremy Foster***
**Barton Slatko**
*Division of Parasitology, New England Biolabs*
*240 County Road, Ipswich, Massachusetts 01938*

**Claudio Bandi**
*DIPAV, University of Milan*
*Via Celoria 10, 20133 Milan, Italy*

**Sanjay Kumar**
*Division of Parasitology, New England Biolabs*
*240 County Road, Ipswich, Massachusetts 01938*

*Phone: (978) 380-7223
Fax: (978) 921-1350
E-mail: foster@neb.com


***Ed. Note:*** *The authors of the published article did not respond.*

† The authors have paid a fee to allow immediate free access to this article.