Abstract As one of the most successful intracellular symbiotic bacteria, *Wolbachia* can infect many arthropods and nematodes. *Wolbachia* infection usually affects the reproduction of their hosts to promote their own proliferation and transmission. Currently, most of the studies focus on the mechanisms of *Wolbachia* interactions with host reproduction. However, in addition to distribution in the reproductive tissues, *Wolbachia* also infect various somatic tissues of their hosts, including the brain. This raises the potential that *Wolbachia* may influence some somatic processes, such as behaviors in their hosts. So far, information about the effects of *Wolbachia* infection on host behavior is still very limited. The present review presents the current literature on different aspects of the influence of *Wolbachia* on various behaviors, including sleep, learning and memory, mating, feeding and aggression in their insect hosts. We then highlight ongoing scientific efforts in the field that need addressing to advance this field, which can have significant implications for further developing *Wolbachia* as environmentally friendly biocontrol agents to control insect-borne diseases and agricultural pests.

Key words aggression; insect hosts; learning and memory capacity; mating; sleep; *Wolbachia*

Introduction

*Wolbachia* is the most widespread group of intracellular bacteria belonging to the alpha-proteobacteria. They can infect numerous of insects, isopods, spiders, as well as filarial nematodes (Werren, 1997; Zug & Hammerstein, 2012; Zhang et al., 2013; Turelli et al., 2018). *Wolbachia* are maternally transmitted and have evolved several strategies to manipulate host reproduction including parthenogenesis, feminization, male killing and cytoplasmic incompatibility to facilitate their own proliferation and transmission (Weeks & Breeuwer, 2001; Zheng et al., 2011a; Beckmann et al., 2017; LePage et al., 2017; Miyata et al., 2017; Harumoto et al., 2018). In the last decade, several lines of evidence have suggested that *Wolbachia* can be used as environmentally friendly biocontrol agents to control insect pest populations and disease vectors (McMeniman et al., 2009; Hoffmann et al., 2011; Moreira et al., 2011; Nguyen et al., 2015; Zhou & Li, 2016; Aliota et al., 2016a, 2016b; Garcia et al., 2019).

*Wolbachia* have been estimated to be the most ubiquitous endosymbiont, and can be used as an ideal model for studying the interactions between microbials and their hosts (Werren, 1997; Wu et al., 2016; Bhattacharya et al., 2017; Pan et al., 2018). So far, most studies on *Wolbachia* focus on the regulatory mechanisms by which *Wolbachia* mediate host reproduction (Zheng et al., 2011a, 2011b; Liu et al., 2014; Beckmann et al., 2017; LePage et al., 2017; Bonneau et al., 2018; Shropshire et al., 2018). However, it has been reported that *Wolbachia* distribute not only in the reproductive system, but also in various somatic tissues of their hosts where they might play a role in regulating host physiology and behavior (Casper-Lindley et al., 2011; Albertson et al., 2013; Rohrscheib et al., 2015; Pietri et al., 2016). Symbiotic bacteria often
alter the behavior of their hosts in various ways. Studies on arthropods that have horizontally acquired parasites show that behavioral changes may be induced by altered levels of gene expression in the central nervous system of the hosts (Thomas et al., 2005; Perrot-Minot & Cézilly, 2010). Although the precise mechanisms are not well understood, the behavioral change is inferred to be the consequence of selection acting on the parasite to promote the host’s transmission. Behavioral changes are also found in hosts due to infection with vertically acquired intracellular bacteria such as Wolbachia. These modifications in host behavior may be caused by altered gene expression levels not only in the central nervous system but also in other places in the hosts (Richard, 2017; Bi et al., 2019; Schneider et al., 2019), and be driven by selection acting to promote bacterial transmission (Goodacre & Martin, 2012). This review presents recent advances in studies on the influences of Wolbachia infection on the behavior of their insect hosts and the current molecular mechanisms by which the endosymbiont modifies hosts’ behaviors.

The distribution of Wolbachia in host somatic cells

Numerous researchers have documented that Wolbachia present not only in host gonads, but also in a wide array of somatic tissues of hosts. For instance, studies have demonstrated the presence of Wolbachia in the brains of Drosophila melanogaster and D. simulans (Min & Benzer, 1997; Dobson et al., 1999; Albertson et al., 2009; Albertson et al., 2013; Strunov et al., 2013), Collomembola (springtails) (Czarnetzki & Tebbe, 2004), and Eurema hecabe (butterfly) (Narita et al., 2007). This indicates that Wolbachia have the potential to influence host behaviors. In addition to the nervous tissues, Wolbachia also infect many other somatic tissues such as the gut, salivary glands, fat body, hemocytes, and Malpighian tubules of their hosts where they may affect host immunity and behavior (Dobson et al., 1999; Casper-Lindley et al., 2011; Albertson et al., 2013; Rohrscheib et al., 2015; Pietri et al., 2016; Pan et al., 2018; Saijo et al., 2018; Da Silva Gongalves et al., 2019). Furthermore, Wolbachia has been found in muscle and wing tissue of some host species (Andersen et al., 2012). Pietri et al. (2016) have summarized the distribution of Wolbachia in different tissues of various hosts. Moreover, Wolbachia localization is variable. In Drosophila, it was revealed that the distribution of the pathogenic Wolbachia strain, wMelPop, in the nervous system of adults was temperature-dependent: Wolbachia would move from the central brain to peripheral areas when temperature is increased (Strunov et al., 2013).

The effect of Wolbachia on the sleep behavior of hosts

Sleep behavior in Drosophila has similar features to mammalian sleep, such as prolonged reversible inactivity, increased arousal thresholds and homeostatic influence (Hendricks et al., 2000; Shaw et al., 2000). The timing of sleep is controlled by a circadian system, which recurs approximately every 24 h in flies. Locomotor activity in Drosophila is organized in a 12 : 12 h light : dark cycle. Drosophila exhibits peaks of activity during dawn and dusk. Sleep homeostasis is traditionally measured by actively disrupting sleep, typically using mechanical stimuli. Sleep latency is the length of time to the first sleep bout after the arousing stimulus. When flies recover sleep after sleep loss, they do so by increasing its duration and/or by enhancing its intensity (Allada et al., 2017).

Currently, sleep in flies is normally monitored using the Drosophila Activity Monitoring System. An infrared beam directed through the midpoint of each tube (with inner diameter 5 mm and length 60 mm) measures an “activity event” each time a fly crosses the beam. Locomotor activity counts were collected every minute, and sleep was defined by zero activity counts for at least five consecutive minutes (Huber et al., 2004). By quantifying the average level of locomotor activity during 5-min periods, one may compare locomotor activities of flies in different statuses when they are awake. Several studies have demonstrated that Wolbachia influence the sleep behavior of their hosts (Table 1). Albertson et al. (2013) once analyzed whether Wolbachia affected behavior and physiology, and showed a significant correlation of Wolbachia infection status with differences in sleep time during the day in Drosophila. Consistently, by testing the effect of Wolbachia infection on activity of D. melanogaster researchers revealed that Wolbachia infection decreased the percentage of time spent being active in both male and female flies, which suggests an increase in sleep time in flies of both sexes (Vale & Jardine, 2015). We recently found that Wolbachia-infected flies increased sleep time in both male and female D. melanogaster when compared to the uninfected flies, which is due to the increased number of sleep bouts during nighttime (Bi et al., 2018).

Wolbachia infection led to a significant decrease in the arousal threshold of flies (Bi et al., 2018), meaning that Wolbachia-infected flies are easily disturbed by a minimal stimulus relative to uninfected flies. This is consistent with the observation that Wolbachia infection resulted in...
an increase of the number of nighttime sleep bouts (Bi et al., 2018), indicating that Wolbachia infection could decrease the sleep quality in D. melanogaster. Morioka et al. (2018) also demonstrated that D. melanogaster carrying Wolbachia exhibited an increase in nocturnal activities relative to uninfected flies, which may reflect a decrease in arousal threshold during the nighttime. Moreover, we also found that Wolbachia infection caused an increase in sleep latency as the night progresses (Bi et al., 2018). This seems to be opposed to our common sense that arousal thresholds increase, and sleep latency decrease as the night progresses, which is how humans and other diurnally active animals behave. However, the overall pattern of the arousal threshold results is in accordance with previous observation in flies where arousal responses were lowest when flies are sleeping at maximal consolidated levels (Faville et al., 2015). In our study, flies showed peak sleep times between ZT 14–16 (ZT = zeitgeber time, where ZT 0 is lights-on, and ZT 12 is lights off), which corresponded exactly to the times when flies showed the lowest responsiveness to an arousing stimulus and shortest sleep latency. Together, these results suggest that Wolbachia-infected flies are incapable of experiencing deep and high-quality sleep, or alternatively are more sensitive to the environment.

Complex interactions between circadian and homeostatic processes ensure that sleep occurs at right times in a day (Dijk & Czeisler, 1995). Plants and animals have these built-in cycles which allow them to flower at the right time, sleep at the right time, whereas the homeostatic process determines the time and quality of sleep, and the body need to sleep when facing sleep deprivation (Donlea et al., 2014; Cavanaugh et al., 2016; Dubowy et al., 2016). Circadian rhythms are “built-in” so that, without time signals from the environment, they keep time at the rate of about 24-h periods. It is important that the rhythms be re-set regularly to the natural light/dark cycle (Zheng & Sehgal, 2012). We found that Wolbachia did not disturb the circadian rhythm and sleep rebound after deprivation of D. melanogaster hosts (Bi et al., 2018). This suggests that Wolbachia infection does not break this internal rhythm under the regular 24 h clock and normal environment.

To explore the mechanisms of sleep behavior change, our further experiments showed that the interference of sleep caused by Wolbachia infection may be related to the dopamine pathway, as the two key genes in dopaminergic signaling pathway, Pale and Ddc, are both upregulated in Wolbachia-infected flies compared to uninfected ones (Bi et al., 2018). Wu et al. (2018) showed that juvenile hormone (JH) signaling pathway was important for maintenance of sexually dimorphic sleep by regulating sex-relevant genes, such as fruitless (fru) and doublesex (dsx) in males and sex-lethal (sxl), transformer (tra) and doublesex (dsx) in females. Several studies have revealed that Wolbachia infection may make sterile Sxl mutant female hosts capable of producing mature eggs (Ote et al., 2016), and Wolbachia can also interfere with dsx gene expression in their insect host, such as expressing the female-specific isofrom of dsx in genetical males (Sugimoto et al., 2010). These suggest that Wolbachia may interact with sex-relevant genes in their insect hosts. Dalton et al. (2013) identified that dopamine receptor 2 was upregulated in response to overexpression of male-specific fru, indicating that sex-relevant genes may regulate the dopamine pathway. Furthermore, JH Inducible-21 (JHI-21) has been reported to be required for sleep/wake regulation, since downregulation of JHI-21 in dopaminergic neurons reduced the sensitivity to L-DOPA, thus leading to an increase in daily sleep and sleep bout duration during the night. (Aboudhiaf et al., 2018). Correspondingly, our previous work found that Wolbachia could stimulate the JH signaling pathway in male Drosophila (Liu et al., 2014). Hence, it is reasonable that Wolbachia could affect
sleep behavior of Drosophila through the JH/sex-relevant gene/dopamine pathway.

Most animals would reduce activity or increase sleep when they get infections, which may therefore be considered as a general reflection of infection (Adelman & Martin, 2009; Kuo et al., 2010; Kuo & Williams, 2014). Wolbachia do not have the complete set of metabolic pathways present in free-living bacteria (Wu et al., 2004). Studies have indicated that Wolbachia could affect host metabolic pathways to obtain the nutrients and energy for their own survival and proliferation (Brownlie et al., 2009; Evans et al., 2009; Yuan et al., 2015; Saucereau et al., 2017). Therefore it is reasonable to suggest that the increase of sleep in the hosts caused by Wolbachia could be an adaptive strategy to conserve limited resources and energy for reproductive output, which is in favor of both the maternally transmitted bacteria and the host. This could be the result of co-evolution of Wolbachia and their hosts.

Effect of Wolbachia on learning and memory capacity (LMC) in hosts

Wolbachia are known to accumulate in nervous tissues of their hosts, including the central brain. The central brain includes the antennal lobes, which are responsible for receiving input from the olfactory sensory neurons, and the mushroom bodies which provide sensory learning and memory capacities. Therefore, this distribution of Wolbachia raises the possibility that Wolbachia may influence host LMC. Indeed, some researchers showed that one kind of Wolbachia, wVulC could decrease the LMC of its host Armadillidium vulgare, a terrestrial isopod. wVulC-infected A. vulgare are less likely to remember the correct direction after training when compared to the Wolbachia-free group (Templé & Richard, 2015). This suggests that Wolbachia can affect cognitive processes in A. vulgare, resulting in a decrease of host adaptation. Kishani Farahani et al. (2017) reported that Wolbachia infection decreased the memory retention of host wasps in new environments relative to uninfected ones, thus increasing the spread of the endosymbiont through regulating wasps to forget the information about previous environments.

Recently, we found that Wolbachia infection significantly enhanced LMC in both D. melanogaster and D. simulans (Bi et al., 2019). This result is different from that reported by Templé and Richard (2015). We can interpret these opposite findings in two ways. (i) There could be differences in experimental design. They examined the effect of Wolbachia on short-term memory (memory tested <1 h post-training), while we tested at 24 h after training which is a long-term memory. Moreover, they used only the chemical cues from the opposite sex as the “reward”, while we applied conditioning and testing protocols with sugar reward. (ii) It could be due to different Wolbachia/host combinations. Documents have revealed that different Wolbachia strains could induce distinct phenotypes in one host, and one Wolbachia strain may have various effects on different hosts (Dean, 2006; Chafee et al., 2011; Russell et al., 2018). In their case, wVulC Wolbachia is pathogenic and over-replicates reducing the lifespan of its host. It also induces feminization of the host A. vulgare (Templé & Richard, 2015). While in our study, wMel and wRi Wolbachia are not pathogenic, they could induce sperm-egg cytoplasmic incompatibility in D. melanogaster and D. simulans, respectively. Furthermore, the different taxonomic groups of the hosts (Crustacea and Insecta) may also contribute to the contrast results. A. vulgare have a gregarious lifestyle and a strong tendency for individuals to aggregate (Broly et al., 2013). This lifestyle may lead to a danger of being fully attacked by predators. The reduction of LMC in A. vulgare induced by wVulC Wolbachia may decrease the risk of being fully preyed upon, thus ensuring the survival and transmission of Wolbachia. However, Drosophila is solitary. The improved LMC caused by Wolbachia may be favorable for flies to find food and escape from dangerous environments. Evidence has suggested that LMC is likely to be important for fitness in a diverse set of taxa (Rice & McQuillan, 2018). For example, in grasshopper Schistocerca americana, insects that could employ associative learning had exhibited higher growth rates than insects that were prevented from learning (Dukas & Bernays, 2000). Similarly, in African striped mice Rhabdomys pumilio, higher spatial memory ability and faster reaction times are associated with increased survival rates (Maille & Schradin, 2016). From this aspect, the enhanced LMC due to Wolbachia infection could increase the fitness of the hosts and also benefit the survival and spread of the bacteria.

It remains largely unknown how Wolbachia influence the LMC of their hosts. We recently explored the molecular mechanisms and demonstrated that Wolbachia infection reduced the expression of a microRNA (miRNA) — dme-miR-92b in D. melanogaster, thus increased the expression of its target gene crebA in the head of flies by binding to the 3' untranslated region (Bi et al., 2019). CrebA encodes cyclic-adenosine monophosphate response element binding protein A and has been shown to have a role in dendrite development in Drosophila (Iyer et al., 2013). Evidence has shown that CrebA knockdown in Drosophila neurons affected dendritic terminals and total length, causing an overall reduction in branch complexity, indicating functions of CrebA in promoting higher order branching and dendritic growth (Iyer et al., 2013).
Furthermore, other studies have revealed that changes in dendritic complexity impaired LMC (Freymuth & Fitzsimons, 2017; Lee et al., 2017). Both the microinjection of dme-miR-92b antagonirs and overexpression of crebA in D. melanogaster did result in significant increase of LMC (Bi et al., 2019), further supporting a miRNA-target pathway that regulates the change of LMC in Drosophila induced by Wolbachia infection. Future investigations on the effects of Wolbachia infection and crebA mutation on fly dendrite development will further reinforce this argument.

The changes in host LMC could potentially contribute in evolutionary strategies. For the pathogenic strain of Wolbachia, the infection causes a decrease in host fitness. Thus the alteration in LMC could aim to avoid or slow down the parasites’ transmission in the population. However, for the Wolbachia strains that are not pathogenic, the adaptive alteration of the hosts induced by Wolbachia may be a kind of co-evolution between the endosymbiont and their hosts. Wolbachia infection improves LMC in hosts which may help hosts to find food, mates and suitable habitats for offspring, and sometimes to avoid danger more efficiently. The benefits to the host also favor the proliferation and propagation of the maternal transmitted bacteria. These findings open a new approach for understanding host/endosymbionts interactions and in particular on Wolbachia impact on signal interpretation, learning and memory consequences in various hosts. As Wolbachia-infected arthropods and nematodes could be intermediate hosts during infection of vertebrates, including humans, the behavioral and ecological consequences of arthropod infection should be taken into consideration while developing strategies for controlling pests and insect-borne diseases.

Effect of Wolbachia on the mating behavior of hosts

The role of Wolbachia in mediating mating behavior of their hosts remains controversial. Zhao et al. (2013) reported that in the two-spotted spider mite Tetranychus urticae, Wolbachia-infected and uninfected males did not show different mating behaviors when mated with uninfected females. Likewise, there is no evidence of male mate preferences in the butterfly Acraea edcedon (Jiggins et al., 2002) and D. innubila (Sullivan & Jaenike, 2006), although Wolbachia can kill male offspring. However, numerous researchers have documented Wolbachia could influence the mating preferences, mating time, mating frequency of their hosts (de Crespigny et al., 2006; Panteleev et al., 2007; Goodacre & Martin, 2012). For example, de Crespigny et al. (2006) showed that infected males mate at a higher rate than uninfected males in both D. melanogaster and D. simulans. Additionally, the females of D. paulistorum semispecies show strong mating choice for intra-semispecific (homogamic) males harboring the same (compatible) symbiont variant. Such preference is lost when the titer of Wolbachia was significantly reduced in females, resulting in random mating with either per se incompatible, heterogamic males or compatible males (Miller et al., 2010). Correspondingly, the decrease of Wolbachia titer in male D. paulistorum led to a rejection of mating by homogamic wild-type females (Schneider et al., 2019). This indicates that it is Wolbachia that are able to affect host mating behavior and thus can trigger pre- and post-mating isolation. Furthermore, Fortin et al. (2018) showed that A. vulgare males spent more time near the Wolbachia-free females than the Wolbachia-infected females, when both females were virgin. This finding supported the result reported by Moreau et al. (2001) that A. vulgare males performed more insemination events with uninfected females. Consistently, Fortin et al. (2019) showed that Wolbachia-infected A. vulgare females were less likely to be gravid in populations exhibiting an excess of females. Recently, we investigated the influence of Wolbachia infection on post-mating responses (PMRs) of D. melanogaster and observed that after mating with male D. melanogaster infected with Wolbachia, the females (regardless of the Wolbachia status) exhibited significantly decreased sexual receptivity to remating compared to those mated with uninfected males (Liu et al., 2014; He et al., 2018).

The different chemical profiles from female or male hosts according to the presence or absence of Wolbachia could explain the altered mating behavior caused by Wolbachia. In Drosophila, commensal bacteria altered the profile of cuticular hydrocarbons and influence mate choice (Sharon et al., 2010; Ringo et al., 2011). Recently, Schneider et al. (2019) have shown that Wolbachia-knockdown D. paulistorum males express altered sexual pheromone profiles, with decrease between 9- and 23-fold in several male-specific compounds when compared to wild-type levels. This is in accord with the finding in A. vulgare, where females presented variations in the relative proportion of chemical compounds according to the presence or the absence of Wolbachia (Richard, 2017; Fortin et al., 2018). In many insects, sexual pheromones play an important role in recognition cues for mate choice between and within species. These suggest that Wolbachia might affect the mating
behavior through alteration of some special sexual chemicals in their hosts. As to PMRs changed by *Wolbachia*, since insect seminal fluid proteins (Sfps) that are transferred to females with sperm during copulation have been demonstrated to play a role in modulating the physiological processes and behavior in the mated females (Avila et al., 2011; Denis et al., 2017), we assayed expressions of some genes coding for Sfps. Quantitative reverse-transcription polymerase chain reaction (qRT-PCR) analyses revealed that *Acp26Aa*, *CG1656*, and *CG42474* were significantly downregulated in *Wolbachia*-infected males, compared to uninfected controls (He et al., 2018). By comparing the protein profiles of spermathecae and seminal receptacles from females mated with *Wolbachia*-infected and uninfected male flies, we once identified a number of differentially expressed proteins, including some Sfps (Yuan et al., 2015). Most of them showed downregulation in the presence of *Wolbachia*. These suggest that *Wolbachia* may result in the changes in PMRs through changing the expressions of some Sfps in their insect hosts.

The altered mating behavior caused by *Wolbachia* might be a result of co-evolution. That the infected males mate at a higher rate than uninfected males may increase the opportunity for *Wolbachia* propagation, or may be a behavioral adaptation employed by males to increase male reproductive success (de Crespygni et al., 2006). For those obligate *Wolbachia*–insect associations, such as the *D. paulistorum*–*Wolbachia* system, the changed mating bias in hosts is most likely for avoiding detrimental reproductive consequences like hybrid mortality, male sterility, producing descendants with reduced fitness (Ehrman, 1968; Kim & Ehrman, 1998; Moreau et al., 2002; Miller & Schneider, 2012). The altered female behavior and physiology after mating may be in favor of males while potentially hurting females (Chapman et al., 1995; Wigby & Chapman, 2005; Sirot et al., 2015; Smith et al., 2017). These opposed interests between the sexes can cause sexual conflict. However, females are not passive responders, instead they may utilize Sfps to produce physiological reactions as a trade-off for mating costs. We have observed that female flies mated with *Wolbachia*-infected males showed a significant decrease in sexual receptivity relative to those mated with uninfected males. This reaction may benefit female flies by reducing the costs of mating. This is supported by the enhanced median life span of the females mated with *Wolbachia*-infected males compared to the mates of uninfected males (He et al., 2018). Hence, *Wolbachia* could modulate the sex conflicts of their insect hosts, and thus also favor *Wolbachia* because these bacteria can be transmitted only by female hosts. The observations that the female *D. paulistorum* semispecies prefer to mate with intra-semispecific (homogamic) males harboring the same (compatible) *Wolbachia* variant (Miller et al., 2010) also benefit the transmission of the bacteria.

**Effect of *Wolbachia* on feeding behavior of hosts**

Many pathogens, such as dengue virus have to undergo a significant period of development in their insect vector before they can be transmitted to a new host, including humans. This indicates that only older insects are vectors of epidemiological importance. Hence, reducing mosquito lifespan has the potential to decrease disease transmission (Sinkins & O’Neill, 2000; McMeniman et al., 2009). One *Wolbachia* strain wMelpop, which can cause host cells being like a bag filled with popcorn due to its massive proliferation, has been found to shorten the lifespan of adult *Drosophila* by up to 50% (Min & Benzer, 1997), and thus has been first inducted to the mosquito vector *Aedes aegypti* (McMeniman et al., 2009). Mosquitoes carrying wMelPop-CLA (cell-line-adapted) showed around a 50% reduction in lifespan of adults compared to uninfected mosquitoes (McMeniman et al., 2009). In addition, Turley et al. (2009) tested the feeding behavior of the mosquito and found that the presence of wMelPop-CLA in *A. aegypti* did not affect the response time to humans, but reduced the number and size of blood meals taken. Especially in old mosquitoes, wMelPop-CLA also led to reduced blood-feeding success because when they tried to insert their styles into a host skin their proboscises repeatedly bent. We recently compared the feeding behaviors between females after mating with *Wolbachia*-infected and uninfected male *D. melanogaster*. We observed that females mated with infected males exhibited reduced feeding frequency compared to those mated with uninfected males, while the female *Wolbachia* status had no effects on their own feeding frequency (He et al., 2018), indicating that *Wolbachia*-infected males could induce a notable reduction in food uptake of their mates.

The mechanisms by which *Wolbachia* affect the feeding behavior of insect hosts are not well known. To investigate whether the alterations in feeding behaviors caused by wMelPop-CLA were due to the manipulation of neuroamines, Moreira et al. (2011) detected the expression of genes involved in the dopamine biosynthetic pathway and measured the amount of dopamine in mosquitoes. They did find *Wolbachia*-infected mosquitoes have higher dopamine levels in their heads, but the effect was not present at all ages. Unexpectedly, there is no obvious
correlation between higher dopamine levels and the abnormal behavioral phenotypes “shaky” and “bendy” shown by the older mosquitoes. Considering that in their original host D. melanogaster, wMelPop undergo massive proliferation in the adult, resulting in widespread degeneration of tissues, including brain and muscle, leading to early death (Min & Benzer, 1997), the altered feeding behavioral phenotypes “bendy” proboscis in mosquitoes is likely due to the degeneration of neurons caused by over-proliferation of wMelPop. As to Wolbachia-infected males inducing a decreased feeding frequency of their mates, this could be regulated by the Sfps derived from Wolbachia-infected males as discussed above for PMRs.

Blood-feeding on humans is just the time when pathogens like dengue virus are transmitted, hence damages in feeding behavior can decrease the transmission rate of viruses. Wolbachia-infected mosquitoes are less able to obtain blood meals especially in old age. In addition, Wolbachia may interfere in transinfected mosquitoes with a wider range of pathogens including nematodes (Kambris et al., 2009), and dengue and Zika viruses (Moreira et al., 2009; Aliota et al., 2016a). Therefore, the World Health Organization has recommended the utilization of Wolbachia to counter the growing problem to human health caused by mosquito-transmitted pathogens (WHO, 2016).

**Effect of Wolbachia on locomotive and aggressive behavior of hosts**

It was reported that Wolbachia affected locomotion in Drosophila in response to olfactory cues, although the effects vary according to host background or environmental conditions (Peng et al., 2008; Peng & Wang, 2009; Caragata et al., 2011). Some studies have shown that Wolbachia infection may increase the locomotor activity of their insect hosts (Fleury et al., 2000; Evans et al., 2009; Peng & Wang, 2009). For example, the wRi Wolbachia can increase the basal activity level of its native host D. simulans. Similarly, transinfection with the virulent wMelPop strain from D. melanogaster into A. aegypti results in an increase in locomotor activity in the mosquito (Evans et al., 2009). In contrast, wMel and wMelPop were not able to cause any alterations in the basal activity levels of their native host D. melanogaster, although they cause slight decreases in responsiveness to food cues (Peng et al., 2008). In addition, our previous work also demonstrated that under laboratory conditions, wRi-infected D. simulans increased both locomotive activity and olfactory responsiveness (Peng & Wang, 2009) when compared to those uninfected controls. Moreover, we found that the olfactory responsiveness of the flies positively correlate to Wolbachia density in D. simulans, that is, the higher the Wolbachia density is in the flies, the faster the hosts’ olfactory responsiveness is, showing shorter time it took to be captured by the food trap. qRT-PCR showed that the expression of an important odorant receptor gene or83b was significantly upregulated in flies with fast olfactory response relative to those with slow olfactory response (Peng & Wang, 2009).

Rohrscheib et al. (2015) once compared the male aggression in D. melanogaster infected with different strains of Wolbachia. They observed that wMelPop infection caused a significantly reduced initiation of aggressive encounters in male flies when compared against the behavior of the uninfected controls. But once starting, the duration of a fighting bout was comparable to that of uninfected control males. However, for the other two Wolbachia strains, wMel and wMelCS, they did not exhibit any effects on D. melanogaster male aggression. Further analyses showed that the octopamine biosynthesis pathway was significantly downregulated in wMelPop-infected male flies, which could explain the reduction in aggressive behavior (Rohrscheib et al., 2015). Since male aggression behavior is critically important in mate competition, the decreased male aggression caused by wMelPop infection may reduce the fitness in their hosts. Furthermore, wMelPop has a larger fecundity cost as well as other potential costs associated with lifespan reduction, feeding and probing behavior, quiescent eggs (in a dried state the wMelPop-infected eggs tend to lose viability over time) (Nguyen et al., 2015). Thus the established wMelPop-CLA infected A. aegypti mosquitoes are not applicable for biocontrol because they displayed reduced fitness in field releases, while wMel-infected mosquito lines were successfully established and released, and have been demonstrated to be effective at blocking local insect-borne disease transmission (Walker et al., 2011; Hoffmann et al., 2014; Aliota et al., 2016a, 2016b; O’Neill et al., 2018; Achee et al., 2019).

**Perspectives**

Wolbachia bacteria are best known for their ability to manipulate host reproduction, despite their widespread distributions in somatic tissues, including brain, and consequently they have the potential to affect host behaviors. Recently, extensive studies have contributed to our understanding the influences of the bacteria on insect behaviors, including sleep, learning and memory, mating, feeding, locomotion and aggression (Fig. 1). With the practice of Wolbachia in controlling insect-borne diseases, more
attention should be paid to the effects of *Wolbachia* on hosts’ behaviors so as to evaluate the influences on the environment of releasing these *Wolbachia*-infected insects into the fields. However, because there are various inherent difficulties to dissect complex behavior, most of the behavioral studies were performed to date under the laboratory conditions. Questions about what influence *Wolbachia* infection has on the host behavior in field conditions and consequently on the ecosystem remain to be further studied. The molecular mechanisms by which *Wolbachia* affect host behaviors need to be more thoroughly and deeply elucidated. The effect of *Wolbachia* on host fitness traits may be multidimensional, and a number of host genes, miRNAs and proteins were modified by *Wolbachia* infection. As the development of technologies of bioinformatics and molecular biology continues, identifying effector molecules and their functional pathways associated with *Wolbachia* and their influence on host behaviors is also a main priority.

**Fig. 1** A simple model of the effects of *Wolbachia* infection on the behavior of insect hosts.

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**Disclosure**

The authors declare no conflicts of interest.

**References**

Aboudhiaf, S., Alves, G., Parrot, S., Amri, M., Simonnet, M.M., Grosjean, Y. *et al.* (2018) LAT1-like transporters regulate dopaminergic transmission and sleep in *Drosophila*. *Sleep*, 41 (10). https://doi.org/10.1093/sleep/zsy137/5054580.

Achee, N.L., Grieco, J.P., Vatandoost, H., Seixas, G., Pinto, J., Ching-NG, L. *et al.* (2019) Alternative strategies for
mosquito-borne arbovirus control. *PLoS* Neglected Tropical Diseases, 13, e0006822.

Adelman, J.S. and Martin, L.B. (2009) Vertebrate sickness behaviors: Adaptive and integrated neuroendocrine immune responses. *Integrative and Comparative Biology*, 49, 202–214.

Albertson, R., Casper-Lindley, C., Cao, J., Tran, U. and Sullivan, W. (2009) Symmetric and asymmetric mitotic segregation patterns influence Wolbachia distribution in host somatic tissue. *Journal of Cell Science*, 122, 4570–4583.

Albertson, R., Tan, V., Leads, R.R., Reyes, M., Sullivan, W. and Casper-Lindley, C. (2013) Mapping Wolbachia distributions in the adult *Drosophila* brain. *Cellular Microbiology*, 15, 1527–1544.

Aliota, M.T., Peinado, S.A., Velez, I.D. and Osorio, J.E. (2016a) The wMel strain of *Wolbachia* reduces transmission of Zika virus by *Aedes aegypti*. *Scientific Reports*, 6, 28792.

Aliota, M.T., Walker, E.C., Uribe Yepes, A., Dario Velez, I., Christensen, B.M. and Osorio, J.E. (2016b) The wMel strain of *Wolbachia* reduces transmission of Chikungunya virus in *Aedes aegypti*. *PLoS* Neglected Tropical Diseases, 10, e0004677.

Allada, R., Cirelli, C. and Sehgal, A. (2017) Molecular mechanisms of sleep homeostasis in flies and mammals. *Cold Spring Harbor Perspectives in Biology*, 9, a027730.

Andersen, S.B., Boye, N., Nash, D.R. and Boomsma, J.J. (2012) Dynamic *Wolbachia* prevalence in acromyrmex leaf-cutting ants: Potential for a nutritional symbiosis. *Journal of Evolutionary Biology*, 25, 1340–1350.

Avila, F.W., Sirot, L.K., Laflamme, B.A., Rubinstein, C.D. and Wolfler, M.F. (2011) Insect seminal fluid proteins: identification and function. *Annual Review of Entomology*, 56, 21–40.

Beckmann, J.F., Ronau, J.A. and Hochstrasser, M. (2017) *A Wolbachia* deubiquitylating enzyme induces cytoplasmic incompatibility. *Nature Microbiology*, 2, 17007.

Bhattacharya, T., Newton, I.L.G. and Hardy, R.W. (2017) *Wolbachia* elevates host methyltransferase expression to block an RNA virus early during infection. *PLoS* Pathogens, 13, e1006427.

Bi, J., Sehgal, A., Williams, J.A. and Wang, Y.F. (2018) *Wolbachia* affects sleep behavior in *Drosophila melanogaster*. *Journal of Insect Physiology*, 107, 81–88.

Bi, J., Zheng, Y., Wang, R.F., Ai, H., Haynes, P.R., Brownlie, J.C. et al. (2019) *Wolbachia* infection may improve learning and memory capacity of *Drosophila* by altering host gene expression through microRNA. *Insect Biochemistry and Molecular Biology*, 106, 47–54.

Bonneau, M., Landmann, F., Labbé, P., Justy, F., Weill, M. and Sicard, M. (2018) The cellular phenotype of cytoplasmic incompatibility in *Culex pipiens* in the light of *cidB* diversity. *PLoS* Pathogens, 14, e1007364.

Broly, P., Deneubourg, J.L. and Devigne, C. (2013) Benefits of aggregation in woodlice: a factor in the terrestrialization process? *Insectes Sociaux*, 60, 419–435.

Brownlie, J.C., Cass, B.N., Rieger, M., Witsenburg, J.J., Iturbe-Ormaetxe, I., McGraw, E.A. et al. (2009) Evidence for metabolic provisioning by a common invertebrate endosymbiont, *Wolbachia* piipientis, during periods of nutritional stress. *PLoS* Pathogens, 5, e1000368.

Caragata, E.P., Real, K.M., Zalucki, M.P. and McGraw, E.A. (2011) *Wolbachia* infection increases recapture rate of field-released *Drosophila melanogaster*. *Symbiosis*, 54, 55–60.

Casper-Lindley, C., Kimura, S., Saxton, D.S., Essaw, Y., Simpson, I., Tan, V. et al. (2011) Rapid fluorescence-based screening for *Wolbachia* endosymbionts in *Drosophila* germ line and somatic tissues. *Applied and Environmental Microbiology*, 77, 4788–4794.

Cavanaugh, D.J., Vigderman, A.S., Dean, T., Garbe, D.S. and Sehgal, A. (2016) The *Drosophila* circadian clock gates sleep through time-of-day dependent modulation of sleep-promoting neurons. *Sleep*, 39, 345–356.

Chafee, M.E., Zecher, C.N., Gourley, M.L., Schmidt, V.T., Chen, J.H., Bordenstein, S.R. et al. (2011) Decoupling of host-symbiont-phage coadaptations following transfer between insect species. *Genetics*, 187, 203–215.

Chapman, T., Liddle, L.F., Kalb, J.M., Wolfer, M.F. and Partridge, L. (1995) Cost of mating in *Drosophila melanogaster* females is mediated by male accessory gland products. *Nature*, 373, 241–244.

Czarnetzki, A.B. and Tebbe, C.C. (2004) Detection and phylogenetic analysis of *Wolbachia* in Collombola. *Environmental Microbiology*, 6, 35–44.

Dalton, J.E., Fear, J.M., Knott, S., Baker, B.S., McIntyre, L.M. and Arbeitman, M.N. (2013) Male-specific Fruitless isoforms have different regulatory roles conferred by distinct zinc finger DNA binding domains. *BMC Genomics*, 14, 659. https://doi.org/10.1186/1471-2164-14-659.

de Crespoigny, F.E.C., Pitt, T.D. and Wedell, N. (2006) Increased male mating rate in *Drosophila* is associated with *Wolbachia* infection. *Journal of Evolutionary Biology*, 19, 1964–1972.

Da Silva Gonçalves, D., Iturbe-Ormaetxe, I., Martins-da-Silva, A., Telleria, E.L., Rocha, M.N., Traub-Csekô, Y.M. et al. (2019) *Wolbachia* introduction into *Lutzomyia longipalpis* (Diptera: Psychodidae) cell lines and its effects on immune-related gene expression and interaction with *Leishmania infantum*. *Parasites & Vectors*, 12, 33.

Dean, M.D. (2006) A *Wolbachia*-associated fitness benefit depends on genetic background in *Drosophila simulans*. *Proceedings of the Royal Society B: Biological Sciences*, 273, 1415–1420.

Denis, B., Claisse, G., Le Rouzic, A., Wicker-Thomas, C., Lepennetier, G. and Joly, D. (2017) Male accessory gland proteins affect differentially female sexual receptivity and remating in
closely related Drosophila species. Journal of Insect Physiology, 99, 67–77.

Dijk, D.J. and Czeisler, C. (1995) Contribution of the circadian pacemaker and the sleep homeostat to sleep propensity, sleep structure, electroencephalographic slow waves, and sleep spindle activity in humans. The Journal of Neuroscience, 15, 3526–3538.

Dobson, S.L., Bourtizs, K., Braig, H.R., Jones, B.F., Zhou, W., Rousset, F. et al. (1999) Wolbachia infections are distributed throughout insect somatic and germ line tissues. Insect Biochemistry and Molecular Biology, 29, 153–160.

Donlea, J.M., Pimentel, D. and Miesenböck, G. (2014) Neuronal machinery of sleep homeostasis in Drosophila. Neuron, 81, 860–872.

Dubowy, C., Moravecvek, K., Yue, Z., Wan, J.Y., van Dongen, H.P.A. and Sehgal, A. (2016) Genetic dissociation of daily sleep and sleep following thermongenic sleep deprivation in Drosophila. Sleep, 39, 1083–1095.

Dukas, R. and Bernays, E.A. (2000) Learning improves growth rate in grasshoppers. Proceedings of the National Academy of Sciences USA, 97, 2637–2640.

Ehman, L. (1968) Antibiotics and infections hybrid sterility in Drosophila paulistorum. Molecular Genetics and Genomics, 103, 218–222.

Evans, O., Caragata, E.P., McMeniman, C.J., Woolfit, M., Green, D.C., Williams, C.R. et al. (2009) Increased locomotor activity and metabolism of Aedes aegypti infected with a life-shortening strain of Wolbachia pipientis. The Journal of Experimental Biology, 212, 1436–1441.

Faville, R., Kottler, B., Goodhill, G.J., Shaw, P.J. and van Swinderen, B. (2015) How deeply does your mutant sleep? Probing arousal to better understand sleep defects in Drosophila. Scientific Reports, 5, 8454.

Fleury, F., Vavr, F., Ris, N., Fouillet, P. and Bouletreau, M. (2000) Physiological cost induced by the maternally-transmitted endosymbiont Wolbachia in the Drosophila parasitoid Leptopilina heterotoma. Parasitology, 121(Pt S), 493–500.

Fortin, M., Debenest, C., Souty-Grosset, C. and Richard, F.J. (2018) Males prefer virgin females, even if parasitized, in the terrestrial isopod Armadillidium vulgare. Ecology and Evolution, 8, 3341–3353.

Fortin, M., Meunier, J., Lavrèrè, T., Souty-Grosset, C. and Richard, F.J. (2019) Joint effects of group sex-ratio and Wolbachia infection on female reproductive success in the terrestrial isopod Armadillidium vulgare. BMC Evolutionary Biology, 19, 65.

Freyms, P.S. and Fitzsimons, H.L. (2017) The ERM protein Moesin is essential for neuronal morphogenesis and long-term memory in Drosophila. Molecular Brain, 10(1), 41. https://doi.org/10.1186/s13041-017-0322-y.

Garcia, G.A., Sylvestre, G., Aguilar, R., da Costa, G.B., Martins, A.J., Lima, J.B.P. et al. (2019) Matching the genetics of released and local Aedes aegypti populations is critical to assure Wolbachia invasion. PLoS Neglected Tropical Diseases, 13, e0007023.

Goodacre, S.L. and Martin, O.Y. (2012) Modification of insect and arachnid behaviours by vertically transmitted endosymbionts: Infections as drivers of behavioural change and evolutionary novelty. Insects, 3, 246–261.

Harumoto, T., Fukatsu, T. and Lemaître, B. (2018) Common and unique strategies of male killing evolved in two distinct Drosophila symbionts. Proceedings of the Royal Society B: Biological Sciences, 285(1875), 20172167. https://doi.org/10.1098/rspb.2017.2167.

He, Z., Zhang, H.B., Li, S.T., Yu, W.J., Biwow, J., Yu, X.Q. et al. (2018) Effects of Wolbachia infection on the postmatting response in Drosophila melanogaster. Behavioral Ecology and Sociobiology, 72, 146.

Hendricks, J.C., Finn, S.M., Panckeri, K.A., Chavkin, J., Williams, J.A., Sehgal, A. et al. (2000) Rest in Drosophila is a sleep-like state. Neuron, 25, 129–138.

Hoffmann, A.A., Montgomery, B.L., Popovicji, J., Iturbe-Ormaetxe, I., Johnson, PH, Muzzi, F. et al. (2011) Successful establishment of Wolbachia in Aedes populations to suppress dengue transmission. Nature, 476, 454–457.

Hoffmann, A.A., Iturbe-Ormaetxe, I., Callahan, A.G., Phillips, B.L., Billington, K., Axford, J.K. et al. (2014) Stability of the wMel Wolbachia infection following invasion into Aedes aegypti populations. PLoS Neglected Tropical Diseases, 8, e3115.

Huber, R., Hill, S.L., Holladay, C., Biesiadecki, M., Tononi, G. and Cirelli, C. (2004) Sleep homeostasis in Drosophila melanogaster. Sleep, 27, 628–639.

Iyer, S.C., Ramachandran Iyer, E.P., Meduri, R., Rubaharan, M., Kuntimaddi, A., Karamsetty, M. et al. (2013) Cut, via CrebA, transcriptionally regulates the COPII secretory pathway to direct dendrite development in Drosophila. Journal of Cell Science, 126, 4732–4745.

Jiggins, F.M., Randerson, J.P, Hurst, G.D.D. and Majerus, M.E.N. (2002) How can sex ratio distorters reach extreme prevalences? Male-killing Wolbachia are not suppressed and have near-perfect vertical transmission efficiency in Acraea encedon. Evolution, 56, 2290–2295.

Kambris, Z., Cook, P.E., Phuc, H.K. and Sinkins, S.P. (2009) Immune activation by life-shortening Wolbachia and reduced filarial competence in mosquitoes. Science, 326(5949), 134–136.

Kim, Y.K. and Ehman, L. (1998) Developmental isolation and subsequent adult behavior of Drosophila paulistorum. IV. Courtship. Behavior Genetics, 28, 57–65.

Kishani Farahani, H., Ashouri, A., Goldansaz, S.H., Shapiro, M.S., Pierre, J.S. and van Baaren, J. (2017) Decrease of
memory manipulation by Wolbachia? Insect Science, 24, 569–583.

Kuo, T.H., Pike, D.H., Beizaeeipour, Z. and Williams, J.A. (2010) Sleep triggered by an immune response in Drosophila is regulated by the circadian clock and requires the NFkappaB Relish. BMC Neuroscience, 11, 17.

Kuo, T.H. and Williams, J.A. (2014) Increased sleep promotes survival during a bacterial infection in Drosophila. Sleep, 37, 1077–1086.

Lee, J.Y., Lee, L.J., Fan, C.C., Chang, H.C., Shih, H.A., Min, M.Y. et al. (2017) Important roles of Vilse in dendritic architecture and synaptic plasticity. Scientific Reports, 7, 45646. https://doi.org/10.1038/srep45646.

LePage, D.P., Metcalf, J.A., Bordenstein, S.R., On, J., Perlmutter, J.I., Shropshire, J.D. et al. (2017) Prophage WO genes recapitulate and enhance Wolbachia-induced cytoplasmic incompatibility. Nature, 543, 243–247.

Liu, C., Wang, J.L., Zheng, Y., Xiong, E.J., Li, J.J., Yuan, L.L. et al. (2014) Wolbachia-induced paternal defect in Drosophila is likely by interaction with the juvenile hormone pathway. Insect Biochemistry and Molecular Biology, 49, 49–58.

Maille, A. and Schradin, C. (2016) Survival is linked with reaction time and spatial memory in African striped mice. Biology Letters, 12, 20160346.

McMeniman, C.J., Lane, R.V., Cass, B.N., Fong, A.W.C., Sidhu, M., Wang, Y.F. et al. (2009) Stable introduction of a life-shortening Wolbachia infection into the mosquito Aedes aegypti. Science, 323, 141–144.

Miller, W.J., Ehrman, L. and Schneider, D. (2010) Infectious speciation revisited: impact of symbiont-depletion on female fitness and mating behavior of Drosophila paulistorum. PLoS Pathogens, 6, e1001214.

Miller, W.J. and Schneider, D. (2012) Endosymbiotic microbes as adaptive manipulators of arthropod behaviour and natural driving sources of host speciation. Host Manipulation by Parasites (eds. D. Hughes, J.J. Brodeur & F. Thomas), pp. 119–139. University Press, Oxford.

Min, K.T. and Benzer, S. (1997) Wolbachia, normally a symbiont of Drosophila, can be virulent, causing degeneration and early death. Proceedings of the National Academy of Sciences USA, 94, 10792–10796.

Miyata, M., Konagaya, T., Yukuhiro, K., Nomura, M. and Kageyama, D. (2017) Wolbachia-induced meiotic drive and feminization is associated with an independent occurrence of selective mitochondrial sweep in a butterfly. Biology Letters, 13, 20170153. https://doi.org/10.1098/rsbl.2017.0153.

Moreau, J., Bertin, A., Caubet, Y. and Rigaud, T. (2001) Sexual selection in an isopod with Wolbachia-induced sex reversal: Males prefer real females. Journal of Evolutionary Biology, 14, 388–394.

Moreau, J., Seguin, S., Caubet, Y. and Rigaud, T. (2002) Female remating and sperm competition patterns in a terrestrial crustacean. Animal Behaviour, 64, 569–577.

Moreira, L.A., Iturbe-Ormaetxe, I., Jeffery, J.A., Lu, G., Pyke, A.T., Hedges, L.M. et al. (2009) A Wolbachia symbiont in Aedes aegypti limits Infection with Dengue, Chikungunya, and Plasmodium. Cell, 139, 1268–1278.

Moreira, L.A., Ye, Y.H., Turner, K., Eyles, D.W., McGraw, E.A. and O’Neill, S.L. (2011) The wMelPop strain of Wolbachia interferes with dopamine levels in Aedes aegypti. Parasites & Vectors, 4, 28.

Morioka, E., Oida, M., Tsuchida, T. and Ikeda, M. (2018) Nighttime activities and peripheral clock oscillations depend on Wolbachia endosymbionts in flies. Scientific Reports, 8, 15432.

Narita, S., Nomura, M. and Kageyama, D. (2007) Naturally occurring single and double infection with Wolbachia strains in the butterfly Eurema hecabe: transmission efficiencies and population density dynamics of each Wolbachia strain. FEMS Microbiology Ecology, 61, 235–245.

Nguyen, T.H., Nguyen, H.L., Nguyen, T.Y., Vu, S.N., Tran, N.D., Le, T.N. et al. (2015) Field evaluation of the establishment potential of wMelPop Wolbachia in Australia and Vietnam for dengue control. Parasites & Vectors, 8, 563.

O’Neill, S.L., Ryan, P.A., Turley, A.P., Wilson, G., Retzki, K., Iturbe-Ormaetxe, I. et al. (2018) Scaled deployment of Wolbachia to protect the community from dengue and other Aedes transmitted arboviruses. Gates Open Research, 2, 36.

Ote, M., Ueyama, M. and Yamamoto, D. (2016) Wolbachia protein TomO targets nanos mRNA and restores germ stem cells in Drosophila sex-letal mutants. Current Biology, 26, 2223–2232.

Pan, X., Pike, A., Joshi, D., Bian, G., McFadden, M.J., Lu, P. et al. (2018) The bacterium Wolbachia exploits host innate immunity to establish a symbiotic relationship with the dengue vector mosquito Aedes aegypti. ISME Journal, 12, 277–288.

Panteleev, D.I., Goriacheva, I.I., Andrianov, B.V., Reznik, N.L., Lazebnyi, O.E. and Kulikov, A.M. (2007) The endosymbiotic bacterium Wolbachia enhances the nonspecific resistance to insect pathogens and alters behavior of Drosophila melanogaster. Genetika, 43, 1277–1280.

Peng, Y., Nielsen, I.E., Cunningham, J.P. and McGraw, E. (2008) Wolbachia infection alters olfactory-caused locomotion in Drosophila spp. Applied and Environmental Microbiology, 74, 3943–3948.

Peng, Y. and Wang, Y.F. (2009) Infection of Wolbachia may improve the olfactory response of Drosophila. Chinese Science Bulletin, 54, 1369–1375.

Perrot-Minnot, M.J. and Cézilly, F. (2010) Parasite-induced behavioral change: Mechanisms. Encyclopedia of Animal Behavior (eds. M. Breed & J. Moore), pp. 618–627. Elsevier Press, Oxford, UK.
Pietri, J.E., DeBruhl, H. and Sullivan, W. (2016) The rich somatic life of Wolbachia. *Microbiology Open*, 5, 923–936.

Rice, A.M. and McQuillan, M.A. (2018) Maladaptive learning and memory in hybrids as a reproductive isolating barrier. *Proceedings of the Royal Society B: Biological Sciences*, 285, 20180542.

Richard, F.J. (2017) Symbiotic bacteria influence the odor and mating preference of their hosts. *Frontiers in Ecology and Evolution*, 5, 143. https://doi.org/10.3389/fevo.2017.00143.

Ringo, J., Sharon, G. and Segal, D. (2011) Bacteria-induced sexual isolation in *Drosophila*. *Fly*, 5, 310–315.

Rohrscheib, C.E., Bondy, E., Josh, P., Riegler, M., Eyles, D., van Swinderen, B. et al. (2015) *Wolbachia* influences the production of octopamine and affects *Drosophila* male aggression. *Applied and Environmental Microbiology*, 81, 4573–4580.

Russell, J.E., Nunney, L., Saum, M. and Stouthamer, R. (2018) Host and symbiont genetic contributions to fitness in a Trichogramma–Wolbachia symbiosis. *PeerJ*, 6, e4655.

Saijo, T., Jiyoti, V., Megan, W., O’Neill, S.L. and Marco, V. (2018) *Wolbachia*-mediated virus blocking in mosquito cells is dependent on xrr1-mediated viral RNA degradation and influenced by viral replication rate. *PLoS Pathogens*, 14, e1006879.

Saucereau, Y., Valiante Moro, C., Dieryckx, C., Dupuy, J.W., Tran, F.H., Girard, V. et al. (2017) Comprehensive proteome profiling in *Aedes albopictus* to decipher Wolbachia-arthrovirus interference phenomenon. *BMC Genomics*, 18, 635.

Schneider, D.I., Ehrman, L., Engl, T., Kaltenporth, M., Huan-Van, A., Le Rouzic, A. et al. (2019) Symbiont-driven male mating success in the neotropical *Drosophila paulistorum* superspecies. *Behavior Genetics*, 49, 83–98.

Sharon, G., Segal, D., Ringo, J.M., Hefetz, A., Zilber-Rosenberg, I. and Rosenberg, E. (2010) Commensal bacteria play a role in mating preference of *Drosophila melanogaster*. *Proceedings of the National Academy of Sciences USA*, 107, 20051–20056.

Shaw, P.J., Cirelli, C., Greenspan, R.J. and Tononi, G. (2000) Correlates of sleep and waking in *Drosophila melanogaster*. *Science*, 287, 1834–1837.

Shropshire, J.D., On, J., Layton, E.M., Zhou, H. and Bordenstein, S.R. (2018) One prophage WO gene rescues cytoplasmic incompatibility in *Drosophila melanogaster*. *Proceedings of the National Academy of Sciences USA*, 115, 4987–4991.

Sinkins, S.P. and O’Neill, S.L. (2000) *Wolbachia as A Vehicle to Modify Insect Populations*. Boca Raton, FL, USA: CRC Press.

Sirot, L.K., Wong, A., Chapman, T. and Wolfner, M.F. (2015) Sexual conflict and seminal fluid proteins: a dynamic landscape of sexual interactions. *Cold Spring Harbor Perspectives in Biology*, 7, a017533.

Smith, D.T., Clarke, N.V.E., Boone, J.M., Fricke, C. and Chapman, T. (2017) Sexual conflict over remating interval is modulated by the sex peptide pathway. *Proceedings of the Royal Society B: Biological Sciences*, 284, 20162394.

Strunov, A., Kiseleva, E. and Gottlieb, Y. (2013) Spatial and temporal distribution of pathogenic *Wolbachia* strain wMelPop in *Drosophila melanogaster* central nervous system under different temperature conditions. *Journal of Invertebrate Pathology*, 114, 22–30.

Sugimoto, T.N., Fujii, T., Kayukawa, T., Sakamoto, H. and Ishikawa, Y. (2010) Expression of a doublesex homologue is altered in sexual mosaics of *Ostrinia scapulalis* moths infected with *Wolbachia*. *Insect Biochemistry and Molecular Biology*, 40, 847–854.

Sullivan, J. and Jaenike, J. (2006) Male-killing *Wolbachia* and male mate choice: a test with *Drosophila innubila*. *Evolutionary Ecology Research*, 8, 91–102.

Templé, N. and Richard, F.J. (2015) Intra-cellular bacterial infections affect learning and memory capacities of an invertebrate. *Frontiers in Zoology*, 12, 36.

Thomas, F., Adamo, S. and Moore, J. (2005) Parasitic manipulation: Where are we and where should we go? *Behavioural Processes*, 68, 185–199.

Turelli, M., Cooper, B.S., Richardson, K.M., Ginsberg, P.S., Pecknappaugh, B., Antelope, C.X. et al. (2018) Rapid global spread of wRi-like Wolbachia across multiple *Drosophila*. *Current Biology*, 28, 963–971.

Turley, A.P., Moreira, L.A., O’Neill, S.L. and McGraw, E.A. (2009) *Wolbachia* infection reduces blood-feeding success in the dengue fever mosquito, *Aedes aegypti*. *PLoS Neglected Tropical Diseases*, 3, e516.

Vale, P.F. and Jardine, M.D. (2015) Sex-specific behavioural symptoms of viral gut infection and *Wolbachia* in *Drosophila melanogaster*. *Journal of Insect Physiology*, 82, 28–32.

Walker, T., Johnson, P.H., Moreira, L.A., Iturbe-Ormaetxe, I., Frentiu, F.D., McMeniman, C.J. et al. (2011) The wMel *Wolbachia* strain blocks dengue and invades caged *Aedes aegypti* populations. *Nature*, 476, 450–453.

Weeks, A.R. and Breeuwer, J.A.J. (2001) *Wolbachia* – induced parthenogenesis in a genus of phytophagous mites. *Proceedings of the Royal Society B: Biological Sciences*, 268, 2245–2251.

Werren, J.H. (1997) Biology of *Wolbachia*. *Annual Review of Entomology*, 42, 587–609.

WHO (2016) Mosquito (vector) control emergency response and preparedness for Zika virus. https://www.who.int/neglected_diseases/news/mosquito_vector_control_response/en/

Wigby, S. and Chapman, T. (2005) Sex peptide causes mating costs in female *Drosophila melanogaster*. *Current Biology*, 15, 316–321.

Wu, B., Ma, L., Zhang, E., Du, J., Liu, S., Price, J. et al. (2018) Sexual dimorphism of sleep regulated by juvenile hormone signaling in *Drosophila*. *PLoS Genetics*, 14, e1007318.
Wu, C.H., Zong, Q., Du, A.L., Zhang, W., Yao, H.C., Yu, X.Q. et al. (2016) Knockdown of Dynamitin in testes significantly decreased male fertility in *Drosophila melanogaster*. *Developmental Biology*, 420, 79–89.

Wu, M., Sun, L.V., Vamathevan, J., Riegler, M., Deboy, R., Brownlie, J.C. et al. (2004) Phylogenomics of the reproductive parasite *Wolbachia pipientis* wMel: a streamlined genome overrun by mobile genetic elements. *PLoS Biology*, 2, 327–341.

Yuan, L.L., Chen, X., Zong, Q., Zhao, T., Wang, J.L., Zheng, Y. et al. (2015) Quantitative proteomic analyses of molecular mechanisms associated with cytoplasmic incompatibility in *Drosophila melanogaster* induced by *Wolbachia*. *Journal of Proteome Research*, 14, 3835–3847.

Zhang, K.J., Han, X. and Hong, X.Y. (2013) Various infection status and molecular evidence for horizontal transmission and recombination of *Wolbachia* and *Cardinium* among rice planthoppers and related species. *Insect Science*, 20, 329–344.

Zhao, D.X., Zhang, X.F., Chen, D.S., Zhang, Y.K. and Hong, X.Y. (2013) *Wolbachia*-host interactions: host mating patterns affect *Wolbachia* density dynamics. *PLoS ONE*, 8, e66373.

Zheng, X. and Sehgal, A. (2012) Speed control: cogs and gears that drive the circadian clock. *Trends in Neurosciences*, 35, 574–585.

Zheng, Y., Ren, P.P., Wang, J.L. and Wang, Y.F. (2011a) *Wolbachia*-induced cytoplasmic incompatibility is associated with decreased Hira expression in male *Drosophila*. *PLoS ONE*, 6, e19512.

Zheng, Y., Wang, J.L., Liu, C., Wang, C.P., Walker, T. and Wang, Y.F. (2011b) Differentially expressed profiles in the larval testes of *Wolbachia* infected and uninfected *Drosophila*. *BMC Genomics*, 12, 595.

Zhou, X.F. and Li, Z.X. (2016) Establishment of the cytoplasmic incompatibility-inducing *Wolbachia* strain wMel in an important agricultural pest insect. *Scientific Reports*, 6, 39200.

Zug, R. and Hammerstein, P. (2012) Still a host of hosts for *Wolbachia*: analysis of recent data suggests that 40% of terrestrial arthropod species are infected. *PLoS ONE*, 7, e38544.

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