



Bacterial sequences in an invertebrate genome

W*olbachia pipientis* is the most prolific intracellular endosymbiont on earth. These bacteria infect not only 70% of insects, but also the most abundant animal phyla, including nematodes and arthropods.

The arthropod-infecting *Wolbachia* exert unusual effects on host reproduction, including: (1) parthenogenesis, whereby infected virgin females produce infected female offspring, (2) male killing, whereby infected male embryos fail to develop, (3) feminization, whereby genetic males develop

into reproductively capable females, and (4) cytoplasmic incompatibility, the most common phenotype, whereby the offspring of uninfected females and infected males fail to develop. *Wolbachia* are maternally inherited, being transferred through the egg cytoplasm. Therefore, these reproductive phenotypes favouring *Wolbachia*-infected females increase the proliferation of *Wolbachia*-infected arthropods. *Wolbachia* are parasitic endosymbionts, since the interaction benefits *Wolbachia* while exerting a negative effect on the host by limiting genetic exchange. However, a mutualistic role benefiting both organisms cannot be excluded.

The genomes of many nematodes and arthropods contain bacterial sequences. How did they get there? **Julie C. Dunning Hotopp** and **Jason Rasgon** explain.



▲ *Wolbachia* infect the most abundant animal phyla including nematodes and arthropods. This includes some bees and butterflies like those shown here. J.C. Dunning Hotopp

Unlike infections in arthropods, treatment of nematodes with antibiotics that are targeted at eliminating the *Wolbachia* infection also kills the host. This suggests that *Wolbachia* form an obligate mutualistic symbiosis with filarial nematodes, since neither organism can survive without the other. The exact nature of the mutualistic interaction is not known, but it has been proposed

that *Wolbachia* provide the host with necessary nucleotides, cofactors and vitamins.

Despite maternal inheritance in arthropods, arthropod-borne *Wolbachia* do not evolve with the host. Instead, the bacteria are transmitted horizontally and infections are lost, although the mechanisms are not understood. In contrast, filarial nematodes and *Wolbachia* evolve together, reflecting

the obligate symbioses between these bacteria and their hosts.

Interdomain lateral gene transfer

In 2001, Natsuko Kondo and colleagues described a variant of a bean beetle, *Callosobruchus chinensis*, where *Wolbachia* genes had moved into the insect chromosome. This movement of DNA from an organism

to an unrelated one is called lateral gene transfer (LGT). In filarial nematodes, an LGT event is responsible for the presence of two degenerate *Wolbachia* gene fragments in the nematodes *Onchocerca volvulus* and *O. ochengi*. It has also been proposed that mosquitoes acquired a *Wolbachia* gene involved in the resistance of *Anopheles* mosquitoes to *Wolbachia* infection. However, this claim is supported only by homology searches, which are inadequate to assign the directionality of LGT events. In fact, these proteins have only been found in *Wolbachia* and mosquitoes, making it impossible to assign directionality.

These are examples of interdomain LGT events, where DNA moves between two of the three domains of life (eukaryotes, eubacteria, archaea). Most described LGT events occur within a single domain of life. LGT is most common in eubacteria where it is responsible for movement of genes for antibiotic resistance, pathogenicity and bioremediation. A very striking example of interdomain LGT is in the hyperthermophilic eubacterial lineage *Thermotoga maritima*, which has 81 archaeal genes clustered in fifteen 4–20 kbp islands.

Interdomain transfers in higher multicellular eukaryotes are thought to be uncommon and unimportant, but several important cases have been documented. Most notably, the plant pathogen *Agrobacterium tumefaciens* transfers 10–30 kbp of T-DNA from the Ti plasmid (200–800 kb) to plants. Upon transfer, the T-DNA is targeted to the nucleus, incorporated into the plant chromosome by illegitimate recombination, and the genes are transcribed from eukaryotic promoters encoded in the T-DNA. Although normally associated with crown gall disease and subsequently the plant's death, this has also been used to introduce novel genes in plants (e.g. genetically modified crops).

Lateral gene transfers between *Wolbachia* and host chromosomes

In every genome sequencing project, sequences remain that do not end up in the final assembly and are often considered chaff or garbage. When combing through the chaff of the fruit fly *Drosophila ananassae* genome, we discovered numerous segments of DNA that belong to both *Drosophila* and *Wolbachia* genes. We verified the structure of some of these regions by PCR and demonstrated by *in situ* hybridization of polytene chromosomes that a large part of the *Wolbachia* genome has been integrated into the *D. ananassae* Hawaii 2L chromosome. Variably sized *Wolbachia* inserts were detected in four lines of *D. ananassae* from Asia and the Pacific, indicating that the insert may be widely distributed and degenerating. Lastly, we have found transcription of at least 28 *D. ananassae* genes of *Wolbachia* origin.

In the filarial nematode *Brugia malayi*, we characterized inserts in the whole-genome shotgun-sequencing project. As *Wolbachia* infection is required for the worm's fertility

and development, the genomes of both organisms were sequenced simultaneously, complicating assemblies and leading to the removal of many *Wolbachia* reads during genome assembly (>98% identity over 90% of the read length on the basis of the independent BAC-based genome sequence of wBm, the *Wolbachia* endosymbiont of *B. malayi*). Despite this, the genome of *B. malayi* contains 249 contigs with *Wolbachia* sequences; PCR and end-sequencing confirmed nine of these inserts. The transfer of at least one region containing a degenerate fragment of the *Wolbachia* aspartate aminotransferase gene (Wbm0002), predates the divergence of three species, *B. malayi*, *B. timori* and *B. pahangi*. While the *D. ananassae* nuclear insert appears to be in one large piece, the *B. malayi* genome seems to have multiple inserts scattered through the genome with some large inserts of numerous *Wolbachia* genes, and other smaller inserts of a single gene fragment.

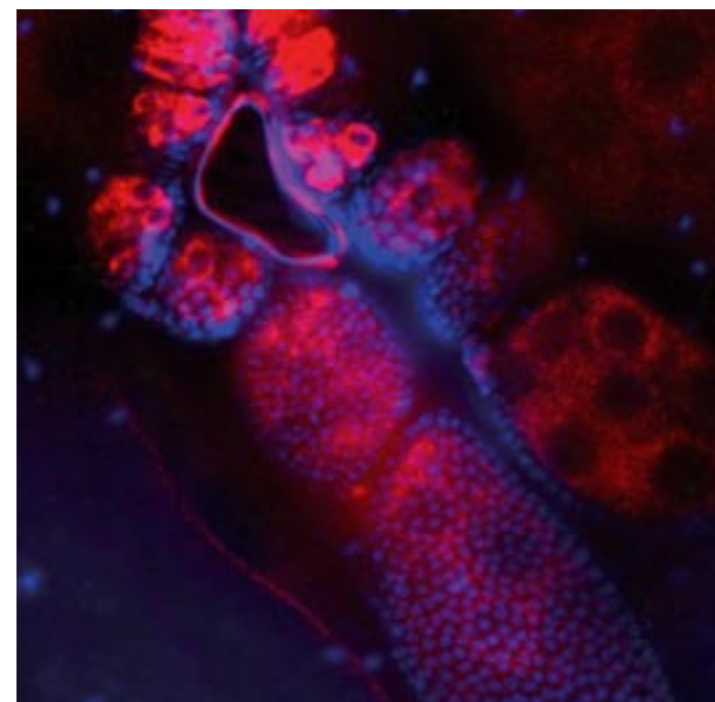
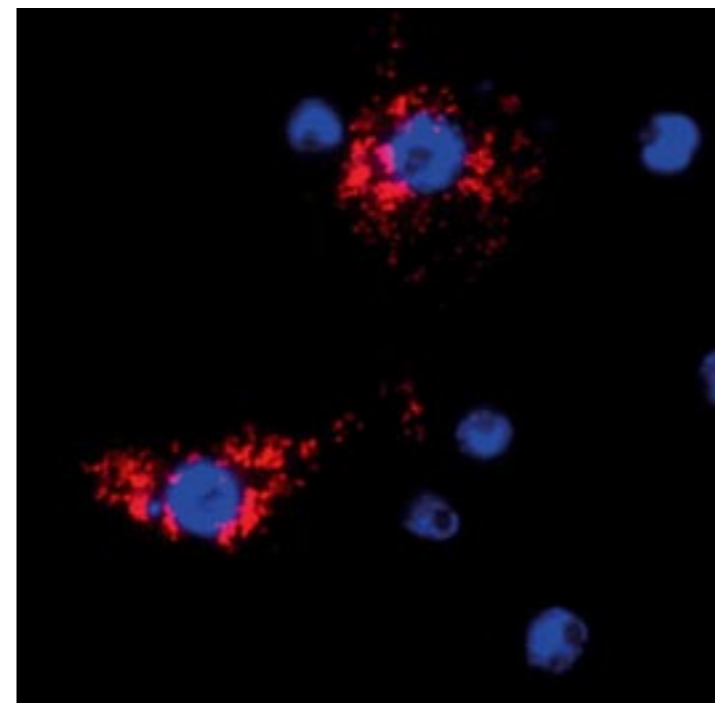
We have found numerous *Wolbachia* LGT inserts in the genomes of diverse invertebrate animals, including fruit flies, wasps and nematodes. Of 26 arthropod and nematode genomes in the trace repositories, 11 contain *Wolbachia* sequences. Of the *Wolbachia*-containing genomes, eight show evidence of having LGT between the endosymbiont genome and the host chromosomes. We have characterized host–endosymbiont LGT in five of these hosts. These results suggest that such inserts are common. Given that *Wolbachia* are among the most abundant endosymbionts, and their hosts represent the most abundant animal phyla, the view that interdomain transfers are uncommon and unimportant in multicellular organisms needs to be re-evaluated.

Endosymbiont lateral gene transfer ratchet

A lateral gene transfer ratchet has been proposed by W. Ford Doolittle to explain the accumulation of nuclear genes of organelle origin. The idea is that at some low frequency organelle genes will be inserted into the nuclear genome, and with some even lower frequency this will occur in positions that allow expression of the protein with an appropriate leader sequence for organelle targeting. Once this occurs, either the nuclear copy or the organelle copy will be lost. If the organelle copy is lost, the gene will become fixed in the nuclear genome, and as such becomes a successful transfer. Otherwise, the process can repeat itself, until all the genes that can insert in the nuclear genome have done so.

It seems reasonable to extend this theory to endosymbionts and their hosts. In the case of endosymbionts, a second option is available upon transfer of the gene to its host. If the genes transferred now fulfil the host's needs, the endosymbiont might become obsolete and can be lost. In the case of an obligate endosymbiont, this would result in the presence of endosymbiont-free lineages in clades of organisms with co-evolving obligate symbionts.

Such a situation exists in filarial nematodes that form an



▲ Top. These cultured *Anopheles gambiae* Sua58 mosquito cells are infected with the *Wolbachia* strain wRi. The *Wolbachia* are red while the mosquito cell nuclei are stained blue. Jason Rasgon

▲ Bottom. These *Drosophila simulans* ovaries are infected with the *Wolbachia* strain wRi. The *Wolbachia* are red while the *Drosophila* cell nuclei are stained blue. Jason Rasgon

obligate mutualistic symbiosis with their *Wolbachia* strains. The origin of the symbiosis is thought to have arisen about 50 million years ago and occurred in the ancestor of the Onchocercinae and the Dirofilarinae, the *Wolbachia*-infected clades. However, since that time there appears to have been at least six independent losses of *Wolbachia* infection. Although *Wolbachia* infections can successfully be transferred to *Wolbachia*-free lineages, the infection is lost on culturing in animals, suggesting that the association is no longer stable. The nature of the instability has not been established. But it is plausible that an LGT event from the endosymbiont to the filarial nematode may have conferred the nematode with the ability to live without its once-obligate symbiont.

Julie C. Dunning Hotopp

Institute for Genome Sciences, Department of Microbiology & Immunology, University of Maryland Baltimore, 20 Penn Street, Room S203B, Baltimore, MD 21201, USA (t +1 410 706 5673; f +1 410 706 1482; e jdhotopp@som.umaryland.edu)

Jason L. Rasgon

The W. Harry Feinstone Department of Molecular Microbiology and Immunology, The Johns Hopkins Malaria Research Institute, Bloomberg School of Public Health, Johns Hopkins University, 615 N Wolfe Street, Room E4626, Baltimore, MD 21205, USA (t +1 410 502 2584; f +1 410 955 0105; e jrasgon@jhsph.edu)

Further reading

- Doolittle, W.F. (1998). You are what you eat: a gene transfer ratchet could account for bacterial genes in eukaryotic nuclear genomes. *Trends Genet* 14, 307–311.
- Dunning Hotopp, J.C. & others (2007). Widespread lateral gene transfer from intracellular bacteria to multicellular eukaryotes. *Science* 317, 1753–1756.
- Keeling, P.J. & Palmer, J.D. (2008). Horizontal gene transfer in eukaryotic evolution. *Nat Rev Genet* 9, 605–618.
- Kondo, N., Nikoh, N., Ijichi, N., Shimada, M. & Fukatsu, T. (2002). Genome fragment of *Wolbachia* endosymbiont transferred to X chromosome of host insect. *Proc Natl Acad Sci U S A* 99, 14280–14285.
- Stouthamer, R., Breeuwer, J.A. & Hurst, G.D. (1999). *Wolbachia pipiensis*: microbial manipulator of arthropod reproduction. *Annu Rev Microbiol* 53, 71–102.