Titer regulation in arthropod-*Wolbachia* symbioses

Sergio López-Madrigal[1], Elves H Duarte

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ABSTRACT

Symbiosis between intracellular bacteria (endosymbionts) and animals are widespread. The alphaproteobacterium *Wolbachia pipientis* is known to maintain a variety of symbiotic associations, ranging from mutualism to parasitism, with a wide range of invertebrates. *Wolbachia* infection might deeply affect host fitness (e.g. reproductive manipulation, antiviral protection), which is thought to explain its high prevalence in nature. Bacterial loads significantly influence both the infection dynamics and the extent of bacteria-induced host phenotypes. Hence, fine regulation of bacterial titers is considered as a milestone in host-endosymbiont interplay. Here we review both environmental and biological factors modulating *Wolbachia* titers in arthropods.

INTRODUCTION

Symbiotic associations between bacteria and animals are widespread, significantly affecting animals' fitness and hence critically influencing their evolutionary dynamics (Douglas 2014). Most of these bacteria colonize external or internal surfaces of the host's body. Moreover, some of them deeply integrate into animals' biology by stably colonizing intracellular niches (Fisher *et al*. 2017). Symbiosis with intracellular bacteria (endosymbionts) is particularly common in invertebrates, whose huge diversification partially relies on bacteria-derived fitness advantages (Mandel and Dunn, 2016; Sudakaran *et al*. 2017; Pollock *et al*. 2018). Endosymbionts are either essential or facultative. Essential endosymbionts (also known as primary, P-endosymbionts) are strictly intracellular bacteria required for proper host development and reproduction. They are fixed in the host population and get vertically transmitted (i.e. from mothers to offspring). In contrast, facultative symbionts (also known as secondary, S-symbionts) typically colonize a broader range of tissues both intra- and extra-cellularly. S-symbionts might behave as parasites and/or supply their host a variety of non-essential fitness advantages. They infect a subset of individuals in a host population and can be transmitted vertically and horizontally. Endosymbionts should be abundant enough to play their symbiotic role, limit their infection cost and ensure at least, their vertical inheritance between host generations (Vigneron *et al*. 2014; Simonet *et al*. 2016; Campbell *et al*. 2018).

The alphaproteobacterium *Wolbachia pipientis* (Proteobacteria: Anaplasmataceae) is among the most widespread bacterial endosymbionts in nature, infecting filarial nematodes and arthropods (Gerth *et al*. 2014). *Wolbachia* evolutionary success relies on its vertical transfer between host generations. Accordingly, it colonizes the germ line early during host development (Serbus *et al*. 2008). Horizontal transfer of *Wolbachia* also

[1] Corresponding author:
Sergio López-Madrigal. Instituto Gulbenkian de Ciência. Rua da Quinta Grande, 6. 2780-156 Oeiras, Portugal; Tel: +351-214464516; Fax: +351-214407970; Email: slopez@igc.gulbenkian.pt

occurs, as suggested by discrepancies between bacteria and host phylogenetic topologies (Ahmed *et al*. 2016). It might be favored by common cellular mechanisms allowing for host cell infection (White *et al*. 2017a) and seems to take place in a variety of ecological scenarios (Huigens *et al*. 2004; Le Clec'h *et al*. 2013; Ahmed *et al*. 2015; Brown and Lloyd 2015).

Wolbachia is generally seen as an opportunistic reproductive parasite which is able to favor infection persistence and spread by manipulating its host's reproduction in several ways (i.e. cytoplasmic incompatibility, parthenogenesis, male-killing, feminization of genetic males) (Werren *et al*. 2008). Nevertheless, *Wolbachia* can play a variety of roles in symbiotic associations. For instance, it supplies non-essential fitness advantages to many invertebrate species, including behavioral modifications and resistance against bacteria, viruses and *Plasmodium* parasite infection (de Crespigny *et al*. 2006; Hedges *et al*. 2008; Teixeira *et al*. 2008; Hughes *et al*. 2011; Furihata *et al*. 2015; Rohrscheib *et al*. 2015; Braquart-Varnier *et al*. 2015a). Moreover, *Wolbachia* behaves as an obligate nutritional mutualist in some insect and nematode lineages (Hellemans *et al*. 2019; Nikoh *et al*. 2014; Balvín *et al*. 2018; Shaikevich and Ganushkina, 2018). In this context, *Wolbachia* genome size varies according to its host dependency, ranging from 0.96 Mb (*w*Ov and *w*Oo strains, Darby *et al*. 2012; Desjardins *et al*. 2013) to 1.80 Mb (*w*Fol strain, Faddeeva-Vakhrusheva *et al*. 2017). Thus, with very few exceptions (Kampfraath *et al*. 2019), the genomes of obligate mutualistic strains are generally smaller than those of S-symbionts and/or reproductive manipulators.

Both *Wolbachia*-induced host phenotypes and transmission potential are highly influenced by *Wolbachia* loads. Given its broad phylogenetic distribution (Hilgenboecker *et al*. 2008), *Wolbachia*-involving symbioses represent an excellent target for the study of mechanisms driving the evolution of bacteria-animal symbioses. Here we review the current knowledge on factors and mechanisms shaping *Wolbachia* titers in arthropods and its relevance for the stability of symbiotic associations.

WOLBACHIA LOADS MATTER

Titers critically influence the stability of *Wolbachia* infection and the extent of *Wolbachia*-induced host phenotypes (Boyle *et al*. 1993, Dedeine *et al*. 2001, Veneti *et al*. 2004, Unckless *et al*. 2009, Osborne *et al*. 2012, Pan *et al*. 2018; Baião *et al*. 2019). Therefore, titer is a key parameter for the understanding of *Wolbachia*-host evolutionary dynamics and for the efficient implementation of *Wolbachia* as a control tool against insect pests and vector-borne diseases (Hoffmann *et al*. 2015).

Wolbachia transmission

Bacterial titers should be high enough to allow for the infection of tissues mediating *Wolbachia* vertical transfer between host generations. Consistently, *Wolbachia* is highly abundant in ovaries and early developmental stages of *Drosophila* (Diptera: Drosophilidae) oocytes, promoting colonization of embryo's germ line via mass action and strategic subcellular localization (Veneti *et al*. 2004). Low titers might impede *Wolbachia* vertical transmission yielding cured animals or, in the case of co-infection, leading to the stochastic loss of particular *Wolbachia* strains. For instance, the parasitoid wasp *Nasonia vitripennis* (Hymenoptera: Pteromalidae) infected with multiple

Wolbachia strains yields both mono-infected and aposymbiotic progeny after prolonged larval diapause (Perrot-Minnot *et al*. 1996), which has been linked to titers decline in *Aedes albopictus* (Diptera: Culicidae) (Ruang-areerate *et al*. 2004). On the other hand, *Wolbachia* horizontal transfer might also be critically influenced by titers, tissue tropism and host biology, which would explain differences in infection acquisition/loss rates among different taxonomic groups (Bailly-Bechet *et al*. 2017). High bacterial density in highly abundant/accessible tissues is expected to favor horizontal spread upon certain ecological scenarios (e.g. cannibalism, predation) (Le Clec'h *et al*. 2013). In support of this hypothesis, a significant rise of bacterial titers in the hemolymph was detected after several forced *Wolbachia* horizontal passage events in *Armadillidium vulgare* (Isopoda: Armadillidiidae) (Le Clec'h *et al*. 2017). Along the same lines, *Wolbachia* infection enhances the host-searching ability of the parasitoid wasp *Asobara japonica* (Hymenoptera: Braconidae), so that *Drosophila melanogaster* (Diptera: Drosophilidae) larvae are more actively parasitized by infected female wasps (Furihata *et al*. 2015). Taking into account evidence suggesting that parasitoids can mediate *Wolbachia* horizontal transfer between unrelated hosts (Ahmed *et al*. 2015), *Wolbachia* impact on the ovipositioning behaviour of *A. japonica* might promote infection spread. Whether such a phenotype is linked or not to *Wolbachia* density remains unclear though.

Besides ensuring infection spread, high bacterial titers might favor infection persistence by allowing *Wolbachia* to overcome stressing environmental conditions or host's defenses (Braquart-Varnier *et al*. 2008; Le Clec'h *et al*. 2013; Pan *et al*. 2018).

Wolbachia-induced host phenotypes

Reproductive manipulation. *Wolbachia* is primarily known as a reproductive manipulator, since infection can affect its host fertility, mating behavior and/or the sex-ratio of host progeny (Dedeine *et al*. 2001; Kremer *et al*. 2009). Cytoplasmic incompatibility (CI) is the most widespread *Wolbachia*-induced phenotype causing reproductive manipulation. It induces embryonic lethality in crosses involving *Wolbachia*-infected males and uninfected females, thus favoring the spread of *Wolbachia* infection by enhancing the fitness of *Wolbachia*-infected females (Boyle *et al*. 1993; Veneti *et al*. 2004; Hughes and Rasgon, 2014). The strength of CI has been linked to *Wolbachia* titers either directly or indirectly (i.e. CI levels correlate with environmental or biological factors previously associated with *Wolbachia* titers variation) (Clancy and Hoffmann, 1998; Noda *et al*. 2001; Calvitti *et al*. 2015). Nevertheless, CI strength/*Wolbachia* density association is not universal, varying between different species or even at the population level within the same host species (Mouton *et al*. 2006; Duron *et al*. 2006; Richardson *et al*. 2019; Yang *et al*. 2019). Moreover, multiple *Wolbachia*-induced phenotypes might co-occur at the same time, so that infection effects on *Wolbachia* population dynamics are complex. For example, *Wolbachia* influence on the mating behavior of male flies seems to buffer the effect of CI. In this context, males of *D. melanogaster* and *Drosophila simulans* (Diptera: Drosophilidae) mate at a higher rate when infected with *Wolbachia* (de Crespigny *et al*. 2006; Awrahman *et al*. 2014). Higher male promiscuity restores the reproductive compatibility with uninfected females, potentially undermining the CI-based transmission advantage of *Wolbachia*. Lower infection frequencies yielded by this effect might favor infection losses in the host population, regardless of *Wolbachia* titers within individual hosts.

Many other *Wolbachia*-induced phenotypes leading to reproductive manipulation have been reported (Werren *et al*. 2008). Phenotype strength/*Wolbachia* density association is mostly unclear though.

Pathogen protection. *Wolbachia* is known to protect their hosts from pathogen infection. Molecular mechanisms driving protection likely involve both *Wolbachia* and host factors (Ford *et al*. 2019). They remain unclear though, possibly varying between different symbiotic models. *Wolbachia*-mediated activation of the immune system (Pan *et al*. 2018) or *Wolbachia*/pathogen competition for space and/or resources (Caragata *et al*. 2013; Chrostek *et al*. 2014; Caragata *et al*. 2014; Rossi *et al*. 2015; Lindsey *et al*. 2018), both tightly related to bacterial titers, have been considered as suitable mechanisms. Consistently, several studies have already linked *Wolbachia* loads to the extent of *Wolbachia*-mediated protective phenotypes, so that highly protective strains use to occur at higher titers (Osborne *et al*. 2012; Chrostek *et al*. 2013; Chrostek *et al*. 2014; Martinez *et al*. 2014; Martinez *et al*. 2017).

Infection cost. *Wolbachia* infection might significantly affect many life-history traits of the host (e.g. fecundity, lifespan). Association between infection cost and *Wolbachia* loads (i.e. the higher the titers the stronger the *Wolbachia*-induced phenotype) have been suggested (Suh *et al*. 2009; Henry and Newton, 2018; Baião *et al*. 2019), although it needs to be further analyzed in most of the reported cases (Hamm *et al*. 2014; Suh *et al*. 2017). For instance, lifespan shortening of infected hosts was noted early in the research field and has been shown in several arthropod lineages (e.g. *Aedes*, *Armadillidium*, *Drosophila*) (Min and Benzer 1997; Suh *et al*. 2009; Chrostek *et al*. 2013; Chrostek *et al*. 2014; Le Clec'h *et al*. 2017). Nevertheless, lifespan shortening/*Wolbachia* titers association is not universal, as already seen for other *Wolbachia*-induced host phenotypes (Braquart-Varnier *et al*. 2008).

FACTORS AND MECHANISMS DRIVING WOLBACHIA LOADS

Environmental factors

Temperature. Environmental temperature displays a great impact over *Wolbachia* titers and titer-dependent host phenotypes (e.g. shortening of host lifespan) (Figure 1). In this context, temperatures over 30ºC lower *Wolbachia* titers or even remove the infection (Opijnen and Breeuwer 1999) while temperatures below 20ºC deeply compromise its proliferation rate. In between, titers seem to positively correlate with temperature as suggested by infection cost variations in *D. melanogaster* (Reynolds *et al*. 2003). Strikingly, *Wolbachia*-associated variations in host thermal preference have been reported (Truitt *et al*. 2018) (see *Wolbachia-induced host phenotypes* section for further examples of *Wolbachia*-induced host phenotypes). It is important to keep in mind that temperature effects on *Wolbachia* titers can be indirect, affecting other members of the host microbiota (see *Host microbiota* section) or the host biology itself. For instance, increased temperature affects *Wolbachia* proliferation in the brain of adult *D. melanogaster* but not in late larvae (Strunov *et al*. 2013). Sex-dependent, developmental stage-dependent or even cross-generational effects of temperature on *Wolbachia* titers have been reported so far (Clancy and Hoffmann, 1998; Foo *et al*. 2019).

Host diet. As for any other endosymbiont, the host physiology defines *Wolbachia* chemical environment. In this context, diet composition is known to influence both host physiology and *Wolbachia* titers (Figure 1). For instance, several natural sugars (i.e. sucrose, galactose, lactose, maltose and trehalose) increase *Wolbachia* titers in developing oocytes while dietary yeast reduces it (Serbus *et al*. 2015; Camacho *et al*. 2017). Interestingly, dietary yeast effect is tissue-dependent, leading to redistribution of *Wolbachia* titers within the host body (Serbus *et al*. 2015; Christensen *et al.* 2019). Importantly, concerns have been recently riced on relative quantification of *Wolbachia* titers in this field, since diet-derived effects on the host might lead to distorted conclusions (Ponton *et al*. 2015; Christensen *et al*. 2019). Finally, antibiotics intake can significantly affect *Wolbachia* titers within very few host generations. Susceptibility may depend on the *Wolbachia* strain, the antibiotic type, concentration and ingestion regime (Fenollar *et al*. 2003; Liu *et al*. 2014; Fallon *et al*. 2018).

Host microbiota. *Wolbachia* is not alone within its host. Multiple *Wolbachia* strains may co-infect a single individual (Ijichi *et al*. 2002; Hiroki *et al*. 2004; Mouton *et al*. 2006; Valette *et al*. 2013). Moreover, *Wolbachia* usually co-exists with other endosymbiotic bacteria or members of the gut microbiota (Gómez-Valero *et al*. 2004; Dittmer *et al*. 2016). Since *Wolbachia* occupies a within-host niche and feeds on resources therein, co-infection of multiple bacterial lineages might translate into competition for space and nutrients (Figure 1) (Caragata *et al*. 2014; Geoghegan *et al*. 2017; Jiménez *et al*. 2019). For example, *Spiroplasma* sp. strain NSRO (Firmicutes: Spiroplasmataceae) negatively affects *Wolbachia* titers in *D. melanogaster* (Goto *et al*. 2006). Moreover, competitive exclusion of *Wolbachia* by *Asaia* sp. (Proteobacteria: Acetobacteraceae) occurs in *Anopheles* (Diptera: Culicidae), so that *Wolbachia* maternal transmission is inhibited (Hughes *et al*. 2014). *Asaia* is also a vertically transmitted endosymbiont colonizing mosquito germ line (Favia *et al*. 2007). Therefore, *Asaia* and *Wolbachia* may compete for the same niche in the female gonads (Rossi *et al*. 2015).

Host-based mechanisms

Distortion of bacterial titers in hybrid hosts strongly suggest that infected animals are actively controlling *Wolbachia* population dynamics. For instance, a dramatic increase of *Wolbachia* titers is observed in hybrids between members of the tsetse fly *Glossina morsitans* (Diptera: Glossinidae) group compared to non-hybrid hosts (Schneider *et al*. 2013). Along the same lines, transinfection-based approaches (i.e. *Wolbachia* strains transfer into an heterologous host) have suggested that host might play a key role in titer regulation (Boyle *et al*. 1993; Hughes and Rasgon, 2014). Consistently, transinfection has shown the impact of exogenous bacterial strains on the recipient host to be broader than that of endogenous strains (Christensen *et al*. 2016). For instance, major perturbation of symbiosis was observed when infecting the naïve wasp *Nasonia giraulti* (Hymenoptera: Pteromalidae) with a *Wolbachia* strain from its close relative *N. vitripennis* (Chafee *et al*. 2011; Funkhouser-Jones *et al*. 2018). Authors showed that most of *Wolbachia* density suppression capabilities can be mapped to two regions of the *Nasonia* genome and identified the *Wolbachia* density suppressor (*wds*) gene, which dominantly hinders bacterial transmission from mothers to offspring (Funkhouser-Jones *et al*. 2018) (Table 1). Similarly, the mosquito *Aedes aegypti* (Diptera: Culicidae) metalloprotease m41 ftsh was shown to foster *Wolbachia* strain *w*MelPop-CLA proliferation via *Wolbachia*-

induced expression of the mosquito microRNA (miRNA) aae-miR-2940 (Hussain *et al.* 2011) (Table 1). Overexpression of miRNA aae-miR-12 in infected *A. aegypti* seems also relevant for replication/maintenance within host cells (Osei-Amo *et al*. 2012). Along the same lines, introgression analysis between two strains of *Culex quinquefasciatus* (Diptera: Culicidae) showing consistent differences in *Wolbachia* density led to the identification of two major-effect quantitative-trait loci (QTL) controlling bacterial loads in nongonadal tissues (Emerson and Glaser, 2017) (Table 1).

Developmental dynamics. Both host physiology and tissue repertoire vary significantly during animal development. Consistently, *Wolbachia* loads and *Wolbachia*-induced host phenotypes are tightly modulated by host ontogeny (Figure 1) (Ijichi *et al*. 2002; Reynolds *et al*. 2003; Ruang-areerate *et al*. 2004; Unckless *et al*. 2009; Gutzwiller *et al*. 2015; Voronin *et al*. 2012; Fisher *et al*. 2018). For instance, the fly axis-determinant gene Gurken (*grk*), displays a cumulative, dosage-sensitive effect on *Wolbachia* titers at *D. melanogaster* oogenesis (Serbus *et al*. 2011) (Table 1). Likewise, *Wolbachia* is known to accumulate in polar cells but not in lateral follicle cells during oogenesis (Kamath *et al*. 2018). Along the same lines, host cell autophagy has been shown to be a key negative regulator of *Wolbachia* titers along host ontogeny (Voronin *et al*. 2012). Consistently, developmental stage-dependent shifts in *Wolbachia* gene expression are observed along *D. melanogaster* development, even when considering total within-host population (Gutzwiller *et al*. 2015; Rice *et al*. 2017). Affected loci include ankyrin repeat domain (ANK)-containing genes as well as genes with predicted membrane or secretion system function, suggesting that *Wolbachia*-host cross-talk is influenced by host development.

Tissue tropism. Immune responses play an important role in the host-based modulation of bacteria tissue tropism (Masson *et al*. 2016). Both *in vivo* and *in vitro* studies have shown *Wolbachia*-mediated activation of immune genes and processes (Xi *et al*. 2008; Voronin *et al*. 2012; Grobler *et al*. 2018; Baião *et al*. 2019). Boosting of host immune system may also favor *Wolbachia* infection (Rio *et al*. 2006; Pan *et al*. 2018). For example, *Wolbachia* is known to activate the basal immune response of *A. aegypti* via the immune deficiency (IMD)- and Toll-pathway. Curiously, silencing of these immune pathways leads to the reduction of *Wolbachia* titers (Pan *et al*. 2018). Immune activation might help *Wolbachia*, which apparently lacks specific targets for the corresponding antimicrobial peptides, to exclude sensitive microbial competitors. Moreover, it might also actively promote *Wolbachia* proliferation via parallel production of antioxidants (Pan *et al*. 2012; Brennan *et al*. 2012; Zug and Hammerstain, 2015). In spite of this, *Wolbachia* infection/transinfection does not necessarily lead to host immune activation. In this context, transfer of strain *w*Au, native to *D. simulans,* into *D. melanogaster* led to stronger *Wolbachia*-induced phenotypes in comparison with strain *w*Mel performance with no apparent activation of host immune system (Chrostek *et al*. 2014).

Tissue tropism affects bacterial sensitivity to environmental stress and influences the extent of titer-dependent host phenotypes (Osborne *et al*. 2012; Strunov and Kiseleva, 2016; Shan *et al*. 2017). High titers and broad tissue tropism normally co-occur with stronger *Wolbachia*-induced host phenotypes (Mouton *et al*. 2004; Duron *et al*. 2006; Osborne *et al*. 2012; Baton *et al*. 2013). Moreover, tissue tropism is central to nutritional symbioses. Mutualist and potentially mutualist *Wolbachia* strains *w*Cle and *w*Ctub, from *Cimex lectularius* (Hemiptera: Cimicidae) and *Cavitermes tuberosus* (Isoptera:

Termitidae) respectively, mostly reside in an organ-like structure called bacteriome rather than maintain a more ubiquitous tissue distribution (Hosokawa *et al*. 2010; Hellemans *et al*. 2019). Finally, infection tropism involving both germ and somatic cell lines influences *Wolbachia* spread within and between individual hosts (Frydman *et al*. 2006; Braquart-Varnier *et al*. 2015b).

Host tissues colonized by *Wolbachia* define the nature of the molecular cross-talk between host and endosymbiont, including gene networks allowing for colonization, maintenance and proliferation of *Wolbachia*. In this context, vertical inheritance-associated bottlenecking of *Wolbachia* infection seems reinforced in *D. melanogaster* heterozygous for cytoskeletal mutations in profilin or villin (i.e. $chic^{221}$, $chic^{1320}$, qua^{6-396}) (Table 1). Titers decrease co-occurs with stochastic loss of *Wolbachia* infection, which suggests that *Wolbachia* utilizes the host actin cytoskeleton for persistence within and transmission between fly generations (Newton and Sheehan, 2015; Newton *et al*. 2015). Moreover, genome-wide RNAi screenings on *Wolbachia*-infected *D. melanogaster* cell line JW18 have recently identified a wide set of genes as positive- or negative-regulators of *Wolbachia* titers, respectively (White *et al*. 2017b; Grobler *et al*. 2018). Negative regulators include many components of the ribosome, the proteasome and the regulatory proteins network. On the other hand, most of positive regulators were genes involved in metabolism and metabolite transportation, including loci involved in lipid metabolism and mitochondrial function. Strikingly, positive covariation of mitochondria and *Wolbachia* titers has been shown in *D. melanogaster* (Henry and Newton, 2018). Moreover, some genes involved in ubiquitin and proteolysis pathways were shown to behave as positive regulators of *Wolbachia* titers. Further functional studies have suggested that *Wolbachia* relies on host proteolysis via ubiquitination and Endoplasmic Reticulum (ER)-associated protein degradation pathway (ERAD) for essential amino acids provisioning (White *et al*. 2017b). Consistently, tightly coupled *Wolbachia*/ER dynamics during cell colonization has been recently shown, although gene expression and immunostaining studies did not reveal ER stress or enhanced ERAD-driven proteolysis (Fattouh *et al*. 2019). This apparent discrepancy is hard to interpret though, since different experimental approaches and/or cell lines were used. Finally, genome-wide RNAi screening of *Wolbachia*-infected JW18 cells also showed up to 166 genes to affect *Wolbachia* and host cell proliferation in the same way, either positive or negative, which might contribute to the *in vivo* coordination of bacteria/host dynamics (Grobler *et al*. 2018).

Endosymbiont-based mechanisms

Substantial variations have been observed in *Wolbachia* titers within the same host species in nature (Unckless *et al*. 2009). Variability remains even when rearing infected animals under controlled environmental conditions or when considering different *Wolbachia* strains within the same host individual, which suggests bacteria-derived factors to influence titer dynamics (Ijichi *et al*. 2002; Veneti *et al*. 2004; Osborne *et al*. 2012; Dittmer *et al*. 2014). Differences in titers do not necessarily reflect differences in proliferation rates. On the contrary, different tissue tropisms might contribute via competition with co-infective microbes or susceptibility to environmental and/or host-derived factors (Figure 1) (Ijichi *et al*. 2002; Veneti *et al*. 2004; Osborne *et al*. 2012; Dittmer *et al*. 2014). Strikingly, closely related *Wolbachia* strains might show similar

titers and/or induce similar titer-dependent host phenotypes, which suggests titer regulation by shared molecular mechanisms (Veneti *et al*. 2004; Osborne *et al*. 2012; Chrostek *et al*. 2013). As for the study of host-based mechanisms, transinfection of different *Wolbachia* strains into the same host species has yielded great insight into bacteria-derived factors modulating infection density. In this context, host/*Wolbachia* shuffling for a variety of *Drosophila* species showed titers and titer-dependent phenotype (i.e. antiviral protection) to be mostly influenced by *Wolbachia* genotype (Martinez *et al*. 2014; Martinez *et al*. 2017). Furthermore, *Wolbachia*-based control of titers and titer-dependent antiviral protection differs even within different variants of *w*Mel strain (Chrostek *et al*. 2013). Genomic analysis of the highly proliferative *Wolbachia* strain *w*MelPop (Min and Benzer 1997; Strunov and Kiseleva 2016) showed the amplification of an eight gene-containing region named Octomom to determine both *Wolbachia* titers and virulence (i.e. *Wolbachia*-induced shortening of *D. melanogaster* lifespan) (Chrostek *et al*. 2013; Chrostek and Teixeira, 2015) (Table 1). Octomom expansion, likely enhanced by selection at the intra-host level (Chrostek and Teixeira, 2018), is the first described link between *Wolbachia* genotype and phenotype. More recently, overexpression of the *Wolbachia* actin-localizing effector 1 (WalE1)-encoding gene has shown to increase bacterial titers in *D. melanogaster* (Sheehan *et al*. 2016). WalE1 is a candidate Type IV effector likely mediating *Wolbachia* interaction with host cell actin cytoskeleton. This gene expression is upregulated in late larval development of flies, peaking at early pupation when adult organogenesis takes place. It might affect *Wolbachia* titers either through bacterial proliferation or bacterial transmission (Sheehan *et al*. 2016) (Table 1).

CONCLUSION

Wolbachia is a maternally-inherited bacterium that stably infects a broad range of animal lineages, being therefore an excellent model for the study of mechanisms driving the evolution of bacteria-animal symbioses. *Wolbachia* needs to guarantee infection persistence between host generations, either supplying the host with any fitness advantage and/or actively enhancing infection spread via reproductive manipulation. Both the extent of infection-induced host phenotypes and its spread potential, either vertical or horizontal, might be highly influenced by *Wolbachia* titers. Thus, fine regulation of *Wolbachia* loads is a key milestone in the evolution of *Wolbachia*-host symbioses.

Titers may vary when considering different *Wolbachia* strains, host species or host populations. Significant variations are frequent even within the same host population, at the individual level, for the same *Wolbachia* strain. Altogether, it suggests that *Wolbachia* density dynamics is driven by a wide spectrum of factors, either extrinsic or intrinsic, and their potential interplay. Thus, model- or context-dependent traits rather than universal mechanisms operating all over *Wolbachia*/arthropod symbioses have been reported.

Extrinsic factors represent environmental conditions in a broad sense, thus considering physical, chemical and biological parameters. In contrast, intrinsic factors include *Wolbachia*- and host-based mechanisms shaping bacteria density and distribution. The impact of extrinsic factors on *Wolbachia* titers has been extensively studied. Available data should be carefully interpreted, since direct effects on *Wolbachia* are hard to prove. In contrast, very little is still known on the nature and role of intrinsic factors, which likely involve a great variety of genes. So far several loci, either prokaryotic or eukaryotic, are

known to play a role in the *in vivo* modulation of *Wolbachia* titer. Furthermore, plenty of candidate loci awaiting for experimental validation have been shown by a variety of high-throughput screenings.

Genes driving the molecular cross-talk between *Wolbachia* and its host are expected to evolve under simultaneous selective pressures both at individual- and population-level. Evolutionary dynamics of these loci may be highly complex, since (a) selective pressures within- and between-host individuals might oppose each other (i.e. favoring *Wolbachia*-driven infection spread or host-driven infection control, respectively) and (b) the impact of *Wolbachia* on host fitness may greatly depend on the interplay of extrinsic and intrinsic factors. Regardless of the ecological scenario, a fine trade-off between *Wolbachia* and host fitness is required for the symbiosis to be stable over time.

CONFLICTS OF INTEREST

Authors declare no conflict of interest

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REFERENCES

Ahmed MZ, Breinholt JW, Kawahara AY. Evidence for common horizontal transmission of *Wolbachia* among butterflies and moths. *BMC Evol Biol* 2016;**16**:118.

Ahmed MZ, Li S-J, Xue X *et al.* The intracellular bacterium *Wolbachia* uses parasitoid wasps as phoretic vectors for efficient horizontal transmission. *PLOS Pathog* 2015;**11**:e1004672.

Awrahman ZA, Champion de Crespigny F, Wedell N. The impact of *Wolbachia*, male age and mating history on cytoplasmic incompatibility and sperm transfer in *Drosophila simulans. J Evol Biol* 2014;**27**:1–10.

Baião GC, Schneider DI, Miller WJ *et al*. The effect of *Wolbachia* on gene expression in *Drosophila paulistorum* and its implications for symbiont-induced host speciation. *BMC Genomics* 2019;**20**:465.

Bailly-Bechet M, Martins-Simões P, Szöllosi GJ *et al*. How long does *Wolbachia* remain on board? *Mol Biol Evol* 2017;**34**:1183-93.

Balvín O, Roth S, Talbot B *et al*. Co-speciation in bedbug *Wolbachia* parallel the pattern in nematode hosts. *Sci Rep* 2018;**8**:8797.

Baton LA, Pacidônio EC, Gonçalves DS *et al. w*Flu: Characterization and evaluation of a native *Wolbachia* from the mosquito *Aedes fluviatilis* as a potential vector control agent. *PLoS One* 2013;**8**:e59619.

Boyle L, O'Neill S, Robertson H *et al.* Interspecific and intraspecific horizontal transfer of *Wolbachia* in *Drosophila*. *Science* 1993;**260**:1796–9.

Braquart-Varnier C, Altinli M, Pigeault R *et al.* The mutualistic side of *Wolbachia*–isopod interactions: *Wolbachia* mediated protection against pathogenic intracellular bacteria. *Front Microbiol* 2015;**6**:1388.

Braquart-Varnier C, Lachat M, Herbinière J *et al. Wolbachia* mediate variation of host immunocompetence. *PLoS One* 2008;**3**:e3286.

Braquart-Varnier C, Raimond M, Mappa G *et al.* The hematopoietic organ: a cornerstone for *Wolbachia* propagation between and within hosts. *Front Microbiol* 2015b;**6**:1424.

Brennan LJ, Haukedal JA, Earle JC *et al.* Disruption of redox homeostasis leads to oxidative DNA damage in spermatocytes of *Wolbachia*-infected *Drosophila simulans*. *Insect Mol Biol* 2012;**21**:510–20.

Brown AN, Lloyd VK. Evidence for horizontal transfer of *Wolbachia* by a *Drosophila* mite. *Exp Appl Acarol* 2015;**66**:301–11.

Calvitti M, Marini F, Desiderio A *et al. Wolbachia* density and cytoplasmic incompatibility in *Aedes albopictus*: concerns with using artificial *Wolbachia* infection as a vector suppression tool. *PLoS One* 2015;**10**:e0121813.

Camacho M, Oliva M, Serbus LR. Dietary saccharides and sweet tastants have differential effects on colonization of *Drosophila* oocytes by *Wolbachia* endosymbionts. *Biol Open* 2017;**6**:1074–83.

Campbell MA, Łukasik P, Meyer MC *et al.* Changes in endosymbiont complexity drive host-level compensatory adaptations in cicadas. *MBio* 2018;**9**:e02104.

Caragata EP, Rancès E, Hedges LM *et al.* Dietary cholesterol modulates pathogen blocking by *Wolbachia*. *PLoS Pathog* 2013;**9**:e1003459.

Caragata EP, Rancès E, O'Neill SL *et al.* Competition for amino acids between *Wolbachia* and the mosquito host, *Aedes aegypti*. *Microb Ecol* 2014;**67**:205–18.

Chafee ME, Zecher CN, Gourley ML *et al.* Decoupling of host–symbiont–phage coadaptations following transfer between insect species. *Genetics* 2011;**187**:203–15.

Christensen S, Camacho M, Sharmin Z *et al.* Quantitative methods for assessing local and bodywide contributions to *Wolbachia* titer in maternal germline cells of *Drosophila*. *BMC Microbiol* 2019;**19**:206.

Christensen S, Pérez Dulzaides R, Hedrick VE *et al. Wolbachia* endosymbionts modify *Drosophila* ovary protein levels in a context-dependent manner. *Appl Environ Microbiol* 2016;**82**:5354–63.

Chrostek E, Marialva MSP, Esteves SS *et al. Wolbachia* variants induce differential protection to viruses in *Drosophila melanogaster*: a phenotypic and phylogenomic analysis. *PLoS Genet* 2013;**9**:e1003896.

Chrostek E, Marialva MSP, Yamada R *et al.* High Anti-viral protection without immune upregulation after interspecies *Wolbachia* transfer. *PLoS One* 2014;**9**:e99025.

Chrostek E, Teixeira L. Mutualism breakdown by amplification of *Wolbachia* genes. *PLOS Biol* 2015;**13**:e1002065.

Chrostek E, Teixeira L. Within host selection for faster replicating bacterial symbionts. *PLoS One* 2018;**13**:e0191530.

Clancy DJ, Hoffmann AA. Environmental effects on cytoplasmic incompatibility and bacterial load in *Wolbachia*-infected *Drosophila simulans*. *Entomol Exp Appl* 1998;**86**:13-24.

Darby AC, Armstrong SD, Bah GS *et al.* Analysis of gene expression from the *Wolbachia* genome of a filarial nematode supports both metabolic and defensive roles within the symbiosis. *Genome Res* 2012;**22**:2467–77.

de Crespigny F, Pitt T, Wedell N. Increased male mating rate in *Drosophila* is associated with *Wolbachia* infection. *J Evol Biol* 2006;**19**:1964–72.

Dedeine F, Vavre F, Fleury F *et al*. Removing symbiotic *Wolbachia* bacteria specifically inhibits oogenesis in a parasitic wasp. *Proc Natl Acad Sci U S A* 2001;**98**:6247-52.

Desjardins CA, Cerqueira GC, Goldberg JM *et al*. Genomics of *Loa loa*, a *Wolbachia*-free filarial parasite of humans. *Nat Genet* 2013;**45**:495–500.

Dittmer J, Beltran-Bech S, Lesobre J *et al.* Host tissues as microhabitats for *Wolbachia* and quantitative insights into the bacterial community in terrestrial isopods. *Mol Ecol* 2014;**23**:2619–35.

Dittmer J, Lesobre J, Moumen B *et al.* Host origin and tissue microhabitat shaping the microbiota of the terrestrial isopod *Armadillidium vulgare*. *FEMS Microbiol Ecol* 2016;**92**:fiw063.

Douglas AE. Symbiosis as a general principle in eukaryotic evolution. *Cold Spring Harb Perspect Biol* 2014;**6**:a016113.

Duron O, Labbé P, Berticat C *et al.* High *Wolbachia* density correlates with cost of infection for insecticide resistant *Culex pipiens* mosquitoes. *Evolution* 2006;**60**:303–14.

Emerson KJ, Glaser RL. Cytonuclear epistasis controls the density of symbiont *Wolbachia pipientis* in nongonadal tissues of mosquito *Culex quinquefasciatus*. *G3 (Bethesda)* 2017;**7**:2627-35.

Faddeeva-Vakhrusheva A, Kraaijeveld K, Derks MFL *et al*. Coping with living in the soil: the genome of the parthenogenetic springtail *Folsomia candida*. *BMC Genomics* 2017;**18**:493.

Fallon AM. Strain-specific response to ampicillin in *Wolbachia*-infected mosquito cell lines. *Vitr Cell Dev Biol Anim* 2018;**54**:580–8.

Fattouh N, Cazevieille C, Landmann F. *Wolbachia* endosymbionts subvert the endoplasmic reticulum to acquire host membranes without triggering ER stress. *PLoS Negl Trop Dis* 2019;**13**:e0007218.

Favia G, Ricci I, Damiani C *et al*. Bacteria of the genus *Asaia* stably associate with *Anopheles stephensi*, an Asian malarial mosquito vector. *Proc Natl Acad Sci* 2007;**104**:9047–51.

Fenollar F, Maurin M, Raoult D. *Wolbachia pipientis* growth kinetics and susceptibilities to 13 antibiotics determined by immunofluorescence staining and real-time PCR. *Antimicrob Agents Chemother* 2003;**47**:1665–71.

Fisher ML, Watson DW, Osborne JA *et al*. Growth kinetics of endosymbiont *Wolbachia* in the common bed bug, *Cimex lectularius*. Sci Rep 2018;**8**:11444.

Fisher RM, Henry LM, Cornwallis CK *et al.* The evolution of host-symbiont dependence. *Nat Commun* 2017;**8**:15973.

Foo IJ-H, Hoffmann AA, Ross PA *et al*. Cross-generational effects of heat stress on fitness and *Wolbachia* density in *Aedes aegypti* mosquitoes. *Trop Med Infect Dis* 2019;**4**: E13.

Ford SA, Allen SL, Ohm JR *et al*. Selection on *Aedes aegypti* alters *Wolbachia*-mediated dengue virus blocking and fitness. *Nat Microbiol* 2019;**4**:1832-9.

Frydman HM, Li JM, Robson DN *et al.* Somatic stem cell niche tropism in *Wolbachia*. *Nature* 2006;**441**:509–12.

Funkhouser-Jones LJ, van Opstal EJ, Sharma A *et al.* The maternal effect gene *Wds* controls *Wolbachia* titer in *Nasonia*. *Curr Biol* 2018;**28**:1692-1702.e6.

Furihata S, Hirata M, Matsumoto H *et al.* Bacteria endosymbiont, *Wolbachia*, promotes parasitism of parasitoid wasp *Asobara japonica*. *PLoS One* 2015;**10**:e0140914.

Geoghegan V, Stainton K, Rainey SM *et al.* Perturbed cholesterol and vesicular trafficking associated with dengue blocking in *Wolbachia*-infected *Aedes aegypti* cells. *Nat Commun* 2017;**8**:526.

Gerth M, Gansauge M-T, Weigert A *et al.* Phylogenomic analyses uncover origin and spread of the *Wolbachia* pandemic. *Nat Commun* 2014;**5**:5117.

Gómez-Valero L, Soriano-Navarro M, Perez-Brocal V *et al.* Coexistence of *Wolbachia* with *Buchnera aphidicola* and a secondary symbiont in the aphid *Cinara cedri*. *J Bacteriol* 2004;**186**:6626–33.

Goto S, Anbutsu H, Fukatsu T. Asymmetrical interactions between *Wolbachia* and *Spiroplasma* endosymbionts coexisting in the same insect host. *Appl Environ Microbiol* 2006;**72**:4805–10.

Grobler Y, Yun CY, Kahler DJ *et al.* Whole genome screen reveals a novel relationship between *Wolbachia* levels and *Drosophila* host translation. *PLOS Pathog* 2018;**14**:e1007445.

Gutzwiller F, Carmo CR, Miller DE *et al.* Dynamics of *Wolbachia pipientis* gene expression across the *Drosophila melanogaster* life cycle. *G3* 2015;**5**:2843–56.

Hedges LM, Brownlie JC, O'Neill SL *et al*. *Wolbachia* and virus protection in insects. *Science* 2008;**322**:702.

Hamm CA, Begun DJ, Vo A *et al*. *Wolbachia* do not live by reproductive manipulation alone: infection polymorphism in *Drosophila suzukii* and *D. subpulchrella*. *Mol Ecol* 2014;**23**:4871-85.

Hellemans S, Kaczmarek N, Marynowska M *et al.* Bacteriome-associated *Wolbachia* of the parthenogenetic termite *Cavitermes tuberosus*. *FEMS Microbiol Ecol* 2019;**95**:fiy235.

Henry LP, Newton ILG. Mitochondria and *Wolbachia* titers are positively correlated during maternal transmission. *Mol Ecol* 2018;**27**:2634–46.

Hilgenboecker K, Hammerstein P, Schlattmann P *et al.* How many species are infected with *Wolbachia*: a statistical analysis of current data. *FEMS Microbiol Lett* 2008;**281**:215–20.

Hiroki M, Tagami Y, Miura K *et al.* Multiple infection with *Wolbachia* inducing different reproductive manipulations in the butterfly *Eurema hecabe*. *Proc R Soc London Ser B Biol Sci* 2004;**271**:1751–5.

Hoffmann AA, Ross PA, Rašic G. *Wolbachia* strains for disease control: ecological and evolutionary considerations. *Evol Appl* 2015;**8**:751-68.

Hosokawa T, Koga R, Kikuchi Y *et al. Wolbachia* as a bacteriocyte-associated nutritional mutualist. *Proc Natl Acad Sci* 2010;**107**:769–74.

Hughes GL, Dodson BL, Johnson RM *et al.* Native microbiome impedes vertical transmission of *Wolbachia* in *Anopheles mosquitoes*. *Proc Natl Acad Sci* 2014;**111**:12498–503.

Hughes GL, Koga R, Xue P *et al. Wolbachia* infections are virulent and inhibit the human malaria parasite *Plasmodium falciparum* in *Anopheles gambia*e. *PLoS Pathog* 2011;**7**:e1002043.

Hughes GL, Rasgon JL. Transinfection: a method to investigate *Wolbachia*-host interactions and control arthropod-borne disease. *Insect Mol Biol* 2014;**23**:141–51.

Huigens ME, de Almeida RP, Boons PA *et al*. Natural interspecific and intraspecific horizontal transfer of parthenogenesis-inducing *Wolbachia* in *Trichogramma* wasps. *Proc Biol Sci* 2004;**271**:509-15.

Hussain M, Frentiu FD, Moreira LA *et al*. *Wolbachia* uses host microRNAs to manipulate host gene expression and facilitate colonization of the dengue vector *Aedes aegypti*. *Proc Natl Acad Sci U S A* 2011;**108**:9250-5.

Ijichi N, Kondo N, Matsumoto R *et al.* Internal spatiotemporal population dynamics of infection with three *Wolbachia* strains in the adzuki bean beetle, *Callosobruchus chinensis* (Coleoptera: Bruchidae). *Appl Environ Microbiol* 2002;**68**:4074–80.

Jiménez NE, Gerdtzen ZP, Olivera-Nappa Á *et al*. A systems biology approach for studying *Wolbachia* metabolism reveals points of interaction with its host in the context of arboviral infection. *PLoS Negl Trop Dis* 2019;**13**:e0007678.

Kamath AD, Deehan MA, Frydman HM. Polar cell fate stimulates *Wolbachia* intracellular growth. *Development* 2018;**145**:dev158097.

Kampfraath AA, Klasson L, Anvar SY *et al.* Genome expansion of an obligate parthenogenesis-associated *Wolbachia* poses an exception to the symbiont reduction model. *BMC Genomics* 2019;**20**:106.

Kremer N, Charif D, Henri H *et al*. A new case of *Wolbachia* dependence in the genus *Asobara*: evidence for parthenogenesis induction in *Asobara japonica*. *Heredity* 2009;**103**:248-56.

Le Clec'h W, Chevalier FD, Genty L *et al.* Cannibalism and predation as paths for horizontal passage of *Wolbachia* between terrestrial isopods. *PLoS One* 2013;**8**:e60232.

Le Clec'h W, Dittmer J, Raimond M *et al.* Phenotypic shift in *Wolbachia* virulence towards its native host across serial horizontal passages. *Proc R Soc B Biol Sci* 2017;**284**:20171076.

Lindsey ARI, Bhattacharya T, Newton ILG *et al*. Conflict in the intracellular lives of endosymbionts and viruses: a mechanistic look at *Wolbachia*-mediated pathogen-blocking. *Viruses* 2018;**10**:141.

Liu H-Y, Wang Y-K, Zhi C-C *et al.* A novel approach to eliminate *Wolbachia* infections in *Nasonia vitripennis* revealed different antibiotic resistance between two bacterial strains. *FEMS Microbiol Lett* 2014;**355**:163–9.

Mandel MJ, Dunn AK. Impact and influence of the natural *Vibrio*-squid symbiosis in understanding bacterial–animal interactions. *Front Microbiol* 2016;**7**:1982.

Martinez J, Longdon B, Bauer S *et al.* Symbionts commonly provide broad spectrum resistance to viruses in insects: a comparative analysis of *Wolbachia* strains. *PLoS Pathog* 2014;**10**:e1004369.

Martinez J, Tolosana I, Ok S *et al.* Symbiont strain is the main determinant of variation in *Wolbachia*-mediated protection against viruses across *Drosophila* species. *Mol Ecol* 2017;**26**:4072–84.

Masson F, Zaidman-Rémy A, Heddi A. Antimicrobial peptides and cell processes tracking endosymbiont dynamics. *Philos Trans R Soc B Biol Sci* 2016;**371**:20150298.

Min K-TT, Benzer S. *Wolbachia*, normally a symbiont of *Drosophila*, can be virulent, causing degeneration and early death. *Proc Natl Acad Sci* 1997;**94**:10792–6.

Mouton L, Dedeine F, Henri H *et al.* Virulence, multiple infections and regulation of symbiotic population in the *Wolbachia-Asobara tabida* symbiosis. *Genetics* 2004;**168**:181–9.

Mouton L, Henri H, Bouletreau M *et al*. Effect of temperature on *Wolbachia* density and impact on cytoplasmic incompatibility. *Parasitology* 2006;**132**:49-56.

Newton ILG, Savytskyy O, Sheehan KB. *Wolbachia* utilize host actin for efficient maternal transmission in *Drosophila melanogaster*. *PLOS Pathog* 2015;**11**:e1004798.

Newton IL, Sheehan KB. Passage of *Wolbachia pipientis* through mutant *Drosophila melanogaster* induces phenotypic and genomic changes. *Appl Environ Microbiol* 2015;**81**:1032-7.

Nikoh N, Hosokawa T, Moriyama M *et al.* Evolutionary origin of insect-*Wolbachia* nutritional mutualism. *Proc Natl Acad Sci* 2014;**111**:10257–62.

Noda H, Koizumi Y, Zhang Q *et al*. Infection density of *Wolbachia* and incompatibility level in two planthopper species, *Laodelphax striatellus* and *Sogatella furcifera*. *Insect Biochem Mol Biol* 2001;**31**:727-37.

Osborne SE, Iturbe-Ormaetxe I, Brownlie JC *et al.* Antiviral protection and the importance of *Wolbachia* density and tissue tropism in *Drosophila simulans*. *Appl Environ Microbiol* 2012;**78**:6922–9.

Osei-Amo S, Hussain M, O'Neill SL *et al*. *Wolbachia*-induced aae-miR-12 miRNA negatively regulates the expression of MCT1 and MCM6 genes in *Wolbachia*-infected mosquito cell line. *PLoS One* 2012;**7**:e50049.

Pan X, Pike A, Joshi D *et al.* The bacterium *Wolbachia* exploits host innate immunity to establish a symbiotic relationship with the dengue vector mosquito *Aedes aegypti*. *ISME J* 2018;**12**:277–88.

Pan X, Zhou G, Wu J *et al. Wolbachia* induces reactive oxygen species (ROS)-dependent activation of the Toll pathway to control dengue virus in the mosquito *Aedes aegypti*. *Proc Natl Acad Sci* 2012;**109**:E23–31.

Perrot-Minnot MJ, Guo LR, Werren JH. Single and double infections with *Wolbachia* in the parasitic wasp *Nasonia vitripennis*: effects on compatibility. *Genetics* 1996;**143**:961–72.

Pollock FJ, McMinds R, Smith S *et al.* Coral-associated bacteria demonstrate phylosymbiosis and cophylogeny. *Nat Commun* 2018;**9**:4921.

Ponton F, Wilson K, Holmes A *et al.* Macronutrients mediate the functional relationship between *Drosophila* and *Wolbachia*. *Proc R Soc B Biol Sci* 2014;**282**:20142029–20142029.

Reynolds KT, Thomson LJ, Hoffmann AA. The effects of host age, host nuclear background and temperature on phenotypic effects of the virulent *Wolbachia* strain popcorn in *Drosophila* melanogaster. *Genetics* 2003;**164**:1027–34.

Rice DW, Sheehan KB, Newton ILG. Large-scale identification of *Wolbachia pipientis* effectors. *Genome Biol Evol* 2017;**9**:1925-1937.

Richardson KM, Griffin PC, Lee SF *et al*. A *Wolbachia* infection from *Drosophila* that causes cytoplasmic incompatibility despite low prevalence and densities in males. *Heredity* 2019;**122**:428-40.

Rio RV., Wu Y, Filardo G *et al.* Dynamics of multiple symbiont density regulation during host development: tsetse fly and its microbial flora. *Proc R Soc B Biol Sci* 2006;**273**:805–14.

Rohrscheib CE, Bondy E, Josh P *et al. Wolbachia* influences the production of octopamine and affects *Drosophila* male aggression. *Appl Environ Microbiol* 2015;**81**:4573–80.

Rossi P, Ricci I, Cappelli A *et al.* Mutual exclusion of *Asaia* and *Wolbachia* in the reproductive organs of mosquito vectors. *Parasit Vectors* 2015;**8**:278.

Ruang-areerate T, Kittayapong P, McGraw EA *et al. Wolbachia* replication and host cell division in *Aedes albopictus*. *Curr Microbiol* 2004;**49**:10–2.

Schneider DI, Garschall KI, Parker AG *et al.* Global *Wolbachia* prevalence, titer fluctuations and their potential of causing cytoplasmic incompatibilities in tsetse flies and hybrids of *Glossina morsitans* subgroup species. *J Invertebr Pathol* 2013;**112**:S104–15.

Serbus LR, Casper-Lindley C, Landmann F *et al*. The genetics and cell biology of *Wolbachia*-host interactions. *Annu Rev Genet* 2008;**42**:683-707.

Serbus LR, Ferreccio A, Zhukova M *et al.* A feedback loop between *Wolbachia* and the *Drosophila gurken* mRNP complex influences *Wolbachia* titer. *J Cell Sci* 2011;**124**:4299–308.

Serbus LR, White PM, Silva JP *et al*. The impact of host diet on *Wolbachia* titer in *Drosophila*. *PLoS Pathog* 2015;**11**:e1004777.

Shaikevich EV., Ganushkina LA. *Wolbachia* bacteria and filarial nematodes: mutual benefit and the parasite's Achilles' heel. *Biol Bull Rev* 2018;**8**:509–17.

Shan H-W, Deng W-H, Luan J-B *et al.* Thermal sensitivity of bacteriocytes constrains the persistence of intracellular bacteria in whitefly symbiosis under heat stress. *Environ Microbiol Rep* 2017;**9**:706–16.

Sheehan KB, Martin M, Lesser CF *et al.* Identification and characterization of a candidate *Wolbachia pipientis* type IV effector that interacts with the actin cytoskeleton. *MBio* 2016;**7**:e00622.

Simonet P, Duport G, Gaget K *et al.* Direct flow cytometry measurements reveal a fine-tuning of symbiotic cell dynamics according to the host developmental needs in aphid symbiosis. *Sci Rep* 2016;**6**:19967.

Strunov A, Kiseleva E. *Drosophila melanogaster* brain invasion: pathogenic *Wolbachia* in central nervous system of the fly. *Insect Sci* 2016;**23**:253–64.

Strunov A, Kiseleva E, Gottlieb Y. Spatial and temporal distribution of pathogenic *Wolbachia* strain *w*MelPop in *Drosophila melanogaster* central nervous system under different temperature conditions. *J Invertebr Pathol* 2013;**114**:22-30.

Sudakaran S, Kost C, Kaltenpoth M. Symbiont acquisition and replacement as a source of ecological innovation. *Trends Microbiol* 2017;**25**:375–90.

Suh E, Mercer DR, Dobson SL. Life-shortening *Wolbachia* infection reduces population growth of *Aedes aegypti*. *Acta Trop* 2017;**172**:232–9.

Suh E, Mercer DR, Fu Y *et al.* Pathogenicity of life-shortening *Wolbachia* in *Aedes albopictus* after transfer from *Drosophila melanogaster*. *Appl Environ Microbiol* 2009;**75**:7783–8.

Teixeira L, Ferreira Á, Ashburner M *et al.* The bacterial symbiont *Wolbachia* induces resistance to RNA viral infections in *Drosophila melanogaster*. *PLoS Biol* 2008;**6**:e1000002.

Truitt AM, Kapun M, Kaur R *et al. Wolbachia* modifies thermal preference in *Drosophila melanogaster*. *Environ Microbiol* 2018;**21**:3259-68.

Unckless RL, Boelio LM, Herren JK *et al. Wolbachia* as populations within individual insects: causes and consequences of density variation in natural populations. *Proc R Soc B Biol Sci* 2009;**276**:2805–11.

Valette V, Bitome Essono P-Y, Le Clec'h W *et al.* Multi-infections of feminizing *Wolbachia* strains in natural populations of the terrestrial isopod *Armadillidium vulgare*. *PLoS One* 2013;**8**:e82633.

van Opijnen T, Breeuwer JA. High temperatures eliminate *Wolbachia*, a cytoplasmic incompatibility inducing endosymbiont, from the two-spotted spider mite. *Exp Appl Acarol* 1999;**23**:871–81.

Veneti Z, Clark ME, Karr TL *et al.* Heads or tails: host-parasite interactions in the *Drosophila-Wolbachia* system. *Appl Environ Microbiol* 2004;**70**:5366–72.

Vigneron A, Masson F, Vallier A *et al.* Insects recycle endosymbionts when the benefit is over. *Curr Biol* 2014;**24**:2267–73.

Voronin D, Cook DAN, Steven A *et al.* Autophagy regulates *Wolbachia* populations across diverse symbiotic associations. *Proc Natl Acad Sci* 2012;**109**:E1638–46.

Werren JH, Baldo L, Clark ME. *Wolbachia*: master manipulators of invertebrate biology. *Nat Rev Microbiol* 2008;**6**:741–51.

White PM, Pietri JE, Debec A *et al.* Mechanisms of horizontal cell-to-cell transfer of *Wolbachia* spp. in *Drosophila melanogaster*. *Appl Environ Microbiol* 2017;**83**:e03425.

White PM, Serbus LR, Debec A *et al.* Reliance of *Wolbachia* on high rates of host proteolysis revealed by a genome-wide RNAi screen of *Drosophila* cells. *Genetics* 2017b;**205**:1473–88.

Xi Z, Gavotte L, Xie Y *et al.* Genome-wide analysis of the interaction between the endosymbiotic bacterium *Wolbachia* and its *Drosophila* host. *BMC Genomics* 2008;**9**:1.

Yang K, Xie K, Zhu YX *et al*. *Wolbachia* dominate *Spiroplasma* in the co-infected spider mite *Tetranychus truncatus*. Insect Mol Biol 2019;**29**:19-37.

Zug R, Hammerstein P. *Wolbachia* and the insect immune system: what reactive oxygen species can tell us about the mechanisms of *Wolbachia*–host interactions. *Front Microbiol* 2015;**6**:1201.

FIGURES AND TABLES

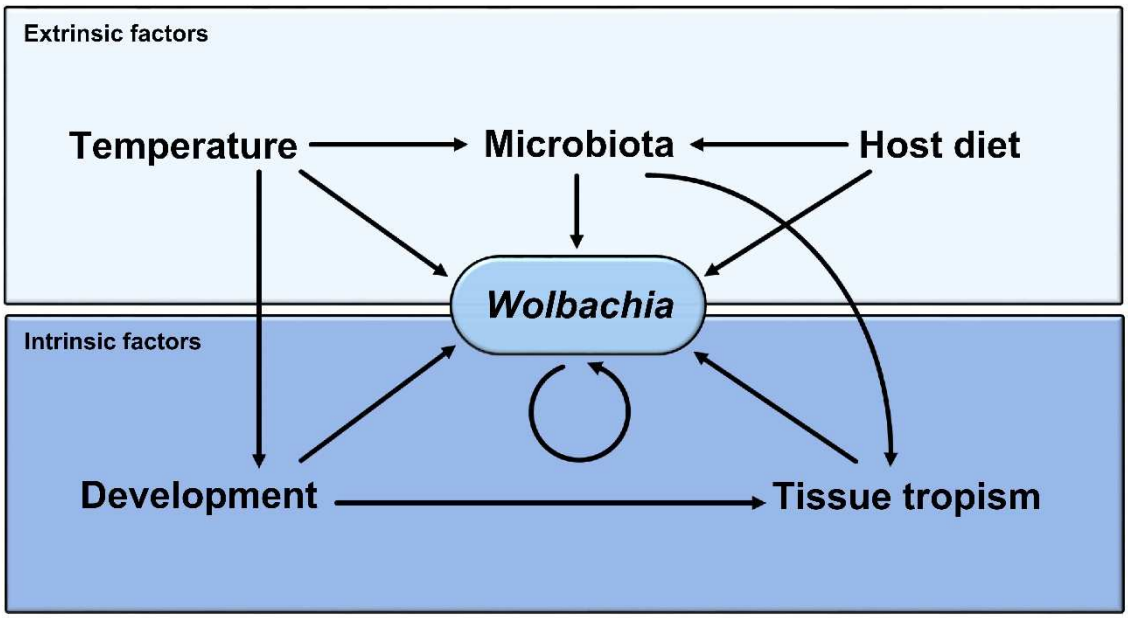

Figure 1. Factors shaping *Wolbachia* titers. Extrinsic (top panel) and intrinsic (bottom panel) factors and their potential interactions (arrows) are indicated. Additional interactions among extrinsic/intrinsic factors may occur (e.g. host diet/development, microbiota/development) but their impact on *Wolbachia* titer dynamics is yet unclear.

Table 1. Genetic basis of *Wolbachia* titer regulation. Loci shown to *in vivo* influence *Wolbachia* titers.

Loci	Target	Model	Reference
Metalloprotease^{h}	Proliferation	*A. aegypti*/*w*MelPop-CLA	Hussain *et al*. 2011
MicroRNA aae-miR-2940^{h}	Proliferation	*A. aegypti*/*w*MelPop-CLA	Hussain *et al*. 2011
Gurken^{h}	Proliferation/Trafficking	*D. melanogaster*/*w*Mel	Serbus *et al*. 2011
MicroRNA aae-miR-12^{h}	Proliferation/Maintenance	*A. aegypti*/*w*MelPop-CLA	Osei-Amo *et al*. 2012
Octomom^{w}	Proliferation	*D. melanogaster*/*w*MelPop	Chrostek *et al*. 2013
Chickadee^{h}	Trafficking	*D. melanogaster*/*w*Mel	Newton *et al*. 2015
Quail^{h}	Trafficking	*D. melanogaster*/*w*Mel	Newton *et al*. 2015
Wolbachia actin-localizing effector 1^{w}	Proliferation/Trafficking	*D. melanogaster*/*w*Mel	Sheehan *et al*. 2016
Ubiquitin conjugating enzyme 6^{h}	Proliferation/Maintenance	*D. melanogaster*/*w*Mel	White *et al*. 2017b
Quantitative-trait loci 1^{h}	Proliferation/Maintenance	*C. quinquefasciatus*/*w*Pip	Emerson and Glaser, 2017
Quantitative-trait loci 2^{h}	Proliferation/Maintenance	*C. quinquefasciatus*/*w*Pip	Emerson and Glaser, 2017
Wolbachia density suppressor^{h}	Trafficking	*N. vitripennis*/*w*VitA	Funkhouser-Jones *et al*. 2018
Ribosomal protein L27A^{h}	Proliferation/Maintenance	*D. melanogaster*/*w*Mel	Grobler *et al*. 2018
Ribosomal protein S3^{h}	Proliferation/Maintenance	*D. melanogaster*/*w*Mel	Grobler *et al*. 2018

^{h}Host encoded; ^{w}*Wolbachia* encoded