Symbiosis, 29 (2000) 151–161
Balaban, Philadelphia/Rehovot

Minireview

First International *Wolbachia* Conference *Wolbachia* 2000

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Received August 2, 2000; Accepted August 26, 2000

From June 7 to 12, 2000, the "First International *Wolbachia* Conference" took place at the Orthodox Academy, Kolymbari, Crete, Greece. More than 100 researchers from four continents gathered to exchange their latest findings on the somewhat enigmatic bacterium *Wolbachia pipientis*. The talks of this meeting gave a thorough overview over the current trends in *Wolbachia* research and an outlook on a new development that promises to provide researchers with new tools and enable them to approach as yet unanswered questions of *Wolbachia*-host interactions.

Wolbachia has taken a slow route to its present popularity. It was first identified in the mosquito *Culex pipiens* in 1924 (Hertig and Wolbach, 1924) and described in 1936 as the new genus and species *Wolbachia pipentis* (Hertig, 1936). Scientific progress in modern biology nearly always turns out to be technology-driven. And so with the advent of PCR techniques, it became possible to easily probe for the presence of *Wolbachia* in the cytoplasm of its host organisms and to obtain the necessary sequence information to allow the construction of molecular phylogenies. In the early 90's, O'Neill and colleagues

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0334-5114/2000/$05.50

described a 16S *rDNA*-based PCR assay (O'Neill et al., 1992) which detects *Wolbachia* infections in a variety of arthropod species (insects, terrestrial crustaceans, mites and arachnidae) and also filarial nematodes. Using the same gene as a molecular marker, they classified *Wolbachia* as a close relative of mammalian pathogens such as *Rickettsia, Ehrlichia, Cowdria* and *Anaplasma,* all of which belong to the same group, α-Proteobacteria, from which the modern mitochondria are descended. Since then, *Wolbachia* have been intensively studied by a growing number of researchers. Recent reviews summarise the development of the field, current knowledge and open issues (Werren, 1997; Bourtzis and O'Neill, 1998; Bourtzis and Braig, 1999; Stouthamer et al., 1999). What has increasingly attracted the interest of researchers are the effects that, in many cases, *Wolbachia* exert on their host's reproduction. *Wolbachia*-associated reproductive alterations include: the induction of parthenogenetic development; overriding chromosomal sex determination to convert infected genetic males into functional females; male-killing; hybrid-breakdown, which was first reported at the conference; and most commonly the induction of cytoplasmic incompatibility. Each of these reproductive effects favors transmission of this maternally inherited bacterial agent. In the following, we will very briefly introduce some aspects of the *Wolbachia*-host biology and illustrate them with examples from presentations given at the "First International *Wolbachia* Conference".

Wolbachia-induced reproductive phenotypes: Just variants of each other?

It is presently not clear if the different *Wolbachia*-induced host phenotypes are based on separate modifications caused by the bacteria or if they are variations of the same endobacterium-host interaction. In this context, it might be worth to recall that *Wolbachia* phylogeny shows no congruence with the induced reproductive phenotypes (van Meer et al., 1999). There is, of course, the possible explanation that the respective traits evolve easily and hence repeatedly. It became evident from several presentations at the conference that the *Wolbachia*-induced phenotypes are not restricted, as previously accepted, to specific host groups and that more than one phenotype might be expressed in a given host group (see examples below). Also, a given *Wolbachia* strain can induce different phenotypes in different host backgrounds, as was reported for the wSca strain by Tetsuhiko Sasaki (University of Tokyo). This bacterial strain, when present in its natural host *Ostrinia scapularis,* induces feminization. However, artificially transferred into another moth, *Ephestia kuehniella,* leads to male killing (for definition, see below). Surprisingly,

naturally infected *Ephestia kuehniella* with the wKue *Wolbachia* strain express cytoplasmic incompatibility.

Cytoplasmic incompatibility

Cytoplasmic incompatibility (CI) is the best-studied reproductive effect of *Wolbachia* infections. CI has been documented in diverse insect taxa including Coleoptera, Diptera, Hemiptera, Hymenoptera, Orthoptera and Lepidoptera, as well as in the terrestrial isopod *Porcellio dilatatus* and in mites. In short, CI appears as a form of embryonic lethality in crosses between infected males and uninfected females. In haplo-diploids, where unfertilised eggs develop into haploid males, CI crosses result in male-biased offspring. Bidirectional CI is observed between host strains that are infected with *Wolbachia* of different incompatibility classes.

Very little is known about how *Wolbachia* induces CI. Based on the current genetic and cytogenetic evidence, the mechanism of CI involves a dual action: first, a 'modification' of sperm chromosomes and second, a 'rescue' process in the eggs (Breeuwer and Werren, 1993; Bourtzis et al., 1998). There have been many suggestions as to how modification and rescue in CI is achieved molecularly. There are no *Wolbachia* in mature sperms. Thus, it seems clear that the chromosomes are modified during spermatogenesis. This can be either understood as the excretion of a product from *Wolbachia*, which modifies chromatin, or as depletion by the bacterium of a factor which is necessary for proper sperm maturation. A modification of this idea is that *Wolbachia* induces the host to produce or deplete/suppress (Bourtzis and O'Neill, 1998) such factors. Infected eggs (in which bacteria are present) then somehow alleviate this modification, uninfected eggs do not.

In this context, the identification (Shinji Masui, University of Tokyo) of a type IV secretion system in a *Wolbachia* strain from *Teleogryllus taiwanemma*, a cricket, may be illuminating, since secretion systems of this type are used by pathogenic bacteria as different as *Helicobacter*, *Agrobacterium* and virulent *Escherichia coli* strains to secrete factors which modify the host-metabolism.

A complementary way to gain insight into *Wolbachia*-induced alterations of the host- metabolism is not to look at the signal(s) given by the endocellular bacterium, but instead at host genes induced or repressed upon *Wolbachia* infection. Cort Anderson (University of Chicago, USA) told the audience that there is an increase of zipper-RNA, encoding non-muscle myosin heavy chain (MHC) in the testes of infected *D. simulans* males. This is intriguing, as MHC plays a role in cell division and oogenesis. Whether it also has a function in sperm development, however, remains to be seen.

Parthenogenesis

Wolbachia-induced parthenogenesis (PI) had previously only been reported for various wasps (Hymenoptera), where males are normally haploid and develop from unfertilised eggs (arrhenotokous parthenogenesis). It has been shown that *Wolbachia* somehow induces genome duplication, giving rise to diploid unfertilized eggs which then develop as parthenogenetic females (Stouthamer and Kazmer, 1994). At the conference, there were first reports for PI outside the Hymenoptera. Andrew Weeks (University of Amsterdam, Netherlands) presented data about *Wolbachia*-induced parthenogenesis in a genus of haplo-diploid phytophagous Tetranychidae mites, the *Bryobia*. Hiroaki Noda (National Institute of Sericultural and Entomological Science, Ibaraki, Japan) reported on a parthenogenesis-inducing *Wolbachia* strain in a species of predatory thrips, *Franklinothrips vespiformis*. There was also a presentation about *Wolbachia*-induced parthenogenesis in the springtail *Folsomia candida* (Hexapoda, Collembola) by Tom Vandekerckhove (University of Gent, Belgium).

Feminisation

Wolbachia-induced feminisation (F) has been previously only found in isopod crustaceans where *Wolbachia*-infected genetic males develop into functional females. The default development of isopods takes the female route. It is the action of the androgenic gland that effects development into males (Suzuki and Yamasaki, 1991). Gilbert Martin (CNRS Poitiers, France) presented work on the woodlouse *Armadillion vulgare*. If injected into male *A. vulgare*, *Wolbachia* produce so called neofemales whose androgenic glands are hypertrophied with an increased production of androgenic hormone. Following up the etiology of this hypertrophy might give a lead to the molecular action of *Wolbachia*, which, in this case appears to be mechanistically very different from the effect on insects or mites. The meeting also provided the first report of *Wolbachia* inducing feminization outside Isopoda. Daisuke Kageyama (University of Tokyo, Japan) described the trait in two moth species, *Ostrinia furnacalis* and *O. scapularis* (Lepidoptera: Crambidae).

Male killing

Michael Majerus (University of Cambridge, United Kingdom) gave a survey of male killing (MK) bacteria, reporting that this phenotype can be caused by a variety of intracellular eubacteria, among them *Spiroplasma* (Hurst et al., 1999b), *Flavobacteria* (Hurst et al., 1997), *Rickettsia* (Werren et al., 1994) and *Wolbachia* (Hurst et al., 1999a). Male embryos fail to develop into adults. He

then presented the novel finding of a nuclear suppresser of male killing in a dipolid species, the ladybird beetle *Cheilomenes sexmaculatus*. Greg Hurst (University College, London) reported on *Wolbachia*-induced male killing in *Drosophila bifasciata*. The data suggest that the penetrance of MK correlates with the density of bacteria in the eggs. This system might be a step forward in experimental elucidation of the mechanism of MK because of the particular suitability of a *Drosophila* species as model system. Interestingly, the same *Wolbachia* strain also induces CI in the same host.

Hybrid breakdown

Filipa Vala (University of Amsterdam, The Netherlands) reported the first case of a *Wolbachia* infection causing a hybrid breakdown phenotype in populations of the phytophagous mites *Tetranychus urticae*. Hybrid breakdown results in an increased F2 recombinant male offspring mortality. Data presented indicate that there is genetic variation of the effects associated with *Wolbachia* infection, and that this variation is host determined. It has to be noted that hybrid breakdown is an important post-zygotic isolation mechanism and thus, *Wolbachia* may be responsible for reproductive incompatibilities observed between different populations of *T. urticae*.

Wolbachia in filarial nematodes

One of the most exciting developments in *Wolbachia* research is the identification of this multi-faceted bacterium in filarial nematodes which are major human and animal pathogens. In nematodes, *Wolbachia* appears to have a truly mutualistic relationship with its hosts. This mutualistic symbiosis is evident from the congruence of nematode and *Wolbachia* phylogenies (Bandi et al., 1998). Also, the nematodes appear to depend on the bacteria, as antibiotic treatment, which abolishes the *Wolbachia*, leads to defective development and retarded growth of their nematode hosts (Genchi et al., 1998; Hoerauf et al., 1999).

There is now even strong evidence that *Wolbachia* contributes to the pathogenicity of their nematode host. M. Taylor and H. Cross (Liverpool School of Tropical Medicine, UK) and Norbert Brattig (Bernhard Nocht Institute for Tropical Medicine, Germany) reported that lipopolysaccharides of Wolbachia origin are responsible for inflammatory reactions suffered by nematode carriers treated with chemotherapy against the nematodes (Brattig et al., in press; Taylor et al., 2000). Interestingly, Claudio Bandi (University of Milano, Italy) found that the major *Wolbachia* surface protein is recognized as an antigen by carriers.

Wolbachia phylogeny and horizontal transmission

Analysis based on the 16S *rDNA*, *ftsZ* and *wsp* genes allow four groups of *Wolbachia* to be distinguished (Werren et al., 1995; Bandi et al., 1998; Zhou et al., 1998). Groups A and B occur only in arthropods, C and D only in filarial nematodes, indicating that there is no frequent horizontal transmission of *Wolbachia* between arthropods and nematodes. Sometimes, the phylogeny of endobacteria matches that of their hosts. This is the case with the *Wolbachia* and their nematode hosts (Bandi et al., 1998; Bazzocchi et al., 2000). In arthropods, however, there are significant deviations between the respective evolutionary trees. Closely related species often carry very different *Wolbachia*, indicating substantial horizontal transmission on an evolutionary scale (Cook and Butcher, 1999; Masui et al., 1997; Schilthuizen and Stouthamer, 1997; Vavre et al., 1999; Werren et al., 1995a, b). Examples of horizontal transfer on a more limited time scale, however, are rare. Two reports at the "First International *Wolbachia* Conference" are therefore in this context of particular interest. Hiroaki Noda (National Institute of Sericultural and Entomological Science, Ibaraki, Japan) presented evidence that two species of planthoppers, *Laodelphax striatellus* and *Sogatella furcifera*, share the same *Wolbachia* strain with their common endoparasite *Elenchus japonicus*, indicating horizontal transfer through the parasite. Richard Stouthamer (Wagening Agricultural University, Netherlands) reported on a significant rate of transmission of *Wolbachia* between larvae of parasitic wasps in a shared host. *Wolbachia* transmission from infected to uninfected wasp larvae occurred in up to 30% of shared hosts (Huigens et al., 2000).

Wolbachia phylogenies are becoming even more complex since it was reported at the conference that these bacteria can recombine. Francis Jiggins (University College of London, United Kingdom) presented data indicating that the sequences of the *ftsZ* and *wsp* genes isolated from the same set of *Wolbachia* strains yielded incompatible phylogenies.

Wolbachia – endoparasite or endosymbiont?

Wolbachia as an obligatory intracellular bacterium depends on its host to provide it with as yet unknown factors. The benefit, if any, for the arthropod host species is not obvious in all cases. Thus, there will not always be a clear distinction between an endoparasitic or endosymbiotic role. If *Wolbachia* infection has no benefit for the host organism and vertical transmission is less than 100%, one should see gradual loss of the bacterium from a population, unless there is efficient horizontal transmission. For this, however, there is scant evidence (but see the previous paragraph). It is thus assumed that

vertical transmission is mainly responsible for maintenance of an infection in most populations so far studied. This would imply that in many cases of *Wolbachia* maintenance in host populations, a benefit to the host has to be implicated even if it has so far been elusive.

Tracy Reynolds (La Trobe University, Australia) reported such a case. There are stable *Wolbachia* infections of *D. melanogaster* in Queensland with a frequency of greater than 80%. No CI is exhibited under field conditions and fecundity of infected females is lower than that of uninfected ones. No obvious factor explaining maintenance of infection could be identified. Where CI is exhibited, however, infected populations can gain a reproductive advantage over uninfected ones and eventually replace them (Turelli and Hoffman, 1991).

In filarial nematodes, however, *Wolbachia* has developed into an obligatory symbiont (see above). The nature of their essential role for the host has yet to be determined.

Wolbachia and speciation

There are several studies providing clues that *Wolbachia* might have an effect or even be a driving force in speciation (Laven, 1967; Giordano et al., 1997; Bordenstein and Werren, 1998; Shoemaker et al., 1999). Jack Werren (University of Rochester, USA) reviewed the possible mechanisms by which *Wolbachia* could promote speciation, while Seth Bordenstein presented data suggesting that *Wolbachia*-induced bidirectional incompatibility may be associated with incipient speciation in the genus *Nasonia*. Although there is growing evidence for the involvement of *Wolbachia* in speciation events, the extent of this involvement still remains controversial.

Genomics

As mentioned above, *Wolbachia* belongs to the α-Proteobacteria, the bacterial division from which modern mitochondria are descended. It has been suggested that their ability to manipulate the reproduction of their hosts so successfully is due to their ability to "communicate" with mitochondria. Chris Bazinet (St. John's University, NY, USA) reported interference with mitochondrial pathways by rickettsial endosymbionts, while Charles Kurland (University of Uppsala, Sweden) pointed out that the genomic evolution of mitochondria is an extreme example of what happens to endosymbionts in time. The genome of mitochondria, with far less than 100 ORF's, seems most related to that of α-Proteobacteria. Thus, it is a likely assumption that they evolved from a *Rickettsia*-like parasitic ancestor. They have many metabolic

reductions in common with the typhus bacterium *Rickettsia prowazekii* (Andersson et al., 1998), an obligate intracellular parasite with just 834 protein encoding genes, which one could envisage as an analog to a mitochondrial ancestor undergoing reductive genome evolution.

Hajime Ishikawa (University of Tokyo, Japan) and Siv Andersson (University of Upssala, Sweden) presented the genomic structure of two *Buchnera* species. This bacterium is another example of an obligate endocellular bacterium. However, it lives in a mutualistic relationship. Even though it contains, with 583 ORFs, even fewer genes than *Rickettsia*, it still provides amino acids and vitamins to its host. Characteristically, it possesses the genes for synthesis of all essential amino acids.

The specific characteristics of different obligate endocellular bacteria and mitochondria tempts to speculate on the evolution and adaptation of *Wolbachia*. It will also show similar genome reduction as *Rickettsia*, probably less advanced and thus correspond to an even earlier stage of mitochondrial evolution. As *Wolbachia* strains C and D are obligate symbionts of their nematode hosts, they might have retained anabolic pathways that the arthropod strains A and B have already dispensed with. Comparative genomic analysis will show.

The genetics and biochemistry of *Wolbachia* are, due to their fastidious nature (*in vitro* culture has not yet been achieved), as good as nonexistent. Now that the age of genomics has truly come upon us, genetics will change for good. "Bacterial genomics have", as James D. Watson said, "absolutely made microbiology exciting again" (Watson, 1999). There is little room for doubt that this will hold particularly true for *Wolbachia* research. Genomics permit, to some extent, the bypassing of traditional genetics and biochemistry. Biochemical functions can be inferred from coding and regulatory sequences, while comparisons between related genomes allow the identification of specific metabolic differences.

Two projects to identify the complete genome of several *Wolbachia* strains have been presented at the conference. Scott O'Neill (Yale University, USA) reported on a project jointly funded by the NIH and New England Biolabs to sequence two *Wolbachia* strains at the Institute for Genomic Research (Rockville, USA). The first is present in *Drosophila melanogaster* while the second is found in the filarial nematode *Brugia malayi*. Kostas Bourtzis (University of Ioannina and IMBB, Heraklion, Greece), the host of the Conference, introduced the "European Wolbachia Project". The project is funded by the European Union and is a collaborative effort of eight laboratories from six European countries. The aim of the project is to generate not just the complete and annotated genome sequence of three *Wolbachia* strains, respectively responsible for the induction of cytoplasmic incompatibility (from *Drosophila simulans*), parthenogenesis (from *Muscidifurax uniraptor*) and feminization

(from *Armadillidium vulgare*), but also to provide tools for the analysis of parasite-host interactions. Genome analysis will be complemented by proteomics and DNA microarrays of the *Drosophila* host and its parasite. It is furthermore intended to develop a genetic transformation system for *Wolbachia* as a basis for manipulation and mechanistical studies of specific bacterial functions. The ultimate goal of this project is to identify and characterize *Wolbachia* genes involved in the induction of the three phenotypes as well as host (*Drosophila melanogaster*) genes involved in host-*Wolbachia* interaction. Identification of these genes will be a major breakthrough in deciphering the biology of this unculturable bacterium, understanding *Wolbachia*-host symbiotic associations and uncovering the evolution of intracellular symbiosis. In addition, this will be the first step towards development of environmentally-friendly technologies that will have wide applications in arthropod management.

The "Second International *Wolbachia* Conference" is scheduled for July, 2002 at the same venue. Participants should then hear that the genomes of the abovementioned *Wolbachia* strains are available. With the help of functional genomics, some of the bewildering variety of *Wolbachia* induced reproductive phenotypes might already have found a satisfactory molecular explanation.

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