Wolbachia host shifts: routes, mechanisms, constraints and evolutionary consequences

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Abstract

Wolbachia is one of the most abundant endosymbionts on earth, with a wide distribution especially in arthropods. Effective maternal transmission and the induction of various phenotypes in their hosts are two key features of this bacterium. Here, we review our current understanding of another central aspect of *Wolbachia*'s success: their ability to switch from one host species to another. We build on the proposal that *Wolbachia* host shifts occur in four main steps: 1) physical transfer to a new species, 2) proliferation within that host, 3) successful maternal transmission, and 4) spread within the host species. Host shift occasions can fail at each of these steps, and the likelihood of ultimate success is influenced by many factors. Some stem from *Wolbachia* properties (different strains have different abilities for host switching), others on host features such as genetic resemblance (e.g. host shifting is likely to be easier between closely related species), ecological connections (donor and recipient host need to interact with each other), or the resident microbiota. Host shifts have enabled *Wolbachia* to reach its enormous current incidence and global distribution among arthropods in an epidemiological process shaped by loss and acquisition events across host species. The ability of *Wolbachia* to transfer between species also forms the basis of ongoing endeavours to control pests and disease vectors, following artificial introduction into uninfected hosts such as mosquitoes. Throughout, we emphasise the many knowledge gaps in our understanding of *Wolbachia* host shifts, and question the effectiveness of current methodology to detect these events. We conclude by discussing an apparent paradox: how can *Wolbachia* maintain its ability to undergo host shifts given that its biology seems dominated by vertical transmission?

Keywords: Ecological connection, endosymbiont, host switching, host shift steps, horizontal transmission, transmission route, epidemiology, phylogenetics

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I. Introduction

The genus name *Wolbachia* denotes a diverse group of α-proteobacteria that live as maternally inherited endosymbionts in many arthropods and nematodes (Hertig, 1936; Sironi et al., 1995). During the last decades, these bacteria have received much attention from researchers and the general public for three main reasons. First, they induce a wide range of fascinating phenotypes in their hosts, often detrimental and with wide-ranging evolutionary consequences (Charlat et al., 2003; Werren et al., 2008; Engelstädter & Hurst, 2009). Second, *Wolbachia* is one of the most abundant symbionts, with around 50% of all arthropod species being infected (Hilgenboecker et al., 2008; Zug & Hammerstein, 2012; Weinert et al., 2015; Bailly-Bechet et al., 2017). Finally, *Wolbachia* can be adopted as a controlling agent against vector-borne pathogens such as dengue, as well as pest species (Kambris et al., 2009; Iturbe-Ormaetxe et al., 2011; Asgari, 2017; Ross et al., 2019) .

The diversity of phenotypes induced by these bacteria include reproductive manipulations (Yen & Barr, 1971; Rousset et al., 1992; Hurst et al., 2000), physiological and behavioural modifications (Min & Benzer, 1997; Beltran-Bech & Richard, 2014; Rohrscheib et al., 2015; Truitt et al., 2018; Bi & Wang, 2020) and changes in their susceptibility to pathogens (Hedges et al., 2008; Teixeira et al., 2008; Kambris et al., 2009; Ekwudu et al., 2020). *Wolbachia* can also contribute to nutrient synthesis (e.g. Brownlie et al., 2009; Moriyama et al., 2015). Reproductive manipulations are common and take various forms. In cytoplasmic incompatibility (CI), *Wolbachia* induces embryonic death in the offspring of uninfected females mated with infected males. In the case of male killing, the bacteria cause the death of sons in the offspring of infected females. Finally, parthenogenesis and feminisation induction both stem from the transformation of potential males into females, which effectively leads to parthenogenesis in host species where zygotes can develop without mating.

From an evolutionary standpoint, these various effects can all be explained as adaptations enhancing the bacteria's fitness through that of the infected hosts, or more specifically the infected "matrilines", that is, the *Wolbachia*-carrying cytoplasmic lineages (Cosmides & Tooby, 1981; Werren, 1997; Stouthamer et al., 1999). This reasoning is straightforward in the case of direct positive effects such as protection

against pathogens or nutrients provision, but possibly less so when it comes to reproductive manipulations, where maternal (as opposed to biparental) transmission is the critical feature (Werren et al., 2008). In the case of CI, the relative fitness of infected embryos is only indirectly increased by the elevated death in the offspring of uninfected females mated with infected males (Hoffmann et al., 1990; O'Neill et al., 1997). Male-killing also has indirect fitness consequences in the sense that the death of infected females' sons does not immediately (and possibly not always) benefit their sisters: only reduced competition for food, or even consumption of their dead brothers, can generate some "fitness compensation" that will give an advantage to the infected cytoplasmic lineage (Hurst & Majerus, 1993). Finally, in species where *Wolbachia* induces parthenogenesis or feminisation, its benefits are most evident and strong: in both cases, *Wolbachia* effectively turn sons into daughters, thus doubling its chances of transmission to the next generation.

Wolbachia have been reported in several major groups of terrestrial arthropods (hexapods, arachnids and isopods) as well as filarial nematodes (Taylor et al., 2005; Weinert et al., 2015). Not surprisingly given this high incidence and wide host range, the *Wolbachia* clade also exhibits a large genetic diversity. Within the only officially named *Wolbachia* species (*Wolbachia pipientis*), hundreds of molecularly distinct lineages, usually referred to as "strains" have been reported. They may be distinguished on the basis of single or multiple genetic markers, some of which have been proposed as standards in a "Multilocus Sequence Typing" scheme (MLST) (Baldo et al., 2006), providing informative (albeit incomplete) knowledge on divergence between *Wolbachia* lineages. These strains are distributed in several large clades denoted "supergroups", having likely diverged for over a hundred million years (Lo et al., 2002; Bordenstein & Rosengaus, 2005; Glowska et al., 2015; Gerth & Bleidorn, 2017; Lefoulon et al., 2020). On the basis of microbial taxonomy standards, these large groups could in principle take a species status, which is a matter of an ongoing debate (Ramírez-Puebla et al., 2015; Gerth, 2016; Lindsey et al., 2016; Bleidorn & Gerth, 2018). *Wolbachia* strains falling in supergroups A and B, and to a lesser extent, F and E, are widely distributed across terrestrial arthropods, presumably mostly as reproductive parasites. *Wolbachia* from supergroups C and D are found in filarial nematodes where they are suggested as essential mutualists (Fenn & Blaxter, 2004). The remaining supergroups are mostly limited to specific and smaller arthropod clades.

How can such a high incidence, large host range and phylogenetic diversity be explained? The present-day distribution of any symbiont is driven by the interplay between processes that increase the number of carrier species and others that decrease it: co-speciation events and host shifts make to the first category while host extinction and symbiont losses form the second (e.g., Thompson, 1987; Charleston & Perkins, 2006; Engelstädter & Fortuna, 2019). Co-speciation occurs when symbionts are retained in both daughter lineages following a host speciation event, leading to symbiont divergence among host lineages (Hafner & Nadler, 1990; de Vienne et al., 2013). (In what follows, we will equivalently use the term "co-divergence"; in this broad sense, "co-speciation" does not specifically imply that the daughter symbiotic lineages are attributed a "species" status.) Strict co-divergence of hosts and symbionts is expected to lead to congruent host and symbiont phylogenies, as indeed observed in a wide variety of symbiotic associations (Ashen & Goff, 2000; Hosokawa et al., 2006; Thrall et al., 2007). Congruent phylogenies of *Wolbachia* and their hosts are also observed in nematodes (supergroup C and D *Wolbachia*) (Ferri et al., 2011; Bandi et al., 1998; Fenn & Blaxter, 2004) and bedbugs (supergroup F) (Balvín et al., 2018), in accordance with the finding that the symbiont behaves as an obligate mutualist in these groups. By contrast, mirror phylogenies are much less common for the vast majority of other *Wolbachia* strains, indicating they often infect new species through host shifts, and also get lost.

Host shifts, also referred to as "horizontal transfers", are defined here as the transmission of a symbiont to a new host species that is successful at the population level, i.e. producing a new stable association. Early work on *Wolbachia* from A and B supergroups established that the phylogenies of the symbiont and its arthropod hosts are rarely congruent, ruling out the hypothesis that an ancestral *Wolbachia* infection underwent faithful co-speciation and strictly co-diversified with arthropods. Instead, these pioneering studies indicated that *Wolbachia* was subject to frequent host shift and extinction events (Schilthuizen & Stouthamer, 1997; Meer et al., 1999; Bourtzis & O'Neill, 1998; Werren et al., 1995; Heath et al., 1999; O'Neill et al., 1992; Rousset et al., 1992; Hurst et al., 1992). Moreover, in several studies, very closely related *Wolbachia* strains were detected in distantly related host species (from different genera to orders), suggesting that host shifts can cover large phylogenetic distances (Cordaux

et al., 2001; Baldo et al., 2008; Noda et al., 2001; Stahlhut et al., 2010; Stouthamer et al., 1999). Evidence has accumulated since that the current distribution of *Wolbachia* is mostly governed by recent host shifts and infection losses (Bailly-Bechet et al., 2017). The host switching ability of *Wolbachia* has also been corroborated by the possibility to artificially transfer the bacteria across species through so-called "transinfection" experiments (reviewed in Hughes & Rasgon 2014). Taken together, the available evidence indicates that *Wolbachia* has a great ability to move between host species (Huigens et al., 2004a; Cordaux et al., 2001; Heath et al., 1999; Sintupachee et al., 2006; Baldo et al., 2008; Russell, 2012).

A special case of host shifting arises when *Wolbachia* enters a new species via hybridisation. Limited gene flow (introgression) between closely related species through the production of hybrids is a common phenomenon (Mallet, 2005). *Wolbachia* strains inducing bidirectional CI (embryonic death of hybrids caused by two incompatible *Wolbachia* strains) may contribute to reproductive isolation and thus foster speciation (Bordenstein et al., 2001). But hybridisation may also facilitate the passage of *Wolbachia* if only one of the populations is infected. Following mating between an infected female from species A and an uninfected male from species B, maternal transmission the symbiont and recurrent backcrossing to B males may lead to *Wolbachia* introgression into species B, especially if the symbiont carries its own driving force through reproductive manipulations or fitness benefits (Rousset & Solignac, 1995; Raychoudhury et al., 2009; Turelli et al., 2018). Hybridisation-mediated host shifts have been suggested in the *Nasonia* species complex (Raychoudhury et al., 2009), *Acraea*, *Hypolimnas* and *Eurema* butterflies (Jiggins, 2003; Charlat et al., 2009; Miyata et al., 2020), in the grasshopper *Chorthippus parallelus* (Martínez-Rodríguez & Bella, 2018) as well as in *Drosophila* species (Turelli et al., 2018; Cooper et al., 2019).

Host shifts though introgression are peculiar for a number of reasons. In contrast to genuine horizontal transfer, introgression occurs by regular maternal inheritance, that is, through vertical transmission. As a result, the mitochondrial haplotype associated with *Wolbachia* in the donor species spreads along with the symbiont into the recipient species. Such events therefore leave a signature of incongruency between nuclear and mitochondrial evolutionary histories, in terms of divergence time or even tree topology,

but no incongruence between the *Wolbachia* and mitochondrial trees. For example, in the butterfly genus *Hypolimnas*, a shared infected mitochondrial haplotype has been observed in two different species, indicating a case of introgression that would not have been identified on the basis of *Wolbachia* and mtDNA data alone, that is, without prior knowledge on the species boundaries (Charlat et al., 2009; Sahoo et al., 2018). Hybridisation-mediated host shifts also represent a special case in that their rates of success are expected to be much higher than that of other transfer routes. This is because introgression releases the bacteria from the challenges of facing an extracellular environment and having to find their way to the germ line. Moreover, as discussed below, the high genetic similarity between the donor and recipient species means that the long-term maintenance of the new infection is also more likely. Host shifts by introgression may thus be more common than genuine host shifts via horizontal transfer, but it should be emphasized that they cannot be seen as a general explanation for the wide distribution of *Wolbachia*, considering that genetically similar *Wolbachia* strains are often observed in phylogenetically distant hosts. For this reason, in what follows, we will focus our discussion on host shifts mediated by horizontal transmission in a strict sense, excluding the special case of hybridisation.

II. Steps involved in host shifts

It is useful to conceptionally break down the process of host-shifting into several distinct steps (Combes, 2001; Woolhouse et al., 2005; Bright & Bulgheresi, 2010), an approach that has previously been suggested for *Wolbachia* (Vavre et al., 2003; Riegler et al., 2004). Here, we put forward the following four steps: 1) physical transfer of the bacteria to a female of a new species, 2) proliferation within this new individual host, 3) successful maternal transmission to its offspring, and 4) spread within the new host population. Each of these steps presents its own challenges that may or may not be overcome, depending on both host and bacterial factors, as summarised in Figure 1.

Step 1: Physical transfer

This very first step may take place via a vector or directly, with more or less prolonged exposure to the external environment (Breeuwer & Jacobs, 1996; Baldo et al., 2008; Cordaux et al., 2001; Heath et al., 1999). As *Wolbachia* is adapted to the intracellular lifestyle, facing the environment may constitute a critical challenge (reviewed by Sicard

et.al., 2014). Below we discuss four routes identified as plausible, among an obviously immense set of ecological possibilities.

Predator-prey interactions

Virtually any living organism is part of a prey-predator interaction, and arthropods are no exception to that rule (Hassell, 1978; Sabelis, 1992). In principle, predators may receive a new *Wolbachia* infection from their prey, an hypothesis that has sometimes been put forward to explain the sharing of similar infections in preys and predators (Kittayapong et al., 2003; Enigl & Schausberger, 2007; Wiwatanaratanabutr & Zhang, 2016). A few cases of direct experimental evidence for successful *Wolbachia* transmission from prey to predator have also been reported (e.g., between woodlice (Le Clec'h et al., 2013)).

Although the majority of insects harbour various bacteria in their gut as part of their microbiome (Engel & Moran, 2013; Dillon & Dillon, 2004), the lumen secretions can be strong enough to digest prey cells and their intracellular bacteria (Dow, 1987; Terra, 1990; Terra & Ferreira, 2012). Therefore, there is a challenge for *Wolbachia* to first survive in the lumen, and then to enter the host tissue through the gut epithelium (Sicard et al., 2014). Hosts with an already established *Wolbachia* infection sometimes also harbour these bacteria in their gut lumen, indicating some ability of *Wolbachia* to overcome the first of these challenges (Cheng et al., 2000; Ye et al., 2017; Osborne et al., 2012; Kikuchi & Fukatsu, 2003; Andersen et al., 2012). Predaceous mites that consume infected phytophagous mites have been reported as *Wolbachia* positive (by PCR) for up to 48 hours (Johanowicz & Hoy, 1996; Enigl et al., 2005) and on rare occasions, the infection was shown to become established in the new host (Johanowicz & Hoy, 1996). Therefore, it appears that *Wolbachia* can somehow and sometimes escape the initial digestion in the midgut.

How often is *Wolbachia* transferred to a new host species through predation? Surveys provide little evidence for shared *Wolbachia* infections in predators and their hosts and therefore limited support for this route of transfer (Cordaux et al., 2001; Enigl et al., 2005; Yun et al., 2011; Hurst et al., 2012). For example, in a screening of mosquitos and their natural predators in China and Thailand, no cases of *Wolbachia* strains shared between prey and predators were reported (Wiwatanaratanabutr & Zhang, 2016).

Moreover, if predation-mediated transfers were common, one would expect that, all else being equal, predator taxa such as spiders may exhibit a higher *Wolbachia* incidence than other groups, which does not seem to be the case (Rowley et al., 2004; Baldo et al., 2007; Wang et al., 2010; Yun et al., 2011; Bailly-Bechet et al., 2017). *Wolbachia* host shifts *per se* were also not found to occur more frequently in spiders than in other arthropod orders (Bailly-Bechet et al., 2017). A recent study indicated that most of the microbiome of spider preys can be found in the gut of their predators, although the presence of *Wolbachia* in the spiders themselves was rarely observed (Kennedy et al., 2020). Thus, although some arthropods predators are presented with ample opportunities to acquire *Wolbachia* from their prey, such transfers do not seem to occur frequently.

Host-parasitoid/parasite interactions

Parasitoids comprise around 20%–25% of all insect species and most terrestrial arthropods can be parasitised by one or several of them (Godfray, 1994; Mills, 2009). In theory, all parasitic strategies, including castration, parasitoidism, micropredation (feeding on a host individual without killing it, e.g. in mosquitos) and phoresis (attaching to a host individual for transportation), may expose both sides to microbial exchange. Moreover, compared to prey-predator interactions, the physical association between host and parasite individuals typically lasts longer, occurs at various developmental stages, and directly puts *Wolbachia* in contact with a wide variety of tissues, and not just the gut lumen). All of these features may increase the probability of parasitism-mediated host shifts. Not surprisingly therefore, these pervasive ecological interactions have been found to open a door for horizontal transfer, notably in the case of *Wolbachia* and host-parasitoid interactions (Cook & Butcher, 1999; Heath et al., 1999; Vavre et al., 1999; Hunter et al., 2003; Kittayapong et al., 2003; Huigens et al., 2004b; Raychoudhury et al., 2009; Kageyama et al., 2010; Morrow et al., 2014; Ahmed et al., 2015; Klopfstein et al., 2018; Hou et al., 2020).

In contrast to prey-predator interactions, the direction of *Wolbachia* exchange can also be from the parasite to its host, at least when it is not always lethal (Vavre et al., 1999). When a parasite and its host are infected with the same strain, understanding the direction of the transfer can be challenging. One strategy can be to assess the presence of close *Wolbachia* relatives in close host relatives on either side. Unless infections

have been recently acquired in the donor, or subject to a recent sweep, larger diversity and shared infection with sister groups are expected on the donor side (Johannesen, 2017).

Complex infection routes may exist between parasites and hosts. A single common parasitoid species can be a source of infection for multiple shared hosts (Dedeine et al., 2005; Noda et al., 2001) or a single host may be a source of infection for multiple parasites. For example, natural inter- and intraspecific horizontal transfers of parthenogenesis-inducing *Wolbachia* have been reported between *Trichogramma* parasitoid wasps sharing host eggs (Huigens et al., 2000). Parasites may also play a vector role between infected and uninfected host without themselves being infected. In support of this notion, Ahmed et al. (2015) reported that the mouthparts and ovipositors of an *Aphelinid* parasitoid become contaminated with *Wolbachia* when this wasp feeds on *Wolbachia*-infected *Bemisia tabaci,* which in turn can be a source of infection for the next parasitised host.

In summary, parasitic interactions may well be one of the most common routes of *Wolbachia* host shifts. If this is the case, we would predict that hosts attacked by diverse parasites and, reversely, generalist parasites, should display a high *Wolbachia* incidence. Some results suggest such a trend may hold (Kittayapong et al., 2003; Klopfstein et al., 2018), but this prediction remains to be thoroughly tested.

Shared plant and other food sources

Shared food sources create important ecological links between species within a community (Paine, 1980) and may constitute a route of transmission for microbes that can survive either within or on the surface of the food. Plants are one of the best examples of such an ecological platform (Chrostek et al., 2017). *Wolbachia* has been found in the salivary glands of several herbivore and non-herbivore insects (Dobson et al., 1999). By physical contact between arthropod mouthparts and plant tissue, their bacteria may be transferred to the plants. The ability of *Wolbachia* to tolerate an extracellular environment was demonstrated in cell-free medium (Schneider's medium), where the *w*AlbB strain could survive for up to 7 days (Rasgon et al., 2006). Some strains of *Wolbachia* may have the ability to survive within plants for even longer

time, during which they may be transferred to new hosts (Burke et al., 2020). In a recent study, one strain has been reported to survive in cotton leaf phloem vessels for up to 50 days, retaining the potential to infect whiteflies (Li et al., 2017a). Similarly, the high abundance of *Wolbachia* in pollen and broods of a carpenter bee (*Ceratina calcarata*) (McFrederick & Rehan, 2016), and the existence of identical *Wolbachia* strains in a hoverfly species (*Merodon luteihumerus*) and its host plant's bulb tissue (sea squill) suggested plants as a plausible platform for *Wolbachia* transmission in this system (Zorić et al., 2019). As a result of plant-mediated host shifts, taxonomically diverse arthropod species feeding on the same plants may share common or closely related *Wolbachia* strains, as has been reported for the agricultural setting of a pumpkin farm (Sintupachee et al., 2006). Sharing a common host plant may also explain high host shift rates among various species of fig wasps (Shoemaker et al., 2002; Haine & Cook, 2005; Yang et al., 2013). Horizontal transmission via plant tissue is not limited to *Wolbachia*. A study focusing on *Rickettsia* showed that transmission of this symbiont from a cotton plant to whiteflies was plausible (Li et al., 2017b). It is also suggested that *Cardinium* can survive in both artificial food and plant tissues and thereby infect leafhoppers (Gonella et al., 2015).

Insect food sources that may mediate *Wolbachia* host shifts are not restricted to plants. In fungus growing ants, fungal gardens represent a likely source of *Wolbachia* horizontal transmission between the ants and their social parasites (Tolley et al., 2019). The transfer of food between ants and crickets inhabiting their nests through trophallaxis appears to have mediated some *Wolbachia* host shifts (Tseng et al., 2020). Sharing the same dung patches may also have led to *Wolbachia* shifts between two Malagasy dung beetle species (Miraldo & Duplouy, 2019). Ingestion of corpses may also lead to horizontal transmission within or between species. For example, laboratory experiments showed that *Tyrophagus putrescentiae* mites can transfer their *Wolbachia* infection to corpses of *Drosophila*, which are later ingested by *Drosophila* larvae, which results in the establishment of a new infection (Brown & Lloyd, 2015).

Step 2: Survival and proliferation in the new host

Before reaching the germline cells and undergoing successful vertical transmission, *Wolbachia* first have to survive in their new environment. The host's intra and extracellular immunity is an inevitable part of the arthropod's physiology, one that

usually prevents infections by any invading microbe (Zug & Hammerstein, 2015). How can *Wolbachia* survive such a threat and proliferate in the new host?

Once a bacterium colonises a new arthropod host, the immune system is often triggered by specific pathogen-associated molecular patterns (PAMPs) such as peptidoglycans (Zaidman-Rémy et al., 2006; Otten et al., 2018). These may then activate the innate immune responses which are categorized into cellular and humoral types (Salt & Salt, 1970; Boman & Hultmark, 1987; Lavine & Strand, 2002). The transcription of antimicrobial peptides such as Defensin was indeed observed in *Aedes aegypti* following transinfection with *w*AlbB (Bian et al., 2010), *w*Mel (Kambris et al., 2009) and *w*MelPop-CLA (Rancès et al., 2012), which usually leads to *Wolbachia* titre reduction or complete loss of the bacteria. However, in some *Wolbachia* transinfection studies, expression of the major host antibacterial immunity genes was only slightly altered or not changed at all (Bourtzis et al., 2000; Chevalier et al., 2012). One plausible explanation is that *Wolbachia* have a unique functional peptidoglycan amidase ($AmiD^{wol}$) that cleaves its own bacterial cell wall so that it may remain hidden from both humoral and cellular immune responses (Eleftherianos et al., 2013; Wilmes et al., 2017; Otten et al., 2018). Indeed, this has been suggested as a potential explanation for the higher incidence of *Wolbachia* than that of other symbionts such as *Cardinium* and *Rickettsia*, in arthropods that do mount immune responses to Gram negative bacteria, but not in groups that lack components of the gram negative innate immune pathway, such as some Hemiptera and Acari (Waxman et al., 2014; Morand et al., 2015; Weinert et al., 2015). In addition, the absence of $AmiD^{wol}$ in mutualist *Wolbachia* strains of nematodes is in line with the hypothesis that the evolution of $AmiD^{wol}$ may be causally linked to the host shifting ability in *Wolbachia* supergroups A and B (Wilmes et al., 2017).

However, *Wolbachia*'s ability to modify its own cell wall may not be sufficient to prevent detection of the bacteria in all situations. Besides the peptidoglycans, cell-to-cell movements of *Wolbachia* (probably based on the activation of cell phagocytic and clathrin/dynamin-dependent endocytic machinery (White et al., 2017)) can cause oxidative stress to the host and consequently lead to a diverse regulation of immune-related genes (Caragata et al., 2017). *Wolbachia* shows a feature that can be interpreted as another layer of defence against this threat: similar to other α-proteobacteria that are

facultative symbionts (such as *Brucella* and *Anaplasma*), *Wolbachia* is always observed in the host cell within a triple layer vacuole, that can partly protect it from cellular immune responses (reviewed in Sicard et al., (2014)). Such a mechanical shield provides a basic protection against the host immune response.

Even though *Wolbachia* may often escape general immune responses from the host, we would expect resistance against the bacteria to be selected for in many systems (Koehncke et al., 2009; Hornett et al., 2010; Salunkhe et al., 2014) because of the direct or indirect negative fitness effects often associated with the infection (Fleury et al., 2000; Koehncke et al., 2009; Le Clec'h et al., 2012; Charlat et al., 2003). There are no high-rank taxonomic groups of terrestrial arthropods where extensive screening has failed to identify any infected species. (One possible exception to that rule is the order Phasmatodea (stick insects), where 247 individuals from 29 species have been screened for *Wolbachia* without a single positive specimen (Werren & Windsor, 2000; Weeks et al., 2003; Perez Ruiz et al., 2015).) A possible interpretation of this pattern is that no single general resistance mechanism ever evolved. Alternatively, it may be that *Wolbachia* infections do not always constitute a significant and negative selective pressure. Some control strategies have however been reported that are specific to particular host or *Wolbachia* lineages (reviewed by López-Madrigal & Duarte (2019). Transinfection experiments indicate that different host species usually have different immune reactions or physiological response against similar *Wolbachia* strains (Rancès et al., 2012; Herbert & McGraw, 2018). Conversely, a given host usually does not react in the same way to all *Wolbachia* strains (e.g. the different immune response of *A. aegypti* or *Lutzomyia longipalpis* to *w*Mel and *w*MelPop-CLA strains (Rancès et al., 2012; da Silva Gonçalves et al., 2019)). In response to the severity of the *Wolbachia* fitness cost, such strain-specific host reactions can be selected in a very short evolutionary time scale (Li et al., 2018). For example, a comparative study of the *Nasonia* species complex led to the identification of a *Wolbachia* density suppressor (*Wds*) gene in *N. vitripennis* that tends to decrease the titre of the native *w*VitA strain and consequently its vertical transmission (Funkhouser-Jones and van Opstal et al. 2018). It appears that this strain-specific controlling strategy has evolved through the change of only a single amino acid in *Wds* (Funkhouser-Jones and van Opstal et al. 2018).

Step 3: Vertical transmission

Effective transmission of the infection to the host's offspring requires reaching the germ line cells and being maintained in the zygote. This ability of *Wolbachia* to initiate vertical transmission may arise from its tropism toward somatic stem cells niche, that the bacteria may first occupy as a stable reservoir before reaching the germline itself (Frydman et al., 2006). Toomey et al., (2013) showed that the high concentration of *Wolbachia* in the somatic stem cells is not only a key factor for vertical transmission but also a conserved feature observed in diverse strains of various *Drosophila* species. From the somatic stem cells, *Wolbachia* may utilize the host's vitellogenin transovarial transportation system to enter the oocyte (Guo et al., 2018). Regulation of such transportation routes or any other mechanisms to control the *Wolbachia* transmission to the oocyte may constitute evolved host adaptations to control the *Wolbachia* titre in *Nasonia vitripennis* (Funkhouser-Jones and van Opstal et al 2018) and *Armadillidium vulgare* (Rigaud & Juchault, 1992; Cordaux et al., 2011). Once the bacteria have successfully entered the zygote, they need to reach important host tissues (including but not limited to the germ line stem cells) without disrupting the embryo's development. This may be achieved through the utilisation of the host cytoskeleton, which appears to be achieved by bundling of *Wolbachia* protein WD0830 to host actin filaments (Newton et al., 2015; Sheehan et al., 2016) as well as by increasing the division rate of germ line stem cells (Fast et al., 2011) to localize and enhance their titre (for details see (Pietri et al., 2016; Guo et al., 2019; Landmann, 2019)).

How often do *Wolbachia* fail at the transmission step of the host shift process? Some light can be shed on this question by transinfection studies again, although it is often difficult to distinguish between failure to proliferate and failure to be transmitted (i.e., steps 2 vs 3). Most reported transinfection attempts resulted in an infection that was stable over many generations (reviewed by Hughes and Rasgon, (2014)) indicating that, at least under these artificial conditions involving high initial *Wolbachia* titres, transmission rates in a new host can be high. However, these data almost certainly involve a publication bias, both towards successful transinfection outcomes and towards transinfection attempts between closely related donor and recipient host species (see below for a discussion of the phylogenetic distance effect). Several studies also reported inefficient transmission and/or loss of *Wolbachia* after a few generations. For example, (Clancy & Hoffmann, 1997) achieved successful transinfection of a

Wolbachia strain from *D. simulans* to *D. serrata* but transmission rate was low (~90%). Rigaud et al., (2001) reported similar transmission rates following transinfection of *Wolbachia* between two closely related woodlice species and very low ones (<10%) between more distantly related woodlice species. Artificial transfer of a double *Wolbachia* infection from the cherry fruit fly *Rhagoletis cerasi* to *D. simulans* led to the loss of one strain from the first generation, and inefficient vertical transmission of the other (Charlat et al., 2004; Riegler et al., 2004). Finally, transinfection of *Wolbachia* from a parasitoid wasp to *D. simulans* was successful but transmission rates were also low and the infection was lost by the seventh generation (Meer & Stouthamer, 1999). These results lead us to think that under natural conditions, where initial infection titres must be much lower than in most transinfection experiments, and where transferred *Wolbachia* strains will often come from more distantly related hosts, efficient maternal transmission constitutes a challenging step in *Wolbachia* host shifts.

Step 4: Spread within the host population

Without inducing a phenotype driving its spread, *Wolbachia* may easily become lost from a new host species. This is because maternal transmission itself does not entail any epidemiological drive: in the absence of positive fitness effects on its host, *Wolbachia* would not be expected to increase in frequency. Even with perfect transmission fidelity *Wolbachia* would just be equivalent to a neutral new allele, with limited chances of spreading. Deleterious fitness effects and imperfect transmission pose further restrictions on the spread of *Wolbachia* within a population. Invasion of a new population thus likely stems from specific phenotypic effects, including reproductive manipulations in the first place (such as CI, feminisation, male killing and parthenogenesis), and/or providing direct fitness benefits to their female hosts (e.g. synthetising nutrients, or perhaps more commonly, protect against pathogens) (Werren, 1997; Stouthamer et al., 1999; Fenton et al., 2011; Zug & Hammerstein, 2014). Moreover, the intensity of the induced phenotypes and consequently the strength of drive needs to be strong enough to enable spread (Breeuwer & Werren, 1993; Unckless et al., 2009; Koga et al., 2012; Osborne et al., 2012).

Although we know little about how often these conditions are met following natural host shifts, transinfection experiments can again be informative. These have shown that *Wolbachia* may sometimes retain its original phenotypic effects upon transfer into a new host, sometimes induce a different phenotype, and sometimes have no detectable

effect at all. For example, four distantly related strains (wRi, wAlbA, wAlbB, and wPip) and three close relatives (from the wMel group), that all induced CI in their original host, also induced CI upon introduction in *A. aegypti* (reviewed in Sicard et al., 2019). A strain inducing male-killing in its original host (the moth *Cadra cautella*) induced CI instead in a novel host (the moth *Ephestia kuehniella*) (Sasaki et al., 2002). The *w*Inn strain, inducing male killing in its original host (*D. innubila*) (Dyer & Jaenike, 2004) had no observable phenotype (either CI or male killing) in transinfected *D. simulans* and *D. melanogaster* (Veneti et al., 2012). The *Hypolimnas bolina* butterfly is also informative with regard to phenotypic switches: host suppression of male-killing revealed the ability of the same *Wolbachia* strain to also induce CI in this species (Hornett et al., 2008). No obvious patterns regarding these changes in phenotypes, e.g. a tendency to preferentially change from one particular phenotype to another, have been uncovered yet. For more examples, including examples where the strength of CI changed in either direction upon transinfection, we refer to (Poinsot et al., 1998; Hughes & Rasgon, 2014; Hoffmann et al., 2015; Ross et al., 2019).

Among all reproductive manipulations, CI is probably the most prevalent *Wolbachia*-associated phenotype and may thus often contribute to its establishment in a host population. Here, in the simplest case, infected females have a relative reproductive advantage due to the embryonic death of uninfected individuals fertilised by infected males. This advantage is said to be "positive frequency-dependent", because it increases as infected males become more frequent in the population. Upon arrival of CI-inducing *Wolbachia* in a new host species, the bacteria are likely to exhibit imperfect maternal transmission and may often have negative effects on host fitness. In such a scenario, theory predicts, and experimental studies have confirmed, that there will be an invasion threshold frequency below which *Wolbachia* is lost from the population and above which it should spread to a high and stable prevalence (Fine, 1978; Hoffmann et al., 1990; Turelli & Hoffmann, 1995; Xi et al., 2005; Hancock et al., 2016; Kriesner et al., 2016). The invasion threshold depends on the strength of CI, the fitness cost or benefits associated with *Wolbachia* and the fidelity of vertical transmission (Turelli, 1994; Rasgon & Scott, 2004; Li & Wan, 2019). For example, in the *A. aegypti* system where *Wolbachia* has been artificially introduced, a 20-30% invasion threshold has been estimated (Axford et al., 2016; Turelli & Barton, 2017).

In release programs of *Wolbachia* infected individuals, the threshold can be artificially overcome by increasing the number of infected hosts in each release (Hoffmann et al., 2011). But how can *Wolbachia* tackle this obstacle in nature? One possibility is that *Wolbachia* passes the invasion threshold in (locally) small populations where a new infection would represent a substantial proportion of the population and random genetic drift may facilitate *Wolbachia* establishment (Turelli, 1994; Jansen et al., 2008). As mentioned above, another possibility is that *Wolbachia* may provide direct fitness benefits such as provisioning of nutrients (Nikoh et al., 2014; Brownlie et al., 2009; Douglas, 2009; Hosokawa et al., 2010; Darby et al., 2012; Ju et al., 2020), or protection against pathogens (Fenton et al., 2011). Models of *Wolbachia* inducing both CI and fitness benefits predict rapid spread and fixation of *Wolbachia* even when starting from very low initial frequencies, provided that the maternal transmission rate is sufficiently high (Dobson et al., 2004; Fenton et al., 2011; Zug & Hammerstein, 2018). However, the beneficial effects of *Wolbachia* can often depend on environmental conditions (Reynolds et al., 2003; Mouton et al., 2006; Zug & Hammerstein, 2014). Moreover, beneficial effects observed in long-established associations may not have been present initially. This view is supported by the finding that the *w*Ri strain in *Drosophila simulans* evolved from imposing reduced to increased fecundity in infected females over a time span of 20 years (Weeks et al., 2007).

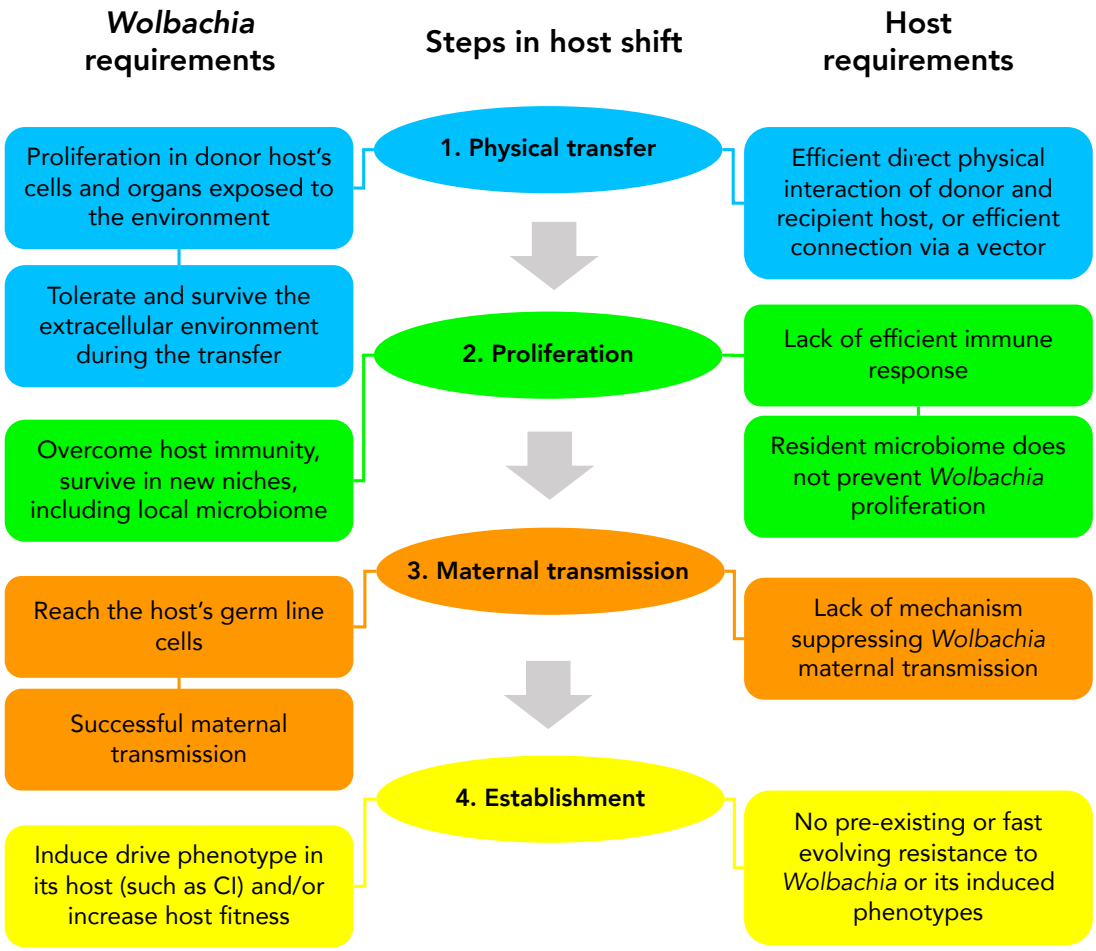

Figure 1: *Wolbachia* and host requirements in each steps of the host shift process.

III. Factors influencing host shifts

Why does a particular *Wolbachia* strain succeed in switching from host A to B, but not to C? Or why does *Wolbachia* *w*A often switch while *w*B does not? We will discuss four main factors influencing host shifts: *Wolbachia* interactions with the host's resident microbiome, variation between different strains in their ability to undergo host shifts, genetic similarity between donor and recipient host, and finally, ecological conditions.

3.1. Host resident microbiome

Interactions between *Wolbachia* and the host's resident microbiome, including exploitative competition for shared resources (Caragata et al., 2013), triggering of host immunity against competitors (Pan et al., 2012; Joubert et al., 2016) or even collaboration, can critically impact the *Wolbachia* titre (Vorburger & Perlman, 2018; Duan et al., 2020). The microbiome may thus influence the last three steps in the host shift process, from *Wolbachia* proliferation to maternal transmission and within-population establishment (Zouache et al., 2009; Chandler et al., 2011; Hughes et al., 2014; Dittmer & Bouchon, 2018; McLean et al., 2018; Fromont et al., 2019). In extreme cases, such interactions may completely prevent the host shift. For example, the native microbiome of *Anopheles* mosquitos has been reported to block *Wolbachia* maternal transmission (Hughes et al., 2014). *Wolbachia*-microbe interactions are not always a restrictive factor and may even facilitate *Wolbachia* establishment, for example through increased host fitness, such as those induced by co-infections of *Wolbachia* and *Spiroplasma* in a spider mite (Xie et al., 2019) and co-infections of *Wolbachia* and *Cardinium* in *Hylyphantes graminicola* spider (Li et al., 2020), or through strengthened reproduction manipulations, as seen in a spider mite where the expression of *Cardinium*-induced CI is strengthen by *Wolbachia* (Zhu et al., 2012) or in a in whitefly where *Cardinium*-*Wolbachia* coinfections enhance male-killing (Lv et al., 2020).

A special but common case arises when the recipient host species is already infected with a different strain of *Wolbachia*. Two scenarios are then conceivable. First, the new variant may enter the new host population as a single infection, that is, in an initially uninfected individual host. In this case there will be a direct competition within the host population between the resident and invader *Wolbachia* matrilines. In the absence of direct fitness benefits, mathematical models indicate that if the resident strain induces any reproductive manipulation, and especially if the two strains induce bidirectional CI, this should inhibit invasion of the new strain (Rousset et al., 1991; Engelstädter et al., 2004; Telschow et al., 2005). If the invasion of the new strain is nevertheless successful, this is generally expected to lead to extinction of the resident strain rather than a stable polymorphism (Rousset et al., 1991; Engelstädter et al., 2004), unless there is a pronounced host population structure (Keeling et al., 2003). An empirical example for such a displacement is provided by the recent spread of the CI-inducing *w*Ri variant in eastern Australia which was accompanied by a marked decline in the frequency of

the *w*Au strain that was initially very prevalent in this region although it does not appear to induce any reproductive manipulation (Kriesner et al., 2013). The second scenario is when the new *Wolbachia* arises in an originally infected host, thus producing a double infection at the individual level. In this case, theory predicts that competition at the population level is less severe and the new strain can spread to form a stable polymorphism with the old strain so that host individuals with either both strains or none may co-occur, although double infections should dominate (Frank, 1998; Engelstädter et al., 2004). Such instances of coinfections with different *Wolbachia* strains are indeed commonly observed in arthropods (e.g. Perrot-Minnot et al., 1996; Kondo et al., 2002).

3.2. Host shift ability of *Wolbachia* strains

Transinfection studies as well as surveys of natural arthropod populations indicate that the ability to undergo host shifts varies widely across *Wolbachia* lineages. Some strains, especially from supergroups C, D and F that comprise obligate symbionts, appear to be characterised by very low host shift rates (Balvín et al., 2018). On the other side of the spectrum, some "superspreaders" have been identified, especially within supergroups A and B. These include strains labelled as ST41 in Lepidoptera (Ilinsky & Kosterin, 2017), *w*Ri in *Drosophila* (Turelli et al., 2018) and HVR-2 in *Acromyrmex* ants (Tolley et al., 2019). Their enhanced host shift ability is indicated by a high number of host species resulting from numerous transfer events in the recent past. For example, it has been estimated that the *w*Ri-like strains spread across eight *Drosophila* species within the last 14,000 years, probably through a mixture of introgression and horizontal transfer in a strict sense (Turelli et al., 2018).

What drives such differences in host shift ability remains largely unknown. It may be that fast evolving genomic regions, such as those associated with mobile elements, contribute to the ability of some strains to rapidly adapt to new hosts. As genomic data accumulate, systematic analyses of genomic variation affecting traits involved in the *Wolbachia* / host interactions, such as sensing, signalling and secretion may help resolve this issue (Lindsey, 2020). *Wolbachia* variations affecting host tissue niche tropism and localisation may also be at play. While the existence of some strains in specific tissues seems related in the first place to their phenotypic effects (Sapountzis et al., 2015; Douglas, 2009), *Wolbachia* localisation in host somatic cells and in the

extracellular environment also affects horizontal transmission (Sicard et al., 2014). For example, the high density of *Wolbachia* in the gut of leaf cutting ants (Andersen et al., 2012) and consequently in their faeces may facilitate intra-species horizontal transmission via trophallaxis behaviour (Frost et al., 2014), and at the same time also boost host shifts. Another example of a plausible link between somatic localisation and horizontal transmission is the proliferation in haemocytes that facilitate rapid transportation to organs exposed to the outside environment, such as the gut and salivary glands (Frost et al., 2014; Braquart-Varnier et al., 2015; Le Clec'h et al., 2017). However, such interpretations of tissue tropisms variations remain speculative at this stage. In particular, it is generally unclear how much tissue distribution will affect host shift abilities, relative to other potential factors, including those related to immune evasion, or the robust induction of reproductive manipulations and other phenotypes across many host species.

3.3. Genetic similarity of the donor and recipient hosts

It is expected that closely related host species are alike in many respects, including their intra- and extracellular environments and immunity (Perlman & Jaenike, 2003; Gilbert & Webb, 2007; Longdon et al., 2011). A symbiont would thus be expected to switch most easily between close relatives (Charleston & Robertson, 2002; Clayton et al., 2003; Tinsley & Majerus, 2007). This expectation, sometimes referred to as the "phylogenetic distance effect" (PDE) (Longdon et al., 2011; Engelstädter & Fortuna, 2019), may be applicable to any entity undergoing host shifts, from transposable elements (Peccoud et al., 2017; Reiss et al., 2019) to *Wolbachia* (Jiggins et al., 2002; Engelstädter & Hurst, 2006; Baldo et al., 2008; Russell et al., 2009).

The existence and strength of the PDE has been investigated both experimentally (through transinfections) and through the comparative analysis of *Wolbachia* and hosts phylogenies. A review of 25 transinfection studies indicated a positive correlation between success rates and relatedness of donor and recipient hosts (Russell et al., 2009). Comparative phylogenetic studies have been less conclusive. Russel et al., (2009) reported a signal indicative of a PDE separately in a group of ants and butterflies. Several host-shifts events among closely related species, as inferred from patterns of *Wolbachia* strain distributions on host phylogenies, support the PDE hypothesis, e.g. in weevils (Lachowska et al., 2010; Sanaei et al., 2019), *Trissolcus* wasps (Guz et al.,

2012), and *Drosophila* (Haine et al., 2005; Turelli et al., 2018). In contrast, in some case studies focused on certain arthropod families or genera, only a negligible part of the data, if any, appeared to indicate a PDE, e.g. in fig wasps (Shoemaker et al., 2002), fungus growing ants (Frost et al., 2010), lepidopterans (Ahmed et al., 2016) and bees (Gerth et al., 2013). Although the PDE may play an important part in explaining the distribution of *Wolbachia* and its host shifts, evaluating its impact is not an easy task. Large and well-resolved trees are required, on both the *Wolbachia* and host sides and rigorous statistical methods need to be applied, that quantify the effect of host relatedness on host-shifts. To date, this has been achieved in other host-parasite systems (e. g. Faria et al., (2013), but not for *Wolbachia*. The interpretation of a PDE is further complicated by the possibility that host phylogenies may correlate not only with physiological but also with ecological features: higher rates of transfers between closely related host species may also stem from niche similarities such as shared food, predators or parasitoids (see following section). It may thus be difficult to tease apart physiological and ecological effects. For example, in the case of *Wolbachia* host shifts among fig wasp species, the importance of the PDE compared to the ecological interactions in the syconium community is not clear (Yang et al., 2012)), and the latter should be taken into account in phylogenetic analyses.

Just as closely related hosts may be similarly permissive to *Wolbachia*, they may share features making them more reluctant to hosting new infections, for example because of phylogenetic inertia in resistance to particular *Wolbachia* strains, or in phenotypic suppression (Longdon et al., 2011; Waxman et al., 2014). For example, *Culex quinquefasciatus* and *C. pipiens* were suggested to control *Wolbachia* density in their gonads in the same way, through a possibly shared ancestral mechanism (Emerson & Glaser, 2017). But such a pattern may not always hold. For example, in the wasp *Nasonia vitripennis*, the *Wds* protein appears to inhibit maternal transmission of the *w*VitA *Wolbachia* strain, possibly by blocking its passage from nurse cells to oocytes (Funkhouser-Jones and van Opstal et al. 2018). This seems to stem from a recent amino acid substitution in this gene, since no such phenomenon is seen in the close relative *Nasonia giraulti* (Funkhouser-Jones and van Opstal et al. 2018). Male killing suppression in *H. bolina* butterflies offers another example of a derived and recent mechanism, stemming in that case from one or more mutations in a single host genomic region (Reynolds et al., 2019). These studies indicate that rather than being polygenic

and conserved ancestral traits, host resistance may often be strain- and host-specific control systems that evolved very recently, and with a simple genetic basis. Such mechanisms, especially if they are costly, may also be relatively short-lived, just as *Wolbachia* infections themselves, as discussed below. The rapid degradation of such control traits following *Wolbachia* losses may also explain the apparent absence of large *Wolbachia*-resistant host clades.

3.4. The role of ecology

Besides resemblance stemming from phylogenetic relatedness, shared ecological features, even extrinsic ones such as climate, may partially explain the current distribution of intracellular microbes (Woodhams et al., 2020). Occupying the same ecological niche by definition enhances interactions between species, e.g. through direct physical contact, shared food, predators or parasitoids. It is therefore not surprising that *Wolbachia* host shifts can be observed between species living in the same habitat, be they closely related or not (Kittayapong et al., 2003; Morrow et al., 2014). For instance, in a particular intertidal ecosystem, two distantly related amphipod species were reported to share the same *Wolbachia* strain (Cordaux et al., 2001). In another study, host ecology (mycophagy vs. non-mycophagy) was predictive of relatedness between different supergroup A *Wolbachia* strains detected in mushroom-associated dipterans, whereas host phylogenetic relatedness was not (Stahlhut et al., 2010).

The presence of shared strains in two host species with significant niche overlap could be explained by frequent physical contact. An alternative but not mutually exclusive explanation could be that host shifts are facilitated because *Wolbachia*'s own niche is aligned with that of their hosts. Although the cellular environment may seem in part disconnected from the host's extracellular physiology and open-environment ecological conditions, many intracellular microorganisms exhibit a level of niche preference oriented to their preferred ecosystem (Isberg et al., 2009; Mertens & Samuel, 2012). For instance, aphids with similar but geographically separated environmental niches tend to harbour closely related bacterial symbionts (Henry et al., 2013). *Wolbachia* strains may also have their own preferential niche (Lemoine et al., 2020). Versace et al., (2014) monitored the infection frequencies of different *Wolbachia* strains in *D. melanogaster* laboratory lines flies subject to hot and cold experimental

environments over 1.5 years. They demonstrated that two strains exhibited reduced fitness in cold temperatures and became lost after 15 generations, whilst the frequency of another increased by 50%. Moreover, the results were reproduced when the hot-adapted *Drosophila* lines were moved again to cold environments. These results indicate that different strains react differently to temperature and thus have different niche preferences. Such associations between *Wolbachia* strains and environmental features may also be detectable in natural systems. For example, several studies indicate that *Wolbachia* density and prevalence in herbivorous arthropods depends on host plants (Ahmed et al., 2010; Toju & Fukatsu, 2011; Guidolin & Cônsoli, 2017). Similarly, some strains are observed at higher infection frequency in higher temperature areas of their host range (Mouton et al., 2006; Toju & Fukatsu, 2011; Zhu et al., 2018). A recent meta-analysis reported complex relationships between climatic conditions and *Wolbachia* prevalence in arthropods, without a clear general trend: while a weak positive relationship between *Wolbachia* incidence and temperature was observed in temperate regions, prevalence was generally lower in the tropics (Charlesworth et al., 2019). Strain specific effects may explain some of this complex pattern.

Host niches may thus impact possibilities of *Wolbachia* host shifts, but the bacteria may also, reciprocally, affect host niches. Many mutualistic endosymbionts can limit their host's niche (Corbin et al., 2017; Perlmutter & Bordenstein, 2020), e.g. by reducing thermal tolerance, as has been reported in *Buchnera* endosymbionts in aphids (Zhang et al., 2019)). This phenomenon may also be at play in hosts where *Wolbachia* is required for survival (e.g. a group of *Wolbachia* strains from supergroup F in bed bugs (Hosokawa et al., 2010)). In other cases, *Wolbachia* may extend their hosts' niche, e.g. by providing protection against pathogens. Finally, and in line with its other manipulative capabilities, *Wolbachia* may modify hosts' ecological preferences toward its own (Lemoine et al., 2020). For example, under laboratory conditions, infected *D. melanogaster* preferred cooler temperature than uninfected flies, which is possibly an indication that *Wolbachia* manipulated its host's thermal preference (Truitt et al., 2018).

IV. Implications of *Wolbachia* host shifts

4.1. Host shifts and between-host epidemiological dynamics

Wolbachia-host co-diversification processes may be considered within a standard epidemiological framework where host species represent individuals, host shifts correspond to transmission events and *Wolbachia* extinction to host recovery. This view makes it clear that the current high global frequency of *Wolbachia* in arthropods is the result of a balance between gain and loss events, that is, between host shifts and *Wolbachia* extinction (Werren & Windsor, 2000). In technical terms, for *Wolbachia* to spread and be maintained within arthropods or any more specific clade, its basic reproductive number R_0 – here, the number of successful host shifts achieved by a *Wolbachia* strain (in an otherwise uninfected clade of hosts) before it goes extinct – needs to exceed one. For example, a simple deterministic model assuming a cycle of susceptible, infected, recovered and susceptible host species (SIRS model) predicts that R_0 is given by the ratio between the rates of host shifts and extinctions (Keeling & Rohani, 2011). Zug et al., (2012) also considered a stochastic model in which host shifts occur on small-world networks, designed to capture the expectation that host shifts take place preferentially between closely related hosts (i.e. the PDE), but also occasionally between distantly related host. Epidemiological models explicitly including host trees and the PDE have also been constructed (Engelstädter & Hurst, 2006; Engelstädter & Fortuna, 2019). Amongst other predictions, these models suggest that species-rich host clades, especially those resulting from recent adaptive radiations, should exhibit a greater incidence of *Wolbachia* infections than more evolutionary inert, species-poor host clades. At least when using taxonomy as a proxy for phylogenetic relationships, this hypothesis does not seem to be supported by data on *Wolbachia* infection incidence (Weinert et al., 2015).

Building on this epidemiological framework, some studies have aimed at estimating the critical parameters underlying the current *Wolbachia* distribution. Early large scale surveys have indicated similar incidence in different regions of the globe (Werren et al., 1995; Werren & Windsor, 2000). This pattern suggests that *Wolbachia* may have reached a steady state, i.e. an epidemiological equilibrium between loss and acquisition rates, the relative values of which may be inferred from the observed global incidence (Zug et al., 2012). More recently, loss and acquisitions rates were independently

estimated from a large-scale comparison between *Wolbachia* and host mitochondrial phylogenies, used to infer likely scenarios of loss and acquisition (Bailly-Bechet et al., 2017). This study suggested that uninfected species acquire *Wolbachia* once every 9 million years on average, whilst infected species lose *Wolbachia* every ~7 million years. These numbers would translate into a global incidence of about 40%, which happens to match the observed global incidence in this data set. This concordance fits the hypothesis that *Wolbachia* has indeed reached a global equilibrium, in line with the geographical stability of its incidence.

Let us keep in mind that these take-home numbers, based on the only attempt made so far to estimate these epidemiological parameters, should be considered with caution, for a number of reasons. On purely technical grounds, uncertainties in the host and symbiont phylogenies, inferred in both cases from single molecular markers, translate into uncertainties in the loss / acquisition scenarios and thus in the estimated rates. In addition, multiple infections have been neglected in this study, which may introduce some bias if strains found in multiple infections tend to follow a peculiar epidemiology. The fact that *Wolbachia* may sometimes recombine at high rates (Bonneau et al., 2018) also introduces the possibility that only some genomic portions, and not entire *Wolbachia* lineages, may be concerned with some of the inferred loss / acquisition events. Variations in loss and acquisition rates between host clades also mean that perhaps no single clade follows the average trend estimated from the entire dataset. Finally, and most critically, these estimations may in part be confounded by difficulties in assessing which events, out of all losses and acquisitions visible on the host tree, correspond to genuine species-level versus mere individual level events (e.g. transient infections acquired from the environment, or loss of infection from one mother to its offspring) that were filtered out in this study, making the rates inferred possibly too conservative (Bailly-Bechet et al., 2017).

Overall, these various sources of uncertainty and potential errors may help explain the apparent discrepancy between these global estimates of *Wolbachia* host shift rates and some recent reports indicating that many *Drosophila* species have acquired new *Wolbachia* infections within the last few thousand years (Turelli et al., 2018; Cooper et al., 2019). It may be that the species group of *Drosophila* investigated in these studies acquire infections much more frequently than the average arthropod species does, or

that the particular *Wolbachia* strains studied are more prone to host shifts than the average *Wolbachia* strain. It may also be that the average arthropod acquires *Wolbachia* more often than suggested by the conservative approach of Bailly-Bechet et al. (2017). Also notable is the fact that the above-mentioned acquisition rate estimations exclude introgression, and only consider horizontal transfers in a strict sense (jumps between distinct cytoplasmic lineages) as acquisition events. More studies, focused on particular clades, are needed to resolve this complex issue.

4.2. Host shifts and their reciprocal effect on *Wolbachia* genetic diversity

Wolbachia exhibits a wide phenotypic diversity but also, at the genomic level, a high degree of instability, stemming from recurrent rearrangements, recombination events, and integrations or losses of mobile elements, that occur at a surprisingly high rate for an intracellular symbiont (Gerth & Bleidorn, 2017). This feature may be understood in relation with *Wolbachia*'s ability to undergo host shifts. *Wolbachia* strains in arthropods tend to harbour larger genomes than in nematode hosts, albeit with substantial variation (Fenn & Blaxter, 2006; Nikoh et al., 2014). This includes diversified mobile elements such as bacteriophages and transposons (Ishmael et al., 2009; Kent & Bordenstein, 2010; Leclercq et al., 2011; Reveillaud et al., 2019; Bing et al., 2020). Although these mobile elements may be generally costly for *Wolbachia* on a short time-scale, as they usually are for any organism, their presence may fuel genomic instability. In that sense, they may be essential in generating the raw heritable variations underlying the evolution of the many phenotypes that *Wolbachia* displays, including its ability to survive in a new host (Licht, 2018). Reciprocally, host shifts may also boost *Wolbachia* genetic diversity by exposing diverse lineages to diverse new environments, that is, diverse selective constraints (Frank, 1997; Read & Taylor, 2001). Host shifts may also contribute to *Wolbachia* genomic variations in a more proximate manner, by generating co-infections by several *Wolbachia* strains, that are indeed of commune occurrence (Perrot-Minnot et al., 1996; Kondo et al., 2002) and open the possibility of recombination, occurring between both close and distant *Wolbachia* lineages (Jiggins Francis M. et al., 2001; Werren & Bartos, 2001; Jiggins, 2002; Baldo et al., 2006; Atyame et al., 2011; Ellegaard et al., 2013). Recombination produces genetic novelty on its own (Klasson et al., 2009; Ilinsky & Kosterin, 2017; Tolley et al., 2019) but also maintains a selective pressure on mobile elements for transposition,

in a positive evolutionary feedback loop that may boost genetic diversification in the long run.

4.3. Applied aspects of *Wolbachia* host shifts

There is an ongoing interest in utilizing *Wolbachia* in many applications such as pest management and vector borne disease control (Townson, 2002; Zabalou et al., 2004; Floate et al., 2006; Vavre & Charlat, 2012; Ritchie et al., 2015, 2018; Berec et al., 2016; Nikolouli et al., 2018; Liu & Guo, 2019). These approaches often involve an artificial host shift by transinfection of *Wolbachia* to new host species. Probably the most prominent example is the successful introduction of dengue-virus suppressing *Wolbachia* into *A. aegypti* mosquitos in ten countries (O'Neill, 2018). A better understanding of natural host shifts may both help implement these efforts and also assess and possibly mitigate their inherent risks (Yen & Failloux, 2020). For example, *Wolbachia* strains with a high host shifting ability may be avoided in such projects, to reduce the risks of spreading infections in non-targeted species, although they may also settle more easily in the targeted one and thus be seen as good candidates in the first place. We currently do not have a general picture of what *Wolbachia* strains should be seen as such "superspreaders", if only because of the small fraction *Wolbachia*'s diversity that has been sampled so far (Detcharoen et al., 2019), and we also do not know what genomic features provide them with such a property. Reciprocally, artificial releases may provide a mean to investigate the dynamics of natural host shifts (Ross et al., 2019). For example, releasing and monitoring the spread of *A. aegypti* infected with CI-inducing strain in Queensland (Australia) provided an opportunity to study its progress and establishment (Hoffmann et al., 2011; Axford et al., 2016). This has highlighted the critical effect of environmental heterogeneity on *Wolbachia* invasion dynamics (Schmidt et al., 2017).

The spread of new infections following host shifts from artificially infected species may have important consequences, including a reduction in host genetic diversity, altered population dynamics and sex ratios and possibly even extinction (Charlat et al., 2003; Engelstädter & Hurst, 2009). Some have argued that in the absence of a thorough understanding of potential onward host shifts of *Wolbachia* and their consequences, these risks should preclude widespread application of *Wolbachia* for disease control (Loreto & Wallau, 2016). However, given that *Wolbachia* already have a high global

incidence, with 40-50% of all arthropod species being infected, any species is likely to be naturally exposed to many new *Wolbachia* infections. The additional risk of new infections caused by human *Wolbachia* release programs has therefore been argued to be negligible, especially in the case of releasing infected male mosquitos (Dobson et al., 2016).

V. Outlook and open questions

The complexity of *Wolbachia* host shifting encompasses the gamut of biology, ranging from molecular genetics, physiology and immunology to ecology, epidemiology and evolutionary biology. As has become clear in the preceding sections, our understanding of this complexity is still in its infancy. For example, while *Wolbachia* surveys suggest that sharing a common parasitoid appears to be a frequent route of transfer, many other routes are plausible and could be more important in particular host groups. Similarly, although we have good data on the process of *Wolbachia* establishment within a population following an artificial release, we know little about the equivalent process following natural host shifts (e.g. how can a new CI-inducing strain overcome the invasion threshold?). Various factors have been identified that may facilitate or hinder host shifts, including ecological and genetical similarity between donor and recipient hosts, but we are largely ignorant about their relative importance. We are also only beginning to understand the long-term dynamics of *Wolbachia* spread across whole clades of host species.

At a quantitative level, we know even less. Building upon the conceptual model of a host shift comprising four consecutive steps, we can partition the probability that such an event effectively occurs into four individual probabilities, P_1 to P_4 (corresponding to steps 1 to 4 in Figure 2). A long-term and ambitious goal would be to estimate these probabilities and how they depend on host and *Wolbachia* groups as well as other factors. At present, we can only suspect that P_3 and P_4 are lower than P_1 and P_2, i.e. that vertical transmission and spread in the population likely represent stronger limitations than physical transfer and proliferation, but there is no hard data to corroborate this conjecture. Although different types of experiments and comparative studies shed some light on these issues, they all come with limitations. Estimating P_1 is

very hard and has rarely been attempted (but see Rigaud & Juchault (1995). Transinfection experiments can be seen as addressing P_2 and, in many cases where several host generations were included, may also provide information pertaining to P_3. However, the presumably large initial titre used in these experiments means they can only be used to estimate upper limits for P_2 and P_3. P_4 can be addressed by field or cage population studies in which few infected individuals are released, but given its presumably low value, it seems virtually impossible to directly estimate P_4 from natural situations where *Wolbachia* needs to spread from a single infected female through a large population. Detailed stochastic models (Jansen et al., 2008) combined with good estimates for parameters such as the transmission rate, strength of CI and host effective population size therefore seem indispensable for this step.

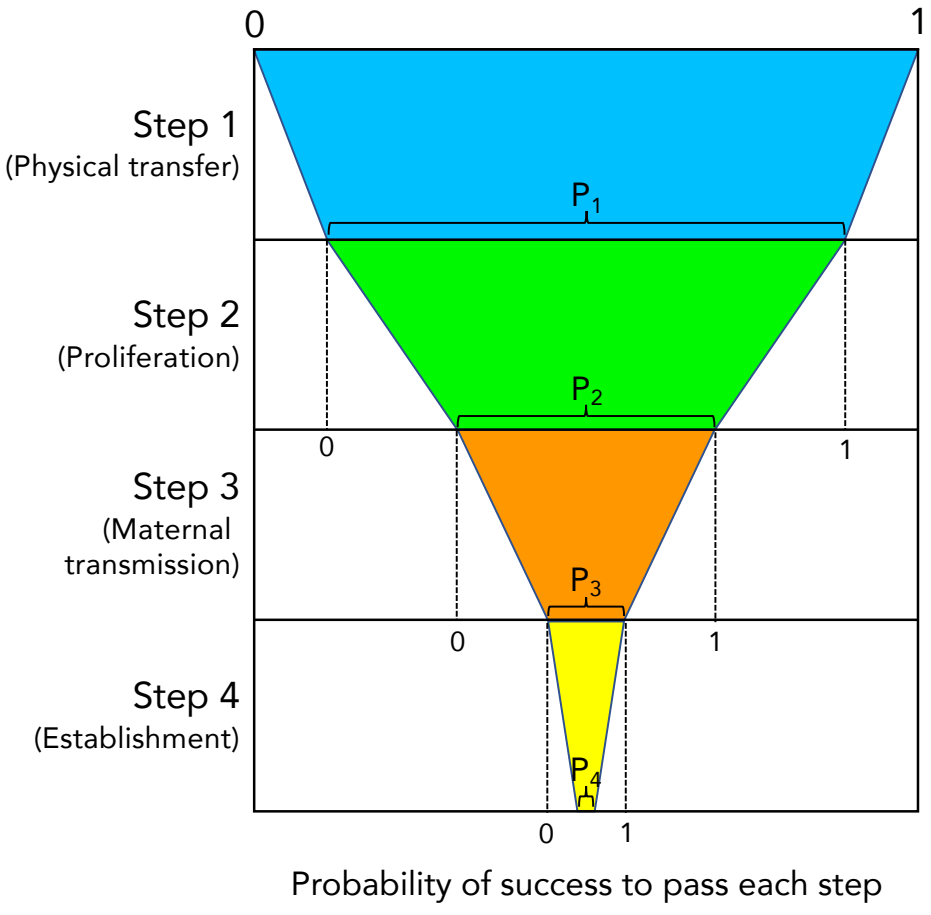

Figure 2. Breaking host shifts into successive events, with various respective probabilities of occurrence. Each step *i* in the process is associated with a probability P_i that it is successfully taken by *Wolbachia*. Graphically, each probability is represented by the bottom width of the respective trapezoid relative to its top width.

Under this framework, the total probability that a host shift occurs is given by the product of the four individual probabilities, $P_{\text{total}} = \prod_i P_i$, graphically represented by the bottom width of the final, yellow trapezoid, relative to the total width.

Comparative studies estimating host shift rates from phylogenetic trees (e.g., Bailly-Bechet et al., (2017)) can be seen as addressing the total probability ($\prod_i P_i$, per unit of time) of successful host shifts, but cannot disentangle the probabilities for the individual steps. However, when powered by sufficiently large and robust trees (ideally built from whole genome sequencing data, as argued below), such comparative studies have considerable potential to estimate not only average host shift rates but uncover variation in these rates between *Wolbachia* strains, host groups or ecosystems.

In order to fill some of the mentioned knowledge gaps, some of the methodologies commonly used in past *Wolbachia* research may require revisions. Putative host shifts are often identified through the detection of very similar *Wolbachia* strains in two different host species via PCR and sequencing of one or a small number of genes. While informative in many regards, this approach also suffers from at least two caveats. First, the presence of *Wolbachia* genes in DNA extracted from a host (detectable by PCR methods) does not necessarily indicate a stable infection in that host (Chrostek & Gerth, 2019). It could merely indicate the presence of *Wolbachia* (or even just *Wolbachia* DNA) on the surface or in the gut of the specimen, or the presence of a truly intracellular but transient infection. This common approach is therefore unable to distinguish between genuine host shifts and either contaminations or unsuccessful spillovers of *Wolbachia* into other species, where not all four steps have been successful. For unequivocal identifications of host shifts, it remains to be determined if *Wolbachia* is indeed established via vertical transmission in both the hypothesised donor and recipient host and has spread to significant prevalence. Potential improvements of screening methodologies may include the detection of *Wolbachia* within host cells through microscopy, sampling from many individuals within a population rather than relying on one or a few, and ideally rearing of individuals in the lab in order to ascertain whether the infection is stably transmitted maternally to the next generation (Chrostek & Gerth, 2019). This integrative approach is obviously not achievable in large surveys and comparative studies, but may usefully complement them in more targeted studies.

The second problem is how to determine the direction of a host shift, and whether it occurred directly between two species or through some intermediates. The identification of the same *Wolbachia* strain in two different host species often represents only two visible spots in the otherwise obscure, large and entangled networks of past and extent hosts and symbionts lineages. The simplest scenario of a direct host

shift between the two species under focus may be misleading. To obtain a more complete picture, one strategy is to increase the number of sampled species within a closed or semi-closed environment (e.g. Bailly-Bechet et al., 2017). But such large scale studies have revealed that often many distinct host lineages carry strains that cannot be distinguished on the basis of one or even a few genes such as those included in the *Wolbachia* MLST scheme (Bleidorn & Gerth, 2018). This lack of phylogenetic signal can certainly be resolved by deeper sequencing that can reveal genomic variation among strains that previously looked virtually identical (Atyame et al., 2011; Bleidorn & Gerth, 2018). When utilized on both the host and *Wolbachia* side, whole genome sequencing approaches can provide highly informative details on the evolutionary histories of all players, including the cytoplasmic host genomes, offering detailed information on the likely direction, timing and mode (hybridisation vs. horizontal transfer) of past host shifts (e.g. Turelli et al., (2018), Cooper et al., (2019)).

A puzzling open question that we have not explicitly discussed yet concerns the evolutionary conservation of the *Wolbachia* traits required for successful host shifts. These events being usually regarded as rare, we would expect that the *Wolbachia* genes involved (e.g., those allowing movement from the gut lumen into host cells, or controlling somatic stem cell niche tropism) to either decay neutrally after transfer events or even be selected against if they are costly. Frequent horizontal transmission within species may represent a source of purifying selection on such traits, and may thus contribute to their evolutionary conservation (Hurst & McVean, 1996). Indeed, through several of the transfer routes discussed here, such as shared parasitoids, predators or food sources, horizontal transmission within species is expected to be much more common than transmission between species. A recent study on the horizontal transmission of *Wolbachia* in a spider indicated indeed that intra-species transmission (by cannibalism or social interactions) is more likely to occur than inter-species transmission (by predation or parasitism) (Su et al., 2019). On the other hand, strong association of particular host mitochondrial haplotypes with *Wolbachia*, as reported in several studies, argues against frequent horizontal transmission within species (e.g. *w*Ri strain in *D. simulans* (Hale & Hoffmann, 1990; Turelli et al., 2018), *w*Bol1b in *H. bolina* (Charlat et al., 2009) and *w*Mel in *D. melanogaster* (Richardson et al., 2012)). The genes involved in host shifts may also be maintained as functional through pleiotropic effects, that is, if they also play significant roles in within-host

Wolbachia dynamics. For example, cell to cell movement of *Wolbachia* involves shifts between different tissues or organs, that is, between contrasting environments (Sicard et al., 2014). The physiological plasticity of *Wolbachia*, required for survival in diverse host cell niches, may also maintain in these bacteria some features required in host shifting. Finally, it may be that host shifts in themselves select for those *Wolbachia* lineages that have retained this capacity. The plausibility of this 'clade selection' hypothesis (Williams, 1992; Hurst & McVean, 1996) will depend on the frequency of host-shift events, and may seem unlikely in the light of the above-mentioned estimates in the order of millions of years (Bailly-Bechet et al., 2017). Notwithstanding the possibility that host shift rates may in fact be more frequent in many parts of the *Wolbachia* tree, it is notable that if jumps into new hosts represent the only way out of extinction, even rare events may constitute a critical and possibly effective selective force.

VI. Conclusions

1. The ability to undergo host shifts is a critical feature of *Wolbachia*, with significant effects on the global incidence and distribution of these bacteria, their genetic diversity, and evolutionary consequences for their arthropod hosts. Evidence for this comes from phylogenetic studies, many successful transinfection experiments where *Wolbachia* has been transferred to a new host, and direct observations of host shifts in experimental settings.
2. Host-shifts can be conceptualized as taking place in four steps: physical transfer to a new host, proliferation within the new host, vertical (maternal) transmission, and establishment in the new population. Physical transfer can potentially occur via a number of routes, of which parasites/parasitoids and shared food sources appear the most plausible. All steps come with their own challenges and requirements from both host and *Wolbachia* that determine whether a host shift is successful.
3. There are many factors that influence the likelihood of successful host shifts of *Wolbachia* to a new host. The resident microbiome of the recipient host plays a large role, including other pre-existing *Wolbachia* strains, as well as different maternally inherited endosymbionts or other microbes. Different strains of

Wolbachia appear to have a different propensity to undergo host shifts, and the probability of host shifts appears to decline with increasing phylogenetic distance between donor and recipient host. Finally, ecological conditions are important, both for ensuring physical contact (direct or indirect) to the new host and, potentially, for providing a suitable niche for *Wolbachia* to thrive in.

4. In the long term, the dynamics of *Wolbachia* host-shifts and losses across many host species can be considered from an epidemiological perspective. Co-phylogenetic studies of *Wolbachia* and their hosts can be used to understand these dynamics and estimate the rates of *Wolbachia* host shifts and extinction events. However, this requires large, well-resolved and robust phylogenetic trees, and we therefore only have a very incomplete understanding of these parameters.
5. Studying natural host shifts can be useful for the application of *Wolbachia* to vector born disease or pest control strategies, for example by identifying strains that can easily switch between hosts ("super-spreaders"). Reciprocally, *Wolbachia* release programs represent a welcome opportunity to study the initial spread and early evolutionary dynamics of *Wolbachia* within a new host species.

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