

NEWS AND VIEWS

PERSPECTIVE

Vertical and horizontal transmission drive bacterial invasion

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A huge variety of Arthropod species is infected with endosymbiotic *Wolbachia* bacteria that manipulate their host's reproduction to invade populations. In addition to vertical transmission from mother to offspring through the egg cytoplasm, it has been demonstrated through phylogenetic analyses and natural transfer experiments that horizontal transmission of *Wolbachia* (i.e. contagion) can occur between Arthropod hosts. More recently, factors influencing horizontal transfer have also been explored. While it is clear that horizontal transmission between species plays a major role in the evolutionary history of *Wolbachia* infections among insects, its role in the spread of a new infection through a host population, notably through within-species transfers, remained unknown. In this issue of *Molecular Ecology*, Kraaijeveld *et al.* (2011) present the first evidence that horizontal transmission played a key role in the early spread of parthenogenesis-inducing *Wolbachia* through the parasitoid wasp *Leptopilina clavipes*. To support their finding, the authors studied genetic variation in three types of markers, including host nuclear DNA, mitochondrial DNA and *Wolbachia* DNA. Specifically, they examined potential associations between their diversity patterns. No diversity was detected in *Wolbachia* genes, indicating that a single *Wolbachia* strain must have infected and spread through *L. clavipes*. In addition, a correlation between substantial variation in mitochondrial and nuclear genotypes suggested that horizontal transmission played an important role in the current clonal genetic variation in this wasp. Such horizontal transmission could be facilitated by a specific host ecology (e.g. parasitoid wasp sharing the same host resource) and potentially impact co-evolution between host and symbiont.

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Keywords: clonal genetic variation, co-evolution, horizontal transmission, parthenogenesis, superparasitism, *Wolbachia*

Received 24 May 2011; revision received 6 June 2011; accepted 8 June 2011

Wolbachia are among the most influential bacteria infecting insects. These endosymbionts are both highly incident (70% of insect species could be infected) and master manipulators of their host's reproduction (Werren *et al.* 2008). Alterations of reproduction are diverse and include induction of cytoplasmic incompatibility, male-killing and two manipulations in which males are turned into females either phenotypically (feminization of genetic males) or genetically (parthenogenesis induction). All strategies favour the production of infected over uninfected females and allow the symbionts, which are vertically transmitted from mother to offspring through the cytoplasm of the egg, to spread through populations. The current study by Kraaijeveld *et al.* (2011) on a novel role of horizontal transmission of *Wolbachia* represents a valuable addition to our understanding of the spread of these remarkable bacteria through host populations. *Leptopilina clavipes* is a solitary parasitoid of *Drosophila* larvae (Fig. 1). This western European wasp reproduces either sexually (south of the French Pyrenees, e.g., Spain) or asexually (north of the Pyrenees, e.g., the Netherlands), as a result of an infection with parthenogenesis-inducing (PI) *Wolbachia*. Consequently, infected female wasps have lost the ability to reproduce sexually. This model system provides an excellent



Fig. 1 A female *Leptopilina clavipes* attacking a *Drosophila* larva. Unlike southern populations (e.g. Spain) that reproduce sexually, northern populations (e.g. the Netherlands) reproduce asexually in response to an infection with parthenogenesis-inducing *Wolbachia*. Image: Nina Fatouros (<http://www.bugsinthepicture.com>).

opportunity to test the role of horizontal transmission in the early spread of a *Wolbachia* infection, as (i) clonal genetic variation was detected in asexual populations, suggesting that horizontal transfer occurred, (ii) induction of parthenogenesis by *Wolbachia* renders the nuclear transmission clonal, facilitating the follow-up of genetic markers and (iii) the infection is fixed (i.e. all individuals are infected) in northern populations and absent from southern ones, which allows a direct comparison of the genetic association patterns.

The occurrence of horizontal transfer across hosts is generally highlighted by the absence of congruence between *Wolbachia* and mtDNA diversity (Baldo *et al.* 2008) or between *Wolbachia* and host nuclear diversity (Schilthuizen & Stouthamer 1997; Frost *et al.* 2010). The elegant experiment designed by Kraaijeveld *et al.* (2011) allowed them to test different evolutionary scenarios using analysis of genetic association patterns from the host nucleus, the mitochondria and the symbiont (Fig. 2). Only one *Wolbachia* genotype was found, and variation in mitochondrial mitotypes correlated with variation in host genotypes, suggesting that horizontal transfer events occurred during the

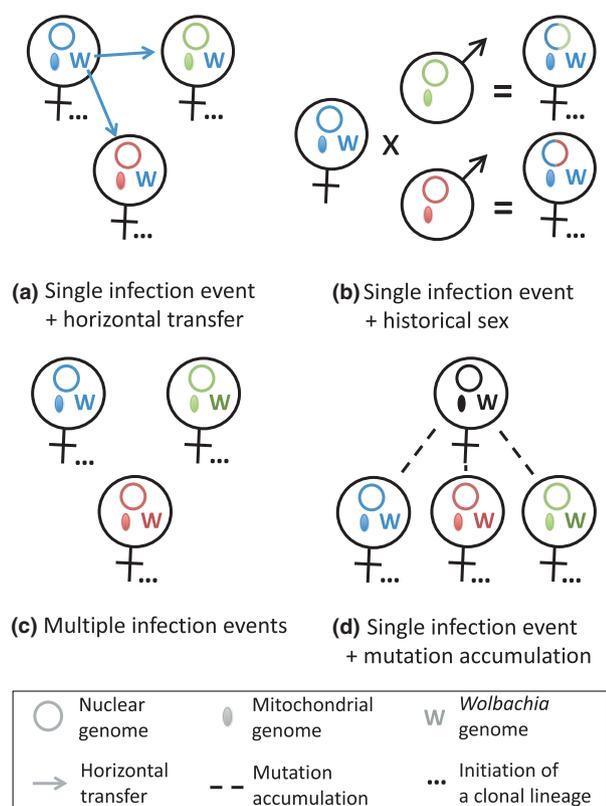


Fig. 2 Main hypothetical scenarios (a–d) explaining the evolution towards clonal genetic variation in a population infected with parthenogenesis-inducing *Wolbachia*. Each colour corresponds to a specific genotype. Combinations of the different scenarios are also possible (i.e. horizontal transfer + historical sex, multiple infection + historical sex, *etc.*) but are not drawn here.

initial spread of *Wolbachia* in *L. clavipes* (Fig. 2a). Historical sexual reproduction by infected females during the spread of *Wolbachia* cannot explain this pattern alone, as such reproduction should have led to an absence of variation in both *Wolbachia* and mtDNA (Fig. 2b). However, we cannot exclude that horizontal transmission and historical sex have played a role together during the early spread. Indeed, the loss of sexuality should have been selected for during the spread of the infection in response to the resolution of a nuclear-cytoplasmic conflict (Stouthamer *et al.* 2010) or after the infection has reached fixation in response to counter-selection of costly reproductive traits (Pijls *et al.* 1996). Alternatively, it is possible that novel mutations accumulated after fixation of a single *Wolbachia* strain, thereby initiating different lineages (Fig. 2d). Mutations should then accumulate in all three genomes, which is not the case in the study presented by Kraaijeveld *et al.* (2011). However, the nuclear (microsatellites) and mitochondrial (CO1) genes investigated have a much higher mutation rate than the genes characterized in the *Wolbachia* genome (MLST and WSP), which could potentially bias the interpretation.

Interestingly, Kraaijeveld *et al.* (2011) also highlighted that clonal diversity in asexual strains of *L. clavipes* is maintained over many generations; strains were even collected side-by-side in the field. This observation raises the question of whether clonal genetic variation is common among other parasitoid wasp species with populations completely infected with PI-*Wolbachia* [e.g. *Apoanagyrus diversicornis*, *Asobara japonica*, *Telonomus nawaii* and *Trichogramma pretiosum* (Pijls *et al.* 1996; Kremer *et al.* 2009; Jeong & Stouthamer 2005; Russell & Stouthamer 2011)]. One might also wonder how genetic variation can be maintained in such populations. Indeed, genetic drift is expected to reduce this diversity, except in the absence of clonal competition or in response to selective processes, such as specialization. In the latter case, specialization might result from 'pre-adaptations' in some hosts or from different compensatory mechanisms in response to the infection. Furthermore, we cannot exclude the potential role of genetic variation in limiting the negative effect of asexuality (loss of recombination) on the host's adaptive potential. Conversely, from a phylogenetic point of view, maintenance of such lineages for a long period of time is unexpected. Neither PI-*Wolbachia* strains nor hosts infected with PI-*Wolbachia* form monophyletic groups, whereas PI-*Wolbachia* infect many haplodiploid species like mites, thrips and hymenopterans (Huigens & Stouthamer 2003).

The host's ecology appears to play a key role in the horizontal transmission of symbionts (Russell *et al.* 2009; Stahlhut *et al.* 2010), especially in the *Drosophila*-parasitoid community (Heath *et al.* 1999; Vavre *et al.* 1999). Parasitism of *Drosophila* larvae by hymenopterans is associated with an intimate contact between the two partners, which could facilitate horizontal transfer of symbionts. A recent study revealed that *Arsenophonus nasoniae*, another insect symbiont, is horizontally transmitted through multiparasitism with high efficiency (Duron *et al.* 2010). Evidence of

intra- and interspecific horizontal transfer after super- and multiparasitism, respectively, has also been experimentally shown in parasitoid wasps infected with PI-*Wolbachia* (Huijgens *et al.* 2000, 2004). Thus, the efficiency of horizontal transmission in *L. clavipes* might be measured in *Drosophila* larvae superparasitized by *Wolbachia*-free and *Wolbachia*-infected wasps. Surprisingly, some symbionts are known to manipulate the superparasitism behaviour of their host to increase their horizontal transmission (Varaldi *et al.* 2003). Such manipulation suggests that selection could favour a mixture of vertical and horizontal transmission, and also higher efficiency of horizontal transfer. If *Wolbachia* strains have a high potential for horizontal transfer and are competitive, frequent selective sweeps might also contribute to an absence of genetic variation in *Wolbachia*, as observed in *L. clavipes*.

Interestingly, clonal reproduction of PI-*Wolbachia*-infected females is thought to maximize the alignment of interests between host and symbiont, thus selecting for symbiont benevolence and even mutualism. Horizontal transmission could rupture this alignment of interest and hence impact the co-evolution between partners by favouring more conflictive interactions. These conflicts would be particularly important when higher virulence is correlated with increased competitiveness, especially when different symbionts coexist within a given host.

In addition to experimental and phylogenetic evidence for horizontal transmission, the present study by Kraaijeveld *et al.* (2011) suggests that transmission of *Wolbachia* and other arthropod symbionts should not be considered as purely vertical, but as a mixture of vertical and horizontal transmission, at least during invasion. The impact of this mixed strategy on invasion and co-evolution will highly depend on the efficiency of horizontal transmission, and whether selection favours strains that are better invaders than others.

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doi: 10.1111/j.1365-294X.2011.05194.x