Evolutionary consequences of *Wolbachia* infections

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The past decade has revealed the bacterium *Wolbachia* as the most widespread symbiont of arthropods and nematodes. Behind this evolutionary success is an remarkable variety of effects on host biology, ranging from manipulation of reproduction in favor of females to more classical mutualistic interactions. Here we discuss the potential of *Wolbachia* for promoting evolutionary changes in its hosts.

The intracellular bacterium *Wolbachia* causes an impressive range of effects on its hosts: it can kill males, turn them to females, sterilize uninfected females (Box 1) or behave as a mutualistic symbiont. Being present in numerous arthropod and filarial nematode species (reviewed in [1]), this symbiont is attracting attention from cell, developmental and evolutionary biologists. Excellent overviews of *Wolbachia* biology have been presented elsewhere [2–4], and in this article, we concentrate on the significance of *Wolbachia* for the evolution of its hosts. We first discuss the involvement of *Wolbachia* in the function and design of core biological process, such as sex determination, cell cycle and gametogenesis. We then show that *Wolbachia* can provide material for evolutionary novelty, by contributing to host functions, or even transferring genes to the nucleus. Finally, we turn to consider higher-level *Wolbachia* effects, ranging from sex role-reversal to birth, and death, of new host species. The unpublished results we discuss were presented at the Second International *Wolbachia* Conference (Crete, Greece, 9–15 July, 2002)

Wolbachia–host conflicts and the diversification of core process

Sex determination

Most arthropods produce males and females, but the mechanism of sex determination varies among species. The chromosomal basis of sex determination can change, with female heterogamety, male heterogamety, genic and haplodiploid systems all occurring within insect species [5]. In addition, the particular genes involved in sexual differentiation vary. For example, the *Sex-lethal* (*Sxl*) gene is present in many Diptera, but only in *Drosophila* is it the key switch gene of sex determination [6]. Also, *msl-3*, is involved in dosage compensation in *Drosophila*, but not in *Sciara* [7].

As a frequent manipulator of host reproductive biology (Box 1), *Wolbachia* must interact with the host sex-determination system. Male-killing (MK, Box 1) bacteria must either detect host sex and then act to kill males, or interfere directly with sex determination to produce male-specific death. Given that death occurs during embryogenesis, they must therefore interact with upstream components of sex-determination pathways. Feminizing bacteria (Box 1) must also alter the sex-determination pathway in fundamental ways, although this might occur in more downstream elements of the system.

The host sex-determination system is therefore at the heart of the interaction between host and reproductive parasite. Given that male-killing or feminizing manipulations are deleterious to the host in the vast majority of cases (Box 2), selection will favor host mutations that prevent the action of the parasite. If parasite prevalence is high (as is often the case), then the selection pressure for modifiers of sex determination that circumvent the action of the *Wolbachia* will be very strong. In such conditions, fairly revolutionary modifications in sex-determination system can be selected; changes that otherwise would be costly to the host [8,9].

The role of the feminizing *Wolbachia* in the modification of the sex-determination system of the woodlouse is now well recognized [10]. The pill woodlouse *Armadillidium vulgare* is ancestrally female heterogametic, and some populations remain this way today. In these populations, sex determination is straightforward, with ZZ individuals developing as males following development of the androgenic gland. Androgenic gland formation is induced by androgenic hormone, as proven by the development of ZW individuals (usually female) into functional males following injection with purified extracts of the hormone [11]. In other populations of this species, there is a strain of *Wolbachia* that feminizes its hosts. Thus, ZZ individuals infected with *Wolbachia* do not form androgenic glands, despite their karyotype, and therefore develop as females. The spread of the feminizing *Wolbachia* has caused the loss of the female-determining W chromosome from infected populations, and all individuals in these populations are ZZ. The female-determining factor has switched from being the W chromosome to the feminizing *Wolbachia*: individuals are female if *Wolbachia* is present and active, and male if it is either absent or inactive. Selection on the host to promote the production of sons has in turn favored

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Box 1. *Wolbachia* misdeeds

Wolbachia is intracellular and is passed from one generation to the next by females only, through the egg cytoplasm. It is therefore subject to different selective pressures from nuclear genes: its effect on males does not affect its own reproductive success, because males are 'dead ends' through which they are not transmitted. By contrast, any *Wolbachia* variant that makes infected females produce more daughters, or daughters that survive or reproduce better than uninfected ones, will invade uninfected host populations, whatever the effects on males. This rationale probably explains why *Wolbachia* evolved the 'reproductive manipulations' described here.

Male killing (MK)

In Coleoptera [55,56], Lepidoptera [41,57,58] and Diptera [59] *Wolbachia* kills the sons of infected females' (reviewed in Refs. [1,60]). This is not deleterious to the bacterium, because it is transmitted only by females. It is advantageous if the hosts' daughters benefit from their brothers' death. Benefits might include eating their brothers (which happens in ladybirds and flour beetles), a reduced probability of inbreeding or reduced intensity of antagonistic interactions between siblings. Infected females produce daughters with a higher probability of survival than uninfected ones, allowing their cytoplasm to be more efficiently transmitted, and the infection to spread.

Feminization in diploids (FD)

In isopod crustaceans [61,62] and Lepidoptera [13,63], *Wolbachia* turns males into females (reviewed in Ref. [10]). Infected females produce twice as many daughters as uninfected ones, allowing their cytoplasm to be transmitted to twice as many granddaughters.

Parthenogenesis induction (PI)

In haplodiploid species (Hymenoptera, thrips and mites), *Wolbachia* also turns males into females (reviewed in [21]). In these organisms, males normally develop from unfertilized haploid eggs (arrhenotokous parthenogenesis), whereas females develop from fertilized diploid eggs. The bacterium effects a doubling of chromosome number in the unfertilized haploid eggs, rendering them diploid. This leads to development as an asexually produced female, so that infected females produce twice as many daughters as uninfected ones, allowing their cytoplasm to be transmitted to twice as many granddaughters.

Cytoplasmic incompatibility (CI)

Reproductive incompatibility between populations of the mosquito *Culex pipiens* was reported in the 1950s [64], but it was not until the 1970s that *Wolbachia* was identified as the causative agent [65]. It is now well known that embryonic mortality occurs when males that bear *Wolbachia* mate with uninfected females, but if the female is infected, then the same cross is perfectly viable. *Wolbachia* here 'utilizes' males to make uninfected females' offspring inviable, and thus gain a relative fitness benefit. Incompatibility also occurs if the two partners bear different *Wolbachia* variants. It is then referred to as bidirectional CI, because both directions of cross are lethal. CI has now been described in numerous host species, making it the most widespread *Wolbachia*-induced manipulation (reviewed in Refs [66,67]). It also seems to be the only *Wolbachia*-specific phenomenon: other bacteria or unicellular eukaryotes are known to cause MK (reviewed in Ref. [60]), FD (reviewed in Ref. [10]) and PI [68,69].

Who's got the control?

Both host and bacterial factors seem to be important in determining which type of manipulation is expressed, and with which efficiency. In terms of quantitative variation, the strength of CI (the rate of embryo death in incompatible crosses) is known to depend on bacterial properties as well as host genomic background [18,19]. Host genes also seem to modulate CI effects in *Nasonia* wasps (S. Bordenstein, unpublished).

In terms of qualitative variation, different *Wolbachia* in the wasp *Asobara tabida* have totally different phenotypic effects, suggesting that bacterial factors are determining which type of manipulation is achieved (F. Dedeine, unpublished). Accordingly, in terrestrial isopods, Moret *et al.* [70] observed that a CI-inducing *Wolbachia* retained its ability to induce CI after transfer into a host feminized by its natural infection. However, similar experiments in Lepidoptera led to very different conclusions; Fujii *et al.* [58] showed that the feminizing *Wolbachia* from *Ostrinia scapulalis* [13,63] induces MK after transfer in the new host *Ephestia kuehniella*, although CI is induced by the natural infection in this host [71]. Furthermore, a *Wolbachia* strain that does not appear to cause sex ratio distortion in its native host, *Cadra cautella*, becomes a male killer on transfection to *Ephestia kuehniella*, where CI is induced by the natural infection [72]. These results highlight that the phenotype depends on bacterial and host factors, and on the interaction between these two.

host genes that prevent either the action or transmission of the bacterium [12]. In summary, the spread of the feminizing *Wolbachia* has caused a change in sex determination from the ancestral system of female heterogamety to one where sex is determined by *Wolbachia* as the female-determining element, balanced against host genes that affect *Wolbachia* transmission and action, which are the new male-determining elements, with no karyotypic differences between male and female.

The *Armadillidium* case study establishes that *Wolbachia* can be important in the design and function of host sex-determination system. The influence of feminizing *Wolbachia* on sex determination is unlikely to be confined to *Armadillidium*, as indicated by the creation of ZZ females by feminizing *Wolbachia* in some populations of the moth *Ostrinia furnacalis* and the butterfly *Eurema hecale* [13,14]. Furthermore, the low prevalence of the infection in these populations suggests there could be host modifiers of *Wolbachia* action, as found in *Armadillidium* (as theory predicts very high prevalence of the parasite in the absence of host genes modifying the parasite action).

The case for feminizing bacteria driving sex-determination system evolution is strong. However, a widespread influence of inherited bacteria on the evolution of arthropod sex determination will depend on the importance of male-killing bacteria in the process, as these are present much more widely. Much less is known about the mechanism of the interaction between male-killers and the host sex-determination system, and it will be important to delineate which parts of sex determination are key in the recognition of host sex, and therefore the potential focus of selection. When we know the level in the sex-determination cascade at which male-killers detect sex, we will be able to appraise the potential role of these bacteria in the evolution of sex-determining systems, and the levels at which they could have been important.

Sexual differentiation

Recent studies indicate that *Wolbachia* can also be important in downstream processes of sexual differentiation, such as germline development. Starr and Cline [15] observed that the phenotypes of certain loss-of-function mutations of *Sxl*, which normally produce

Box 2. *Wolbachia* and their hosts: conflicting or not?

Wolbachia are often referred to as reproductive parasites or selfish genetic elements [4,9]. These terms assume that *Wolbachia* and its hosts are conflicting and that reproductive manipulations decrease the host's fitness. Here we delimit situations where *Wolbachia* and their hosts are actually conflicting, and therefore, where evolution of resistance mechanisms is to be expected. Before starting, we should point out that host fitness refers here to the reproductive success of nuclear autosomal genes. Mitochondrial genes always have common interests with *Wolbachia*, and are thus not considered.

Sex-ratio distortion

Male killing (MK), feminization in diploids (FD) and parthenogenesis induction (PI) result in female-biased sex ratio: males get rarer as infection frequency increases. Because the rarest sex always has the highest reproductive success, producing females rather than males in female-biased populations is costly for host nuclear genes [73,74]. In other words, any nuclear gene that would eliminate the infection or repress its effect would increase in frequency. The intensity of the conflict (or the strength of selection for resistance genes) depends on the frequency of infection: the more biased the sex ratio (i.e. the more frequent the infection), the stronger the cost of sex-ratio distortion.

MK bacteria are more costly than other sex-ratio distorters: added to the cost of sex-ratio distortion is the fact that infected females produce fewer offspring than uninfected ones, because their sons die. Thus, for equal infection frequencies, selection for resistance genes is always the strongest with male killers.

Regarding the intensity of conflicts, turning males to females in diploids (i.e. FD) or in haplodiploids (i.e. PI) also have different consequences. In diploids, males can get so rare and sperm so limiting that not all eggs will be fertilized; in haplodiploids, females do not need males. Thus, with extreme infection frequencies, FD is more costly than PI. One should not conclude, however, that resistance is more likely to evolve in diploids. Indeed, although the conflict caused by PI is less intense, it can last longer, because populations where infection is fixed do not go extinct. The conflict will vanish only when genes preventing sexual reproduction have invaded, which can occur through two main processes, as discussed in the main text. At that stage, producing females only is not costly anymore, because males are sterile.

Cytoplasmic incompatibility

The costs of CI are not straightforward to predict. This question was investigated by Turelli [75]. For females, bearing *Wolbachia* is advantageous, because it protects the eggs from CI-induced mortality. By contrast, bearing *Wolbachia* is deleterious for males, because it reduces fertility in crosses with uninfected females. The direction of selection thus depends on infection prevalence: when *Wolbachia* prevalence is low, the cost suffered by infected males is far stronger than the benefit to infected females; when it is high, costs suffered by infected males will be much lower than benefits to infected females. Overall, costs and benefits of bearing *Wolbachia* will equilibrate when infected and uninfected individuals are equally frequent, which is only a transient stage. When infection frequency passes 50%, selection will favor nuclear genes increasing female transmission rates. By contrast, nuclear genes reducing levels of embryonic mortality in crosses between infected males and uninfected females are selected for in most conditions. More precisely, if infection frequency is lower than 100%, host factors that would allow infected males to exclude *Wolbachia* from testes, or to resist the mechanism of CI in embryos, are advantageous.

aberrations of oogenesis, were rescued by *Wolbachia*. This study is complemented by the study of Dedeine *et al.* [16], which indicated *Wolbachia* is essential for oogenesis in the parasitoid wasp *Asobara tabida*. Collectively, they indicate that the influence of *Wolbachia* in processes of female sexual differentiation goes beyond altering the basic system of sex determination to the process of gametogenesis. Surprisingly, the study by Starr and Cline indicates that *Wolbachia* interacts with *Sxl*, the key gene in sex determination and gametogenesis [15]. Thus, important interactions can occur between *Wolbachia* and key genes in host sex determination and sexual differentiation.

Wolbachia can also have a role in the design of the male germline. In the case of *Wolbachia* that induce cytoplasmic incompatibility (CI, Box 1), host genes preventing *Wolbachia* entry into testes are selected for, as CI lowers male host fitness (Box 2), and infection can also lower spermatogenesis efficiency [17]. A role of host genes in regulating the entry of *Wolbachia* into the testes is suggested by *trans*-infection experiments. For instance, the strain of *Wolbachia* in *Drosophila melanogaster* causes weak CI, but following *trans*-infection to *Drosophila simulans* induces strong CI [18]. Reciprocally, a natural strain from *D. simulans* shows high CI in *D. simulans*, but low CI on *trans*-infection to *D. melanogaster* [19]. The *Wolbachia* strains in *D. melanogaster* achieve only very low densities in sperm cysts compared with those in *D. simulans* ([18,20], Z. Veneti, unpublished). These differences are attributable to the host and not the bacterium, and could be interpreted as selection on *D. melanogaster* having favored alterations in testes biology to exclude *Wolbachia* from this tissue.

Interaction with the cell cycle

Beyond the processes of sex determination and sexual differentiation, *Wolbachia* also interacts with cell-cycle processes. Parthenogenesis induction (PI, Box 1) in haplodiploid species involves alterations of mitosis or meiosis. In these organisms, where normally males develop from unfertilized haploid eggs and females from diploid eggs, *Wolbachia* induces female development by restoring diploidy, most often through gamete duplication [21], but more rarely by preventing normal meiosis [22].

Cell-cycle disruption is also observed in *Wolbachia*-induced CI. In incompatible crosses of *Drosophila*, paternal chromosomes are undercondensed at the first embryonic mitosis, which results in their loss or improper segregation [23]. In addition, by observing early development in living embryos of the wasp *Nasonia*, Tram and Sullivan showed that nuclear envelope breakdown, an important stage of the first mitosis, is delayed [24]. This suggests that *Wolbachia* targets cell-cycle regulator(s) acting upstream of both chromosome condensation and nuclear envelope breakdown (e.g. possibly the Cdk1/cyclin B complex) [24].

Prospects

Better understanding the consequences of *Wolbachia* on the evolution of its hosts' core traits will require us to determine the *Wolbachia* genes involved in reproductive manipulation, as well as their targets. Characterization of *Drosophila* mutants mimicking *Wolbachia*'s CI effects, such as *ms(3)K81* [25,26] and maternal haploid (*mh*) [27,28], could give useful insights. In the near future, genomic approaches will also be valuable. The first full

Wolbachia sequence has now been obtained (strain wMel from *D. melanogaster*; S. O'Neill, unpublished) and three other strains from different arthropods are currently being sequenced (K. Bourtzis, unpublished). This will provide the basis for analysis of *Wolbachia* factors involved in host manipulation. As an example, different *Wolbachia* strains from *D. melanogaster* (namely wMel and wMelPop) that differ in virulence in adult hosts, have been found to differ by an important deletion/insertion (S. O'Neill, unpublished). The genome sequence is also the basis for the development of microarray techniques, allowing comparison of the transcription patterns of *Wolbachia* displaying different properties. For example, two very closely related *Wolbachia* variants of *D. simulans* differ with regard to their ability to induce CI: wNo does, and wMa does not (reviewed in [29]). Identifying differences in the transcriptome of these variants might provide candidate pathways underlying the genetic basis of phenotypic differences. Comparison of the proteome of spermatozoa and eggs from infected and uninfected individuals using 2D gel electrophoresis will also identify candidate genes involved with incompatibility (H. Harris, unpublished). The next step is testing the function of these loci. Here, ectopic expression in *Drosophila* will be important. Finally, transformation of the *Wolbachia* genome, hopefully feasible in the near future, will prove to be a powerful approach.

Providing novelty

Domestication

Domestication (or co-option) refers to the use by the host of some properties of selfish genetic elements. Now well recognized as occurring with transposable elements [9,30,31], this can also occur with inherited microorganisms. In its filarial nematode hosts, *Wolbachia* does not obviously manipulate reproduction, but experiments based on antibiotic therapy revealed that *Wolbachia* is necessary to nematode embryogenesis and other developmental stages. In this case, therefore, *Wolbachia* is best regarded as an essential partner in host function (reviewed in [1]). This role is further emphasized by the congruence of *Wolbachia* and filarial phylogeny for more than 100 million years, which is typical of 'partnership' interactions. The precise role of *Wolbachia* in nematode function is unclear, but it has been suggested that in addition to its contributions to nematode physiology, *Wolbachia* could help to evade oxidative damage caused by the mammalian host's immune system in response to nematode infection. Indeed, *Wolbachia* produces a catalase enzyme that is functional in the detoxification of hydrogen peroxide [32].

Wolbachia is also involved the function of other hosts; for example in mosquito, where subtle increases of host fitness have been observed [33]. More dramatically, Dedeine *et al.* [16] discovered that eliminating *Wolbachia* from the parasitic wasp *Asobara tabida* prevents correct development of the female germline: antibiotic-treated hosts do not produce eggs. Although other interpretations are not ruled out [34], this result is best explained by assuming that *Wolbachia* takes part in host oogenesis. Presumably, the wasp has lost some component of oogenesis because *Wolbachia* was providing something better, or at least not worse, than the host itself. The wasp phenotype after antibiotic treatment strikingly resembles that of the above-mentioned *Sxl* loss-of-function mutants in *D. melanogaster* [15], and suggests a common interaction between *Wolbachia* and germline function.

Gene acquisition

Wolbachia and mitochondria both belong to the α-proteobacteria clade, and are both maternally inherited symbionts [35,36]. One striking aspect of the eukaryote–mitochondria symbiosis is that numerous mitochondrial genes have been transferred to the nuclear genome [37]. Until very recently, gene transfer between *Wolbachia* and the host genome was only speculation, but now there is hard evidence [38]. Based on sequence data, three *Wolbachia* variants were initially described in the adzuki bean beetle *Callosobruchus chinensis* [39], with most individuals being triply infected. Aiming to understand the respective phenotypic effects of the three *Wolbachia*, Kondo *et al.* undertook to separate them through limited antibiotic treatment, and observed that one variant (namely wBruAus) was never lost. Quantitative PCR revealed that wBruAus has a lower titer than the two other variants in triply infected individuals. Most surprisingly, wBruAus was found to be transmitted not only by females, as expected, but also by males, and to segregate like an X-linked trait. The authors also observed that females present twice as many copies of wBruAus than males. Together, these results strongly suggest that wBruAus is not a bacterium, but a bacterial genome fragment inserted on the X chromosome. Confirming this, they found that a eukaryotic transposable element flanks 'wBruAus' sequences.

Higher levels evolutionary consequences

We now turn to consider the consequences of *Wolbachia* on higher-level biological traits, ranging from sex role-reversal to speciation and extinction.

Sexual selection and population sex ratio

Sexual selection theory states that males compete for females because males increase their reproductive success through multiple copulations. Conversely, female reproductive success mainly depends on the quality, rather than quantity, of males fathering their offspring. Thus, males compete for access to females, and females are choosy [40]. As is the case for other sex-ratio distorters, *Wolbachia* has the potential to perturb this rule, by making males rare. When males are rare, competition between males is reduced, and competition between females can occur. Traits associated with male–male competition and female choosiness are thus expected to be lost in female-biased populations, and traits associated with female–female competition and male choice should be visible. Very high frequencies of infection by an MK *Wolbachia* were reported in the butterfly *Acraea encedon* [41], and large number of virgin females were observed, suggesting that reproduction is sperm limited. This is apparently behind a peculiar mating behavior where females tend to group together and to mate readily, which is typically a male mating strategy.

In this species, and others where extreme sex-ratios are observed [42], many sexually selected traits would be worth investigating. Those associated with sperm competition could be of particular interest: in populations where males are rare, ejaculate size is expected to be reduced, first because sperm competition is reduced, and second because the number of matings achieved by any male is increased.

The loss of sex

Wolbachia that induce parthenogenesis (PI strains) can invade haplodiploid species without eventually inducing population extinction, because females can reproduce without males. In some species, males are indeed absent in natural populations (or very rare), but can be obtained by removing *Wolbachia* with antibiotics. However, in several cases these males either fail to mate successfully or to fertilize females (reviewed in [21]), which has been interpreted as sexual traits having degenerated in species where selection on such traits is relaxed.

An interesting alternative interpretation was recently put forward (R. Stouthamer, unpublished). In female-biased populations, producing males is advantageous. In haplodiploids, this means that remaining a virgin (or at least preventing eggs from being fertilized) is beneficial for uninfected females, because uninfected unfertilized eggs develop into males. The same is true for infected females if some of their unfertilized eggs can develop into males (i.e. if gamete duplication does not affect 100% of infected eggs, or transmission efficiency is less than 100%). Selection on nuclear genes can thus drive 'virginity genes' to fixation. Such genes do actually appear to exist in the parasitic wasp *Telenomus nawai* (G. Jeong, unpublished).

Speciation

As discussed above, *Wolbachia* alterations of host reproduction might induce adaptive changes in host nuclear genes. Such nucleo-cytoplasmic coevolution has the potential to accelerate divergence, and thus reproductive isolation, between populations of different infection status. Aside from this, CI can have direct consequences on gene flow between populations, making it a potentially important speciation agent. First, incompatibility between males from infected populations and females from uninfected populations (unidirectional incompatibility) can reduce gene flow in one direction. This seems to be important in the isolation between two closely related *Drosophila* species [43], but in other *Wolbachia*-infected insects, unidirectional incompatibility appears to be independent of CI itself [44]. Possibly more powerful is bidirectional incompatibility, occurring between populations infected by different *Wolbachia* variants. The potential of bidirectional CI in inducing, or contributing to speciation has been discussed and debated in detail elsewhere [45–48]. Theory suggests this phenomenon can be efficient in promoting divergence [49], but compelling empirical evidence is lacking so far. In *Nasonia* wasps, bidirectional incompatibility appears to have arisen early in speciation, but might not be the causal agent [50]. In *D. simulans* and in the birdnest blowfly *Protocalliphora siala*, two species where different populations are infected with incompatible *Wolbachia* strains, the bacterium does not seem to cause detectable divergence between populations at the nuclear level ([51], E. Baudry and J. Werren, pers. commun.).

Extinction

Aside from giving birth to new species, *Wolbachia* might also cause their death. First, *Wolbachia* can increase extinction risks directly by decreasing population productivity (the number of offspring produced at every generation). In diploid species, sex-ratio bias toward females can decrease population productivity if extremely high infection frequencies are reached. (By contrast, limited female biased sex-ratio can increase population productivity as a small number of males can insure fertilization of many females.) Cases are known where sex-ratio is too biased for all females to be fertilized [41], but the effects on population size have not been assessed. In addition to sex-ratio distortion, CI can also have heavy consequences on population productivity. During the process of invasion of an uninfected population, numerous crosses are incompatible and thus lead to inviable progeny. Dobson *et al.* [52] investigated this issue and emphasized the possible use of serial introduction of different CI strains for reducing population size of pest species.

In the long term, *Wolbachia* can also increase extinction risk by reducing genetic diversity. Effective population size is greatly reduced by sex-ratio bias: genetically speaking, population size is close to that of the rarest sex [53]. Finally, in parthenogenetic populations, the lack of sex leads to the accumulation of deleterious mutations, and lower evolvability [54].

Conclusion

In many ways, *Wolbachia* has come of age. It is now emerging as a potent evolutionary force. Its interactions with host sex-determination systems and the cell cycle place it at the heart of organismal biology, and its effect on host populations can frame sexual behaviors and species diversity. Moreover, *Wolbachia* can become indispensable to its hosts, suggesting reproductive parasitism as a possible pathway for the emergence of evolutionarily stable and intimate associations. The complete genome will give new impetus to understanding the mechanistic basis of *Wolbachia*/host interactions, which will in turn provide a fuller understanding of the degree to which this bacterium has framed its hosts' biology.

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