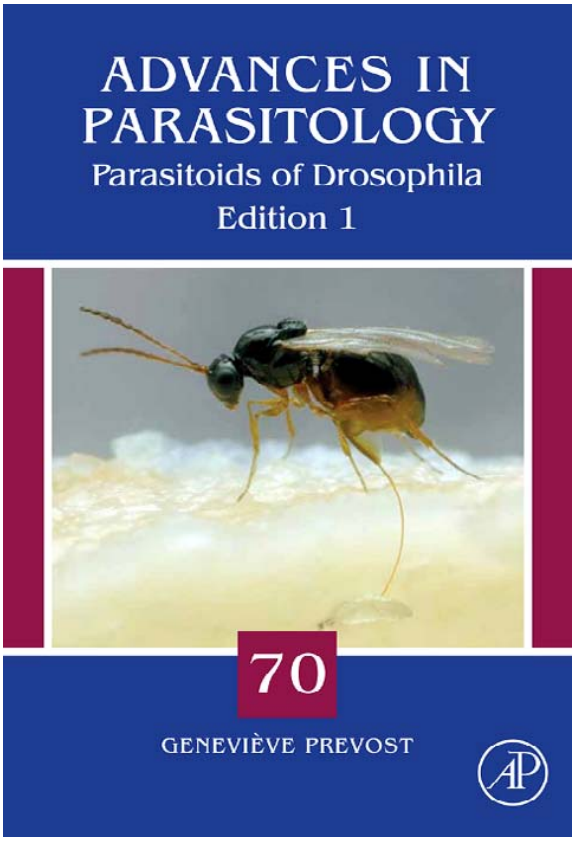

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CHAPTER 12

Drosophila–Parasitoid Communities as Model Systems for Host–*Wolbachia* Interactions

Fabrice Vavre,* Laurence Mouton,* and **Bart A. Pannebakker†**

Contents

* Université Lyon 1; CNRS, UMR5558, Laboratoire de Biométrie et Biologie Evolutive, F-69622, Villeurbanne, France
† Evolutionary Genetics, Centre for Ecological and Evolutionary Studies, University of Groningen, P.O. Box 14, NL-9750 AA Haren, The Netherlands

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Abstract *Wolbachia* bacteria are cytoplasmic endosymbionts that infect a wide range of arthropod and nematode hosts. They are transmitted from mother to offspring via the eggs (vertical transmission) and enhance their transmission to the next generation by manipulating the reproductive system of their hosts. These manipulations occur in many forms, such as the induction of cytoplasmic incompatibility, feminization, male killing and parthenogenesis induction. *Wolbachia* is estimated to occur in up to 66% of all insect species, but the greatest diversity of reproductive manipulations is found in the order of the Hymenoptera. Studies of *Wolbachia* in *Drosophila–parasitoid* communities have allowed for important insights into different aspects of *Wolbachia* biology. The extensive knowledge available on *Drosophila* parasitoids provides a solid base on which to test new hypotheses on host–Wolbachia interactions. The large range of *Wolbachia* phenotypes present in *Drosophila* parasitoids, combined with the recent acquisition of the bacteria from their Drosophilid hosts, make them an ideal model system to study the evolution and dynamics of *Wolbachia* infections, both in the laboratory as in the field. In this chapter, we aim to review the current knowledge on the associations between *Wolbachia* and *Drosophila* parasitoids, and identify open questions and specify new research directions.

12.1. INTRODUCTION

Wolbachia bacteria are cytoplasmic endosymbionts (α-proteobacteria) that infect a wide range of arthropod and nematode hosts (Duron et al., 2008; O'Neill et al., 1997; Stouthamer et al., 1999; Werren et al., 2008). They are

transmitted from mother to offspring via the eggs (vertical transmission). *Wolbachia* enhance their transmission to the next generation by manipulating the reproductive system of their hosts. This bacterial reproductive parasitism occurs in many forms, such as the induction of cytoplasmic incompatibility (Boyle et al., 1993), feminization (Rousset et al., 1992), male killing (Hurst et al., 1999) and parthenogenesis induction (Stouthamer et al., 1999). All these manipulations result in an increase in the number of infected females in the host population and maximize the transmission of the bacteria in the host population. These direct effects of *Wolbachia* on host reproduction can also have important indirect ecological and evolutionary consequences for the host, from structuring communities to mediating parasitoid–host interactions and life-history strategies.

Even though *Wolbachia* is estimated to occur in up to 66% of all insect species (Hilgenboecker et al., 2008), the greatest diversity of reproductive manipulations is found in the order of the Hymenoptera. Haplodiploid sex determination, where females develop from fertilized diploid eggs, and males from unfertilized haploid eggs, makes Hymenoptera especially prone to (bacterial) manipulation of their reproductive system. Among the Hymenoptera, *Drosophila* parasitoids are one of the best studied groups for host–symbiont interactions. Other Hymenopteran genera in which host–symbiont interactions are well described are *Nasonia* (Hymenoptera: Pteromalidae) (Bordenstein and Werren, 2007; Bordenstein et al., 2001; Breeuwer and Werren, 1990, 1995; Breeuwer et al., 1992; Tram et al., 2003, 2006) and *Trichogramma* (Hymenoptera: Trichogrammatidae) (Pintureau et al., 1999, 2002; Stouthamer and Kazmer, 1994; Stouthamer and Luck, 1993; Stouthamer et al., 1990). Here we present *Drosophila* parasitoids as a model system for studying host–Wolbachia interactions, with a focus on the *Leptopilina* and *Asobara* genera. Studies of *Wolbachia* in *Drosophila* parasitoid communities have allowed for important insights in different aspects of *Wolbachia* biology, such as the dynamics of *Wolbachia* in insect communities, the diversity of interactions between hosts and bacteria, the regulation of bacterial populations, and the evolutionary consequences of infection for the host. The knowledge available on *Drosophila* parasitoids, which is reflected in this issue and covers fields as diverse as developmental biology, immunology, physiology, ecology and evolution, forms a solid base on which to test new hypotheses on a wide array of host–Wolbachia interactions. In addition, the large range of *Wolbachia* phenotypes present in *Drosophila* parasitoids (Section 12.3), and the recent transfer of the bacteria from their Drosophilid hosts (Section 12.2) make them an ideal model system in which to study the evolution and dynamics of *Wolbachia* infections, both in the laboratory and in the field. In the next sections, we aim to (1) review the current knowledge on the associations between *Wolbachia* and *Drosophila* parasitoids, and (2) identify open questions and specify new research directions.

12.2. PATTERN OF INFECTION AND PHYLOGENETIC DIVERSITY OF *WOLBACHIA* IN *DROSOPHILA* PARASITOIDS

One of the most striking results obtained from phylogenetic studies of *Wolbachia* is the almost complete absence of congruence between the *Wolbachia* and the host phylogenies. This is interpreted as the possibility of *Wolbachia* to be horizontally transmitted from one species to another, which means *Wolbachia* has the ability to invade new host species regularly. Because of their intimacy, parasitoid–host interactions have immediately been suggested as a possible route for these transfers, a hypothesis that has been supported by some isolated cases (Werren et al., 1995).

While case studies are interesting to understand under which conditions horizontal transmission may occur, they provide only limited information on the general mechanisms that may favor or limit horizontal transmission. A more powerful way of tackling these questions is to study patterns of infection in many species simultaneously that share either ecological connections or phylogenetic ancestry. Both types of studies have been performed in *Drosophila* parasitoids and they shed light on important factors that determine the probability of horizontal transmission among species.

12.2.1. *Drosophila* parasitoids are highly susceptible to *Wolbachia* infection

While *Wolbachia* infection has been detected in about 20% of all insect species, 11 out of 16 *Drosophila* parasitoid species (around 69%) have been found infected (Table 12.1). This higher incidence of *Wolbachia* infection in *Drosophila* parasitoids may be caused by small sample sizes in most global surveys (often only one or two individuals per species), which undoubtedly underestimates the real *Wolbachia* incidence. Statistical inferences on these global surveys have recently estimated that *Wolbachia* incidence may be as high as 66% (Hilgenboecker et al., 2008), which is close to the incidence found in *Drosophila* parasitoids. However, in *Drosophila* parasitoids, infection is fixed or near fixation in most species, even though polymorphism has been detected in some of them, notably in *Leptopilina victoriae* and *Pachycrepoideus dubius* (Vavre et al., 2000, 2002). This means that either the incidence of *Wolbachia* is higher in *Drosophila* parasitoids than in other species, or that infection within species is more prevalent.

Another result that suggests a high susceptibility of *Drosophila* parasitoids to *Wolbachia* infection is that many species are infected by multiple *Wolbachia* strains. In many cases, these multiple strains are hosted within

TABLE 12.1 *Wolbachia* infection in *Drosophila* parasitoids

Genus	Species	Infection	Phenotype
Leptopilina	*heterotoma*	wLhet1, wLhet2, wLhet3	CI, CI, CI
	victoriae	wLvic	CI
	guineaensis	wLgui1, wLgui2	CI, unknown
	boulardi	–	
	freyae	–	
	orientalis	–	
	clavipes	wLcla	PI
	australis	wLaus	PI
Asobara	*tabida*	wAtab1, wAtab2, wAtab3	CI, CI, oogenesis
	rufescens	wAruf	CI suspected
	japonica	wAjap	PI
	persimilis	–	
	citri	–	
Trichopria	*Drosophilae*	wTdro	?
	nr. Drosophilae	wTsp1, wTsp2	CI
Pachycrepoideus	*dubius*	wPdub	No effect detected

Table shows species, *Wolbachia* strain, *Wolbachia* phenotype and infection type (CI-cytoplasmic incompatibility, PI-parthenogenesis induction, and oogenesis-obligatory for oogenesis.)

the same individuals, like in *Trichopria nr. drosophilae* that is infected by two *Wolbachia* strains, and *Leptopilina heterotoma* and *Asobara tabida* where three *Wolbachia* strains have been detected (Vavre et al., 1999). In *Leptopilina guinaensis*, however, two *Wolbachia* strains have been detected, but each of these occur in different populations (Vavre, unpublished data). Taken together, these results suggest that *Drosophila* parasitoids are quite susceptible to *Wolbachia* infection.

Finally, *Drosophila* parasitoids are also susceptible to other vertically transmitted bacteria. For example, *P. dubius* has been shown to host *Arsenophonus* (Gueguen and Duron, personal communication) and a *Rickettsia* has been recently detected in newly collected individuals in *A. tabida* (Zouache and Mavingui, personal communication), but their effects remain unknown to date. In addition, it has recently been shown that *Leptopilina boulardi* is host to a virus that can be either vertically or horizontally transmitted and that manipulates the strategy of host exploitation by increasing superparasitism by infected females (Varaldi et al., 2003; Chapter 13 by Varaldi et al.).

Taken together, these results show that symbionts, and especially *Wolbachia*, are frequent partners of *Drosophila* parasitoids and, because of their potential impact on the evolutionary trajectories of their hosts, they should not be ignored in these insects.

12.2.2. Phylogenetic analyses reveal frequent horizontal transmission

Because of their intimate links, hosts and parasitoids are especially prone to the occurrence of horizontal transmission. *Drosophila* and their parasitoids have been the first insect communities where a clear pattern of horizontal transmission has been detected at a community level (Vavre et al., 1999). In many cases, the same *Wolbachia* strain is found in *Drosophila* and their parasitoids (Fig. 12.1). For example, the strain wLhet1, infecting

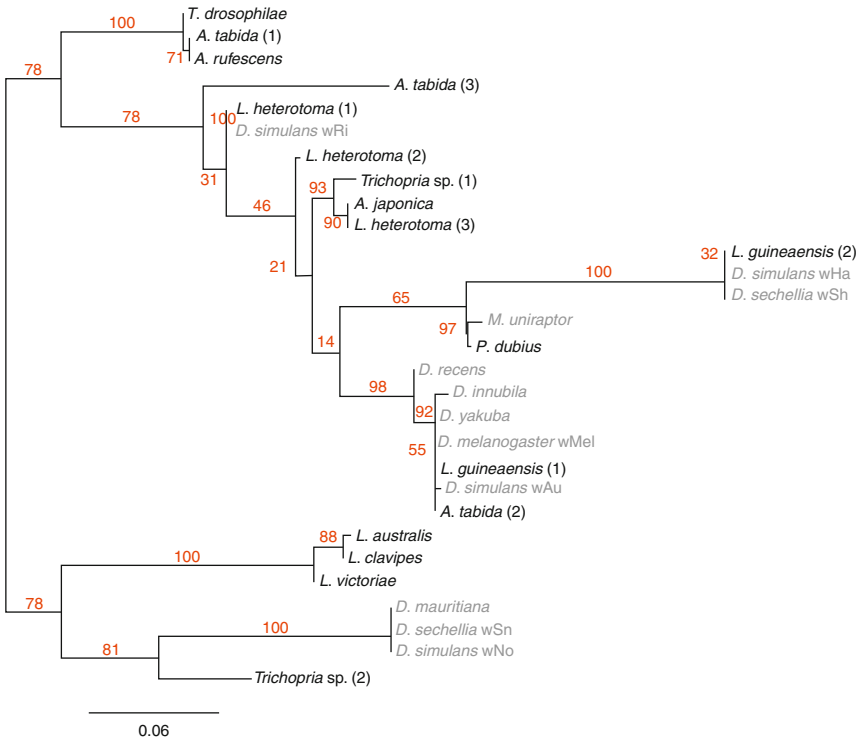

FIGURE 12.1 Phylogenetic tree of *Wolbachia* reconstructed by maximum likelihood using the wsp gene. Each *Wolbachia* strain is represented by the name of its host species and by a number or the strain name for multiply infected species. In grey are the *Wolbachia* in *Drosophila* hosts; in black, the *Wolbachia* in *Drosophila* parasitoids. *Note: Adapted from Vavre et al. (1999).*

L. heterotoma, has the same WSP sequence as the strain wRi infecting *Drosophila simulans* in continental areas. The same is true for the strain wAtab2 infecting *A. tabida* and the strain wMel infecting *Drosophila melanogaster*. The situation in *L. guinaensis* is also striking: populations from West Africa harbor a *Wolbachia* strain similar to a strain found in *D. melanogaster*, while populations from East Africa harbor a *Wolbachia* strain similar to a strain harbored by *D. simulans* in islands of the Indian Ocean. Parasitoids may also share the same infection, like for instance *A. tabida* and *Trichopria drosophilae*, *L. heterotoma* and *T. nr drosophilae* and *Pachycrepoideus dubius* and *Muscidifurax uniraptor*, a generalist parasitoid of Diptera (Vavre et al., 1999).

One important restriction of these data was that they were obtained using only the *wsp* gene, which has been shown to recombine frequently among *Wolbachia* strains (Baldo et al., 2005). Preliminary additional data confirm some of these strains have been recently horizontally transmitted. Using a multiple locus strain typing (MLST) approach (Baldo et al., 2006b), no variation was found for wLhet1 and wRi, and wAtab2 and wMel, confirming that these strains are very closely related. However, the close relationships observed between wAjap and WLhet3 on wsp was not sustained by MLST analysis, suggesting recombination events among these strains rather than horizontal transmission of the bacterium. Clearly, ongoing characterization of *Wolbachia* strains infecting *Drosophila* parasitoids will allow a more thorough analysis of strain exchanges and/or recombination among strains infecting interconnected species at a community level.

Overall, these results strongly suggest that interactions at the community level favor horizontal transmission of *Wolbachia* in *Drosophila–parasitoid* communities. Interestingly, Heath et al. (1999) experimentally transferred *Wolbachia* from *D. simulans* to *L. boulardi* with a success rate of 0.7%, showing that transfers do occur during parasitism. However, infection was lost in *L. boulardi* during subsequent generations, suggesting this species is somehow resistant to *Wolbachia* infection. Similar experiments using *L. heterotoma* and *D. melanogaster* could not detect any horizontal transfer (Vavre, unpublished data). This can be due either to insufficient sampling effort (only 250 wasp lines were tested) or to the fact that density of *Wolbachia* in *D. melanogaster* is lower than in *D. simulans* (Boyle et al., 1993), which would reduce the efficiency of transfer. Thus, while horizontal transmission is common at an evolutionary scale, it does not occur at high frequency at the individual level in these systems.

12.2.3. Pattern of infection in the genus *Leptopilina*: Coincidence or constraint

Additional species have recently been described in the *Leptopilina* genus (Allemand et al., 2002), allowing for a thorough analysis of the pattern of *Wolbachia* infection at the scale of the entire genus (Vavre and Henri,

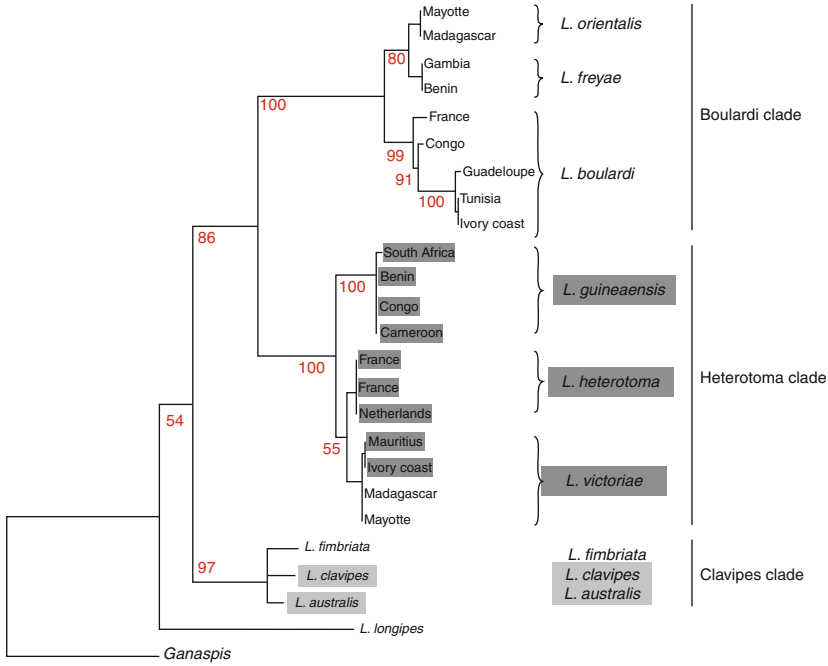

FIGURE 12.2 Phylogenetic tree of the *Leptopilina* genus reconstructed by maximum likelihood using ITS1. In dark grey, populations and species infected with CI-inducing *Wolbachia*; in light grey, species infected with PI-inducing *Wolbachia*. For each species origin of the individuals is indicated.

unpublished data). The *Leptopilina* genus is composed of three clades: Heterotoma, Boulardi and Clavipes, each composed of three species (Fig. 12.2). The three species in the Heterotoma clade are infected with *Wolbachia* strains inducing CI, and two are infected by more than one strain. In many cases, these strains are closely related to the strains that also infect *Drosophila* species. Within the Clavipes clade two of the three species are singly infected with closely related *Wolbachia* strains inducing parthenogenesis, which could suggest cospeciation. Finally, three species belong to the Boulardi clade, but none of them is infected. The probability that *Wolbachia* infection and phenotypes cluster as they do is only 0.007, which suggests that susceptibility to *Wolbachia* infection is determined at the phylogenetic level.

12.2.4. What determines the infection status of a species?

For horizontal transmission to occur, different factors must be fulfilled, which can be considered as a series of filters the infection must pass through (Vavre et al., 2003). First, the *encounter* filter must be passed,

where an uninfected species must come into contact with an infected species, so that the transfer can occur. Second, the newly acquired *Wolbachia* must be able to pass a *compatibility* filter that corresponds to its ability to escape the novel hosts immune system and to multiply in this new host in order to get transmitted to the next generation. Third, the infection must pass an *invasion* filter and spread into the host population. This is an especially important step for CI-*Wolbachia* because infection must reach a certain threshold in order to be maintained in the population (Turelli, 1994).

The parasitoid way of life obviously opens up the encounter filter, and facilitates the transfer of *Wolbachia* from the *Drosophila* host to the wasp as each wasp develops in a potentially infected *Drosophila* host. The reverse, however, is not so easy. First, most *Drosophila* that reach the adult stage have not been parasitized. Second, those that have been parasitized but that were resistant to parasitism have mounted an immune response leading to the encapsulation of the wasp egg. This physical barrier probably limits the ability of *Wolbachia* to reach the *Drosophila* tissues. Hence, while parasitoid–host interaction certainly increases the chance that wasps are infected, parasitoids may not be major vectors of *Wolbachia* for their *Drosophila* hosts.

Given that all *Drosophila* parasitoids share the same way of life, all of them should be equally susceptible to *Wolbachia* primary infection. Why then do we observe important variations for infection among the *Leptopilina* clades? A first obvious possibility is that the *Drosophila* hosts differ among these various species and that these hosts differ for their infection status. It has been proposed that fungivorous *Drosophila* may be less infected than frugivorous *Drosophila* because of the presence of natural antibiotics produced by the substrate on which fungivorous *Drosophila* live (Haine et al., 2005; Jaenike et al., 2006). Interestingly, parasitoids developing in these hosts are mostly found in the Clavipes group where no obvious case of horizontal transmission could be detected. However, parasitoids form the Heterotoma and Boulardi clades share similar hosts, in particular *D. melanogaster* and *D. simulans*, but these two clades show very different susceptibility to *Wolbachia* infection. These differences can only be accounted for by differences in the compatibility or invasion filters. Experimental infections of *L. boulardi* by *Wolbachia* showed that infection was lost in a few generations in this species (Heath et al., 1999). This suggests that *Wolbachia* is able to infect *L. boulardi* but is not able to proliferate or be transmitted in this species, which could reflect a closure of the compatibility filter. The origin of this is unknown, and it would be difficult to investigate. The recent discovery of a virus in *L. boulardi* (Varaldi et al., 2003), which is not found in the sister species *L. heterotoma* that does harbor *Wolbachia*, opens up the possibility that resistance is mediated by a third party. Interestingly, two independent studies have

recently shown that *Wolbachia* protects *Drosophila* against infection with RNA viruses (Hedges et al., 2008; Teixeira et al., 2008). Whether viruses can also protect from *Wolbachia* infection would thus be interesting to test. Another possibility is that the ancestor of the Boulardi group has been infected previously by *Wolbachia* and subsequently acquired resistance to *Wolbachia*. Finally, it is suspected that the effective population size of *L. boulardi* is higher than in *L. heterotoma*, which could also limit the spread of CI-*Wolbachia* in the former species, by closing the invasion filter. Unfortunately, ecological data are missing for the other species of these two clades.

Finally, other communities have been studied to detect patterns of horizontal transmission. While some have found such patterns (Dedeine et al., 2005a; Kittayapong et al., 2003), others have failed to detect them (Schilthuizen and Stouthamer, 1998; West et al., 1998). *Drosophila*–parasitoid communities and their variations in *Wolbachia* susceptibility may help to explain these variations among communities.

12.3. PHENOTYPIC DIVERSITY OF *WOLBACHIA* IN *DROSOPHILA* PARASITOIDS

12.3.1. Cytoplasmic incompatibility and its variability

Wolbachia-induced cytoplasmic incompatibility is a postzygotic isolation mechanism, which in its simplest form occurs in the crosses between infected males and uninfected females (unidirectional CI, Fig. 12.3). While the molecular mechanism of CI remains unknown, cytological observations indicate that in this cross, male chromosomes are improperly condensed precluding their normal participation to karyogamy (reviewed in Poinsot et al. 2003). The proposed model is that *Wolbachia* ''imprint'' chromosomes during spermatogenesis of infected males (the so-called modification (*mod*) function). These chromosomes can only get properly condensed when the *Wolbachia* present in the eggs rescue them (*resc* function). In diploids, CI results in the death of embryos. In haplodiploids, CI has first been described in the genus *Nasonia* (Breeuwer and Werren, 1990). Based on these results, it was thought that CI resulted in male-biased sex-ratio without reduction in offspring production (we will refer to this type of CI as the male development (MD) type of CI, Fig. 12.3). This could be explained by two factors. First, unfertilized eggs are obviously not exposed to CI and develop normally into males. Second, complete elimination of the paternal chromosome set from fertilized eggs restores haploidy, and hence allow their male development. Breeuwer (1997), however, showed that in the haplodiploid mites *Tetranychus urticae* and *T. turkestanica*, CI resulted in a male-biased sex-ratio, but that was

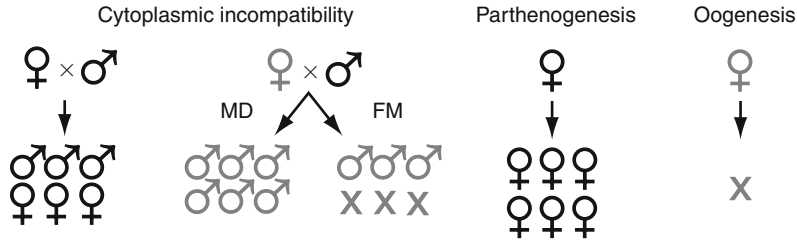

FIGURE 12.3 Schematic representation of the effects of *Wolbachia* found in *Drosophila* parasitoids. In black, infected individuals; in grey, uninfected individuals. MD, male development CI type; FM, female mortality CI type.

accompanied by a reduction in offspring production. In fact, male production in incompatible crosses was only due to the normal development of unfertilized eggs, while fertilized eggs died, as in diploids. We will refer to this CI type as the female mortality (FM) type (Fig. 12.3). The proposed mechanism was that male chromosomes were not entirely eliminated, leading to aneuploid, unviable eggs, a hypothesis recently confirmed by precise cytological analyses (Tram et al., 2006). Two hypotheses were proposed to explain this phenomenon: either this was due to the holokinetic structure of mite chromosomes, or to a reduced efficiency of the *mod* function in males.

Observations in *Drosophila* parasitoids, and especially the description of the CI phenotype in *L. heterotoma*, allowed for important advances in the description of CI in haplodiploids. First, in crosses between triply infected males and uninfected females, a FM phenotype was observed for the first time in Hymenoptera (Vavre et al., 2000). Second, crosses between males infected by a subset of these three *Wolbachia* strains showed that FM and MD phenotypes are only the extreme of a continuous gradient between FM and MD phenotypes (Mouton et al., 2005; Vavre et al., 2001), suggesting that the CI type is a quantitative rather than a qualitative trait. These two results reinforce the hypothesis that the variability was due to quantitative variations in the *mod* intensity, which is also corroborated by some indications that reduced *Wolbachia* density in males could lead to aneuploid unviable eggs in incompatible crosses in *Nasonia* (Breeuwer and Werren, 1993). Since these first observations, all new reported cases of bacterial-induced CI in haplodiploids (*Wolbachia* or *Cardinium)* have been shown to be of the FM phenotype (Hunter et al., 2003; Mochiah et al., 2002; Perlman et al., 2006), among which two in *Drosophila* parasitoids, in *A. tabida* (Dedeine et al., 2004) and in *Trichopria nr. drosophilae* (Vavre et al., 2002). Even in the *Nasonia* genus, a reanalysis of the CI phenotypes showed that the MD phenotype is only restricted to *N. vitripennis*, while a FM phenotype is expressed in *N. giraulti* and *N. longicornis* (Bordenstein et al., 2003).

Interestingly, studies in *L. heterotoma* and in the *Nasonia* genus highlight that both host and *Wolbachia* can affect the CI type. In *L. heterotoma*, CI type was measured using a single host genotype, but various compositions of *Wolbachia* strains. These experiments showed that the fraction of dying fertilized eggs (FM type) increased when the number of *Wolbachia* strains inducing CI is smaller (Fig. 12.4), thus showing that variation in the bacterial community alone is sufficient to affect the CI phenotype (Mouton et al., 2005; Vavre et al., 2001). On the contrary, in *Nasonia*, crosses between the different sister species showed that the host genotype plays a crucial role in the CI phenotype in this genus (Bordenstein et al., 2003). Understanding the CI diversity in haplodiploids and the evolutionary forces acting on this phenotype thus requires to take into account both partners.

From a mechanistic point of view, the results obtained on *L. heterotoma* are counter-intuitive. One hypothesis to explain the different phenotypes was that a reduced *mod* function could limit the ''imprinting'' of paternal chromosomes resulting in an incomplete destruction of the chromosomes and subsequent to aneuploid unviable embryos. Because bacterial density can be related to the intensity of CI (e.g., Boyle et al., 1993; Veneti et al., 2004), it has been proposed that a reduced bacterial density might result in a FM phenotype (Breeuwer and Werren, 1993). However, in *L. heterotoma* an increase in bacterial density, as observed when more *Wolbachia* strains are present, induces an increase in the number of dying embryos,

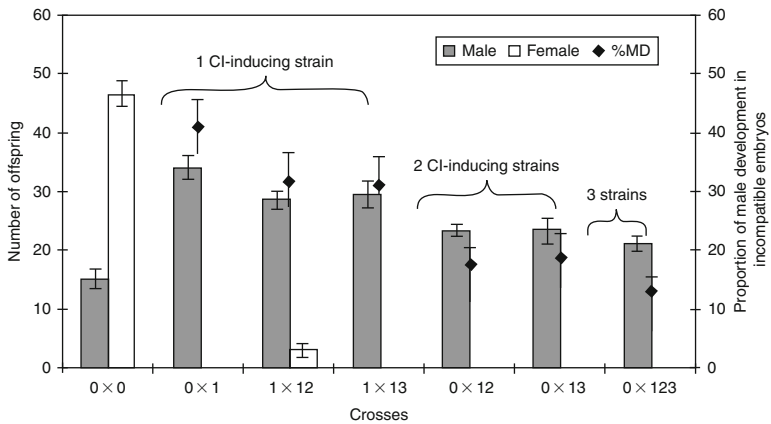

FIGURE 12.4 Male and female offspring production (left axis) and proportion of incompatible embryos developing into males (right axis) in crosses between *Leptopilina heterotoma* strains infected by different compositions of *Wolbachia* strains. Crosses are classified according to the number of strains that induce the CI phenotype.
Note: Adapted from Mouton et al. (2005).

thus in the FM phenotype (Mouton et al., 2005). Unfortunately, it is impossible to distinguish between the effect of bacterial diversity versus the effect of bacterial density in these experiments. However, the *Wolbachia* strains each exhibit a specific CI phenotype, and no direct interaction could be observed among them. This unexpected result makes *L. heterotoma* a suitable species to further investigate the mechanism of CI in haplodiploids, and cytological studies as those performed in *Nasonia* (Tram et al., 2006) would be interesting to conduct in this species.

The diversity of CI phenotypes in haplodiploids is not only important to understand the molecular basis of CI, but also has important consequences on the epidemiology and stability of *Wolbachia* infection, which could explain the higher incidence of the FM CI type. Indeed, spread and maintenance of *Wolbachia* in natural populations depend on the counter-selection exerted on uninfected females by incompatible crosses, whose frequency in turn depend on the frequency of infected males in the population. In haplodiploids, incompatible crosses result in the production of uninfected males, but more so under the MD CI type. As a consequence, CI in haplodiploids is less efficient than in diploid species, and among haplodiploids, the MD type is less efficient than the FM CI type (Egas et al., 2002; Vavre et al., 2000, 2003). This raises the possibility that at the start of a new *Wolbachia* infection, *Wolbachia* inducing the FM type has more chance to spread in the population than a *Wolbachia* inducing the MD type, that is, the invasion filter is less restrictive to the FM type. This selection for more invasive *Wolbachia* strains has been referred to as clade selection by Hurst and McVean (1996). In addition, it is more and more accepted that *Wolbachia* infections do not necessarily last for long periods in the same host. Theoretical models have shown that the MD type is more susceptible to infection loss than the FM type, and this could also explain why the MD type has not been as commonly found (Vautrin et al., 2007; Vavre et al., 2003).

12.3.2. Parthenogenesis induction and sexual degradation

Parthenogenesis-inducing *Wolbachia* (Fig. 12.3) are known in three *Drosophila* parasitoid species: *Leptopilina clavipes* (Schidlo et al., 2002), *Leptopilina australis* (Werren et al., 1995) and *Asobara japonica* (Kremer et al., 2009). Since PI-*Wolbachia* was only recently discovered in the latter species, and the infection is poorly described in *L. australis*, the focus in this section will be on *L. clavipes*, for which most information is available. *L. clavipes* is a parasitoid of *Drosophila* larvae that has a pan-European distribution (Nordlander, 1980). It occurs in woodlands where it parasitizes *Drosophila* larvae living in fungal fruit bodies (Driessen et al., 1990; Vet, 1983). In northern-western Europe *D. phalerata* is its main host, but larvae of *D. kuntzei*, *D. transversa* and *D. subobscura* are also parasitized

(Driessen et al., 1990). Southern European *L. clavipes* parasitize *D. melanogaster* larvae as well, a species that mainly breeds in fermenting fruits (Pannebakker et al., 2004c, 2008). Two modes of reproduction occur within Europe: all north-western European populations (Denmark, Sweden, Germany, The Netherlands, England and France) reproduce thelytokously (Nordlander, 1980; Pannebakker et al., 2004c; Vet, 1983). In contrast, populations south of the Pyrenees reproduce arrhenotokously (Pannebakker et al., 2004c). Thelytoky in *L. clavipes* is induced by infection with *Wolbachia* bacteria (Schidlo et al., 2002; Werren et al., 1995). The southern European arrhenotokous populations are uninfected by *Wolbachia*.

Thelytoky in *L. clavipes* is induced by diploidization of the haploid eggs through anaphase restitution during the first somatic mitosis (Pannebakker et al., 2004b). This mechanism is a form of gamete duplication that results in the generation of completely homozygous offspring (Suomalainen et al., 1987). Because gamete duplication potentially reduces infected populations to clones without genetic exchange, it can have large consequences for the population genetic structure of the wasps. However, *Wolbachia*-induced parthenogenesis does not necessarily result in a reduction of genetic variation, since the parthenogenetic populations are initially derived from uninfected populations. An analysis of the population genetic structure of uninfected arrhenotokous and infected thelytokous populations of *L. clavipes* in Europe did show similar levels of genetic variation in the uninfected and infected populations, but also a clear division between the two modes of reproduction (Pannebakker et al., 2004c). The infected wasps show two distinct haplotypes that are present at different collection sites and that sometimes co-occur at the same locality. The coexistence of multiple clones in the same habitat is likely to be ephemeral, as one clone will eventually replace the others through competitive exclusion or clonal drift (Jaenike et al., 1980). The current coexistence of clonal haplotypes is a direct consequence of the infection history of European *L. clavipes*. Because both clonal haplotypes *L. clavipes* are infected by the same *Wolbachia* strain, multiple infection events by different bacterial strains can be excluded (Pannebakker et al., 2004c). Analysis of the mitochondrial DNA of infected wasps revealed the presence of multiple mitochondrial haplotypes, which suggests the initial *Wolbachia* infection was horizontally transmitted from infected to uninfected wasps (Kraaijeveld, personal communication). Horizontal transmission in the initial stages of the infection will effectively ''freeze'' the genetic variation available in the uninfected population, after which only the best adapted clones survive (Simon et al., 2003).

Besides its drastic effects on population structure, PI-*Wolbachia* also has profound effects for the individual hosts. In the short term,

parthenogenetic reproduction can be considered to be more efficient than sexual reproduction as no resources need to be invested in males or costly mating behavior (Maynard Smith, 1978). The long-term effects, however, are not necessarily beneficial. Parthenogenetic reproduction reduces selection on traits involved in sexual reproduction. Genes coding for these traits are no longer maintained by selection, and mutations in these genes can accumulate freely or even be favored by selection either because they improve the performance of the infected females or in response to the nucleo-cytoplasmic conflict that favors male production (Huigens and Stouthamer, 2003; Pijls et al., 1996; Werren, 1998). *Wolbachia*-induced parthenogenesis offers a unique possibility to study these mutational processes, because high temperature or antibiotic treatment can cure PI-*Wolbachia*-infected females from their infection (Stouthamer et al., 1990). This results in the production of uninfected males and females, in which the decay of sexual functionality can then be studied. The presence of uninfected populations in *L. clavipes* allows for a full comparison of both male and female sexual function, as opposed to similar studies in species where infection has gone to fixation (De Barro and Hart, 2001; Gottlieb and Zchori-Fein, 2001; Weeks and Breeuwer, 2001; Zchori-Fein et al., 1992, 1995).

Antibiotic curing of females from thelytokous populations resulted in males that were able to complete courtship behavior with arrhenotokous females, resulting in successful copulation and sperm transfer (Pannebakker et al., 2004a, 2005). Mating by these ''cured'' males also resulted in a full inhibition of female mating receptivity (Reumer et al., 2007) as observed in *L. heterotoma* (van den Assem, 1969) and *L. boulardi* (Kopelman and Chabora, 1986). Interestingly, for all the thelytokous lines tested, the sex ratio (proportion of males) of the offspring resulting from these crosses was significantly higher than that from crosses between arrhenotokous individuals (in both intra- and interpopulation crosses). Because of haplodiploid sex determination, an increase in sex ratio implies a lower fertilization success for the thelytokous males. *Wolbachia*-infected females were willing to mate with arrhenotokous males but they did not use the received sperm (Pannebakker et al., 2005). Hence, restored males from thelytokous populations are sexually only partially functional, and females from thelytokous populations apparently lost their sexual functionality. *Wolbachia* has become an obligate partner for survival and reproduction in thelytokous *L. clavipes* populations, which makes the transition to thelytoky in *L. clavipes* irreversible. Rather than being infected by a facultative symbiont, *L. clavipes* has become dependent on its reproductive parasite (see Section 12.3.4).

A similar situation is observed in *A. japonica*. In this species, populations from the main islands of Japan are thelytokous and *Wolbachia*-infected,

while populations from the subtropical islands are arrhenotokous and uninfected (Kremer et al., 2009; Mitsui et al., 2007). No other symbiont could be detected in this species. Antibiotic treatment allows the restoration of male production in thelytokous females, suggesting that *Wolbachia* is the causative agent of thelytoky (Kremer et al., 2009). Analysis of reproductive behaviors showed that as in *L. clavipes*, females originating from thelytokous populations are not able to reproduce sexually. However, in contrast to *L. clavipes*, this is due to the complete absence of courtship between males (both from arrhenotokous and thelytokous populations) and thelytokous females. As in *L. clavipes*, males originating from thelytokous populations are still able to reproduce sexually with females from arrhenotokous populations, even though a reduction of fertility might occur. This situation reinforces the idea that when a PI-*Wolbachia* spreads and persists in a population, sexual decay is quicker in females than in males, and is a good indication that selective forces act to promote sexual decay in females. It also shows that when a species gets infected with a PI-*Wolbachia*, the evolution of dependence is swift and frequent.

12.3.3. Oogenesis

The involvement of *Wolbachia* in host oogenesis was only recently discovered in *Asobara tabida*, where it is obligate for successful oogenesis (Fig. 12.3; Dedeine et al., 2001) and is thus far the only such case within the *Drosophila* parasitoids. *A. tabida* is infected with three *Wolbachia* strains of which only the wAtab3 strain is involved in host oogenesis, while the other two strains induce CI of the FM type (Dedeine et al., 2004). Removal of this strain reduces female oocyte production in European strains of *A. tabida* from 260 to 300 to zero oocytes, resulting in complete sterility. The pattern is different for North American *A. tabida* strains, where symbiotic females have a lower oocyte number (approximately 220 oocytes) than the European strains, but removal of *Wolbachia* still leaves females capable of producing about 80 oocytes. These oocytes, however, are smaller than the symbiotic oocytes, and the larvae that emerge from these eggs die before completing development (Dedeine et al., 2005b). Thus, although the underlying mechanisms are likely to be different, failure to produce viable offspring makes *Wolbachia* an obligate partner for reproduction in North American *A. tabida* strains like it is for European strains. Introgression experiments have shown that these two distinct ovarian phenotypes are under the sole control of the host genotype.

The mechanisms underlying *Wolbachia*-dependent oogenesis have been explored in further detail by cytological observations. A detailed description and discussion of these observations can be found in Vavre et al. (2009). Briefly, removal of *Wolbachia* results in the occurrence of

extensive programmed cell death (PCD) in the ovarioles of females from both the American and European populations (Pannebakker et al., 2007; Pannebakker, unpublished data). PCD is a vital part of insect oogenesis, both as a structural developmental process, as well as checkpoint processes at early and mid-oogenesis that regulate oocyte production in response to intrinsic or environmental cues (Buszczak and Cooley, 2000; McCall, 2004; see Vavre et al. (2009) for a brief overview of the role of PCD in insect oogenesis). In *A. tabida*, removal of *Wolbachia* does not induce general apoptosis, but apoptosis is restricted to mid-oogenesis egg chambers, suggesting a specific interaction of *Wolbachia* with the mid-oogenesis PCD pathway (Vavre et al., 2009). Because the mid-oogenesis PCD pathway is also dependent on external signals, such as nutrient deprivation, it is not clear whether PCD in *A. tabida* is directly controlled by *Wolbachia*, or whether induction of PCD is the by-product of another manipulation of the wasp. The involvement of the *Wolbachia* outer surface protein (WSP) in the inhibition of apoptosis of human granulocytes (Bazzocchi et al., 2007) combined with the ability of *Rickettsia* bacteria – close relatives of *Wolbachia* – to manipulate PCD of their vertebrate hosts (Clifton et al., 1998), suggests that direct manipulation of PCD in *A. tabida* is possible (but see Braig et al., 2009).

The discovery of bacterial manipulation of PCD could provide some insight into the evolutionary scenario that resulted in *Wolbachia*-dependent oogenesis in *A. tabida*. Immune responses against intracellular bacteria often involve apoptosis of infected cells (Zychlinsky and Sansonetti, 1997) and, in turn, several intracellular bacteria, including close relatives of *Wolbachia* have evolved apoptosis-inhibiting mechanisms to enable and sustain their own growth environment (Batut et al., 2004; Gao and Abu Kwaik, 2000). Bacterial inhibition of apoptosis can severely reduce the functionality of infected host tissues where apoptosis plays a crucial role, such as in the ovaries. In this coevolutionary process, hosts are then selected to compensate for this bacterial manipulation, and adjust their own gene expression and physiology to the presence of the bacteria (Aanen and Hoekstra, 2007; Pannebakker et al., 2007). Both host and bacteria benefit from this status quo, but will suffer equally when *Wolbachia* is removed and PCD is deregulated. Several other hypothesis have been proposed to explain the evolution of *Wolbachia*-dependent oogenesis in *A. tabida*, and we would like to refer the interested reader to the several papers discussing these in detail, that is, Aanen and Hoekstra (2007), Braig et al. (2009), Dedeine et al. (2001), Pannebakker et al. (2007), Vavre et al. (2009). Irrespective of the exact evolutionary pathway, the outcome of the interaction between *A. tabida* and *Wolbachia* shows that the evolution of obligate mutual dependence between host and parasite can be swift and does not have to involve initial fitness advantages to either partner.

12.3.4. Recurrent evolution of dependence

As discussed in the previous sections, dependence of *Drosophila* parasitoids on *Wolbachia* has at least evolved several times independently: in *A. tabida* and *A. japonica*, and in *L. clavipes* and *L. australis*. In the latter two species, close phylogenetic relationships among *Wolbachia* and parasitoids may suggest a cospeciation event, and make it difficult to consider these two cases as an independent event. Interestingly, dependence in these cases is not associated with a new function to the host, but rather to a loss of autonomy of the host to accomplish a function that obviously preexisted the infection by *Wolbachia* (i.e., oogenesis and reproduction). These results suggest that evolution of dependence can occur swiftly through different mechanisms that include the evolution of tolerance, but also possibly the resolution of the nucleo-cytoplasmic conflict associated with sex-ratio distortion by microorganisms. These remarkable cases contrast with the classic scenarios of evolution of insect–symbiont interactions. It is generally thought that host dependence has evolved secondarily to mutualism through specialization and coevolution between hosts and symbionts. On the contrary, we suggest that the evolution of dependence can precede the evolution of mutualism. The arguments for this scenario are the following: while *Wolbachia* bacteria are selected to provide an advantage to their hosts, very few cases of ''true'' mutualism, where *Wolbachia* provides an additional function to the host, have been reported, suggesting evolution of mutualism is not straightforward. One of the clearest cases is in filarial nematodes where *Wolbachia* is obligate and where both partners have cospeciated (Fenn and Blaxter, 2006; Foster et al., 2005). However, even there, the benefit *Wolbachia* provides is not as obvious as it is in long-term mutualisms in insects (Moran and Baumann, 2000). In addition, it remains possible that the evolution of tolerance is at the origin of the patterns observed in nematodes as well. This suggests the evolution towards mutualism is not straightforward. Another interesting pattern that emerges from insect–symbiont associations is that many of them are labile. Host–Wolbachia cospeciation is not the rule, and if horizontal transmission is able to explain the observed pattern, it is clear that host–Wolbachia associations do not persist long enough within a host for cospeciation to occur.

When combining these two observations, that is, the not so straightfoward evolution towards mutualism, and the observed labile associations, the question arises why so many insects depend on symbionts for their reproduction and development. One option is that mutualism has evolved from facultative associations. The alternative scenario that we propose is that dependence precedes the evolution of mutualism. By consolidating the fate of the two partners, dependence can stabilize host–symbiont associations, leaving more time for the evolution of mutualism.

12.4. STABILITY, REGULATION AND CONSEQUENCES OF MULTIPLE *WOLBACHIA* INFECTIONS

Multiple infections have been extensively studied in horizontally transmitted parasites and were shown to play a major role in the evolution of host–parasite interactions and virulence (e.g. Chao et al., 2000; Frank, 1996). The central idea is that hosts provide a limited space and amount of resources, resulting in competition among parasites. Depending on the type of competition (direct competition among parasites or competition by interference) both an increase or a decrease in virulence can be expected. In both cases, the consequence of multiple infections is a departure from the expected optimal level of virulence when infections occur individually.

This issue has not received much attention in vertically transmitted symbionts. One reason for this is that, until recently, multiple infections with maternally inherited symbionts were thought to be rare, notably due to the bottleneck during transmission that should result in the homogenization of the bacterial population within the host. This image has changed drastically in recent years, with more and more descriptions of multiply infected systems involving different strains of *Wolbachia*, but also different bacterial species (reviewed in Vautrin and Vavre, 2009). Numerous questions arise from these observations: how do these multiple infections invade populations, how are they maintained, how are they regulated, and how do they affect the host? Because *Drosophila* parasitoids are frequently infected by multiple *Wolbachia* strains, that is, both *L. heterotoma* and *A. tabida* each harbor three *Wolbachia* strains, they are excellent systems to study these questions.

12.4.1. Invasion and stability of multiple infections

In all the populations of *A. tabida* and *L. heterotoma* studied to date, which originated from France, Spain and the UK, triple infections have been found (Dedeine et al., 2005b; Haine et al., 2005; Vavre et al., 1999). In addition, a recent survey of field-collected individuals in France showed that all *L. heterotoma* individuals are triply infected, regardless of the collection site or the time in the season they were collected (Vautrin, 2008). Multiple infections thus seem very stable in these associations.

In an experimental setup, parasitoid lines infected by different subsets of *Wolbachia* strains were established for these two species, which allowed the determination of the effect of the individual bacterial strains. In *L. heterotoma*, the three strains induce CI, and are mutually incompatible, even though it is still unknown whether wLhet2 and wLhet3 are able to rescue CI induced by wLhet1 because the attempts to remove wLhet1

without eliminating the two other strains failed (Mouton et al., 2005; Vavre et al., 2001). These results demonstrate that these strains are ''real'' strains that differ not only in molecular markers, but also in their phenotype. In addition, the fact that all these strains are mutually incompatible can explain the spread and stability of multiple infections. Only females infected by all three bacterial strains are able to mate with all males in the population, which allows multiple infections to spread in the population (Frank, 1998; Vautrin et al., 2007). In *A. tabida*, wAtab1 and wAtab2 induce CI and are mutually incompatible (Dedeine et al., 2004). wAtab3 is required for oogenesis and is unable to rescue the CI induced by the two other strains (see also Section 12.3.3). Because uninfected females are sterile, the ability of wAtab3 to induce CI cannot be tested. This shows that also in *A. tabida*, each strain has a particular phenotype (different types of CI, oogenesis) and again this can account for the success and stability of these multiple infections. In this case, however, it is not known what mechanism drove wAtab3 in the population. Obviously, *A. tabida* was able to produce eggs before infection with wAtab3. Either this strain was or still is inducing CI, allowing it to spread in the population, and the involvement in oogenesis and dependence evolved only at a later time. Alternatively, wAtab3 was able to confer an advantage to *A. tabida* by increasing its fecundity, thereby allowing its spread and subsequent evolution of dependence (see also Section 12.3.3).

12.4.2. Regulation of multiple infections and phenotypic consequences

When studying host–microbial associations, one must keep in mind that the interaction involves a single host but a population of symbionts. The size of this bacterial population plays a key role on fundamental parameters of the association. Intuitively, sustaining a higher number of symbionts will increase the cost of infection to the host, which indirectly can have consequences for the bacteria. On the other hand, it might also increase the efficiency of transmission and increase the level of CI. Because of this tradeoff between transmission and infection cost, an optimal density is expected. The situation is more complex when multiple symbionts share the same host since, in addition to this tradeoff, competition among symbionts might change the optimal size of the total bacterial population, but also for individual strains.

Using lines with a controlled genetic background and infected with different subsets of *Wolbachia* strains, it was shown in *L. heterotoma* and *A. tabida* that the total bacterial density increases with the diversity of the bacterial community, while in the mean time the specific density of each CI-inducing strain remained constant, regardless of the composition of the bacterial community (Mouton et al., 2003, 2004). For wAtab3, a slight

increase in its density was observed in lines harboring other strains, suggesting a positive effect of other strains on wAtab3. These results indicate that there is no competition among CI-inducing *Wolbachia* strains within a host, and that some cooperation might exist between wAtab3 and the two other strains in *A. tabida*. Differential localization of the three strains is unlikely since the relative proportion of the different strains is constant in different parts of the body (Mouton et al., 2003, 2004). Interestingly, strain-specific regulation has also been found in other insects infected by different CI-inducing *Wolbachia* strains, suggesting a general phenomenon (Ikeda et al., 2003; Rousset et al., 1999; but see Kondo et al., 2005). This result is surprising given that various studies showed variation in the specific densities of each symbiont when hosts are infected with symbionts belonging to different species (e.g., Goto et al., 2006; Oliver et al., 2006).

Analysis of the phenotypic consequences of density variations revealed a positive correlation between density and infection cost in *A. tabida* (Mouton et al., 2004). A similar trend was also observed in *L. heterotoma* (Mouton, 2003). Similar results have also been obtained in *Drosophila* species (McGraw et al., 2002), suggesting a general phenomenon. The pattern suggested by this correlation is that sustaining a higher number of *Wolbachia* strains increases the infection cost, which in turn should be selected against. However, the infection costs are generally low, and their expression under field conditions is probably limited. In addition, loosing one of the *Wolbachia* strains may result in the exposure of females to CI and these females will be strongly selected against in populations where *Wolbachia* reaches high prevalence, such as in *L. heterotoma* and *A. tabida*. Therefore, specific regulation of *Wolbachia* at the strain level could be seen as a way to limit stochastic loss of some *Wolbachia* strains and hence exposure to CI. In addition, by limiting competition among strains this might also limit the evolution of increased levels of virulence.

How specific regulation is achieved remains a complex issue. Bacterial density is influenced by the bacterial genotype, the host genotype, the environment, and the interactions among these factors (Mouton et al., 2006, 2007). In addition, interactions among symbionts might also take place. This seems to be the case for wAtab3, whose density increases when other strains are present. Interactions are also suspected in *L. heterotoma* where infected lines not harboring wLhet1 have never been obtained despite numerous attempts, suggesting that this strain is obligate for the maintenance of the two other strains. A recent theoretical study demonstrated that such positive or dependence relationships among symbionts can evolve in these systems (Vautrin et al., 2008). Indeed, vertical transmission not only locks one symbiont within a host, but also consolidates the different symbiotic genotypes that are

cotransmitted from one generation to the other. This situation creates extreme partner fidelity among symbionts, which is one of the conditions for cooperation or dependence to evolve (Sachs et al., 2004).

12.4.3. Evolution of *Wolbachia* genomes in multiply infected hosts

Recombination in *Wolbachia* genomes have now been found repeatedly (Baldo et al., 2006a). Multiple infections in a single host create favorable conditions for genetic exchanges among *Wolbachia* strains. The results obtained in *L. heterotoma* and *A. tabida* so far did not prove any exchanges between strains, although it should be noted that only few studies have tackled this question. First, for all cases observed so far, there is complete linkage between the *wsp* sequence of a strain and its induced phenotype. However, the most striking result has been obtained on the WO bacteriophage that infects *Wolbachia*. This phage has been shown to be frequently horizontally transmitted between *Wolbachia* strains (Gavotte et al., 2007). However, within *L. heterotoma*, each *Wolbachia* strain harbors a single and specific phage (Gavotte et al., 2004), showing complete linkage between two specific markers (WSP and WO) that are known to be frequently involved in recombination in other systems (Baldo et al., 2006a; Gavotte et al., 2007). Thus, while recombination between strains does occur, its frequency at the individual host level might be rather limited.

In addition, genomes of intracellular bacteria are known to undergo reductive evolution where functions related to the free-living state, or that are provided by the host, are rapidly eliminated from the genome (Wernegreen, 2002). Interestingly, in the case of multiple infections some other functions might also be dispensable because they are provided by coinherited symbionts. This could create dependence among symbionts such has been recently observed in the aphid *Cinara cedri* (Gosalbes et al., 2008). We have no information whether genome erosion is more pronounced in multiply infected hosts, but it would be interesting to test this hypothesis.

12.5. THE ROLE OF *WOLBACHIA* IN THE INTERACTION BETWEEN PARASITOIDS AND HOSTS

Besides playing an important role in the biology of the parasitoid, *Wolbachia* can potentially mediate the *Drosophila–parasitoid* interaction. By manipulating the reproduction of its hosts (both *Drosophila* and parasitoids), *Wolbachia* has the potential to alter the parasitoid–*Drosophila* dynamics. The consequences of *Wolbachia* infection in this dynamics will depend on its fitness effects for both parasitoid and *Drosophila*. In general,

vertically transmitted parasites can only be maintained in host populations if they do not reduce the fitness of their host (Anderson and May, 1982; Ebert and Herre, 1996). By contrast, *Wolbachia* and other reproductive parasites, can spread through their hosts population, even if they induce a physiological cost to their hosts (O'Neill et al., 1997; Turelli, 1994). Across its range of infection, the effect of *Wolbachia* ranges from an increase to decrease in host fitness (e.g., Min and Benzer, 1997; Teixeira et al., 2008). Because of the intimate interaction between parasitoid and its *Drosophila* host, *Wolbachia* mediated alteration in fitness, can potentially impact both of them. Below, we discuss these fitness effects on *Drosophila* parasitoids and put them in the context of parasitoid–host dynamics.

12.5.1. Effects on host physiology and their consequences on the *Drosophila*–parasitoid interaction

The cost of infection with *Wolbachia* can be expressed in many different traits. Across taxa, *Wolbachia* has been found to negatively impact a wide range of traits, such as longevity in *D. melanogaster* (Min and Benzer, 1997), competitive ability in *Trichogramma kaykai* (Huigens et al., 2004), fecundity in *Tetranychus urticae* mites (Perrot-Minnot et al., 2002) or sperm competitive ability in *D. simulans* (de Crespigny and Wedell, 2006). However, the effects are not always strongly expressed (Harcombe and Hoffmann, 2004) and, at least in *Drosophila*, appear to depend on the genotype of the host (Fry et al., 2004).

Within the *Drosophila* parasitoids, the cost of *Wolbachia* infection has been studied most extensively for *L. heterotoma*. Fleury et al. (2000) were the first to investigate the *Wolbachia*-related physiological and behavioral costs in a *Drosophila* parasitoid. They found a negative impact of infection on female fecundity and adult survival, in addition to a strong reduction in locomotor activity in both sexes. Because locomotor activity is a good proxy for the overall physiological state of individuals, the observed reduction suggests a heavy cost of infection (Fleury et al., 2000). Detailed analysis of the *Wolbachia* density further revealed a positive correlation between bacterial density and infection cost, measured as tibia length, fresh weight and longevity (Mouton, 2003). In addition, *Wolbachia* was found to have negative effects on virulence-related traits in *L. heterotoma*. Elimination of *Wolbachia* from the parasitoid resulted in lower encapsulation rates by *D. simulans* (Fytrou et al., 2006). In *Leptopilina*, suppression of the hosts immune response involves the injection of virus-like particles (VLPs) in the host upon oviposition (see also Chapter 5 in this issue). VLPs render the hosts lamellocytes unable to encapsulate the parasitoid egg (Labrosse et al., 2003). Fytrou et al. (2006) proposed that the observed higher encapsulation rates of *Wolbachia*-infected parasitoids could be due to the bacteria influencing the VLP production in the parasitoid. While the

exact mechanism remains to be determined, their suggestion was corroborated with the recent discovery of *Wolbachia*-induced resistance to viral infections in *D. melanogaster* (Hedges et al., 2008; Teixeira et al., 2008), even though the exact nature of VLP is still controversial.

While *Wolbachia* results in a higher encapsulation rate of *L. heterotoma* eggs, the infection is also harmful to *D. simulans* that are less resistant to parasitoid attacks when infected and this likely affects parasitoid–host dynamics in this system (Fytrou et al., 2006). In species where *Wolbachia* is fixed or nearly fixed (e.g., *L. heterotoma*, *A. tabida*, *D. simulans*) the impact on the *Drosophila–parasitoid* community will be constant over all populations. However, in species where infection is polymorphic such as in *D. melanogaster* or *P. dubius*, complex interactions between *Wolbachia* dynamics and host–parasitoids relationship might be expected. In other words, in species where reproductive manipulation is strong, the impact of the infection cost on *Wolbachia* dynamics will be limited to the invasive phase of the symbionts. On the opposite, in species where reproductive manipulation is milder and does not allow fixation of the symbiont, infection cost may play an important role on the prevalence of the infection, and even on the maintenance of the symbiont. In addition, if infection frequency varies among populations, it might locally modify the coevolutionary dynamics of hosts and parasitoids.

The relation between bacterial density and infection costs was studied in more detail in *A. tabida* (see also Section 12.4.2). Bacterial density and diversity were found to be negatively correlated with dry weight, adult survival and locomotor activity (Mouton et al., 2004). The observed link between bacterial density and infection costs can partly be explained by the higher energy requirements of having more symbionts (Thompson, 1988). However, in multiply infected species such as *A. tabida*, different *Wolbachia* strains can induce different fitness costs on their hosts, resulting in complex interactions between the cost of infection and strain-specific and/or total bacterial density.

Infection with *Wolbachia* does not always result in a cost to its host. For instance, in *D. melanogaster*, *Wolbachia* infection enhances the survival and fecundity of some fly strains (Fry et al., 2004) and in other strains the infection can induce resistance to viral infections (Hedges et al., 2008; Teixeira et al., 2008). For the *Drosophila* parasitoids, not many fitness benefits have been described. To our knowledge, the only known case of *Wolbachia*-induced fitness benefits is found in *L. clavipes*. Here, PI-*Wolbachia* infected females show an increase in longevity compared to naturally uninfected females (Reumer et al., 2007). However, the strains used in this study originated from two different localities (northern and southern Europe). The observed differences could also be the result of adaptations to local ecological conditions, rather than be induced by differences in *Wolbachia* infection.

12.5.2. Indirect effects of *Wolbachia* infection

An indirect short-term benefit potentially provided by infection with PI-*Wolbachia* could be a reduction in the cost of sexual reproduction. Because males are absent, parthenogenetically reproducing organisms do not pay the twofold cost of sex (Maynard Smith, 1978) and are able to sustain a higher population growth. Because PI-*Wolbachia* infected populations consist only of females, they are potentially more effective in attacking *Drosophila* host populations than uninfected parasitoids. This can completely modify the structure of the local community through modifications of the coevolutionary arms race between hosts and parasitoids, but also of competitive interactions among parasitoids. On the long term, however, *Wolbachia*-induced parthenogenesis is not necessarily beneficial to the parasitoids. Because of gamete duplication, the mechanism involved in PI-*Wolbachia*, all genetic variation is effectively frozen in the infected females and recombination among genotypes is impossible (see Section 12.3.2). This lack of genetic variation and recombination could potentially be a handicap in the ongoing arms race between parasitoids and their hosts.

A reduction in genetic diversity of the parasitoid is not only expected for PI-*Wolbachia*. Also for the other phenotypes of *Wolbachia*, presence of the reproductive parasite can drive rapid changes in allele frequencies in the host population (Charlat et al., 2007; Hornett et al., 2006, 2008). *Wolbachia* dynamics can result in a selective sweep in the host genome, potentially fixating deleterious alleles, some of which might be involved in parasitoid–host interactions. However, the exact long-term impact of *Wolbachia* on parasitoid–host interactions is hard to predict and requires additional studies.

12.6. CONCLUSION

Drosophila parasitoids provide insight into a variety of questions related to host–*Wolbachia* interactions. These include, among others, patterns of horizontal transmission, the diversity of *Wolbachia* phenotypes and their consequences on host evolution, and the regulation of bacterial populations in complex systems. The facility to combine field and laboratory experiments makes *Drosophila* parasitoids perfect models to tackle questions on these host–symbiont associations ranging from molecules to community levels. However, the parasitoid way of life also has some constraints. For example, cytological studies on embryos are quite difficult to realize and time-consuming, especially in solitary parasitoids. These studies would, however, provide necessary answers on the mechanisms maintaining the variation in CI phenotypes in haplodiploids. In addition,

artificial *Wolbachia* transfers are also very difficult since the most powerful techniques for such artificial transfers is the injection of *Wolbachia* directly in the embryos (Boyle et al., 1993), which is impossible in endoparasitoids such as *Drosophila* parasitoids. Injection in nymphae or adults may, however, be possible as has been demonstrated in *Drosophila* (Frydman et al., 2006) and it is worth to explore this technique for the parasitoids as well. Clearly, studies of the genetic bases of *Wolbachia* phenotypic diversity would gain enormously from the possibility to create new parasitoid–*Wolbachia* associations by artificial transfers. For example, transfer of wAtab3 in different species would allow the determination of the respective roles of the host and bacterial genotypes in the dependence for oogenesis. Similarly, artificial transfers of CI and PI-*Wolbachia* in different species would be extremely interesting.

One other caveat is the current paucity of genomic data available for *Drosophila* parasitoids, limiting detailed functional studies. However, the recent development of new sequencing technologies is expected to rapidly fill that gap. Such information would allow for the study of host–parasitoid interactions at a molecular level, but would also help studying parasitoid–*Wolbachia* interactions. The current sequencing of the *Nasonia* genome will already revolutionize parasitoid research, but more such efforts are required on other parasitoid systems. Such attempt has been started for *A. tabida*, where more than 30,000 ESTs have been sequenced (Vavre, unpublished data) for *Wolbachia* infected and uninfected individuals to study the basis of *Wolbachia* dependence for oogenesis. In return, this extreme phenotype can be used as a mutant to study the genetic pathways underlying parasitoid oogenesis. Similarly, more data are required on the genomics of *Wolbachia* strains infecting *Drosophila* parasitoids. Very few data are available apart from sequences obtained for phylogenetic studies and WO phage infection. A noticeable exception is the full characterization of the genes encoding the type IV secretion system in wAtab3 (Rances et al., 2008) that could also pave the way to determine key effectors mediating the host–symbiont interaction. Access to *Wolbachia* genomes is also becoming easier, with currently two fully annotated *Wolbachia* genomes available and 14 genomes currently undergoing sequencing or full annotation (Werren et al., 2008). In addition, several *Wolbachia* genomes have been discovered as ''by-product'' from genome sequencing projects of their *Drosophila* hosts (Salzberg et al., 2005), further emphasizing the case for the sequencing of *Drosophila* parasitoids. Having genome information on *Wolbachia* infecting *Drosophila* parasitoids may help to study functional aspects of the interactions with the host but also among *Wolbachia* strains. It might also shed light on the consequences of multiple infections in the genome dynamics of symbionts.

Finally, an important effort is needed at the community level. While patterns of horizontal transmission have been well established at the phylogenetic level, explanation of the variation of *Wolbachia* infection at a wider scale is still required. In addition, the impact of *Wolbachia* infection at the community level has not received much attention. A promising question is certainly how the acquisition of thelytoky impacts the composition of communities. Field surveys and/or population cages experiments could provide some insights on this question.

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