

Dispatch

Symbiosis: *Wolbachia* Host Shifts in the Fast Lane

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Summary:

The inherited bacterium *Wolbachia* is an important component of the biology of many arthropods. What makes it so common? An analysis of drosophilids revealed one strain host shifts at a surprisingly high rate, infecting eight species in under 30,000 years.

If you were to go into your garden, pick up an insect and sequence it, the DNA sequence obtained would likely be from two sources. First, there would be the standard nuclear and mitochondrial genomes of the insect host. Second, there is a high likelihood that there would be sequences from heritable microbes — bacteria that pass from a mother to her progeny and represent part of the ‘extended genome’ of the insect [1]. These heritable microbes are important modulators of the biology of their hosts, in some cases parasitic and others beneficial. Heritable bacteria perform a variety of functions. They may synthesise amino acids or B vitamins for their host, defend them against various natural enemies, affect thermal tolerance but may also manipulate reproduction, favouring the survival and production of infected female hosts at the expense of males.

Amongst the many heritable microbes you might have found in your garden insect, *Wolbachia* is by far the most common. Surveys report *Wolbachia* infection in 15–25% of species [2,3], and statistical estimates are higher, suggesting that up to 52% of species may be infected with one or more strains of this microbe [4]. *Wolbachia* contrasts with other heritable bacteria that occur either commonly in a phylogenetically narrow group (for example, *Hamiltonella* in aphids and whiteflies) or exist broadly but in fewer host species (such as *Cardinium*) [2]. *Wolbachia* occurs broadly and commonly. However, the factors underlying the *Wolbachia* pandemic are unclear.

The number of species that carry *Wolbachia* is a product of its capacity to move across into new host species — known as ‘host shifting’. Whilst its transmission is through maternal inheritance alone in all but a few cases, the observation of closely related *Wolbachia* strains in distantly related host species tells us the microbe occasionally host shifts, establishing itself in phylogenetically distant arthropod taxa. These ‘horizontal’ transfers are only very rarely observed during laboratory study but occur sufficiently over the long term to drive *Wolbachia* into 25–52% of host species.

A new study in this issue by Turelli *et al.* [5] demonstrates that particular *Wolbachia* strains may show rates of establishment in new species that are much higher than previously considered. The work focuses on one strain — wRi — in drosophilids. wRi was first observed spreading through Californian *D. simulans* populations in the 1980s and 1990s [6], a seminal observation of how rapidly *Wolbachia* could establish in a population. The present study shows how commonly wRi has

established in new host species. *Wolbachia* strains closely related to wRi were uncovered in *D. ananassae* in the 1990s [7], and recent work revealed wRi infections in two further species [8,9]. The Turelli *et al.* [5] paper reports a further four infected species, bringing the total to eight wRi-infected strains out of 29 *Wolbachia*-infected species so far identified in the *Drosophila melanogaster* species group clade. Notably, the hosts are both phylogenetically disparate and vary from cosmopolitan to highly range-restricted.

Most studies of *Wolbachia* host-shift biology don't resolve the tempo of movement with any accuracy — they observe strains that are closely related using the sequence of one or a few genes and infer that where the host species are distantly related that there was a host shift event. However, the genetic divergence data in a very small gene group is too limited in power to establish the timing of recent events; identity across the five *Wolbachia* genes used in multilocus sequence typing [10] that are commonly used to distinguish *Wolbachia* strains may mean a common ancestor just last year—or 500,000 years ago. In this study, the team resolved the relatedness of wRi strains with much greater precision using whole genome drafts. They established a set of 525 genes that could be aligned across all 8 isolates, enabling a much finer scale analysis of the tempo and pattern of movement. This depth of genetic information — combined with previous work establishing a 'clock' for the wMel *Wolbachia* genome [11] — enabled the team to both establish horizontal transmission events with more accuracy than previously managed, and also estimate the time at which they occurred.

The data revealed that wRi has undergone host shifts at a very high rate. There were only 239 single nucleotide variants out of 506,307 bases examined across the *Wolbachia* from the 8 host taxa. This limited divergence led the authors to estimate that these infections all arose within the last 5,000–27,000 years. There may be some debate about precisely how rapidly the infections spread through the clade — the dating assumes divergence rates at third bases are equivalent to those of the wMel clock. There may also be some uncertainty in the precise pattern of relatedness amongst strains — the work here utilizes single nucleotide variant markers, and these place *subpulchrella Wolbachia* within the *suzukii* clade, contrasting with inference based on dynamic genome elements (insertion sequences) that supports the *subpulchrella Wolbachia* as an outgroup [12]. Nevertheless, the rate of establishment into new host species is much higher than previously considered — these eight infections are all recently derived in evolutionary terms. In addition, the rate of host shifts may be underestimated because wRi may be present undetected outside of the set of host species tested. Further, it is possible that there are 'ghost' host-shift events within the sampled group: cases where wRi arrived, spread, but then declined and is no longer present.

The overall pattern in some ways recapitulates that seen for the *P* element, a transposable element found in members of the genus *Drosophila*. The *P* element has a history of transfer between species followed by global pandemic spread associated with a strong selective drive [13,14]. In terms of transfer, it is possible that transfer through ectoparasitic mites is important for both *P* and *Wolbachia* [15]. In terms of drive, six of the *Wolbachia* strains show cytoplasmic incompatibility, the reproductive manipulation first associated with the wRi wave of advance through California in the 1980s and 1990s. However, two of the wRi-like strains do not exhibit cytoplasmic incompatibility [9], and the driver of spread here is more enigmatic. Importantly, these data suggest that cytoplasmic incompatibility is not necessary for a host shift to be successful.

Host-shift rates as high as this raise an obvious question — why aren't *more* species infected with *Wolbachia*? One possibility is that wRi is unusual amongst *Wolbachia* in its proclivity to host shift, and that rates for other strains are lower. However, other sequence types, as identified by multilocus sequence typing, are very common (for example, the *Wolbachia* strain ST-41 in

Lepidoptera) implying other *Wolbachia* strains may have similarly high rates [16]. A second possibility is that loss events — where *Wolbachia* frequency declines to zero within a species — occur commonly and balance the rapid rate of gain. Loss can occur through displacement — one symbiont driving out another [17]; however, displacement cannot explain the presence of uninfected species. Evolution of host resistance to reproductive parasitic phenotypes, evolution of the microbe to reduce phenotype strength, and loss of benefit of infection may all produce declines in frequency. It is notable that there are evolutionary losses of the cytoplasmic incompatibility phenotype in the wRi clade, and that the genes likely to be associated with the cytoplasmic incompatibility show a high rate of stop codon evolution and/or loss and copy number changes. Finally, there is the possibility that host species vary in whether they support *Wolbachia* infection and spread. There is heterogeneity in *Wolbachia* incidence across arthropod groups that supports the presence of broad scale variation in susceptibility to *Wolbachia* spread.

Much remains to be explained about the *Wolbachia* pandemic. It is not the only heritable microbe to show rapid host shifts — indeed secondary symbionts in aphids show higher rates [18]. However, it is the only one where rapid host shifts are observed across a broad phylogenetic range of host species. One potential driver of *Wolbachia*'s 'success' is its arsenal of phenotypic influences, but these are not very much greater than those exhibited by other broad-range heritable microbes such as *Cardinium* and *Rickettsia* [19]. *Wolbachia*'s commonness is probably a result of its capacity to thrive and vertically transmit in many host species upon first arrival. Given the utility of *Wolbachia* transinfection in disease control — a process in which microinjection is used to place *Wolbachia* into naive focal species as a means to modify their vector competence [20] — understanding the biological basis of host-shift capacity is of more than academic interest.

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In Brief

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