Wolbachia-infected *Drosophila* prefer cooler temperatures

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Abstract

Temperature plays a fundamental role in host-pathogen interactions. *Wolbachia* is an endosymbiont that infects about 40% of arthropod species, which can affect host behaviour and reproduction. The effect of *Wolbachia* on host thermoregulatory behaviour is largely unknown. Here, we used a thermal gradient to test whether *Drosophila melanogaster* infected with *Wolbachia* exhibit different temperature preferences (T_p) to uninfected flies. We found that *Wolbachia*-infected flies preferred a cooler mean temperature (T_p = 25.06±0.25˚C) than uninfected flies (T_p = 25.78±0.24˚C). Our finding suggests that *Wolbachia*-infected hosts might seek out cooler microclimates to reduce exposure to and lessen the consequences of high temperatures.

Keywords: *Drosophila melanogaster*; host-pathogen interaction; temperature preference; thermal gradient; *w*MelCS; *Wolbachia pipientis*

Abbreviations:

T_b : body temperature

T_p : preferred temperature

1. Introduction

Wolbachia pipientis is an endosymbiont bacteria that infects an estimated 40% of terrestrial arthropod species (Zug and Hammerstein, 2012). The association between *Wolbachia* and its hosts has been the subject of a wide array of studies, including the alteration of host behaviours and reproduction (Panteleev et al., 2007; Vala et al., 2004; van Houte et al., 2013; Weeks et al., 2002), cytoplasmic incompatibility for disease vector control (Clancy and Hoffmann, 1998; Mouton et al., 2005), and environmental factors mediating host-pathogen interactions (Murdock et al., 2012). Temperature is a key environmental modulator of host-pathogen interactions, which constrains the rate of biological reactions and sets limits to performance and survival (Thomas and Blanford, 2003).

For the insect host, there is little physiological capacity to differentiate their body temperature (T_b) from the ambient temperature of their surrounding environment (Angilletta, 2009). Physiological rates and performance are strongly affected by T_b in ectotherms, so organisms should aim to maintain their T_b across a range of temperatures that correspond to adequate performance (Huey and Kingsolver, 1989; Sinclair et al., 2016). One strategy that ectotherms can employ to avoid exposure to unsuitable temperatures is to modify their behaviour to seek more suitable microclimates, such as in shade, to find their preferred temperature (T_p) (Sunday et al., 2014). Temperature preference is the perception and neural integration of thermal information, resulting in this crucial thermoregulatory behaviour (Abram et al., 2017). *D. melanogaster* exhibits strong circadian and neutrally controlled temperature preference behaviour, which centres around 24–27°C (Arnold et al., 2015; Kaneko et al., 2012; Sayeed and Benzer, 1996).

Less is known about the thermal biology of *Wolbachia*, however high temperatures appear to be unfavourable. *Wolbachia* density is much higher at lower temperatures (e.g.,

13–19°C (Moghadam et al., 2017)) and is reduced at higher temperatures (e.g., 26°C (Clancy and Hoffmann, 1998; Hurst et al., 2000)). *Wolbachia* can be mostly or completely eliminated by exposure to cyclic heat stress or temperatures above 30°C, which also reduces vertical transmission of the symbiont (Corbin et al., 2016; Ross et al., 2017).

As temperatures ideally suited to *D. melanogaster* are generally higher than those suited to *Wolbachia*, we predict that manipulating their host's behaviour to seek cooler temperatures would be beneficial. Thus, our objective here is to use an established behavioural assay to test the capacity for *Wolbachia* infection to alter host T_p.

2. Methods

2.1 Fly and Wolbachia lines

Drosophila melanogaster from the Oregon RC line were infected with the *w*MelCS line of *Wolbachia pipientis* (hereafter +*Wol*). All flies were reared in 25°C incubators on a standard cornmeal diet and 12 h light/dark cycle. The control (*Wolbachia*-free) fly line was generated from the *w*MelCS-infected line by treating flies with 0.03% tetracycline. These flies were reared on a standard cornmeal diet for at least five generations before use to recover after tetracycline treatment. Male flies were used exclusively in temperature preference assays but both males and females are known to exhibit similar temperature preferences (Sayeed and Benzer, 1996).

2.2 Temperature preference assays

Temperature preference assays used an identical thermal gradient apparatus to that previously described in (Arnold et al., 2015). The apparatus achieved a stable linear gradient of 0.2°C per cm across a temperature range of 17.5–33.5°C (Arnold et al., 2015).

Temperatures were measured throughout the experiment by five K-type thermocouples suspended in the gradient airspace, held by bungs that were fitted into an acrylic cover, recorded by a Squirrel 2040 temperature meter.

Five flies were gently tipped into the centre of the apparatus allowed to freely move about the apparatus for 30 minutes. At the end of the trial period, flies were anaesthetised by CO_2 that was introduced into both ends of the gradient at a low-flow rate to prevent changes to the position of flies. Distance along the gradient was then used to determine the preferred temperature at the position of rest for each fly in the gradient, T_p.

As circadian rhythm affects T_p in *Drosophila*, we always conducted temperature preference assays between 09:30 and 13:30, a time period across which T_p is stable (Kaneko et al., 2012). Assays were also conducted in darkness by covering the apparatus in black material to prevent phototactic behaviour affecting positioning (Dillon et al., 2009).

2.3 Statistical analyses

To determine whether presence or absence of *Wolbachia* affected T_p, we applied Welch's two-sample *t*-test to compare the preferred temperatures of control and +*Wol* flies. We then calculated Cohen's *d* with 95% confidence intervals (CIs) as a measure of effect size, given that *p* is not always robust (Halsey et al., 2015). All analyses were conducted in R v3.4.1 (R Development Core Team, 2017).

3. Results and discussion

We found that flies infected with *Wolbachia* preferred cooler temperatures compared to those without any *Wolbachia* (Fig. 1). The difference between populations was significant at $\alpha = 0.05$ ($t_{1, 114} = 2.123$, $p = 0.036$), which was supported by an effect size and 95% CIs that

did not overlap with zero (Cohen's d = 0.394 [0.189 – 0.563]). Both populations exhibited large variance in T_p, ranging between 18.5 and 29˚C (Fig. 1A). There is some overlap in T_p distributions between the populations (Fig. 1B). In absolute terms, +*Wol* flies preferred a cooler mean (± SE) temperature of 25.06 ± 0.25˚C compared to the control flies which preferred 25.78 ± 0.24˚C.

To the best of our knowledge, this is the first empirical account of *Wolbachia*-infected flies exhibiting a preference for cooler temperatures. This finding is a strong indication that *Wolbachia* can manipulate an important aspect of host thermoregulatory behaviour.

Pathogens can improve their transmission probability and reproductive capacity by inducing host behavioural changes (Lefèvre and Thomas, 2008). Changes in thermoregulation behaviour has been well studied from the perspective of the infected host, particularly behavioural fever, where the host elevates its T_b by behavioural means to rid itself of the pathogen (Kluger, 1979). However, it is less clear whether pathogens manipulate host T_p, especially when the pathogen is not parasitic (i.e., endosymbionts or mutualisms) and for decreases to T_p. *Wolbachia* infects a highly diverse array of arthropod hosts and often has different T_p and temperature limits to that of its host (e.g., Pintureauand and Bolland, 2001).

Variance of temperatures in nature may lead to populations with mixed or incomplete *Wolbachia* infection (Van Opijnen and Breeuwer, 1999), but it is possible that *Wolbachia* could manipulate host T_p to maximise its own fitness without negatively affecting the host. Cyclic heat stress fluctuating between 26˚C and 37˚C at 12 h intervals significantly reduced *Wolbachia* density and cytoplasmic incompatibility of *w*Mel and *w*MelPop-CLA, but not *w*AlB in *Aedes aegypti* (Ross et al., 2017). Thermal biology will likely differ among strains of both host and pathogen, and the strain-specificity of T_p is important for future studies to consider.

The *w*MelCS strain used in the present study likely shares a recent field origin with *w*Mel (Riegler et al., 2005), a widely used dengue-suppressing *Wolbachia* strain. Temperature fluctuations like the cyclic heat stress experiment might well be experienced naturally in tropical regions. This would likely result in incomplete infection, which could explain the erratic temporal and spatial dynamics of *Wolbachia* spread in controlled infected-vector release programs (e.g., Schmidt et al., 2017). Our finding suggests that *Wolbachia*-infected hosts prefer cooler temperatures and might be likely to seek out cooler microclimates, which would reduce exposure to and lessen the fitness consequences of high temperatures.

The absolute decrease in T_p of less than 1˚C that we observed in flies infected with *Wolbachia* provides little buffer to the predicted 2–4˚C increase by 2100 due to climate change (IPCC, 2014). However, *Wolbachia* are maternally inherited and exposure to high temperatures can reduce vertical transmission in only a few generations (Corbin et al., 2016). If the infected host prefers cooler temperatures, then this behaviour would confer a selective advantage for *Wolbachia*. Arthropod hosts of *Wolbachia* have rapid generation times relative to the forecast rate of temperature increase, therefore it is conceivable that a minor change in T_p could be enhanced by selection across generations to mitigate fitness consequences.

This study paves the way for discovering the mechanisms by which *Wolbachia* infection alters host T_p. Whether the observed phenomenon is due to *Wolbachia* directly manipulating host behaviour, a host defense response, or a by-product of infection will need to be determined. The efficacy of introductions of populations of *Wolbachia*-infected vectors may hinge upon a better understanding of complex host-pathogen-environment interactions. Testing for *Wolbachia*-induced changes in thermal preference across multiple host and pathogen strains will elucidate whether unexpected ecological and evolutionary responses might occur in planned vector releases in a changing climate.

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160 Figure

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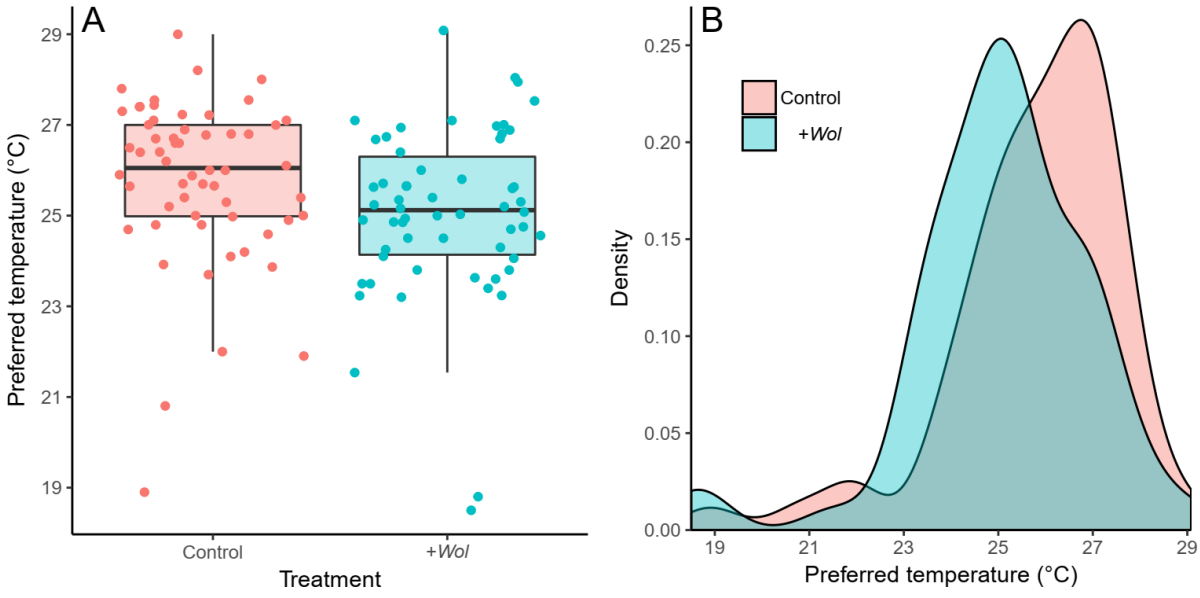

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163 **Figure 1. Preferred temperature of control and +*Wol Drosophila melanogaster*.** (A) Boxplot of

164 preferred temperature including raw data points for control and +*Wol* flies. Each population

165 had *n* = 58 individuals. (B) Density plot showing smoothed distributions of the preferred

166 temperature data for each population.

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