Medical and Veterinary Entomology

Medical and Veterinary Entomology (2014), doi: 10.1111/mve.12063

SHORT COMMUNICATION

Wolbachia infection does not alter attraction of the mosquito *Aedes (Stegomyia) aegypti* to human odours

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Abstract. The insect endosymbiont *Wolbachia pipientis* (Rickettsiales: Rickettsiaceae) is undergoing field trials around the world to determine if it can reduce transmission of dengue virus from the mosquito *Stegomyia aegypti* to humans. Two different *Wolbachia* strains have been released to date. The primary effect of the *w*Mel strain is pathogen protection whereby infection with the symbiont limits replication of dengue virus inside the mosquito. A second strain, *w*MelPop, induces pathogen protection, reduces the adult mosquito lifespan and decreases blood feeding success in mosquitoes after 15 days of age. Here we test whether *Wolbachia* infection affects mosquito attraction to host odours in adults aged 5 and 15 days. We found no evidence of reduced odour attraction of mosquitoes, even for those infected with the more virulent *w*MelPop. This bodes well for fitness and competitiveness in the field given that the mosquitoes must find hosts to reproduce for the biocontrol method to succeed.

Key words. mosquito, odour, symbiont.

Wolbachia is a naturally occurring obligate intracellular bacterium common to many insect species. *Wolbachia* manipulates host reproductive biology to its advantage, most often via the action of cytoplasmic incompatibility that favours the reproductive success of infected females. Because the symbiont is maternally transmitted this leads to spread of *Wolbachia* infections through insect populations. Its self-spreading nature and intimate association with insects make it an attractive platform for biological control strategies against a range of vectors and vector-borne diseases (McGraw & O'Neill, 2013). As part of a strategy to control dengue virus transmission from the mosquito *Stegomyia aegypti* to humans, multiple *Wolbachia* strains have been transinfected from *Drosophila melanogaster* into the naturally uninfected mosquito, where they have formed stable infections (McMeniman *et al.*, 2009; Walker *et al.*, 2011; McGraw & O'Neill, 2013). Two of the strains, *w*Mel and *w*MelPop, also induce pathogen protection (Moreira *et al.*, 2009a; Walker *et al.*, 2011), whereby the presence of the symbiont limits the replication of a range of pathogens and parasites inside the mosquito, including dengue virus and the malaria parasite (Kambris *et al.*, 2009; Moreira *et al.*, 2009a).

The *w*MelPop strain is unique, causing additional fitness effects not associated with the *w*Mel strain. Growing to high densities in the insect, *w*MelPop reduces the average host insect's lifespan by half (McMeniman *et al.*, 2009). This strain was initially selected for transinfection, because reducing the lifespan of the mosquito vector could be used to limit dengue virus transmission. After consumption of a dengue infectious bloodmeal, the virus must disseminate through the mosquito and invade the salivary glands (typically taking > 6 days) before it can be transmitted to another human. This means that the probability of a mosquito transmitting the virus rises with age. Removing older individuals from the mosquito population would therefore reduce the proportion of dengue-transmitting individuals in the population and hence reduce disease transmission rates (McGraw & O'Neill, 2013).

*w*MelPop causes additional phenotypic effects in the mosquito, including reduced fecundity and egg viability

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(McMeniman *et al.*, 2009; McMeniman & O'Neill, 2010) and behavioural changes. Aging mosquitoes infected with *w*MelPop take fewer and smaller bloodmeals (Turley *et al.*, 2009; Moreira *et al.*, 2009b). Close examination of feeding behaviour revealed that these mosquitoes attempted to probe but repeatedly failed, with the proboscis appearing to bend, rather than the stylet and sheath sliding against one another. Mosquitoes also exhibited characteristic jittery movements (Turley *et al.*, 2009; Moreira *et al.*, 2009b). While reduced blood feeding success, particularly after 6 days of age could help to reduce the number of dengue transmission events to humans, young *Wolbachia*-infected mosquitoes need to successfully obtain bloodmeals and produce offspring if the *Wolbachia* are to spread and the biocontrol method is to be effective. Here we determine whether *w*Mel- and *w*MelPop-infected mosquitoes experience any changes in their ability to respond to human odour cues at 5 and 15 days of age using an olfactometer. An understanding of these effects may help to inform future choice of *Wolbachia* strain for open field releases.

Two mosquito lines transinfected with *Wolbachia* previously, *w*MelPop (or PGYP1), and *w*Mel (or MGYP1) (McMeniman *et al.*, 2009; Walker *et al.*, 2011), were used. As is standard, prior tetracycline treatment was used to generate *Wolbachia*-free control lines (McMeniman *et al.*, 2009). Mosquito-rearing procedures were as previously reported (Walker *et al.*, 2011). Adult mosquitoes were held in mixed sex populations to the appropriate age at 26 ± 1 °C, RH 60 ± 5% and LD 12 : 12 h cycle, with access to 10% sucrose solution *ad libitum*. Adults were examined at 5 and 15 days of age.

A dual-choice Y-tube olfactometer was used to test the response of mosquitoes to odour cues. The Y-tube consisted of a transparent acrylic tube (inner diameter 7 cm and wall thickness 0.5 cm) according to Geier and Boeckh (1999) with the exceptions that rotating doors on traps were replaced by mesh funnels (25 mm OD × 10 mm ID) and the rotating door of the release cage was replaced by a removable sliding mesh door (Verhulst *et al.*, 2008). Air from a pressurized air system was cleaned with a charcoal filter before being introduced into the testing apparatus. A jar filled with distilled water and heated to 38 ± 2 °C was used to humidify the air. Surrounding the four sides of olfactometer were white walls (50 cm H × 150 cm L × 50 cm W) marked with four 5-cm black stripes (30 cm apart) for visual cues. The olfactometer was illuminated by two 4.2 W LED lamps (Janjso; IKEA, Leiden, The Netherlands). Conditions in the olfactometer were set at a wind speed 0.2 m/s, temperature 27 ± 1 °C and humidity 80 ± 10%. The olfactometer was cleaned with 0.3% Liqui-nox phosphate-free liquid detergent (Sigma-Aldrich Pty. Ltd, Sydney, NSW, Australia), rinsed with distilled water, cleaned with 30% methanol and rinsed again with distilled water. The odour stimulus tested was a nylon sock (Razzamataz, Kew, Vic, Australia) that had been worn (A.P. Turley) for 24 h and stored in the freezer between uses (Smallegage *et al.*, 2010). In each experiment, an unworn sock was placed in the trap opposite the odour stimulus to provide a control for the visual stimulus. Control trials without socks in either port were performed to test the symmetry and cleanliness of the trapping system. Approximately 18 h before each experiment, 30 mosquitoes were transferred into release cages, where they were starved of sucrose but given access to a water-soaked cotton-wool ball. On the day of experiments, release cages were placed onto the stem of the olfactometer for 5 min before mosquitoes were released and scored for movement into an arm of the tube over a 10-min period. The order of control and treatment trials was randomized each day. All experiments were replicated eight times with each replicate comprising a cage of co-reared mosquitoes on which a set of 5 and 15 days trials were carried out. The effects of mosquito line (*w*Mel, *w*Mel.Tet, *w*MelPop and *w*MelPop.Tet), adult mosquito age (5 and 15 days) and odour (worn sock and unworn sock) or control (no sock, no sock) were examined using a General Linear Model. All data analysis was conducted using SPSS v19 (IBM, Armonk, NY, U.S.A.).

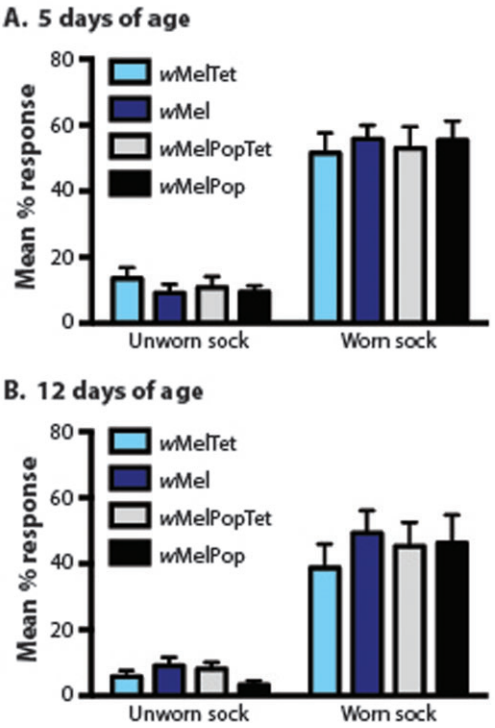

Fig. 1. Mean % response of (A) 5- and (B) 15-day-old mosquitoes to natural host-odour cues +/− sem. General Linear Models showed no significant effect of mosquito line (*w*Mel, *w*MelPop, *w*Mel.Tet, *w*MelPop.Tet) or age but did show differences in response to odour (worn sock and unworn sock).

Using a Y-tube olfactometer and a human worn sock as bait, we examined the relative capture rates of 5- and 15-day-old female mosquitoes with and without *Wolbachia* infection. In all control trials where no socks were placed in either arm of the Y-tube as a test of cleanliness of the apparatus there was no effect of line ($F = 1.1$, d.f. $= 3$, $P = 0.351$) but there was an effect of age ($F = 6.93$, d.f, $= 1$, $P = 0.010$) with younger mosquitoes slightly more responsive in general than old (average 1% and 0%, respectively). In the experimental trials with socks the odour stimulus was a significant factor ($P < 0.001$) with mosquitoes more highly attracted to the worn sock than the unworn (Table 1 and Fig. 1A,B). Mosquito line and age, in contrast, had no effect on mosquito responsiveness (Table 1 and Fig. 1).

Table 1. Summary statistics table comparing the responses of *w*Mel or *w*MelPop-infected *Stegomyia aegypti* to natural host odours.

Factor	Type III sum of squares	d.f.	Mean square	*F*	*P*
Corrected model	6314.71	15	420.98	25.85	0.000
(Intercept)	6641.28	1	6641.28	407.79	0.000
Line	27.156	3	9.05	0.55	0.645
Mosquito age	47.53	1	47.53	2.91	0.900
Odour	6188.28	1	6188.28	379.98	0.000
Line × age	7.03	3	2.34	0.14	0.933
Line × odour	22.40	3	7.46	0.45	0.712
Age × odour	16.53	1	16.53	1.01	0.316
Line × age × odour	5.78	3	1.92	0.11	0.949
Error	1824.00	112	16.28	—	—
Total	14 780.00	128	—	—	—
Corrected total	8138.71	127	—	—	—

General Linear Model factors are significant if $P < 0.05$. Mosquito age (5 or 15 days of age), line (*w*Mel, *w*Mel.Tet, *w*MelPop, *w*MelPop.Tet) and odour stimulus (worn sock and unworn sock) significantly affected the behaviours of mosquitoes.

It is not unexpected that the *w*Mel strain had no effect on mosquito attraction to odour, as there is no evidence from the laboratory of the strain causing direct reductions in host fitness (Walker *et al.*, 2011) and unlike *w*MelPop, *w*Mel is present in fewer tissues and grows to lower densities (Moreira *et al.*, 2009a). The absence of an effect in *w*MelPp-infected mosquitoes is more surprising given the reduced survival, fecundity, egg viability and blood feeding success and increased metabolic rates and locomotion defects with this strain many of which may relate to the strain's tropism and hence greater damage in nervous tissue (Evans *et al.*, 2009; McMeniman *et al.*, 2009; Turley *et al.*, 2009; Moreira *et al.*, 2009b; McMeniman & O'Neill, 2010). Many of these phenotypes, however, only emerge once infected hosts grow older and the case may be similar for odour responsiveness. Mosquitoes infected with *w*MelPop do not begin to exhibit the signs of a reduced lifespan until approximately 15 days of age (McMeniman *et al.*, 2009). While young *w*MelPop-infected mosquitoes do take smaller bloodmeals, poor feeding success and onset of the 'bendy' proboscis and 'jittering' phenotypes were not common until 35 days of age (Moreira *et al.*, 2009b).

The results of this study are encouraging for the use of *Wolbachia* infections to control vector-borne diseases. The ability to seek bloodmeals, mates and oviposition and resting sites all rely on *S. aegypti* being able to successfully find human hosts. These experiments suggest that both *w*Mel- and *w*MelPop-infected mosquitoes should be able to successfully carry out host seeking in young and middle ages. Given that the estimated daily survival of a population of mosquitoes is 90% (10% of the population is expected to die each day), it is the younger cohorts of mosquitoes that are most responsible for contributing to the next generation (McDonald, 1977). In the case of *w*MelPop-infected mosquitoes, this is even more so given their shortened lifespan (McMeniman *et al.*, 2009). Lastly, predictions from modelling indicate that given cytoplasmic incompatibility and maternal transmission efficiencies that *Wolbachia* will spread into host populations in spite of negative consequences for host fitness, particularly if their expression is associated with advanced age (Sinkins & Godfray, 2004). Future experiments that may provide more field-relevant measures of host seeking could include landing catches of *w*Mel-and *w*MelPop-infected mosquitoes in semi-field cages. Since this study was conducted, however, released *w*Mel infections have been shown to successfully spread into wild populations of mosquitoes (Hoffmann *et al.*, 2011) providing an empirical test of this strain's ability to effectively respond to odour cues in the field.

Acknowledgements

The authors wish to thank Nichola Kenny for technical assistance with mosquito rearing and Gradus Leenders for constructing the Y-tube olfactometer. This work was supported by grants through the Foundation for the National Institute of Health, the Grand Challenges in Global Health Initiative of the Bill and Melinda Gates Foundation, The Queensland Government through the Smart State Initiative, and the National Health & Medical Research Council of Australia.

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Accepted 31 January 2014