CHAPTER 9

Wolbachia-Based Technologies for Insect Pest Population Control

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Abstract

Wolbachia are a group of obligatory intracellular and maternally inherited bacteria found in many arthropod species, including insects, mites, spiders, springtails, crustaceans, as well as in certain nematodes. Several PCR-based surveys suggest that over 20% of the arthropod species may be Wolbachia-infected, rendering this bacterium the most ubiquitous intracellular symbiont yet described. Wolbachia have recently attracted attention for their potential as novel and environmentally friendly bio-control agents. Wolbachia are able to invade and maintain themselves in the arthropod species through manipulation of the host's reproduction. Several strategies can be distinguished, one of which is cytoplasmic incompatibility (CI). Wolbachia-induced cytoplasmic incompatibility can be used beneficially in the following ways: (a) as a tool for insect pest population control in a way analogous to the "Sterile Insect technique" (SIT) and (b) as a drive system to spread desirable genotypes in field arthropod populations. In addition, virulent Wolbachia strains offer the potential to control vector species by modifying their population age structure. In the present chapter, I summarize the recent developments in Wolbachia research with an emphasis on the applied biology of Wolbachia and conclude with the challenges that Wolbachia researchers will face if they want to use and/or introduce Wolbachia into pest and vector species of economic, environmental and public health relevance and, through Wolbachia-based technologies, to suppress or modify natural populations.

Introduction

Wolbachia pipientis (Wolbachia for the purpose of this chapter) is a widespread group of obligatory intracellular maternally transmitted bacteria belonging to the α-Proteobacteria. Wolbachia bacteria were initially discovered in the ovaries of the mosquito Culex pipiens complex—hence the name Wolbachia pipientis—and were formally described in 1936. Since then, Wolbachia has been found in several hundreds of arthropod species including all major insect groups, mites, isopods, spiders and filarial nematodes. The results of PCR surveys suggest that 20-70% of insect species may be infected, rendering Wolbachia the most ubiquitous endosymbiont on earth. Wolbachia mainly reside in the reproductive tissues of arthropods and cause a number of reproductive alterations including cytoplasmic incompatibility (CI), thelytokous parthenogenesis, feminization of genetic males, and male-killing.

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Based on 16 rDNA gene analysis, Wolbachia belong to the alpha-2 subdivision of the Proteobacteria, forming a monophyletic group closely related to intracellular bacteria of the genera Ehrlichia, Anaplasma, Rickettsia and Cowdria.\textsuperscript{1,12-13} Many members of these genera are arthropod-borne pathogens of mammals. The phylogenies of Wolbachia isolates so far generated suggest the existence of eight major clades (A-H), which have been named 'super-groups'.\textsuperscript{14-16} Most of the supergroups occur in arthropods (A, B, E, F, G and H), while supergroups C and D are so far only found in filarial nematodes. The majority of insect Wolbachia belong to supergroups A and B.\textsuperscript{3,14,16-18} Relationships among the various supergroups are not well understood because of the absence of a suitable outgroup for rooting the inferred trees.\textsuperscript{14}

In order to resolve the branching order of the supergroups, sequence analysis of a large number of genes is necessary. Several such sequencing projects are currently underway. In addition, two "Multi Locus Sequencing Typing" (MLST) systems were developed, which allow genotyping of any given Wolbachia strain on the basis of a combination of alleles of a sample of housekeeping genes.\textsuperscript{19-20}

A number of studies have conclusively shown that the unique interaction of Wolbachia with the reproductive tissues and organs is responsible for the induction of several reproductive abnormalities such as: (a) feminization, which is the conversion of genetic males into functional females (mainly observed in isopods); (b) parthenogenesis, which is the exclusive production of female offspring by infected females (observed in many parasitoids); (c) male killing, which is the death of male embryos during early embryonic development (observed in insects) and (d) cytoplasmic incompatibility, which is a kind of male sterility observed in all major orders of insects. Each of these reproductive alterations favors the transmission of the bacterium at the expense of the uninfected arthropod population. Treatment of the infected hosts with antibiotics results in restoration of the normal reproductive phenotypes.\textsuperscript{5,8,11}

Wolbachia Induced Cytoplasmic Incompatibility

Reproductive isolation phenomena between different populations of the mosquito C. pipiens were reported as early as in the 1930s and 1950s, but it was only in the early 1970s that the presence of Wolbachia in the gonads of Culex mosquitoes was uncovered by Yen and Barr, who demonstrated that these bacteria are responsible for the expression of cytoplasmic incompatibility.\textsuperscript{9,21-24}

Cytoplasmic incompatibility is the most common and widespread reproductive abnormality Wolbachia infections induce in their arthropod hosts.\textsuperscript{25-26} In most insects, the expression of cytoplasmic incompatibility manifests itself as lethality in the developing embryo. In insects with haplodiploid sex determination (Hymenoptera), the result of cytoplasmic incompatibility is, however, a sex ratio shift to the haploid sex, which is usually male. Cytoplasmic incompatibility can be either unidirectional or bi-directional. Unidirectional cytoplasmic incompatibility is typically expressed when an infected male is crossed with an uninfected female. The reciprocal cross (infected female and uninfected male) is fully compatible, as are crosses between infected individuals. Bidirectional cytoplasmic incompatibility usually occurs in crosses between infected individuals harbouring different strains of Wolbachia. The penetrance of CI depends on the respective host-Wolbachia strain combination and can range from a few to 100%. As a consequence of cytoplasmic incompatibility, Wolbachia infections can spread and persist in nature by replacing uninfected populations.\textsuperscript{27}

Wolbachia-induced cytoplasmic incompatibility has been reported for almost all major insect orders (Coleoptera, Diptera, Hemiptera, Hymenoptera, Orthoptera and Lepidoptera) as well as in some terrestrial isopod species.\textsuperscript{6,20} The mechanism of Wolbachia-induced cytoplasmic incompatibility has not yet been elucidated at the molecular level. Indeed, little is known about the mechanistic basis of cytoplasmic incompatibility, except that the bacterial density and the percentage of infected sperm cysts are positively correlated with the penetrance of the incompatibility phenotype. However, a number of genetic, cytogenetic and cellular studies indicate that Wolbachia somehow modifies the paternal chromosomes during spermatogenesis.