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Tsetse-*Wolbachia* symbiosis: Comes of age and has great potential for pest and disease control

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Abstract

Tsetse flies (Diptera: Glossinidae) are the sole vectors of African trypanosomes, the causative agent of sleeping sickness in human and nagana in animals. Like most eukaryotic organisms, *Glossina* species have established symbiotic associations with bacteria. Three main symbiotic bacteria have been found in tsetse flies: *Wigglesworthia glossinidia*, an obligate symbiotic bacterium, the secondary endosymbiont *Sodalis glossinidius* and the reproductive symbiont *Wolbachia pipientis*. In the present review, we discuss recent studies on the detection and characterization of *Wolbachia* infections in *Glossina* species, the horizontal transfer of *Wolbachia* genes to tsetse chromosomes, the ability of this symbiont to induce cytoplasmic incompatibility in *Glossina morsitans morsitans* and also how new environment-friendly tools for disease control could be developed by harnessing *Wolbachia* symbiosis.

Keywords

Glossina; *Wolbachia*; Insect symbiosis; *Sodalis*; *Wigglesworthia*; Paratransgenesis

1. Introduction

Tsetse flies (*Glossina* spp.) are found throughout tropical sub-Saharan Africa and are the sole vectors of *Trypanosoma* spp., which cause African animal trypanosomiasis (AAT) or nagana in livestock and human African trypanosomiasis (HAT) or sleeping sickness in

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Disclosures

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humans (Leak, 1998; Van den Bossche et al., 2010; Welburn et al., 2001). Current estimates of the World Health Organization (WHO) suggest that epidemics where tens of thousands of people were infected have declined recently, although about 60 million people in Africa continue to be at risk to contract sleeping sickness (Aksoy, 2011; Barrett, 2006; Simarro et al., 2008). In many parts of sub-Saharan Africa, AAT and the presence of tsetse is considered the major obstacle to the development of more efficient and sustainable livestock production systems and one of the most important causes of hunger and poverty (Cattand, 1995; Dyck et al., 2005; Feldmann et al., 2005; Kioy et al., 2004).

Controlling the vector, the tsetse fly, remains theoretically the most efficient and sustainable way of managing AAT (Jordan, 1986; Leak, 1998). There are currently several accepted environment- friendly methods of controlling tsetse: (i) the SAT (sequential aerosol technique) an aerial application of ultralow volume, non-residual insecticides (Jordan, 1974), (ii) stationary bait techniques, i.e. use of insecticide-impregnated targets and traps that can be odor baited (Green, 1994), (iii) the live bait technique, i.e., application of residual insecticides on livestock (Thompson et al., 1991), and (iv) the release of sterile male insects, called the sterile insect technique (SIT) (Oladunmade et al., 1990; Politzar and Cuisance, 1984; Vreysen et al., 2000). Recently, *Wolbachia*-based control strategies have been suggested as a tool to suppress agricultural pests and disease vectors (Apostolaki et al., 2011; Bourtzis, 2008; Bourtzis and Robinson, 2006; Brelsfoard and Dobson, 2009, 2011; Xi et al., 2005; Zabalou et al., 2009, 2004).

The aim of this review is to summarize the currently available knowledge about the presence of *Wolbachia* infections in tsetse flies and to describe how this symbiont could be exploited for the control of the tsetse vector species and the associated diseases.

2. Tsetse symbiotic partners

Microbial symbiotic associations, beneficial and pathogenic in nature, are ubiquitous in the Biosphere, including the insect fauna (Bourtzis and Miller, 2009, 2006, 2003; Zchori-Fein and Bourtzis, 2011). In tsetse flies, three main symbiotic bacteria have been described (Aksoy, 2000). The first symbiont *Wigglesworthia glossinidia* (called Primary endosymbiont) is an obligate symbiotic bacterium harbored by all tsetse flies. Absence of *Wigglesworthia* results in female sterility (Nogge, 1976; Pais et al., 2008). Two distinct populations of *Wigglesworthia* have been reported in female tsetse. The first population are intracellularly restricted in specialized epithelial- like cells called the bacteriocytes that make up the bacteriome organ in the anterior midgut. The bacteriome population is thought to provision the host with essential nutrients such as vitamins that are absent from tsetse's single vertebrate blood-based diet (Akman et al., 2002; Aksoy, 1995; Aksoy et al., 1995; Nogge, 1981; Wang et al., 2009). Extracellular forms of *Wigglesworthia* have been detected in female milk secretion, through which the symbionts are presumably transmitted to the offspring (Attardo et al., 2008; Ma and Denlinger, 1974; Pais et al., 2008). In addition to its nutritional role, *Wigglesworthia* was recently reported to influence host immune maturation processes during the larval juvenile stages (Pais et al., 2008; Weiss et al., 2006).

The second symbiont, genus *Sodalis glossinidius* (called secondary endosymbiont) is present in all individuals in laboratory maintained tsetse lines. It is distributed in many tissues including haemolymph, salivary glands, milk gland, and also in the midgut where it lives in close proximity to where the trypanosomes develop (Aksoy et al., 1997; Cheng and Aksoy, 1999; Dale and Maudlin, 1999). It is also transmitted into tsetse's intrauterine larva in mother's milk secretions (Attardo et al., 2008; Pais et al., 2008). Unlike *Wigglesworthia*, the presence of *Sodalis* in natural populations is heterogenous (Geiger et al., 2005b). Although the genome size of *Sodalis* is similar to that of close free-living relatives (e.g. *Yersinia* and

E. coli), a significant portion of the *Sodalis*'s genome (over 25% of its coding sequences, CDSs) is composed of pseudogenes that have been inactivated, presumably as a consequence of relaxed selection as they no longer play a vital role in the symbiosis (Belda et al., 2010; Toh et al., 2006). The role of this recently acquired symbiont in tsetse flies has not yet been determined, but it has been suggested to influence host longevity and susceptibility to trypanosome infection (Dale and Maudlin, 1999; Dale and Welburn, 2001; Geiger et al., 2007; Geiger et al., 2005a). *Sodalis* can be cultured *in vitro* (Welburn et al., 1987) and genetically transformed (Beard et al., 1993a; Dale et al., 1995). Both intron mutagenesis based and lambda red-mediated genetic modification methods have been applied to *Sodalis* for functional studies (Pontes and Dale, 2011; Runyen-Janecky et al., 2010). A paratransgenic methodology has been developed in order to express trypanocidal products, which can be expressed in tsetse midguts when reconstituted with recombinant *Sodalis* (Beard and Aksoy, 1998).

The third symbiont, *Wolbachia pipientis* (*Wolbachia* for the purposes of this review), belongs to a group of obligatory intracellular and maternally transmitted bacteria, which have been reported in filarial nematode species and are widespread in arthropods, infecting all major orders of insects, mites, spiders, scorpions, isopods and springtails (Hilgenboecker et al., 2008; Saridaki and Bourtzis, 2010; Werren et al., 2008). In insects, the presence of these bacteria in the reproductive tissues is often associated with the induction of parthenogenesis, feminization, male-killing or cytoplasmic incompatibility (abbreviated CI) (Saridaki and Bourtzis, 2010; Werren et al., 2008). CI is the most widespread reproductive effect *Wolbachia* has on its hosts. It can be unidirectional or bidirectional. In diplo-diploid insects, unidirectional CI is expressed as embryonic lethality, when an infected male is crossed with an uninfected female or a female infected with a different *Wolbachia* strain. The reciprocal cross, as well as crosses between individuals with the same infection status, is fully compatible. Bidirectional CI is expressed in crosses between individuals infected with *Wolbachia* strains with different CI properties (Saridaki and Bourtzis, 2010; Werren et al., 2008). *Wolbachia*-induced CI is currently under consideration as a novel environment-friendly tool for the population control of insect pests and disease vectors (Apostolaki et al., 2011; Bourtzis, 2008; Bourtzis and Robinson, 2006; Xi et al., 2005; Zabalou et al., 2009; Zabalou et al., 2004).

3. Prevalence of *Wolbachia* infections in populations of tsetse flies

Wolbachia infections seem to be widespread in tsetse flies and concern both laboratory and natural populations of several *Glossina* species (Table 1). The presence of *Wolbachia* in tsetse flies was first reported in 1993 (O'Neill et al., 1993). Using a *Wolbachia* specific 16S rRNA-based PCR assay, *Wolbachia* was detected in female reproductive tissues of *Glossina morsitans morsitans* (*G. m. morsitans*) and *G. m. centralis*, while it was not found in *G. m. submorsitans*, *G. palpalis palpalis* and *G. p. gambiensis*. Cheng et al. (2000) also detected *Wolbachia* in laboratory and natural populations of *G. austeni* and *G. brevipalpis*.

A recent extensive *Wolbachia*-specific 16S rRNA PCR-based survey of 3750 individuals from both laboratory and natural populations of nine different *Glossina* species originating from 10 African countries confirmed that: (a) *Wolbachia* is widespread in species of the *morsitans* complex: *G. m. morsitans*, *G. m. centralis* and *G. austeni* populations. The report described for the first time *Wolbachia* in *G. pallidipes*, albeit at low prevalence, (b) *Wolbachia* is present in the *fusca* complex in *G. brevipalpis* and (c) *Wolbachia* could not be detected in the species of the *palpalis* complex: *G. p. palpalis*, *G. fuscipes* and *G. tachinoides*, while it was detected in a few individuals of *G. p. gambiensis* (Doudoumis et al., 2012).

Wolbachia infections were heterogeneous in the field, ranging from 0 to 100% in natural populations of *G. austeni* and *G. brevipalpis* and from 9.5 to 100% in natural populations of *G. m. morsitans* (Doudoumis et al., 2012). This observation suggests that geography may affect *Wolbachia* prevalence, as reported previously for field populations of the spider *Hylyphantes graminicola* (Yun et al., 2010), and probably indicates recent sweeps through tsetse populations. Alternatively, this phenomenon could be due to the fact that *Glossina* populations exhibit extensive genetic structuring, which may influence *Wolbachia* infection dynamics (Krafsur, 2009; Ouma et al., 2007; Ouma et al., 2011). Of the laboratory strains, the populations of *G. m. morsitans*, *G. m. centralis*, *G. longipinnis* and *G. swynnertoni* were 100% infected. *Wolbachia* infection was not fixed in *G. brevipalpis* and *G. pallidipes*, and the symbiont was absent from *G. p. palpalis*, *G. p. gambiensis*, *G. f. fuscipes* and *G. tachinoides*.

4. Genotyping and phylogeny of tsetse *Wolbachia* strains

Wolbachia is a highly diverse group of bacteria, which are assigned to a single species, *Wolbachia pipientis* (Lo et al., 2007). The *Wolbachia* strains are currently classified into 13 supergroups, A to N, while the supergroup G is considered as the artificial result of recombination events (Augustinos et al., 2011; Bordenstein and Rosengaus, 2005; Casiraghi et al., 2005; Cheng et al., 2000; Gorham et al., 2003; Lo et al., 2007; Lo et al., 2002; Ros et al., 2009; Rowley et al., 2004; Zhou et al., 1998). Single gene (mainly using the *wsp* gene, which encodes for the major *Wolbachia* surface protein) and/or Multi locus sequence typing (MLST) approaches are the main tools for genotyping *Wolbachia* strains (Baldo et al., 2006; Paraskevopoulos et al., 2006). Using these approaches, nine allelic profiles or Sequence Types (ST) were found in *Wolbachia* strains infecting *G. m. morsitans*, *G. m. centralis*, *G. austeni*, *G. pallidipes*, *G. brevipalpis* and *G. p. gambiensis* (Doudoumis et al., 2012). The *wsp*-based genotyping revealed the presence of nine alleles (Doudoumis et al., 2012).

MLST- and *wsp*-based phylogenetic analysis showed that *Wolbachia* strains infecting *G. m. morsitans*, *G. m. centralis*, *G. austeni*, *G. pallidipes* and *G. brevipalpis* belong to supergroup A, while the *Wolbachia* strain infecting *G. p. gambiensis* is a member of the supergroup B (Cheng et al., 2000; Doudoumis et al., 2012; Zhou et al., 1998). The tsetse *Wolbachia* strains of supergroup A are members of three separate and distantly related groups, which supports the hypothesis that horizontal transmission of *Wolbachia* from unrelated taxa may have occurred, as has been described for other arthropod taxa (Baldo et al., 2008; Raychoudhury et al., 2009; Ros et al., 2009; Salunke et al., 2010). It is worth noting, however, that the sibling species *G. m. morsitans* and *G. m. centralis* carry closely related *Wolbachia* strains, which may be the result of a hostsymbiont co-divergence process.

Schneider and colleagues recently showed that Variable- Number-Tandem-Repeat (VNTR) marker loci can be used from the characterization of *Wolbachia* diversity present in *Glossina* species. Based on this approach, and on the combined PCRblot technique developed by the same research group, it was confirmed: (a) that tsetse fly species harbor multiple cytoplasmic strains, (b) the presence of *Wolbachia* gene insertions onto tsetse host chromosomes and (c) the presence of low-titer infections (Schneider et al., 2013).

5. *Wolbachia* genomics

Since the first fully annotated *Wolbachia* genome of the *Drosophila melanogaster* strain *wMel* (Wu et al., 2004), three additional genomes are currently available. Two of these, *wRi* and *wPip*, are *Wolbachia* strains from insects, *Drosophila simulans* and *Culex pipiens* and the third is from the filarial nematode *Brugia malayi* (Foster et al., 2005; Klasson et al., 2009b, 2008). At least ten genomes are currently being sequenced from a diverse set of hosts, which represents just a small part of the extremely broad phenotypic diversity of

Wolbachia (Genomes Online Database – GOLD and personal communication; Table 2). The majority of these sequencing efforts are in progress with so far incomplete genomes, except for the *wVitB* strain from *Nasonia vitripennis* and the *wPip* strain from *Culex quinquefasciatus*, which are permanent draft genomes (Kent et al., 2011; Salzberg et al., 2005).

Wolbachia strains have small genomes (1.02–1.7 Mb), with the smallest identified so far being that of the *wGmm* strain from *G. m. morsitans* (unpublished data). Although *Wolbachia* genome sizes are within the range of the Rickettsiales (0.8–2.1 Mb), unlike most Rickettsiales they contain a high number of mobile and repetitive elements (Cerveau et al., 2011; Cordaux, 2008; Leclercq et al., 2011; Wu et al., 2004). Insertion sequences, transposons and the *Wolbachia* bacteriophage family (known as WO) can constitute up to 21% of the *Wolbachia* genome (Kent and Bordenstein, 2010; Klasson et al., 2009b, 2008; Wu et al., 2004). This makes *Wolbachia* a likely target for large scale horizontal gene transfer (HGT) of mobile elements, but so far only scant evidence of HGT events exists. Indirect data indicate that gene transfer of minor capsid gene sequences of bacteriophage WO between *Wolbachia* co-infections in the same host is common (Bordenstein and Wernegreen, 2004; Chafee et al., 2010), but evidence of phage sequence transfer beyond this single gene has been demonstrated so far only a 52.2 kb phage and the flanking genes between the *wVitA* and *wVitB* coinfections of *N. vitripennis* (Kent et al., 2011).

6. Horizontal gene transfer events

HGT, also known as Lateral Gene Transfer (LGT), permits the movement of genetic information between distantly related species. In prokaryotes, HGT events are almost universal, occur frequently and are regarded as a driving force of prokaryotic evolution (Andam and Gogarten, 2011; Kurland, 2000). In several *Wolbachia*-host interactions, horizontal gene transfer events from *Wolbachia* to a variety of insects and nematodes have been reported (Table 3). The first reported HGT event involves the adzuki bean beetle *Callosobruchus chinensis*, in which an estimated 380 kb genomic fragment of *Wolbachia*, presumably transcriptionally inactive, was identified in the X chromosome (Kondo et al., 2002; Nikoh et al., 2008). A nearly complete *Wolbachia* genome was found in the second chromosome of the fruit fly *Drosophila ananassae* (Dunning Hotopp et al., 2007); almost 2% of the transferred genes seem to be transcribed. Whether these genes have any biological significance is unclear, as the level of transcription is low (Dunning Hotopp et al., 2007; Nikoh and Nakabachi, 2009; Nikoh et al., 2008). Also in the genomes of parasitoid wasps of the genus *Nasonia* and filarial nematodes of the genera *Onchocerca*, *Brugia* and *Dirofilaria*, a small number of *Wolbachia* genes have been detected (Dunning Hotopp et al., 2007; Fenn et al., 2006). In the longicorn beetle *Monochamus alternatus*, at least 14% of a *Wolbachia* genome, corresponding to more than 170 genes, is located on a chromosome (Aikawa et al., 2009).

Gene transfer events have also been detected in the mosquitoes *Aedes aegypti* (*Ae. aegypti*) and *Ae. mascarensis*, although the direction of transfer is unclear (Klasson et al., 2009a; Woolfit et al., 2009). Interestingly, the transferred genes have been classified as transcriptionally active in the salivary glands of *Ae. aegypti*, which is the most important mosquito vector of human dengue virus and other arboviruses, and naturally not associated with *Wolbachia*. This suggests that important biological functions of *Wolbachia* could have been incorporated into host genomes through HGT even in host species that currently do not harbor cytoplasmic *Wolbachia* infections but apparently did in the past. It is worth noting that only in the absence of *Wolbachia*, the horizontally transmitted genes present expression levels above background level, although this conclusion can not be generalized. (Table 3).

The extent of HGT events has been further strengthened with the detection of *Wolbachia* gene transfers to the *G. m. morsitans* nuclear genome. Initial characterization indicated that at least three genes (*16S rRNA*, *fbpA* and *wsp*) of the naturally infected *Wolbachia* strain of *G. m. morsitans* (wGmm) have been transferred to the host genome (Doudoumis et al., 2012). These transfer events were identified in natural and laboratory populations, which highlight the long co-evolution of the host-*Wolbachia* association. The Whole Genome Sequence of *G. m. morsitans* (<http://www.sanger.ac.uk>) led to the identification of at least two horizontal transfer events with 52% and 47% of the *Wolbachia* genome being transferred to the host (unpublished data). This massive gene transfer from *Wolbachia* to *G. m. morsitans* can provide further insight into the evolution and fate of laterally transferred endosymbiont genes in multicellular host organisms. The retention of large fragments of *Wolbachia* in the *G. m. morsitans* genome may also suggest that this event may be relatively recent and may not have had time to undergo extensive erosions. Interestingly, in the subspecies *G. m. centralis*, no (similar) chromosomal insertions have been identified as yet, which suggests that the HGT event may have taken place after the divergence of these two subspecies again supporting its relatively recent origin. However, it is also possible that the *Wolbachia* chromosomal inserted genes were lost in the *G. m. centralis* lineage (Doudoumis et al., 2012).

Taken together, these findings suggest that symbiont–host gene transfers might be occurring more frequently than previously thought, similar to the gene transfers from organelles to the eukaryotic nucleus. In the HGT events presented, a genomic *Wolbachia* sequence, which can be as large as the whole genome, or as small as a few genes are transferred by an as yet unknown mechanism to the host genome. Once integrated, these genes are pseudogenized through the acquisition of point mutations, insertions and/or deletions. So far in a very few cases, some of the transferred genes survive and acquire novel expression patterns, and are thus incorporated into the gene complement of the host organism. It is likely the localization of *Wolbachia* infections in germ-line tissues promotes the HGT events since no similar gene transfers from symbiont to host genomes have been noted with obligate endosymbionts, including *Buchnera* in aphids or *Wigglesworthia* and *Sodalis* in tsetse (Kurland, 2000).

7. *Wolbachia*-induced CI and tsetse flies

Although *Wolbachia* infections have been reported in somatic tissues of several arthropod hosts, the symbiont is mainly present in the reproductive tissues of tsetse flies (Aksoy, 2000; Cheng et al., 2000; Doudoumis et al., 2012; O'Neill et al., 1993). The occurrence of *Wolbachia* in ovaries and testes has been linked to the induction of reproductive phenotypes, the most common being CI. However, the functional role of the symbiont in tsetse flies had for long time remained elusive, because the use of antibiotics, such as tetracycline, for the establishment of *Wolbachia*-free lines necessary for appropriate genetic crosses, also eliminated the mutualistic *Wigglesworthia* and led to the production of sterile flies. Alam and colleagues (2011) recently reported the successful establishment of *Wolbachia*-free and still fertile *G. m. morsitans* lines by dietary provisioning of tetracycline supplemented blood meals with yeast extract. This allowed the performance of proper mating experiments, which for the first time provided evidence that the presence of *Wolbachia* is responsible for the induction of strong CI in *G. m. morsitans* (Alam et al., 2011). It is worth noting that this was also the first time that *Wolbachia*-induced CI was investigated in a viviparous insect. The cured lines were further used in comparative studies to investigate potential effects of *Wolbachia* infections on host fitness under laboratory conditions. The results were clear: (a) there was no major effect of *Wolbachia* on host eclosion rates and (b) removal of *Wolbachia* did not result in a decrease of the mating capacity of males (Alam et al., 2011).

Wolbachia-induced CI has been proposed as an environmentfriendly mechanism for population control of agricultural pests and disease vectors (Beard et al., 1998, 1993b; Bourtzis, 2008; Bourtzis and O'Neill, 1998; Dobson, 2003; Sinkins and Godfray, 2004). Expression of strong CI in *Wolbachia*-infected *G. m. morsitans* opens new routes for population control of the insect vector and trypanosomosis. This can be done with three potential approaches: First, *Wolbachia*-induced CI can be used as a population suppression mechanism in a way analogous to the SIT. This *Wolbachia*-based approach, known as Incompatible Insect Technique (IIT), has been developed and successfully used for population control of agriculturally important insects and disease vectors under laboratory and field conditions (Apostolaki et al., 2011; Laven, 1967; Zabalou et al., 2009, 2004). IIT could potentially be advantageous over SIT, since it does not rely on the use of irradiation to sterilize males, a process that results in the release of males with reduced fitness. In IIT, sterility is due to the presence of a *Wolbachia* strain generically capable of inducing CI (Bourtzis, 2008; Bourtzis and Robinson, 2006; Vreysen et al., 2011). Second, *Wolbachia*-induced CI could also be used as a spreading/replacement mechanism for desired phenotypes, because infected females have a reproductive advantage over uninfected females, since they can mate with both uninfected and infected males thus invading populations in nature (Bourtzis, 2008; Bourtzis and Robinson, 2006; Bourtzis and O'Neill, 1998; Brelsfoard and Dobson, 2009; Dobson et al., 2002; Hoffmann et al., 1998; Rasgon, 2008, 2007; Sinkins and Gould, 2006). *Wolbachia*-based suppression and/or replacement approaches have been documented with insect vector species (the mosquito species: *Culex pipiens* species complex, *Aedes aegypti*, *Aedes albopictus*) under laboratory, semi-field or field conditions (Atyame et al., 2011; Chambers et al., 2011; Hoffmann et al., 2011; Laven, 1967; Ruang-Areerate and Kittayapong, 2006; Walker et al., 2011; Xi et al., 2005). Most of the above-mentioned examples were based on host transinfection via embryonic cytoplasmic injections. However, such microinjections cannot easily be applied to tsetse flies, because they are viviparous. In this case, infection should be attempted through other methods, for example via maternal intrathoracic injections, as has been done in the case of *Aedes aegypti* (Ruang-Areerate and Kittayapong, 2006). Considering that *Wolbachia* can find their way to the germ line by infecting stem cells, intrathoracic injections may represent an efficient alternative method for the transinfection of viviparous species such as *Glossina* spp. (Fast et al., 2011; Frydman et al., 2006; Sacchi et al., 2010). Finally, *Wolbachia* mediated CI can be harnessed to drive trypanosome resistant paratransgenic tsetse into natural populations to replace their parasite susceptible counterparts (Alam et al., 2011). Based on the fact that all symbionts are maternally transmitted to tsetse's progeny, resistance genes expressed by *Sodalis* can be propagated by CI caused by *Wolbachia* if perfect inheritance of *Sodalis* and *Wolbachia* can be ensured (Aksoy et al., 2008; Aksoy and Weiss, 2007; Rio et al., 2004). Given that natural tsetse populations carry heterogeneous *Wolbachia* infections, naturally infected and uninfected strains can be used to develop novel strains that may be incompatible with indigenous populations. Recolonization of *Wolbachia* infected lines with modified *Sodalis* strains conferring parasite resistance can give rise to tsetse lines that may be used in the field to spread desirable phenotypes.

8. Harnessing and controlling pathogens for pest and disease control

The available tools for the prevention, diagnosis and therapy of HAT and AAT are rather limited or inadequate (Holmes and Torr, 1988; Simarro et al., 2008). It is generally accepted that the reduction of tsetse populations or reduction of the flies' ability to transmit trypanosomes remain the most effective approaches to control disease.

The use of sterile insects as part of an area-wide integrated pest management (AW-IPM) approach is a proven approach in controlling dipteran pests, such as fruit flies and screwworms, as well as in the sustainable eradication of tsetse flies from the Island of

Unguja, Zanzibar (Enkerlin, 2005; Klassen and Curtis, 2005; Vreysen et al., 2000; Wyss, 2000). The sustainable removal of the *Glossina austeni* population from the Unguja Island led to the Pan African Tsetse and Trypanosomosis Eradication Campaign (PATTEC) initiative and to a project aiming to create a zone free of *Glossina pallidipes* in the Southern Rift Valley of Ethiopia (Alemu et al., 2007; Feldmann et al., 2005). This project was based on the establishment of a laboratory colony of the target species at the Insect Pest Control Laboratory (former Entomology Unit) of the Joint FAO/IAEA Programme of Nuclear Techniques in Food and Agriculture, Seibersdorf, Austria, using pupae obtained from the target field population in Ethiopia. Despite its initial successful establishment in 1996, this colony experienced a steady decline over 2 years and collapsed.

Investigations revealed that up to 85% of both male and female flies had salivary gland hypertrophy (SGH), a syndrome first described in wild populations of *G. pallidipes* (Burt, 1945; Whitnall, 1934), but later detected in many tsetse species from different African countries (Ellis and Maudlin, 1987; Gouteux, 1987; Jura et al., 1989; Jura et al., 1988; Minter-Goedbloed and Minter, 1989; Odindo et al., 1982; Otieno et al., 1980; Shaw and Moloo, 1993). Jaenson (1978) was the first to identify a nuclear rod-shaped enveloped DNA virus averaging 70×640 nm in size as the causative agent and named as the Salivary Gland Hypertrophic Virus (SGHV). This virus was also associated with testicular degeneration and ovarian abnormalities (Jura et al., 1988; Kokwaro et al., 1990; Sang et al., 1999; Sang et al., 1998) and affected the development, survival, fertility and fecundity of naturally (Feldmann et al., 1992) or experimentally (Jura et al., 1993; Sang et al., 1997) infected flies. In tsetse populations in nature, mother-to-offspring transmission, either trans-ovum or through infected milk glands, is thought to be the most likely mode of transmission of the SGHV (Jura et al., 1989; Sang et al., 1998, 1996). In laboratory maintained flies, horizontal transmission during membrane feeding (Feldmann, 1994) is the most significant route of virus infection, as each tray of blood may be used to feed up to ten successive sets of fly cages.

Due to the negative impact of SGHV on fly health, it caused the collapse of two colonies of *G. pallidipes* and constrains the expansion of the colony in Ethiopia (Abd-Alla et al., 2010), thus a virus management strategy is required. Management of the virus can be achieved by one of the following approaches, or a combination of: (i) change of the feeding system currently used in colony rearing; (ii) neutralization of the virus released during blood feeding by adding virus-specific antibodies to the blood; (iii) inhibition of viral replication with commercially available antiviral drugs against the DNA polymerase of similar viruses, and (iv) development and application of RNA interference strategies to silence essential virus specific gene(s) (Abd-Alla et al., 2011).

Could *Wolbachia*-based approaches offer an alternative route for the control of SGHV in tsetse flies? *Wolbachia* has recently been shown to protect *Drosophila melanogaster* against infections with RNA viruses (Hedges et al., 2008; Osborne et al., 2009; Teixeira et al., 2008). Although SGHV is a DNA virus, we are currently investigating *Wolbachia*-SGHV interactions in *Glossina* species. Interestingly enough, a series of recent studies showed that *Wolbachia* infections present in insect vector species limit the infection and/ or increase host resistance against several human pathogens. These effects were observed against pathogenic viruses such as Dengue, Chikungunya and West Nile Virus, filarial nematodes and a major eukaryotic microbial pathogen, *Plasmodium* (Glaser and Meola, 2010; Hughes et al., 2011; Kambris et al., 2009; Moreira et al., 2009; Mousson et al., 2010). Laboratory experiments with flies where the obligate symbiont *Wigglesworthia* has been eliminated while retaining *Sodalis* and *Wolbachia* indicate much greater susceptibility to trypanosome infections (Pais et al., 2008). This finding may suggest that unlike the other insect systems, where newly introduced *Wolbachia* infections can limit pathogen transmission through host immune

induction, tsetse symbiosis is dominated by mutually beneficial interactions dominated by *Wigglesworthia*. In the absence of *Wigglesworthia*, various aspects of host physiology, including fecundity and immunity are compromised. Whether *Wolbachia* limit trypanosome infection in natural *Glossina* populations is currently under investigation.

A virulent strain of *Wolbachia*, *popcorn*, was first described in *Drosophila melanogaster* as over-replicating bacteria in somatic tissues, which reduce the life span of their hosts (Min and Benzer, 1997). This property was recently harnessed in order to transinfect and reduce the life span of insect vector species, such as *Aedes aegypti* (McMeniman et al., 2009). It is also worth noting that *Wolbachia* researchers combined several genetic properties of *Wolbachia*, such as induction of CI and limiting pathogen infection, in transinfected *Aedes aegypti* (infected with the *wMel* *Wolbachia* strain). It was shown that the *wMel* infection was able to reduce dengue virus proliferation in the host and when these *Wolbachia*-infected *Aedes aegypti* populations were released into nature, in Australia, successfully blocked dengue transmission (Hoffmann et al., 2011; Walker et al., 2011). In principle, a similar approach, perhaps combined with the virulent properties of some *Wolbachia* strains, could be developed for the control of tsetse flies and trypanosomosis (Alam et al., 2011; Van den Abbeele et al., 2013).

It is also worth noting that recent findings suggest that the *Wolbachia* strains infecting the *Glossina morsitans* could become virulent over-replicating bacterial strains, like the *wMelPopcorn* *Wolbachia* strain (Min and Benzer, 1997), significantly affecting fitness and fecundity of *Glossina* hybrid species. Schneider and colleagues suggested that this pathogenic effect may be used as a new and or complementary tool for the control of tsetse flies and trypanosomosis (Schneider et al., 2013).

9. Conclusions

Tsetse flies are vectors of the trypanosomes, which cause African sleeping sickness in humans (HAT) and nagana in animals (AAT). Given that there are no effective vaccines or drugs against this pathogen, control of trypanosomosis currently relies on vector control methods. There is an urgent need for pesticide-free and environment-friendly methods for vector and disease control. The SIT, which has successfully been used in the past to control *G. austeni* populations, is currently being considered as the method of choice also for the control of other *Glossina* species in sub-Saharan Africa. Recent developments in the field of Insect-*Wolbachia* Symbiosis suggest new tools to combat the vector and the diseases. The detection and characterization of *Wolbachia* infections in both laboratory and natural populations of several *Glossina* species, as well as the ability of these symbionts to induce CI, to reduce the life span of insect vector species and to interfere with major pathogens strongly suggest that *Wolbachia*-based sustainable approaches, in combination with SIT or as stand-alone methods, can be developed for the control of *Glossina* species and trypanosomosis.

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References

- Abd-Alla AM, Kariithi HM, Parker AG, Robinson AS, Kiflom M, Bergoin M, Vreysen MJ. Dynamics of the salivary gland hypertrophy virus in laboratory colonies of *Glossina pallidipes* (Diptera: Glossinidae). *Virus Res.* 2010; 150:103–110. [PubMed: 20214934]
- Abd-Alla AM, Parker AG, Vreysen MJ, Bergoin M. Tsetse salivary gland hypertrophy virus: hope or hindrance for tsetse control? *PLoS Negl Trop Dis.* 2011; 5:e1220. [PubMed: 21912708]
- Aikawa T, Anbutso H, Nikoh N, Kikuchi T, Shibata F, Fukatsu T. Longicorn beetle that vectors pinewood nematode carries many *Wolbachia* genes on an autosome. *Proc Roy Soc B—Biol Sci.* 2009; 276:3791–3798.
- Akman L, Yamashita A, Watanabe H, Oshima K, Shiba T, Hattori M, Aksoy S. Genome sequence of the endocellular obligate symbiont of tsetse flies, *Wigglesworthia glossinidia*. *Nat Genet.* 2002; 32:402–407. [PubMed: 12219091]
- Aksoy S, Weiss BL. Symbiosis-based technological advances to improve tsetse *Glossina spp.* In: Vreysen, MJB., et al., editors. *SIT application. Area-Wide Control of Insect Pests*. Springer; Dordrecht, The Netherlands: 2007. p. 137-148.
- Aksoy S, Pourhosseini AA, Chow A. Mycetome endosymbionts of tsetse flies constitute a distinct lineage related to *Enterobacteriaceae*. *Insect Mol Biol.* 1995; 4:15–22. [PubMed: 7742973]
- Aksoy S, Chen X, Hypsa V. Phylogeny and potential transmission routes of midgut-associated endosymbionts of tsetse (Diptera:Glossinidae). *Insect Mol Biol.* 1997; 6:183–190. [PubMed: 9099582]
- Aksoy S, Weiss B, Attardo G. Paratransgenesis applied for control of tsetse transmitted sleeping sickness. *Adv Exp Med Biol.* 2008; 627:35–48. [PubMed: 18510012]
- Aksoy S. *Wigglesworthia gen. nov.* and *Wigglesworthia glossinidia sp. nov.*, taxa consisting of the mycetocyte-associated, primary endosymbionts of tsetse flies. *Int J Syst Bacteriol.* 1995; 45:848–851. [PubMed: 7547309]
- Aksoy S. Tsetse—a haven for microorganisms. *Parasitol Today.* 2000; 16:114–118. [PubMed: 10689331]
- Aksoy S. Sleeping sickness elimination in sight: time to celebrate and reflect, but not relax. *PLoS Negl Trop Dis.* 2011; 5:e1008. [PubMed: 21364971]
- Alam U, Medlock J, Brelsfoard C, Pais R, Lohs C, Balmand S, Carnogursky J, Heddi A, Takac P, Galvani A, Aksoy S. *Wolbachia* symbiont infections induce strong cytoplasmic incompatibility in the tsetse fly *Glossina morsitans*. *PLoS Pathogens.* 2011; 7(12):e1002415. (Epub. 2011 December 8). [PubMed: 22174680]
- Alemu, T.; Kapitano, B.; Mekonnen, S.; Aboset, G.; Kiflom, M.; Bancha, B.; Woldeyes, G.; Bekele, K.; Feldmann, U. Area-wide control of tsetse and trypanosomosis: Ethiopian experience in the Southern Rift Valley. In: Vreysen, MJB., et al., editors. *Area-Wide Control of Insect Pests: From Research to Field Implementation*. Springer; Dordrecht, The Netherlands: 2007. p. 325-335.
- Andam CP, Gogarten JP. Biased gene transfer in microbial evolution. *Nat Rev Microbiol.* 2011; 9:543–555. [PubMed: 21666709]
- Apostolaki A, Livadaras I, Saridaki A, Chrysargyris A, Savakis C, Bourtzis K. Transinfection of the olive fruit fly *Bactrocera oleae* with *Wolbachia*: towards a symbiont-based population control strategy. *J Appl Entomol.* 2011; 135:546–553.
- Attardo GM, Lohs C, Heddi A, Alam UH, Yildirim S, Aksoy S. Analysis of milk gland structure and function in *Glossina morsitans*: milk protein production, symbiont populations and fecundity. *J Insect Physiol.* 2008; 54:1236–1242. [PubMed: 18647605]
- Atyame CM, Pasteur N, Dumas E, Tortosa P, Tantely ML, Pocquet N, Licciardi S, Bheecarry A, Zumbo B, Weill M, Duron O. Cytoplasmic incompatibility as a means of controlling *Culex pipiens quinquefasciatus* mosquito in the Islands of the South-Western Indian Ocean. *PLoS Negl Trop Dis.* 2011; 5:e1440. [PubMed: 22206033]
- Augustinos AA, Santos-Garcia D, Dionyssopoulou E, Moreira M, Papapanagiotou A, Scarvelakis M, Doudoumis V, Ramos S, Aguiar AF, Borges PAV, Khadem M, Latorre A, Tsiamis G, Bourtzis K. Detection and characterization of *Wolbachia* infections in natural populations of aphids: is the

- hidden diversity fully unraveled? PLoS ONE. 2011; 6 (12):e28695. <http://dx.doi.org/10.1371/journal.pone.0028695>. [PubMed: 22174869]
- Baldo L, Dunning Hotopp JC, Jolley KA, Bordenstein SR, Biber SA, Choudhury RR, Hayashi C, Maiden MC, Tettelin H, Werren JH. Multilocus sequence typing system for the endosymbiont *Wolbachia pipientis*. Appl Environ Microbiol. 2006; 72:7098–7110. [PubMed: 16936055]
- Baldo L, Ayoub NA, Hayashi CY, Russell JA, Stahlhut JK, Werren JH. Insight into the routes of *Wolbachia* invasion: high levels of horizontal transfer in the spider genus *Agelenopsis* revealed by *Wolbachia* strain and mitochondrial DNA diversity. Mol Ecol. 2008; 17:557–569. [PubMed: 18179432]
- Barrett MP. The rise and fall of sleeping sickness. Lancet. 2006; 367:1377–1378. [PubMed: 16650633]
- Beard, CB.; Aksoy, S. Genetic manipulation of insect symbionts. In: Crampton, J.; Beard, BC.; Louis, C., editors. Molecular Biology of Insect Disease Vectors. Chapman and Hall; London: 1998. p. 555-560.
- Beard CB, O'Neill SL, Mason P, Mandelco L, Woese CR, Tesh RB, Richards FF, Aksoy S. Genetic transformation and phylogeny of bacterial symbionts from tsetse. Insect Mol Biol. 1993a; 1:123–131. [PubMed: 8269090]
- Beard CB, O'Neill SL, Tesh RB, Richards FF, Aksoy S. Modification of arthropod vector competence via symbiotic bacteria. Parasitol Today. 1993b; 9:179–183. [PubMed: 15463748]
- Beard CB, Durvasula RV, Richards FF. Bacterial symbiosis in arthropods and the control of disease transmission. Emerg Infect Dis. 1998; 4:581–591. [PubMed: 9866734]
- Belda E, Moya A, Bentley S, Silva FJ. Mobile genetic element proliferation and gene inactivation impact over the genome structure and metabolic capabilities of *Sodalis glossinidius*, the secondary endosymbiont of tsetse flies. BMC Genomics. 2010; 11:449. [PubMed: 20649993]
- Bordenstein S, Rosengaus RB. Discovery of a novel *Wolbachia* super group in Isoptera. Curr Microbiol. 2005; 51:393–398. [PubMed: 16252129]
- Bordenstein SR, Wernegreen JJ. Bacteriophage flux in endosymbionts (*Wolbachia*): infection frequency, lateral transfer, and recombination rates. Mol Biol Evol. 2004; 21:1981–1991. [PubMed: 15254259]
- Bourtzis, K.; Miller, TA. Insect Symbiosis. CRC Press; Boca Raton, Fla: 2003. p. 347
- Bourtzis, K.; Miller, TA. Insect Symbiosis. Vol. 2. CRC Press; Boca Raton, Fla: 2006. p. 276p
- Bourtzis, K.; Miller, TA. Insect Symbiosis. Vol. 3. CRC Press; Boca Raton, Fla: 2009. p. 408
- Bourtzis K, O'Neill S. *Wolbachia* infections and arthropod reproduction – *Wolbachia* can cause cytoplasmic incompatibility, parthenogenesis, and feminization in many arthropods. Bioscience. 1998; 48:287–293.
- Bourtzis K, Robinson AS. Insect pest control using *Wolbachia* and/or radiation. Insect Symbios. 2006; 2:225–246.
- Bourtzis K. *Wolbachia*-based technologies for insect pest population control. Adv Exp Med Biol. 2008; 627:104–113. [PubMed: 18510018]
- Brelsfoard CL, Dobson SL. *Wolbachia*-based strategies to control insect pests and disease vectors. Asia Pac J Mol Biol Biotechnol. 2009; 17:55–63.
- Brelsfoard CL, Dobson SL. An update on the utility of *Wolbachia* for controlling insect vectors and disease transmission. Asia Pac J Mol Biol Biotechnol. 2011; 19:85–92.
- Burt E. Hypertrophied salivary glands in *Glossina*: evidence that *G. pallidipes* with this abnormality is particularly suited to trypanosome infection. Ann Trop Med Parasitol. 1945; 39:11–13.
- Casiraghi M, Bordenstein SR, Baldo L, Lo N, Beninati T, Wernegreen JJ, Werren JH, Bandi C. Phylogeny of *Wolbachia pipientis* based on *gltA*, *groEL* and *ftsZ* gene sequences: clustering of arthropod and nematode symbionts in the F supergroup, and evidence for further diversity in the *Wolbachia* tree. Microbiology. 2005; 151:4015–4022. [PubMed: 16339946]
- Cattand P. The scourge of human African trypanosomiasis. Afr Health. 1995; 17:9–11. [PubMed: 12319651]

- Cerveau N, Leclercq S, Leroy E, Bouchon D, Cordaux R. Short- and longterm evolutionary dynamics of bacterial insertion sequences: insights from *Wolbachia* endosymbionts. *Genome Biol Evol.* 2011; 3:1175–1186. [PubMed: 21940637]
- Chafee ME, Funk DJ, Harrison RG, Bordenstein SR. Lateral phage transfer in obligate intracellular bacteria (*Wolbachia*): verification from natural populations. *Mol Biol Evol.* 2010; 27:501–505. [PubMed: 19906794]
- Chambers EW, Hapairai L, Peel BA, Bossin H, Dobson SL. Male mating competitiveness of a *Wolbachia*-introgressed *Aedes polynesiensis* strain under semi-field conditions. *PLoS Negl Trop Dis.* 2011; 5:e1271. [PubMed: 21829750]
- Cheng Q, Aksoy S. Tissue tropism, transmission and expression of foreign genes in vivo in midgut symbionts of tsetse flies. *Insect Mol Biol.* 1999; 8:125–132. [PubMed: 9927181]
- Cheng Q, Ruel TD, Zhou W, Moloo SK, Majiwa P, O'Neill SL, Aksoy S. Tissue distribution and prevalence of *Wolbachia* infections in tsetse flies, *Glossina spp.* *Med Vet Entomol.* 2000; 14:44–50. [PubMed: 10759311]
- Cordaux R. ISWpi1 from *Wolbachia pipientis* defines a novel group of insertion sequences within the IS5 family. *Gene.* 2008; 409:20–27. [PubMed: 18155858]
- Dale C, Maudlin I. *Sodalis gen. nov.* and *Sodalis glossinidius* sp. nov., a microaerophilic secondary endosymbiont of the tsetse fly *Glossina morsitans morsitans*. *Int J Syst Bacteriol.* 1999; 49 (Pt 1): 267–275. [PubMed: 10028272]
- Dale C, Welburn SC. The endosymbionts of tsetse flies: manipulating host-parasite interactions. *Int J Parasitol.* 2001; 31:628–631. [PubMed: 11334953]
- Dale C, Toleman M, Welburn SC, Crampton JC, Maudlin I. Stable plasmid transformation of *Glossina* midgut symbionts (RLO). *J Cell Bio-Chem.* 1995:223.
- Dobson SL, Fox CW, Jiggins FM. The effect of *Wolbachia*-induced cytoplasmic incompatibility on host population size in natural and manipulated systems. *Proc Biol Sci.* 2002; 269:437–445. [PubMed: 11886634]
- Dobson SL. Reversing *Wolbachia*-based population replacement. *Trends Parasitol.* 2003; 19:128–133. [PubMed: 12643996]
- Doudoumis V, Tsiamis G, Wamwiri F, Brelsfoard C, Alam U, Aksoy E, Dalaperas S, Abd-Alla A, Ouma J, Takac P, Aksoy S, Bourtzis K. Detection and characterization of *Wolbachia* infections in laboratory and natural populations of different species of tsetse flies (genus *Glossina*). *BMC Microbiol.* 2012; 12(Suppl 1):S3. [PubMed: 22376025]
- Dunning Hotopp JC, Clark ME, Oliveira DC, Foster JM, Fischer P, Munoz Torres MC, Giebel JD, Kumar N, Ishmael N, Wang S, Ingram J, Nene RV, Shepard J, Tomkins J, Richards S, Spiro DJ, Ghedin E, Slatko BE, Tettelin H, Werren JH. Widespread lateral gene transfer from intracellular bacteria to multicellular eukaryotes. *Science.* 2007; 317:1753–1756. [PubMed: 17761848]
- Dyck, VA.; Hendrichs, JP.; Robinson, AS. *The Sterile Insect Technique: Principles and Practice in Area-Wide Integrated Pest Management.* Springer; Dordrecht: 2005.
- Ellis DS, Maudlin I. Salivary-gland hyperplasia in wild caught tsetse from Zimbabwe. *Entomol Exp Appl.* 1987; 45:167–173.
- Enkerlin, WR. Impact of fruit fly control programmes using the sterile insect technique. In: Dyck, VA., et al., editors. *Sterile Insect Technique. Principles and Practice in Area-Wide Integrated Pest Management.* Springer; Dordrecht, The Netherlands: 2005. p. 651-676.
- Fast EM, Toomey ME, Panaram K, Desjardins D, Kolaczyk ED, Frydman HM. *Wolbachia* enhance *Drosophila* stem cell proliferation and target the germline stem cell niche. *Science.* 2011; 334:990–992. [PubMed: 22021671]
- Feldmann HU, Barnor H, Acs R. Abweichungen in der Reproduktion von *Glossina morsitans submorsitans* Newstead (Diptera: Glossinidae): Untersuchungen zur Behebung eines gestörten Geschlechterverhältnisses und zum Übertragungsweg von Fertilitäts-reduzierenden Viren an die Nachkommen. *Mitt Dtsch Ges Allg Angew Ent.* 1992; 8:248–251.
- Feldmann, U.; Dyck, VA.; Mattioli, RC.; Jannin, J. Potential impact of tsetse fly control involving the sterile insect technique. In: Dyck, VA., et al., editors. *Sterile Insect Technique. Principles and Practice in Area-Wide Integrated Pest Management.* Springer; Dordrecht, The Netherlands: 2005. p. 701-723.

- Feldmann, U. Guidelines for the rearing of tsetse flies using the membrane feeding technique. In: Ochieng'-Odero, JPR., editor. *Techniques of Insect Rearing for the Development of Integrated Pest and Vector Management Strategies*. ICIPE Science Press; Nairobi, Kenya: 1994. p. 449-471.
- Fenn K, Conlon C, Jones M, Quail MA, Holroyd NE, Parkhill J, Blaxter M. Phylogenetic relationships of the *Wolbachia* of nematodes and arthropods. *PLoS Pathog*. 2006; 2:e94. [PubMed: 17040125]
- Foster J, Ganatra M, Kamal I, Ware J, Makarova K, Ivanova N, Bhattacharyya A, Kapatral V, Kumar S, Posfai J, Vincze T, Ingram J, Moran L, Lapidus A, Omelchenko M, Kyrpides N, Ghedin E, Wang S, Goltsman E, Joukov V, Ostrovskaya O, Tsukerman K, Mazur M, Comb D, Koonin E, Slatko B. The *Wolbachia* genome of *Brugia malayi*: endosymbiont evolution within a human pathogenic nematode. *PLoS Biol*. 2005; 3:e121. [PubMed: 15780005]
- Frydman HM, Li JM, Robson DN, Wieschaus E. Somatic stem cell niche tropism in *Wolbachia*. *Nature*. 2006; 441:509–512. [PubMed: 16724067]
- Geiger A, Ravel S, Frutos R, Cuny G. *Sodalis glossinidius* (Enterobacteriaceae) and vectorial competence of *Glossina palpalis gambiensis* and *Glossina morsitans morsitans* for *Trypanosoma congolense* savannah type. *Curr Microbiol*. 2005a; 51:35–40. [PubMed: 15942697]
- Geiger A, Cuny G, Frutos R. Two Tsetse fly species, *Glossina palpalis gambiensis* and *Glossina morsitans morsitans*, carry genetically distinct populations of the secondary symbiont *Sodalis glossinidius*. *Appl Environ Microbiol*. 2005b; 71:8941–8943. [PubMed: 16332895]
- Geiger A, Ravel S, Mateille T, Janelle J, Patrel D, Cuny G, Frutos R. Vector competence of *Glossina palpalis gambiensis* for *Trypanosoma brucei* s. l. and genetic diversity of the symbiont *Sodalis glossinidius*. *Mol Biol Evol*. 2007; 24:102–109. [PubMed: 17012373]
- Glaser RL, Meola MA. The native *Wolbachia* endosymbionts of *Drosophila melanogaster* and *Culex quinquefasciatus* increase host resistance to West Nile virus infection. *PLoS ONE*. 2010; 5:e11977. [PubMed: 20700535]
- Gorham CH, Fang QQ, Durden LA. *Wolbachia* endosymbionts in fleas (*Siphonaptera*). *J Parasitol*. 2003; 89:283–289. [PubMed: 12760642]
- Gouteux JP. Prevalence of enlarged salivary glands in *Glossina palpalis*, *G. pallicera*, and *G. nigrofusca* (Diptera: Glossinidae) from the Vavoua area, Ivory Coast. *J Med Entomol*. 1987; 24:268. [PubMed: 3035182]
- Green CH. Bait methods for tsetse fly control. *Adv Parasitol*. 1994; 34:229–291. [PubMed: 7976751]
- Hedges LM, Brownlie JC, O'Neill SL, Johnson KN. *Wolbachia* and virus protection in insects. *Science*. 2008; 322:702. [PubMed: 18974344]
- Hilgenboecker K, Hammerstein P, Schlattmann P, Telschow A, Werren JH. How many species are infected with *Wolbachia*? –A statistical analysis of current data. *FEMS Microbiol Lett*. 2008; 281:215–220. [PubMed: 18312577]
- Hoffmann AA, Hercus M, Dagher H. Population dynamics of the *Wolbachia* infection causing cytoplasmic incompatibility in *Drosophila melanogaster*. *Genetics*. 1998; 148:221–231. [PubMed: 9475734]
- Hoffmann AA, Montgomery BL, Popovici J, Iturbe-Ormaetxe I, Johnson PH, Muzzi F, Greenfield M, Durkan M, Leong YS, Dong Y, Cook H, Axford J, Callahan AG, Kenny N, Omodei C, McGraw EA, Ryan PA, Ritchie SA, Turelli M, O'Neill SL. Successful establishment of *Wolbachia* in *Aedes* populations to suppress dengue transmission. *Nature*. 2011; 476:454–457. [PubMed: 21866160]
- Holmes PH, Torr SJ. The control of animal trypanosomiasis in Africa – current methods and future-trends. *Outlook Agric*. 1988; 17:54–60.
- Hughes GL, Ren X, Ramirez JL, Sakamoto JM, Bailey JA, Jedlicka AE, Rasgon JL. *Wolbachia* infections in *Anopheles gambiae* cells: transcriptomic characterization of a novel host-symbiont interaction. *PLoS Pathog*. 2011; 7:e1001296. [PubMed: 21379333]
- Jaenson TG. Virus-like rods associated with salivary gland hyperplasia in tsetse, *Glossina pallidipes*. *Trans R Soc Trop Med Hyg*. 1978; 72:234–238. [PubMed: 675787]
- Jordan AM. Recent developments in the ecology and methods of control of tsetse flies (*Glossina spp*) (Dipt., Glossinidae) – a review. *Bull Entomol Res*. 1974; 63:361–399.
- Jordan, AM. *Trypanosomiasis Control and African Rural Development*. Longman; London: 1986.

- Jura WGZO, Odhiambo TR, Otieno LH, Tabu NO. Gonadal lesions in virus-infected male and female tsetse, *Glossina pallidipes* (Diptera–Glossinidae). *J Invertebr Pathol*. 1988; 52:1–8. [PubMed: 3418131]
- Jura WGZO, Otieno LH, Chintawi MMB. Ultrastructural evidence for trans-ovum transmission of the DNA virus of tsetse, *Glossina pallidipes* (Diptera, Glossinidae). *Curr Microbiol*. 1989; 18:1–4.
- Jura WGZO, Zdarek J, Otieno LH. A simple method for artificial infection of tsetse, *Glossina morsitans morsitans* larvae with the DNA virus of *Glossina Pallidipes*. *Insect Sci Its Appl*. 1993; 14:383–387.
- Kambris Z, Cook PE, Phuc HK, Sinkins SP. Immune activation by lifeshortening *Wolbachia* and reduced filarial competence in mosquitoes. *Science*. 2009; 326:134–136. [PubMed: 19797660]
- Kent BN, Bordenstein SR. Phage WO of *Wolbachia*: lambda of the endosymbiont world. *Trends Microbiol*. 2010; 18:173–181. [PubMed: 20083406]
- Kent BN, Salichos L, Gibbons JG, Rokas A, Newton IL, Clark ME, Bordenstein SR. Complete bacteriophage transfer in a bacterial endosymbiont (*Wolbachia*) determined by targeted genome capture. *Genome Biol Evol*. 2011; 3:209–218. [PubMed: 21292630]
- Kioy D, Jannin J, Mattock N. Human African trypanosomiasis. *Nat Rev Microbiol*. 2004; 2:186–187. [PubMed: 15751187]
- Klassen, W.; Curtis, CF. History of the sterile insect technique. In: Dyck, VA., et al., editors. *Sterile Insect Technique: Principles and Practice in Area-Wide Integrated Pest Management*. Springer; Dordrecht, The Netherlands: 2005. p. 1-34.
- Klasson L, Walker T, Sebaihia M, Sanders MJ, Quail MA, Lord A, Sanders S, Earl J, O'Neill SL, Thomson N, Sinkins SP, Parkhill J. Genome evolution of *Wolbachia* strain wPip from the *Culex pipiens* group. *Mol Biol Evol*. 2008; 25:1877–1887. [PubMed: 18550617]
- Klasson L, Kambris Z, Cook PE, Walker T, Sinkins SP. Horizontal gene transfer between *Wolbachia* and the mosquito *Aedes aegypti*. *BMC Genomics*. 2009a; 10:33. [PubMed: 19154594]
- Klasson L, Westberg J, Sapountzis P, Naslund K, Lutnaes Y, Darby AC, Veneti Z, Chen L, Braig HR, Garrett R, Bourtzis K, Andersson SG. The mosaic genome structure of the *Wolbachia* wRi strain infecting *Drosophila simulans*. *Proc Natl Acad Sci USA*. 2009b; 106:5725–5730. [PubMed: 19307581]
- Kokwaro ED, Nyindo M, Chintawi M. Ultrastructural changes in salivary glands of tsetse, *Glossina morsitans morsitans*, infected with virus and rickettsialike organisms. *J Invertebr Pathol*. 1990; 56:337–346. [PubMed: 2250102]
- Kondo N, Nikoh N, Ijichi N, Shimada M, Fukatsu T. Genome fragment of *Wolbachia* endosymbiont transferred to X chromosome of host insect. *Proc Natl Acad Sci USA*. 2002; 99:14280–14285. [PubMed: 12386340]
- Krafsur ES. Tsetse flies: genetics, evolution, and role as vectors. *Infect Genet Evol*. 2009; 9:124–141. [PubMed: 18992846]
- Kurland CG. Something for everyone. Horizontal gene transfer in evolution. *EMBO Rep*. 2000; 1:92–95. [PubMed: 11265763]
- Laven H. Eradication of *Culex pipiens fatigans* through cytoplasmic incompatibility. *Nature*. 1967; 216:383–384. [PubMed: 4228275]
- Leak, SGA. *Tsetse Biology and Ecology: Their Role in the Epidemiology and Control of Trypanosomiasis*. CABI Publishing; Wallingford: 1998.
- Leclercq S, Giraud I, Cordaux R. Remarkable abundance and evolution of mobile group II introns in *Wolbachia* bacterial endosymbionts. *Mol Biol Evol*. 2011; 28:685–697. [PubMed: 20819906]
- Lo N, Casiraghi M, Salati E, Bazzocchi C, Bandi C. How many *wolbachia* supergroups exist? *Mol Biol Evol*. 2002; 19:341–346. [PubMed: 11861893]
- Lo N, Paraskevopoulos C, Bourtzis K, O'Neill SL, Werren JH, Bordenstein SR, Bandi C. Taxonomic status of the intracellular bacterium *Wolbachia pipientis*. *Int J Syst Evol Microbiol*. 2007; 57:654–657. [PubMed: 17329802]
- Ma WC, Denlinger DL. Secretory discharge and microflora of milk gland in tsetse flies. *Nature*. 1974; 247:301–303.

- McMeniman CJ, Lane RV, Cass BN, Fong AW, Sidhu M, Wang YF, O'Neill SL. Stable introduction of a life-shortening *Wolbachia* infection into the mosquito *Aedes aegypti*. *Science*. 2009; 323:141–144. [PubMed: 19119237]
- McNulty SN, Foster JM, Mitreva M, Dunning Hotopp JC, Martin J, Fischer K, Wu B, Davis PJ, Kumar S, Brattig NW, Slatko BE, Weil GJ, Fischer PU. Endosymbiont DNA in endobacteria-free filarial nematodes indicates ancient horizontal genetic transfer. *PLoS ONE*. 2010; 5:e11029. [PubMed: 20543958]
- Min KT, Benzer S. *Wolbachia*, normally a symbiont of *Drosophila*, can be virulent, causing degeneration and early death. *Proc Nat Acad Sci USA*. 1997; 94:10792–10796. [PubMed: 9380712]
- Minter-Goedbloed E, Minter DM. Salivary gland hyperplasia and trypanosome infection of *Glossina* in two areas of Kenya. *Trans R Soc Trop Med Hyg*. 1989; 83:640–641. [PubMed: 2617626]
- Moreira LA, Iturbe-Ormaetxe I, Jeffery JA, Lu G, Pyke AT, Hedges LM, Rocha BC, Hall-Mendelin S, Day A, Riegler M, Hugo LE, Johnson KN, Kay BH, McGraw EA, van den Hurk AF, Ryan PA, O'Neill SL. A *Wolbachia* symbiont in *Aedes aegypti* limits infection with dengue, Chikungunya, and *Plasmodium*. *Cell*. 2009; 139:1268–1278. [PubMed: 20064373]
- Mousson L, Martin E, Zouache K, Madec Y, Mavingui P, Failloux AB. *Wolbachia* modulates Chikungunya replication in *Aedes albopictus*. *Mol Ecol*. 2010; 19:1953–1964. [PubMed: 20345686]
- Nikoh N, Nakabachi A. Aphids acquired symbiotic genes via lateral gene transfer. *BMC Biol*. 2009; 7:12. [PubMed: 19284544]
- Nikoh N, Tanaka K, Shibata F, Kondo N, Hizume M, Shimada M, Fukatsu T. *Wolbachia* genome integrated in an insect chromosome: evolution and fate of laterally transferred endosymbiont genes. *Genome Res*. 2008; 18:272–280. [PubMed: 18073380]
- Nogge G. Sterility in tsetse flies (*Glossina morsitans* Westwood) caused by loss of symbionts. *Experientia*. 1976; 32:995–996. [PubMed: 986317]
- Nogge G. Significance of symbionts for the maintenance of an optimal nutritional state for successful reproduction in hematophagous arthropods. *Parasitology*. 1981; 82:101–104.
- Odindo MO, Amutalla PA, Turner DA, Kokwaro ED, Otieno WA, Sabwa DM. Morphological variation and incidence of cuticular lesions in the tsetse *Glossina pallidipes* Austen, *G. brevipalpis* Newstead and *G. austeni* Newstead (Diptera: Glossinidae) on the Kenyan coast. *Insect Sci Its Appl*. 1982; 3:65–71.
- Oladunmade, MA.; Feldmann, U.; Takken, W.; Tenabe, SO.; Hamann, HJ.; Onah, JA.; Dengwat, L.; Van der Vloedt, AMV.; Gingrich, RE. Sterile Insect Technique for Tsetse Control and Eradication. I.A.E. Agency; Vienna: 1990. Eradication of *Glossina palpalis palpalis* (Robineau-Desvoidy) (Diptera: Glossinidae) from agropastoral land in central Nigeria by means of the sterile insect technique; p. 5-23.
- O'Neill SL, Gooding RH, Aksoy S. Phylogenetically distant symbiotic microorganisms reside in *Glossina* midgut and ovary tissues. *Med Vet Entomol*. 1993; 7:377–383. [PubMed: 8268495]
- Osborne SE, Leong YS, O'Neill SL, Johnson KN. Variation in antiviral protection mediated by different *Wolbachia* strains in *Drosophila simulans*. *PLoS Pathogens*. 2009; 5
- Otieno LH, Kokwaro ED, Chintawi M, Onyango P. Prevalence of enlarged salivary glands in wild populations of *Glossina pallidipes* in Kenya, with a note on the ultrastructure of the affected organ. *J Invertebr Pathol*. 1980; 36:113–118.
- Ouma JO, Marquez JG, Krafur ES. Patterns of genetic diversity and differentiation in the tsetse fly *Glossina morsitans morsitans* Westwood populations in East and southern Africa. *Genetica*. 2007; 130:139–151. [PubMed: 16897444]
- Ouma JO, Beadell JS, Hyseni C, Okedi LM, Krafur ES, Aksoy S, Caccone A. Genetic diversity and population structure of *Glossina pallidipes* in Uganda and western Kenya. *Parasit Vect*. 2011; 28:122.
- Pais R, Lohs C, Wu Y, Wang J, Aksoy S. The obligate mutualist *Wigglesworthia glossinidia* influences reproduction, digestion, and immunity processes of its host, the tsetse fly. *Appl Environ Microbiol*. 2008; 74:5965–5974. [PubMed: 18689507]

- Paraskevopoulos C, Bordenstein SR, Wernegreen JJ, Werren JH, Bourtzis K. Toward a *Wolbachia* multilocus sequence typing system: discrimination of *Wolbachia* strains present in *Drosophila* species. *Curr Microbiol.* 2006; 53:388–395. [PubMed: 17036209]
- Politzar H, Cuisance D. An integrated campaign against riverine tsetse, *Glossina palpalis gambiensis* and *Glossina tachinoides*, by trapping, and the release of sterile males. *Insect Sci Its Appl.* 1984; 5:439–442.
- Pontes MH, Dale C. Lambda red-mediated genetic modification of the insect endosymbiont *Sodalis glossinidius*. *Appl Environ Microbiol.* 2011; 77:1918–1920. [PubMed: 21216910]
- Rasgon J. Population replacement strategies for controlling vector populations and the use of *Wolbachia pipientis* for genetic drive. *J Vis Exp.* 2007; 225
- Rasgon JL. Using predictive models to optimize *Wolbachia*-based strategies for vector-borne disease control. *Adv Exp Med Biol.* 2008; 627:114–125. [PubMed: 18510019]
- Raychoudhury R, Baldo L, Oliveira DC, Werren JH. Modes of acquisition of *Wolbachia*: horizontal transfer, hybrid introgression, and codivergence in the *Nasonia* species complex. *Evolution.* 2009; 63:165–183. [PubMed: 18826448]
- Rio RV, Hu Y, Aksoy S. Strategies of the home-team: symbioses exploited for vector-borne disease control. *Trends Microbiol.* 2004; 12:325–336. [PubMed: 15223060]
- Ros VI, Fleming VM, Feil EJ, Breeuwer JA. How diverse is the genus *Wolbachia*?. Multiple-gene sequencing reveals a putatively new *Wolbachia* supergroup recovered from spider mites (Acari: Tetranychidae). *Appl Environ Microbiol.* 2009; 75:1036–1043. [PubMed: 19098217]
- Rowley SM, Raven RJ, McGraw EA. *Wolbachia pipientis* in Australian spiders. *Curr Microbiol.* 2004; 49:208–214. [PubMed: 15386106]
- Ruang-Areerate T, Kittayapong P. *Wolbachia* transinfection in *Aedes aegypti*: a potential gene driver of dengue vectors. *Proc Natl Acad Sci USA.* 2006; 103:12534–12539. [PubMed: 16895998]
- Runyen-Janecky LJ, Brown AN, Ott B, Tujuba HG, Rio RV. Regulation of high-affinity iron acquisition homologues in the tsetse fly symbiont *Sodalis glossinidius*. *J Bacteriol.* 2010; 192:3780–3787. [PubMed: 20494987]
- Sacchi L, Genchi M, Clementi E, Negri I, Alma A, Ohler S, Sassera D, Bourtzis K, Bandi C. Bacteriocyte-like cells harbour *Wolbachia* in the ovary of *Drosophila melanogaster* (Insecta, Diptera) and *Zyginidia pullula* (Insecta, Hemiptera). *Tissue Cell.* 2010; 42:328–333. [PubMed: 20817243]
- Salunke BK, Salunkhe RC, Dhotre DP, Khandagale AB, Walujkar SA, Kirwale GS, Ghate HV, Patole MS, Shouche YS. Diversity of *Wolbachia* in *Odontotermes spp* (Termitidae) and *Coptotermes heimi* (Rhinotermitidae) using the multigene approach. *FEMS Microbiol Lett.* 2010; 307:55–64. [PubMed: 20402779]
- Salzberg SL, Dunning Hotopp JC, Delcher AL, Pop M, Smith DR, Eisen MB, Nelson WC. Serendipitous discovery of *Wolbachia* genomes in multiple *Drosophila* species. *Genome Biol.* 2005; 6:R23. [PubMed: 15774024]
- Salzberg SL, Puiu D, Sommer DD, Nene V, Lee NH. Genome sequence of the *Wolbachia* endosymbiont of *Culex quinquefasciatus* JHB. *J Bacteriol.* 2009; 191:1725. [PubMed: 19114486]
- Sang RC, Jura WGZO, Otieno LH, Ogaja P. Ultrastructural changes in the milk gland of tsetse *Glossina morsitans centralis* (Diptera; Glossinidae) female infected by a DNA virus. *J Invertebr Pathol.* 1996; 68:253–259. [PubMed: 8954813]
- Sang RC, Jura WGZO, Otieno LH, Tukei PM, Mwangi RW. Effects of tsetse DNA virus infection on the survival of a host fly, *Glossina morsitans centralis* (Diptera; glossinidae). *J Invertebr Pathol.* 1997; 69:253–260.
- Sang RC, Jura WG, Otieno LH, Mwangi RW. The effects of a DNA virus infection on the reproductive potential of female tsetse flies, *Glossina morsitans centralis* and *Glossina morsitans morsitans* (Diptera: Glossinidae). *Mem Inst Oswaldo Cruz.* 1998; 93:861–864. [PubMed: 9921317]
- Sang RC, Jura WG, Otieno LH, Mwangi RW, Ogaja P. The effects of a tsetse DNA virus infection on the functions of the male accessory reproductive gland in the host fly *Glossina morsitans centralis* (Diptera; Glossinidae). *Curr Microbiol.* 1999; 38:349–354. [PubMed: 10341076]

- Saridaki A, Bourtzis K. *Wolbachia*: more than just a bug in insects genitals. *Curr Opin Microbiol*. 2010; 13:67–72. [PubMed: 20036185]
- Schneider D, Garschall KI, Parker AG, Abd-Alla AAM, Miller WJ. Global *Wolbachia* prevalence and titer fluctuations in tsetse flies and inter-species hybrids of the *Glossina morsitans* subgroup. *J Invertebr Pathol*. 2013; 112:s104–s115. [PubMed: 22516306]
- Shaw MK, Moloo SK. Virus-like particles in rickettsia within the midgut epithelial cells of *Glossina morsitans centralis* and *Glossina brevipalpis*. *J Invertebr Pathol*. 1993; 61:162–166.
- Simarro PP, Jannin J, Cattand P. Eliminating human African trypanosomiasis: where do we stand and what comes next? *PLoS Med*. 2008; 5:e55. [PubMed: 18303943]
- Sinkins SP, Godfray HC. Use of *Wolbachia* to drive nuclear transgenes through insect populations. *Proc Biol Sci*. 2004; 271:1421–1426. [PubMed: 15306342]
- Sinkins SP, Gould F. Gene drive systems for insect disease vectors. *Nat Rev Genet*. 2006; 7:427–435. [PubMed: 16682981]
- Teixeira L, Ferreira A, Ashburner M. The bacterial symbiont *Wolbachia* induces resistance to RNA viral infections in *Drosophila melanogaster*. *PLoS Biol*. 2008; 6:e2. [PubMed: 19222304]
- Thompson JW, Mitchell M, Rees RB, Shereni W, Schoenfeld AH, Wilson A. Studies on the efficacy of deltamethrin applied to cattle for the control of tsetse flies (*Glossina spp*) in southern Africa. *Trop Anim Health Prod*. 1991; 23:221–226. [PubMed: 1796525]
- Toh H, Weiss BL, Perkin SA, Yamashita A, Oshima K, Hattori M, Aksoy S. Massive genome erosion and functional adaptations provide insights into the symbiotic lifestyle of *Sodalis glossinidius* in the tsetse host. *Genome Res*. 2006; 16:149–156. [PubMed: 16365377]
- Van den Abbeele J, Bourtzis K, Weiss B, Cordon-Rosales C, Miller W, Abd-Alla A, Parker AG. Enhancing Tsetse fly refractoriness to Trypanosome infection – a new IAEA coordinated research project. *J Invertebr Pathol*. 2013; 112:s142–s147. [PubMed: 22841950]
- Van den Bossche P, de La Rocque S, Hendrickx G, Bouyer J. A changing environment and the epidemiology of tsetse-transmitted livestock trypanosomiasis. *Trends Parasitol*. 2010; 26:236–243. [PubMed: 20304707]
- Vreysen MJ, Saleh KM, Ali MY, Abdulla AM, Zhu ZR, Juma KG, Dyck VA, Msangi AR, Mkonyi PA, Feldmann HU. *Glossina austeni* (Diptera: Glossinidae) eradicated on the island of Unguja, Zanzibar, using the sterile insect technique. *J Econ Entomol*. 2000; 93:123–135. [PubMed: 14658522]
- Vreysen MJ, Saleh KM, Lancelot R, Bouyer J. Factory tsetse flies must behave like wild flies: a prerequisite for the sterile insect technique. *PLoS Negl Trop Dis*. 2011; 5:e907. [PubMed: 21364965]
- Walker T, Johnson PH, Moreira LA, Iturbe-Ormaetxe I, Frentiu FD, McMeniman CJ, Leong YS, Dong Y, Axford J, Kriesner P, Lloyd AL, Ritchie SA, O'Neill SL, Hoffmann AA. The *Wolbachia* strain blocks dengue and invades caged *Aedes aegypti* populations. *Nature*. 2011; 476:450–453. [PubMed: 21866159]
- Wang J, Wu Y, Yang G, Aksoy S. Interactions between mutualist *Wigglesworthia* and tsetse peptidoglycan recognition protein (PGRP-LB) influence trypanosome transmission. *Proc Natl Acad Sci USA*. 2009; 106:12133–12138. [PubMed: 19587241]
- Weiss BL, Mouchotte R, Rio RV, Wu YN, Wu Z, Heddi A, Aksoy S. Interspecific transfer of bacterial endosymbionts between tsetse fly species: infection establishment and effect on host fitness. *Appl Environ Microbiol*. 2006; 72:7013–7021. [PubMed: 16950907]
- Welburn SC, Maudlin I, Ellis DS. In vitro cultivation of rickettsia-like organisms from *Glossina spp*. *Ann Trop Med Parasitol*. 1987; 81:331–335. [PubMed: 3662675]
- Welburn SC, Fevre EM, Coleman PG, Odiit M, Maudlin I. Sleeping sickness: a tale of two diseases. *Trends Parasitol*. 2001; 17:19–24. [PubMed: 11137736]
- Werren JH, Baldo L, Clark ME. *Wolbachia*: master manipulators of invertebrate biology. *Nat Rev Microbiol*. 2008; 6:741–751. [PubMed: 18794912]
- Whitnall ABM. The Trypanosome infections of *Glossina pallidipes* in the Umfolosi Game Reserve, Zululand. *Onderstepoort J Vet Sci Anim Ind*. 1934; 2:7–21.

- Woolfit M, Iturbe-Ormaetxe I, McGraw EA, O'Neill SL. An ancient horizontal gene transfer between mosquito and the endosymbiotic bacterium *Wolbachia pipientis*. *Mol Biol Evol*. 2009; 26:367–374. [PubMed: 18988686]
- Wu M, Sun LV, Vamathevan J, Riegler M, Deboy R, Brownlie JC, McGraw EA, Martin W, Esser C, Ahmadinejad N, Wiegand C, Madupu R, Beanan MJ, Brinkac LM, Daugherty SC, Durkin AS, Kolonay JF, Nelson WC, Mohamoud Y, Lee P, Berry K, Young MB, Utterback T, Weidman J, Niernan WC, Paulsen IT, Nelson KE, Tettelin H, O'Neill SL, Eisen JA. Phylogenomics of the reproductive parasite *Wolbachia pipientis* wMel: a streamlined genome overrun by mobile genetic elements. *PLoS Biol*. 2004; 2:E69. [PubMed: 15024419]
- Wyss, JH. Screw-worm eradication in the Americas-overview. In: Tan, KH., editor. *Area-Wide Control of Fruit Flies and other Insect Pests*. Penerbit Universiti Sains Malaysia; Penang, Malaysia: 2000. p. 79-86.
- Xi Z, Khoo CC, Dobson SL. *Wolbachia* establishment and invasion in an *Aedes aegypti* laboratory population. *Science*. 2005; 310:326–328. [PubMed: 16224027]
- Yun Y, Lei C, Peng Y, Liu F, Chen J, Chen L. *Wolbachia* strains typing in different geographic population spider, *Hylyphantes graminicola* (*Linyphiidae*). *Curr Microbiol*. 2010; 62:139–145. [PubMed: 20533041]
- Zabalou S, Riegler M, Theodorakopoulou M, Stauffer C, Savakis C, Bourtzis K. *Wolbachia*-induced cytoplasmic incompatibility as a means for insect pest population control. *Proc Natl Acad Sci USA*. 2004; 101:15042–15045. [PubMed: 15469918]
- Zabalou S, Apostolaki A, Livadaras I, Franz G, Robinson AS, Savakis C, Bourtzis K. Incompatible insect technique: incompatible males from a *Ceratitis capitata* genetic sexing strain. *Entomol Exp Appl*. 2009; 132:232–240.
- Zchori-Fein, E.; Bourtzis, K. *Manipulative Tenants: Bacteria Associated with Arthropods*. CRC Press; Boca Raton, Fla: 2011. p. 268p
- Zhou W, Rousset F, O'Neil S. Phylogeny and PCR-based classification of *Wolbachia* strains using *wsp* gene sequences. *Proc Biol Sci*. 1998; 265:509–515. [PubMed: 9569669]

Table 1

Wolbachia prevalence in adult flies of natural populations and laboratory lines of twelve *Glossina* species.

Glossina species	Wolbachia prevalence			References
	Natural	Laboratory	Total	
<i>G. austeni</i>	(355/432) 82.2%	Not analyzed	(355/432) 82.2%	Cheng et al. (2000), Doudoumis et al. (2012) and O'Neill et al. (1993) ^a
<i>G. brevipalpis</i>	(17/218) 7.8%	(24/44) 54.5%	(41/262) 15.6%	Cheng et al. (2000), Doudoumis et al. (2012) and O'Neill et al. (1993) ^a
<i>G. m. centralis</i>	Not analyzed	(13/13) 100.0%	(13/13) 100.0%	Cheng et al. (2000), Doudoumis et al. (2012) and O'Neill et al. (1993) ^a
<i>G. m. morsitans</i>	(519/601) 86.4%	(119/119) 100.0%	(638/720) 88.6%	Cheng et al. (2000), Doudoumis et al. (2012) and O'Neill et al. (1993) ^a
<i>G. f. fuscipes</i>	(0/116) 0.0%	(0/46) 0.0%	(0/162) 0.0%	Cheng et al. (2000), Doudoumis et al. (2012) and O'Neill et al. (1993) ^a
<i>G. p. gambiensis</i>	(2/555) 0.4%	(0/99) 0.0%	(2/654) 0.3%	Cheng et al. (2000), Doudoumis et al. (2012) and O'Neill et al. (1993) ^a
<i>G. p. palpalis</i>	(0/48) 0.0%	(0/46) 0.0%	(0/94) 0.0%	Cheng et al. (2000), Doudoumis et al. (2012) and O'Neill et al. (1993) ^a
<i>G. pallidipes</i>	(19/1800) 1.1%	(13/247) 5.3%	(32/2047) 1.6%	Cheng et al. (2000) and Doudoumis et al. (2012)
<i>G. tachinoides</i>	(0/93) 0.0%	(0/17) 0.0%	(0/110) 0.0%	Cheng et al. (2000), Doudoumis et al. (2012) and O'Neill et al. (1993) ^a
<i>G. longipinnis</i>	(0/47) 0.0%	(10/10) 100.0%	(10/57) 17.5%	Cheng et al. (2000)
<i>G. swynnertoni</i>	(3/27) 11.1%	(10/10) 100.0%	(13/37) 35.1%	Cheng et al. (2000)
<i>G. m. submorsitans</i>			0.0%	O'Neill et al. (1993) ^a
Total	(915/3937) 23.2%	(189/651) 29%	(1104/4588) 24.1%	

^aIn the study by O'Neill et al. (1993), *G. austeni*, *G. brevipalpis*, *G. m. morsitans* and *G. m. centralis* are reported as *Wolbachia*-infected while *G. f. fuscipes*, *G. p. gambiensis*, *G. p. palpalis*, *G. tachinoides* and *G. m. submorsitans* as *Wolbachia*-uninfected; however, the precise number of individuals tested was not mentioned.

Table 2

Wolbachia genome projects.

Host	Strain	Supergroup	Genome size (Kb)	ORFs	Contigs	Status	Reference	NCBI record
<i>Drosophila melanogaster</i>	wMel	A	1267	1312	1	CG	Wu et al. (2004)	AE017196
<i>Drosophila simulans</i>	wRi	A	1445	1187	1	CG	Klasson et al. (2009b)	CP001391
<i>Culex quinquefasciatus Pel</i>	wPip	B	1482	1312	1	CG	Klasson et al. (2008)	AM999887
<i>Brugia malayi</i>	wBm	D	1080	940	1	CG	Foster et al. (2005)	AE017321
<i>Glossina morsitans morsitans</i>	wGmm	A	1021	932	1	CG	Aksoy S.	NK
<i>Culex quinquefasciatus JHB</i>	wPip	B	1542	1415	21	PDG	Salzberg et al. (2009)	ABZA00000000
<i>Nasonia vitripennis</i>	wVitB	A	NK	NK	523	PDG	Kent et al. (2011)	AERW00000000
<i>Muscidifurax uniraptor</i>	wUni	A	867	855	256	DG	Klasson et al. (2009a)	ACFP00000000
<i>Culex pipiens molestus</i>	wPipMol	B	NK	NK	888	DG	Parkhill J	CACK00000000
<i>Drosophila simulans</i>	wSim	A	1063	781	629	DG	Salzberg et al. (2005)	AAGC00000000
<i>Drosophila willistoni</i>	wWil	A	862	992	260	DG	Salzberg et al. (2005)	AAQP00000000
<i>Drosophila ananassae</i>	wAna	A	1440	1834	464	DG	Salzberg et al. (2005)	AAGB00000000
<i>Dirofilaria immitis</i>	wDim	C	NK	NK	NK	IP	Bandi C	NK
<i>Onchocerca volvulus</i>	wOv	C	NK	31100	NK	IP	Parkhill J and Birren B	NK
<i>Diaphorina citri</i>	ND	B	NK	1063	760	IP	Weinstock G	NK
<i>Wuchereria bancrofti</i>	ND	D	NK	NK	NK	IP	Birren B	NK

CG, complete genome; PDG, permanent draft genome; DG, draft genome; IP, in progress; NK, not known; ND, not designated.

Table 3*Wolbachia* acquired genes in insects and filarial nematodes.

Host	Estimated size of genes transferred	Cytoplasmic <i>Wolbachia</i>	Level of expression	References
<i>Callosobruchus chinensis</i>	380 kb	+	–	Kondo et al. (2002) and Nikoh et al. (2008)
<i>Drosophila ananassae</i>	>1 Mb	+	+ ^c	Dunning Hotopp et al. (2007)
<i>Onchocerca volvulus</i>	2 Kb	+	NK	Fenn et al. (2006)
<i>Nasonia vitripennis</i>	491 bp	+	NK	Dunning Hotopp et al. (2007)
<i>Nasonia longicornis</i>	142 bp	+	NK	Dunning Hotopp et al. (2007)
<i>Nasonia giraulti</i>	446 bp	+	NK	Dunning Hotopp et al. (2007)
<i>Brugia malayi</i>	>8 kb	+	NK	Dunning Hotopp et al. (2007)
<i>Dirofilaria immitis</i>	978 bp	+	NK	Dunning Hotopp et al. (2007)
<i>Monochanus alternatus</i>	>180 kb	+	NK	Aikawa et al. (2009)
<i>Aedes aegypti</i> ^a	1278 bp	–	+ ^d	Woolfit et al. (2009) and Klasson et al. (2009a)
<i>Aedes mascarensis</i> ^a	1278 bp	–	+ ^d	Woolfit et al. (2009) and Klasson et al. (2009a)
<i>Acyrtosiphon pisum</i> ^b	1312 bp	–	+ ^d	Nikoh and Nakabachi (2009)
<i>Glossina morsitans morsitans</i>	>500 kb	+	NK	Bourtzis K and Serap A. personal communication
<i>Acanthocheilonema viteae</i>	>30 kb	–	+ ^c	McNulty et al. (2010)
<i>Onchocerca flexuosa</i>	>30 kb	–	+ ^c	McNulty et al. (2010)

NK, not known.

^aDirection of transfer is not clear.^bTransfer from a *Wolbachia*-like bacterium.^cExpression was detected just above background level; biological significance is not clear.^dExpressed above background level.