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The effect of captive breeding upon adult thermal preference in the Queensland Fruit Fly (*Bactrocera tryoni*)

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Abstract

The Queensland fruit fly (*Bactrocera tryoni*) is a generalist pest that poses a significant threat to the Australian horticultural industry. This species has become broadly established across latitudes that encompass tropical to temperate climates, and hence populations occupy diverse thermal niches. Successful expansion across this range may have been brokered by evolutionarily labile features of breeding phenology, physiology and/or behaviour. We explored the potential role of behavioural flexibility by characterizing variation in adult thermal preference using a novel gradient apparatus. Flies oriented within this apparatus essentially at random in the absence of thermal variation, but sought and maintained precise positions when presented with an established gradient. Male and female flies from an 'old' colony (>300 generations) and a 'young' (F7) colony were compared. Whereas we found no difference between the sexes, flies from the young colony preferred higher temperatures (30.93 ± 7.30 °C) and had greater individual variation than their counterparts from the old colony (28.16 ± 5.63 °C). Given that *B. tryoni* are routinely maintained at 25 °C in the laboratory, a lower mean preference of the old colony is consistent with thermal adaptation. This is further supported by their reduced phenotypic variance, which follows as a logical

consequence of stabilising selection given long-term environmental constancy. These results demonstrate that *B. tryoni* seek to thermoregulate via adult behaviour, and that individual temperature preference can be precisely measured using a gradient apparatus. The evidence for adaptive tuning of this behaviour has importance for both the design of captive rearing protocols as well as the prediction of invasive potential and species biogeography under future climatic variation.

Keywords: thermal preference, thermoregulation, domestication, Q-fly, *Bactrocera*, adaptation

Introduction

The Queensland fruit fly (*Bactrocera tryoni* Froggatt) (Diptera: Tephritidae) (henceforth Q-fly) is estimated as the most economically important pest insect in Australia (Clarke et al., 2011; Sutherst et al., 2000). It infests diverse fruit types, and threatens both local and international trade. Whereas this species was initially restricted to tropical and subtropical rainforests along the east coast of Australia (May, 1963), the past century has witnessed a rapid and significant range expansion. Extant populations now occupy the full latitudinal range of mainland Australia. Longitudinally, they range from the eastern coastline to over 1000 km inland, along the Western borders of NSW and the Northern Territory (see Fig. 1. in Dominiak and Mapson, 2017). This distribution encompasses all six of Australia's major climatic zones (equatorial, tropical, sub-tropical, temperate, desert, and grassland; Zillman, 2001), and hence spans great variation in thermal regimes (i.e., averages, maxima and minima, ranges, and extreme weather events).

Unlike endotherms, body temperature in most insect groups is primarily determined by their external environment. This has led to a range of physiological mechanisms for

thermoregulation, including the ability to vary the metabolic capacity of their tissues (Seebacher 2005), and the modulation of cell membranes, their cytoskeleton and nervous tissues (Chown and Terblanche 2007). Such changes underpin the processes of ‘acclimation’ (under gradual temperature change) and ‘hardening’ (in response to a transient change). In both cases, individuals that experience extreme high or low temperature events are better able to survive and recover more quickly from subsequent thermal fluctuations (Hoffman et al. 2003). Overall, the magnitude of physiological adaptation appears greatest under conditions of gradual and sustained thermal change, and/or when individuals experience repeat exposure to extremes (Chown and Terblanche 2007; Colinet and Hoffman 2012).

The physiological capacity for tolerating suboptimal thermal conditions is however only viable given relatively moderate rates or magnitudes of temperature change (Angilletta 2009; Huey and Kingsolver, 1989). Most insects therefore exploit opportunities for behavioural thermoregulation where possible. Behavioural mechanisms encompass strategic microhabitat selection, flexibility in diel activity regimes, heat generation via shivering, and warming by basking in the sun (Heinrich 2013). By selectively using such mechanisms, either singly or in combination, individuals strive to operate as close as possible to their thermal optimum. In this sense, thermotactic behaviours such as microhabitat choice present expressions of underlying thermal preference, and offer an empirical basis for estimating this property.

Tight relatedness between thermal preference and the temperature conducive to optimal performance has been confirmed for multiple insect groups (Halliday and Blouin-Demers, 2015; Steward, 1981; Yamamoto, 1994). Both preference and physiological optima are known to (co)vary across different thermal environments (Huey, 1991), which supports an underlying basis of variation and the ability for adaptive change (Girgius and Lee, 2006; Lah

et al., 2017; Steward, 1981; Yamamoto and Ohba, 1984). Work in model groups such as *Drosophila* has revealed high genetic variances for preference (Yamamoto, 1994a,b), and moreover demonstrated realized responses to artificial selection upon this trait in laboratory populations (Good, 1993; Richmond and Finkel, 1973). *Drosophila* temperature preferences have also been observed to evolve towards prevailing laboratory conditions when maintained in captivity for many generations (McDaniel et al., 1995).

Relative to *Drosophila*, much less is known about the sources, causes and consequences of variation in Q-fly thermal traits. Workers have addressed the question of plastic variation in thermal tolerance, primarily according to the influence of acclimation on survivorship at extreme temperatures. This work indicates reduced mortality at higher temperature when exposed to heat treatments immediately prior to a high temperature event (Beckett and Evans, 1997). A similar pattern was also found for acclimation at lower temperatures, where prior experience to low temperatures decreases mortality during low temperature events (Meats, 1973, 1976, 1984).

Studies have also investigated the fitness consequences of thermal variation in both controlled laboratory environments and field cages. This work has shown that constant temperature regimes influence development time, with colder temperatures prolonging development (Bateman, 1967) and decreasing mating frequency (Fay and Meats 1983; Meats and Fay 2000).

While both high ($\leq 30^{\circ}\text{C}$) and low ($\leq 20^{\circ}\text{C}$) temperatures lead to increased pupal mortality (Bateman, 1967 (laboratory); O’Laughlin et al. 1984 (field cages)) and decreased fecundity (Bateman, 1967). However, for this species behavioural adaptation to temperature change is yet to be explored.

In this study we investigate, validate and apply a novel thermal preference protocol to test for variation between strains of the Q-fly which vary in respect to generation time in captivity. We first validated the ability of the apparatus to present a precisely-controlled thermal gradient by characterizing inter- and intra-day reliability of the gradient, including the degree of uniformity across channels. We then tested the applicability of the apparatus (generally) and the biological salience of the gradient (specifically) by comparing how test flies behaved both in the presence and absence of thermal variation. Following these steps, we used the apparatus to estimate thermal preferences among the two *B. tryoni* colonies. Here we compared individuals from a colony bred in captivity since 2006 (300+ generations; Gilchrist et al., 2006) with those from a more-recently derived colony (“F7”), where both colonies were reared under identical laboratory conditions.

Our contrast among colonies was motivated by the importance of understanding thermal trait variation in both wild and laboratory populations. This is particularly important in *B. tryoni* because mass-reared laboratory populations are used for sterile male release as a biological control method across Australia (Jessup et al. 2007). Evidence suggests that *B. tryoni* can adapt to laboratory conditions (a process we refer to as domestication) over fewer than ten generations, at least in terms of traits such as behavioural activity (Weldon et al. 2010,) mating behaviour and age at maturity (Meats et al. 2004). A loss of genetic diversity due to genetic bottlenecks is also evident in these early generations (Gilchrist et al. 2012). Insects generally become less stress tolerant when laboratory adapted, losing their ability to survive and recover from temperature and humidity extremes (Hoffman and Ross 2018).

By comparing these colonies, we examined the potential role of domestication to laboratory environment via genetic adaptation on thermal preferences – something not yet explored for *B. tryoni*. This kind of adaptation could occur through unintentional selection of flies suited to laboratory conditions, including those with preferences for temperatures close to laboratory environments. It may also result in reduced genetic variation via mechanisms of inbreeding or population bottlenecks.

Examining a potential role of domestication carries the additional benefit of informing the adaptive genetic basis of thermal preference. This is based upon the reasoning that flies which have been maintained under relatively benign/invariant laboratory conditions over long periods should become adapted to those conditions.

However, as we compared only two populations, and not multiple lines of both ‘old’ and ‘new’ colonies, it is possible that inter-population level differences could be the result of genetic drift and / or founder effects rather than domestication. Thus, in order to support the hypothesis that these two populations diverged because of laboratory adaptation (implying a heritable basis of temperature preference in *B. tryoni*), we made two simple predictions: (1) that long-term captive-bred flies will prefer a temperature range closer to the ambient laboratory mean, and (2) that intra-population variance in temperature preference will be reduced in comparison to more recently field-derived populations.

Methods

Populations

We used laboratory-reared flies from three different sources. The first consisted of F12 individuals from a stock laboratory culture initiated by flies sampled in Mareeba (North Queensland). This population was used purely for practical reasons, and exclusively in the formative stages of testing broad parameters of the apparatus and the thermal preference

assay (as described below). While we recognize that data obtained from this population may vary from others in terms of actual parameter values (e.g., mean preference), we consider this largely inconsequential to testing the efficacy of protocols for Q-flies more broadly. The remaining two colonies – hereafter the “experimental” colonies were initiated by flies captured from a common location (Sydney) but which differed markedly in how long they have been cultured in captivity. These encompassed a “young” (F7) colony versus an “old” colony that had been cultured for >300 generations. Studies using neutral genetic markers (microsatellites) demonstrate that Q-flies from around the Sydney basin represent a single, genetically-homogenous population (Yu et al. 2001; Gilchrist et al. 2006). Subsequent studies (Gilchrist et al. 2012) have therefore treated collections from different regions across Sydney as replicate samples of the same source population. We draw upon this precedent to justify our primary comparison as between two sample populations according largely to generation time in captivity, without a regional confound. We used these colonies for our experimental test of whether adaptation under captive rearing may influence thermal preference (i.e., an effect potentially akin to “domestication”). All colonies were maintained according to standard laboratory practice at 25.0 ± 1.0 °C and 75 ± 5 % relative humidity, under a 12:12 light:dark photoperiod, and with larvae raised on a standard carrot diet (Steiner and Mitchell, 1966).

We reared individuals sourced from each experimental colony in mixed-sex cages under standard conditions (as above), and tested thermal preference in adults that ranged between 5 and 30 days post-eclosion.

Thermal Apparatus

Thermal preferences are typically measured using a thermal gradient apparatus, whereby a thermally conductive material is heated and cooled at opposing ends (Casterlin and Reynolds, 1980). Animals are placed upon the apparatus and their association with regions of particular

'preferred' temperature is recorded. Past applications have however revealed several potential problems with this approach. The first, identified by Dillon et al. (2009), is that many studies use a lamp as a heat source, which potentially confounds thermotaxis with phototaxis. Second, many studies have not accounted for the behaviour of organisms in the apparatus when no thermal gradient is present. Some insects have been found to cluster at the edges of the equipment when maintained at uniform temperatures (Deal, 1941; Fogelman, 1979; Murphy and Heath, 1983; Waddington et al., 1954), while others have been found to disperse homogeneously across apparatus at stable temperature (Hong et al., 2006). Third, when an apparatus has multiple channels for testing multiple organisms in concert, the consistency of the thermal gradient across channels must be accounted for, or else differences in the position of individuals may not precisely indicate their actual temperature preference. Many studies use only one channel, with multiple individuals in the channel, presenting a further problem of accounting for group dynamics (e.g. Arnold et al. 2015; Hamada et al. 2008).

To measure thermal preference accounting for these potential issues, we designed a thermal preference apparatus based on that developed by Sayeed and Benzer (1996), where an aluminium block is heated and cooled at each end. Our block measured 1000 x 160 x 25 mm, with two legs (150 x 25 mm) which were submerged in dry ice at one end and hot water at the other (Figure 1). However, the design used by Sayeed and Benzer (1996), and other subsequent studies (Hamada et al. 2008; Arnold et al. 2015) used only a single channel with multiple flies measured at once. Later developments such as that of Goda et al. (2014) divided their apparatus into multiple lanes to allow for replicates within trials, although groups of flies were still measured within a single lane. We developed our apparatus with nine machined channels, measuring 8 x 8 mm ran along the length of the block, and individual flies were placed in a channel each for testing. Marks at 10 mm increments along

the edges of each channel were used to determine fly position along the apparatus. A 3 mm clear Perspex lid was placed along the top of block to restrain the flies in the channels whilst allowing for continuous observation. The ends of each channel were plugged with rubber fit to size (8 x 8 mm). Seven millimetre diameter holes were drilled along the Perspex above each channel 300 mm from the cooled end through which flies were transferred to the arena, and were covered with clear Perspex lids during each trial to prevent escape. In order to validate a stable temperature gradient across the apparatus, smaller holes (2 mm diameter) were drilled along the centre channel at 100, 300, 500, 700, and 900 mm for the insertion of Type-K thermocouple temperature sensors. Thermocouples were connected to a Lutron (BTM 4208SD) data logger which recorded temperature data for each location along the channel every 10 seconds. The apparatus was lit from above with florescent room lighting, and illumination was assessed at positions across the apparatus prior to each day of testing using a Digitech QM1587 luxometer.

We recorded fly position using a Canon EOS 70D camera placed on a tripod which automatically captured images at one or five minute intervals for a period of between 40 and 90 minutes. Photographs were then scored by two independent scorers, with each data set checked by the other scorer for consistency. Fly position was recorded using the 10 mm markers along the channels to the nearest 5 mm. When fly position could not be ascertained or agreed upon by the two scorers, this data point was removed from analysis (6 from the Sydney –no heat trials, and two from the Sydney –old and young trials), and any flies that died, escaped or were injured during the trial were removed from analysis (5 from the Sydney-no heat trials). An additional 16 flies were excluded from the Sydney old and young trials due to a camera malfunction.

Assay Validation

To examine the natural behaviour of flies in the absence of a heat gradient, we tracked the position of individuals from the young and old Sydney populations (young; $n = 19$ F, 17 M, old; $n = 21$ F, 17 M) at five-minute intervals for 40 minutes, with each channel held at a uniform ambient temperature ($25 \pm 1^\circ\text{C}$) throughout. To examine the efficacy of the thermal gradient and determine the optimal time for point-sampling temperature preference, we applied the gradient and tracked the position of flies from the Mareeba population ($n = 16$ F, 16 M) at one minute intervals for a 90-minute period, and recorded temperature data at 10 second intervals using thermocouple temperature sensors placed along an empty channel.

Effect of Sex and Colony

We investigated the influence of sex and colony on temperature preference by loading individual male and female flies into single channel of the apparatus, while a temperature gradient was running through it, and recording individual location at five minute intervals for a 40-minute period. The order of male and female and flies of each colony were randomised across the apparatus for each trial. We sampled temperature data at 10 second intervals using thermocouple temperature sensors placed along an empty channel, and 393 flies ($n = 195$ from the old colony, and $n = 198$ from the young colony) were included in the analysis.

Statistical Analysis

Following our analysis of the stability and linearity of our heat gradient (detailed below), we used temporally-precise linear models to estimate temperature as a function of location within the choice apparatus, and thus to convert a given individual's location into an estimate of their thermal preference at that time point. That is, we used the readings from the thermal probes within each flies' channel at the point in time nearest our recording of the flies'

position, with a maximum difference of ten seconds (e.g. for photographs taken at 25.0 min, we statistically modelled the temperature gradient using thermal readings taken at 25.0 ± 0.17 min).

We derived two related measures to explore both the general effectiveness of our apparatus for identifying the thermal preference of flies, and to estimate the optimal time(s) at which to point-sample individual preferences: (1) the Pearson's' correlation between the thermal preference of individual flies at each minute of the trial and their overall mean preference, and (2) individuals' absolute deviation from their mean thermal preference at each minute of the 90-minute pilot trial. We then used segmented linear regressions fit via maximum-likelihood to separately model these variables as a function of time (Toms and Lesperance, 2003), and Davies' tests to test the significance of non-constant regression parameters (i.e. break-points; Davies 1987) within each model. This reflected our expectation that, assuming the apparatus functions as intended, flies should 'settle' at their preferred location (hence, temperature) within a channel, following an initial period of adjustment. We ran segmented regressions using the package 'segmented' (v. 0.5-2-2; Vitto and Muggeo, 2008) in R (v3.4.0; R Core Team 2017).

For our focal question regarding the effects of colony on thermal preferences, we first used both a Levene's test for homogeneity of variance and an asymptotic test for the equality of coefficients of variation (Feltz and Miller 1996), specifying an interaction of colony and sex, to test the prediction that flies from the 'old' colony should express reduced variation in their thermal preferences. Following this, we used a linear mixed effects model to examine the influence of colony on flies' overall temperature preferences. We included sex and colony, and their interaction, as fixed effects, and individual age as a random covariate. In light of the

heterogeneous variance structure (see results), we allowed the variance components of the colony effect to vary independently. Mixed-effects modelling was run using the package nlme (v. 3.1-131; Pinheiro et al., 2017) in R (v3.4.0; R Core Team 2017). All summary statistics are means \pm s.d. unless otherwise specified.

Results

Thermal Apparatus

Our choice apparatus effectively established and maintained a linear gradient across an ecologically relevant range of temperatures with little variation ($23.13 - 63.91 \pm 2.70$ °C; Fig. 2). The minimal variation present was, at any rate, further ameliorated by our use of temporally-specific regressions to derive point-estimates of temperatures for individual flies within the apparatus. The minimal inter-channel variation allowed us to maintain a breadth of temperature variation in the apparatus over the course of a day of testing. It also meant that we were able to extrapolate temperature data measured in a single channel to other channels in the gradient where flies were positioned.

Fly behaviour

Flies from the ‘validation colony’ behaved randomly and indicated a near-uniform distribution across the length of channels within the apparatus in the absence of a thermal gradient (Fig. 3a). By contrast, when placed within the apparatus in the presence of a thermal gradient, flies expressed clear, directional preferences for particular locations within their channel (Fig. 3b). Under these conditions, the correlation between an individual’s position at a given time point and its overall mean position was high and stable beyond 21.00 ± 0.66 minutes (Davies $p < 0.001$, GLM slope estimates = 0.04 ± 0.002 , -0.002 ± 0.0003 s.e; Fig. 4a). This indicates that flies initially adjusted their position within the apparatus (7.4 ± 0.58

minutes, Davies' $p < 0.001$), before remaining at their putatively preferred temperature for the remainder of the trial (slope estimates = -0.56 ± 0.06 , -0.002 ± 0.0003 s.e; Fig. 4b).

Thermal preferences according to colony age

We found a difference in the variance (Levene's $F_{3,389} = 4.141$, $p = 0.007$) and coefficients of variation ($D'AD = 4.65$, $p = 0.031$) of thermal preferences across experimental colonies, with flies from the old colony expressing reduced variation (males = $28.4 \pm 5.4^{\circ}\text{C}$, females = $28.0 \pm 5.4^{\circ}\text{C}$) relative to flies from the young colony (males = $30.8 \pm 7.2^{\circ}\text{C}$, females = $31.0 \pm 7.3^{\circ}\text{C}$). We also identified a distinct effect of colony age upon thermal preferences (Fig. 5; Table 1), with individuals from the old colony preferring cooler temperatures nearer their uniform laboratory-rearing temperature ($28.16 \pm 5.63^{\circ}\text{C}$, pooled across sexes) than their counterparts from the young colony ($30.93 \pm 7.30^{\circ}\text{C}$). We found no main or interactive effect of sex on the mean or variation in thermal preference (as summarised above).

Discussion

Assay Validation

In the absence of temperature variation, flies dispersed evenly across the apparatus, a phenomenon also observed in *Drosophila* (Hong et al., 2006). Once a temperature gradient was established, however, the spatial distribution of test subjects changed in response. This indicates a behavioural reaction to variation in temperature, indicating an underlying thermal preference. In addition to this, we demonstrated that individual flies reacted differently to a thermal gradient, which lends credence to the conclusion that movement across the apparatus provides a good indication of preferred temperature. This demonstrates that *B. tryoni* behaviourally thermoregulate, when presented with environments of thermal variability,

something that has recently been confirmed under field conditions (Inskeep et al. forthcoming).

The most effective indication of preference was observed from 21 minutes into the trial, after which individuals subsequently remained in a relatively fixed position. This indicates that flies first explore different thermal environments along the gradient, before selecting their preferred temperature. This is an important point, and is over-looked in many gradient-based temperature preference studies. For instance, in *Drosophila*, temperature preference is recorded as corresponding to fly position after 20 minutes (Sayeed and Benzer, 1996), 30 minutes (Hamada et al., 2009; Yamamoto and Obha 1982; 1984; Arnold et al. 2015), and the average of positions at ten minutes and two hours (McDaniel et al., 1995). The choice to sample at these times is not justified in any of these studies. It is important to determine the appropriate 'settling' period before making conclusions about thermal preferences, and this period may differ between species or populations in relation to other phenotypes such as exploratory tendencies or locomotor activity. As our trials ran for a maximum of 90 minutes, we are unable to make any conclusions about behaviour and its relationship to thermal preference after this time. Thus we can recommend between 21 and 90 minutes after being placed in the gradient as an appropriate period for measurement of thermal preference for the Q-fly.

Sex and Colony Effects

No sex differences in thermal preference were found for any of the tested colonies (and no sex-by-colony interactions), indicating that male and female flies respond to the thermal environment in a similar manner. Weldon et al. (2005) also found that more general patterns of activity and behaviour (including the proportion of time spent walking, flying, grooming

etc.) do not differ between male and female Q-flies. Thermal preference did however vary between our two studied colonies, with flies from the old colony preferring temperatures nearer to laboratory rearing conditions (25°C), and flies from the young colony preferring warmer temperatures. Not only does this indicate a trend towards laboratory conditions, but the old colony also exhibited significantly less inter-individual variation in thermal preference behaviour. The data therefore support both a-priori predictions for how Q-flies should evolve under the hypothesis of adaptation to captive conditions.

We conclude that the data best support adaptation shaped by a captive environment, but the lack of replicated lines raises several important caveats. This design cannot, for example, strictly exclude stochastic mechanisms such as founder effects or genetic drift as explanations for population differentiation. Similar caveats have applied to prior studies in Q-flies (Weldon 2005; Weldon and Meats 2010; Gilchrist and Meats 2011) and in studies of adaptation more generally (e.g. Kemp et al. 2009), yet are not always discussed explicitly. For the present study, we consider both possibilities less likely because focal populations derived from large initial samples and were subsequently maintained for high effective size (N_E) in captivity. In regard to drift, an important heuristic comes from work in flour beetles (*Tribolium*; Rich et al. 1979), whereby inadvertent selection due to captivity was shown to drive consistent adaptation among replicate populations except when $N_E < 20$. Although we cannot exclude non-adaptive possibilities, the fact that our populations differed as-per a-priori prediction adds strength to the conclusion for adaptive divergence.

McDaniel et al. (1995) observed a similar trend in three species of *Drosophila*, wherein flies from populations that had been cultured for longer in the laboratory preferred warmer temperatures that were closer to the laboratory conditions of 20°C. As stocks were maintained

for longer periods, this preference became increasingly warmer. Other behaviours in the Q-fly have been shown to alter due to captive-breeding, indicating an effect of domesticity. For instance, female flies mature earlier (Meats et al., 2004), and males begin calling (a courtship behaviour) significantly earlier in the day (Weldon, 2005). Pheromone production also increases as colonies age, indicating an adaptation to crowded rearing conditions (Perez et al., forthcoming). Gilchrist et al. (2012) examined the genetic consequences of domestication using microsatellite markers in multiple replicated lines over time, and found that Q-fly populations undergo a significant loss of genetic diversity due to domestication. This is thought to happen first due to genetic bottlenecks in the early generations and secondly due to selection, on both pupae and adult flies. It must be noted that an initial reduction in genetic variation could result in reduced capacity to adapt to laboratory environments, meaning that the subsequent adaptive process happens more slowly. Future work is needed to ascertain the genetic basis of thermal preference in *B. tryoni*, which could both inform – and be informed by – studies of populations at intervals following establishment in captivity.

The impact of domestication is ecologically relevant as laboratory colonies representing different populations are often used to infer the behaviour and biology of their wild counterparts. This must be taken into account if thermal preferences are estimated for flies from different regions using laboratory colonies. *B. tryoni* are widely dispersed across the Australian continent, and their distribution continues to spread (Dominiak and Mapson, 2017). Knowledge that *B. tryoni* use behavioural mechanisms to thermoregulate informs the way in which they are understood to adapt and disperse to changing environments. This information may be used, in concert with knowledge of thermal vulnerabilities such as their critical maximums and minimums (Bateman, 1967; Beckett and Evans, 1997; Meats, 1973, 1976, 1984; Meats and Fay, 2000; O’Laughlin et al., 1984), to predict their future spread and

dispersal patterns. This is especially relevant given predicted future climate change, where local averages, maximums and minimums, ranges, as well as the occurrence and degree of extreme weather events are forecast to alter rapidly. The reduced variation in response, along with the indication that domestic flies preferred temperatures close to laboratory conditions, suggests that thermal preference has a heritable basis in *B. tryoni*. Knowing the heritability of a temperature-related trait is becoming increasingly relevant, as predictive models are used to forecast evolutionary responses to changing climates (for example see Bush et al., 2016). Future work formally estimating the heritability of thermal preference in the Q-fly could be used to assist efforts to control populations as they spread across different thermal regimes.

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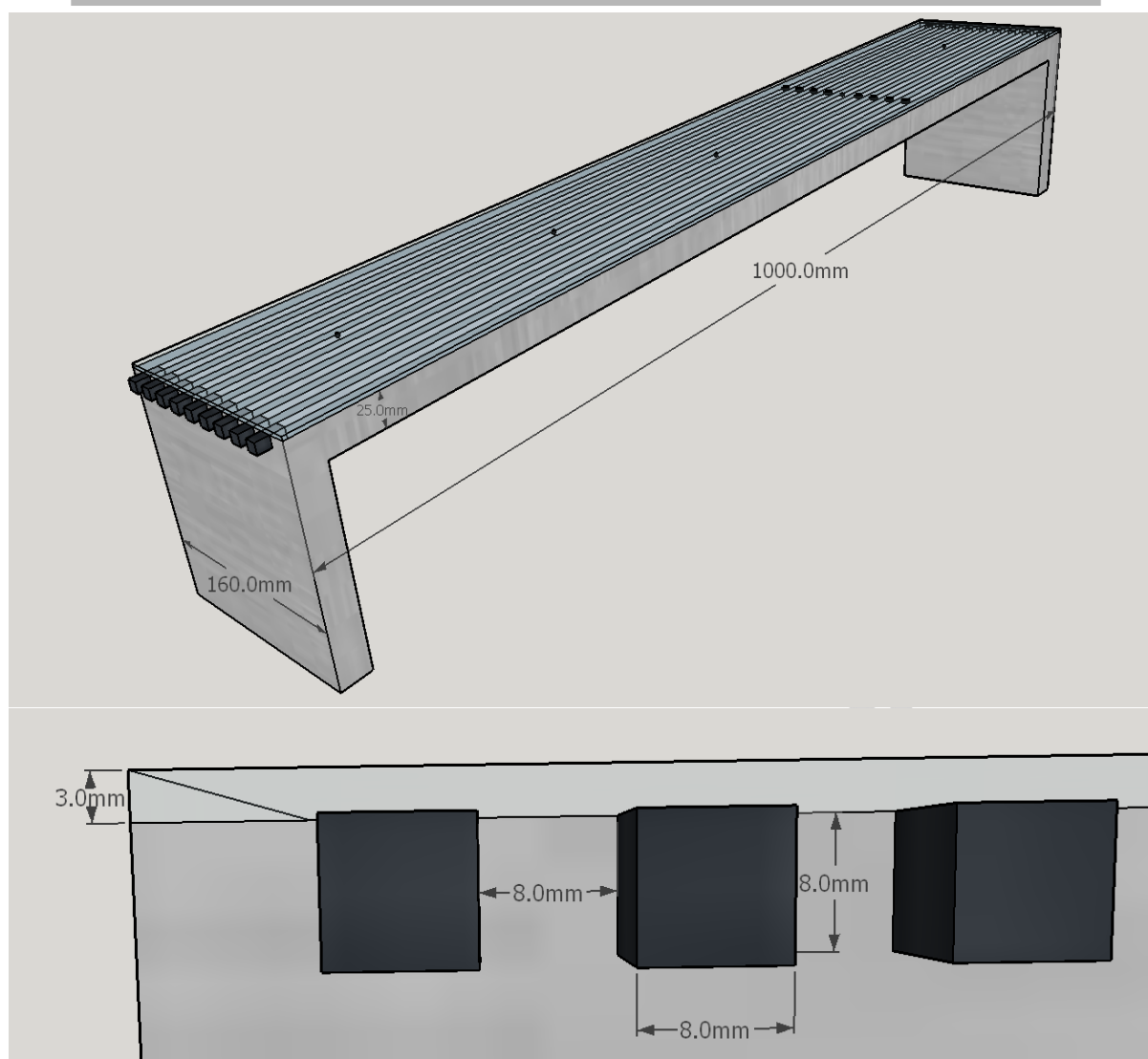


Figure 1: A schematic of the thermal preference apparatus, consisting of aluminium blocks, a clear Perspex top, and fitted rubber plugs.

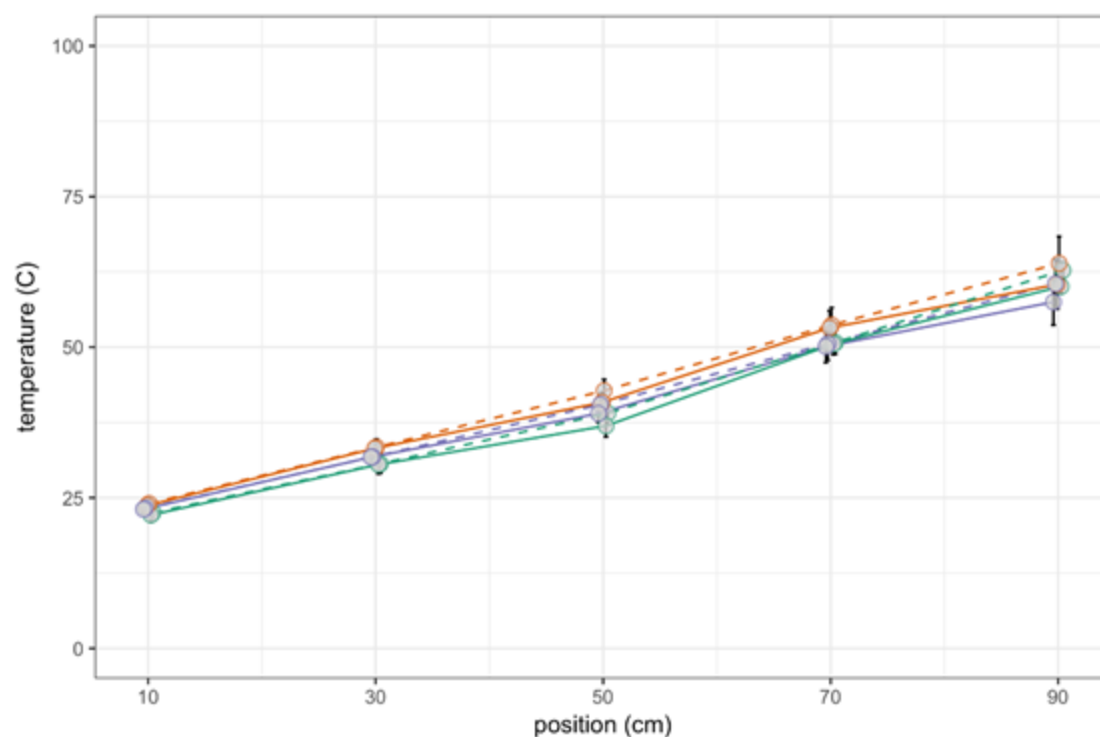


Figure 2: The range and stability of the thermal gradient in our fly-choice apparatus. Line colours indicate pilot-trial days (1-3), line types indicate the physical position of probes on the apparatus (dashed = channels 1-5, solid = channels 6-10, grouped for convenience), and points and error bars denote intra-day (i.e. trial) means \pm standard deviations.

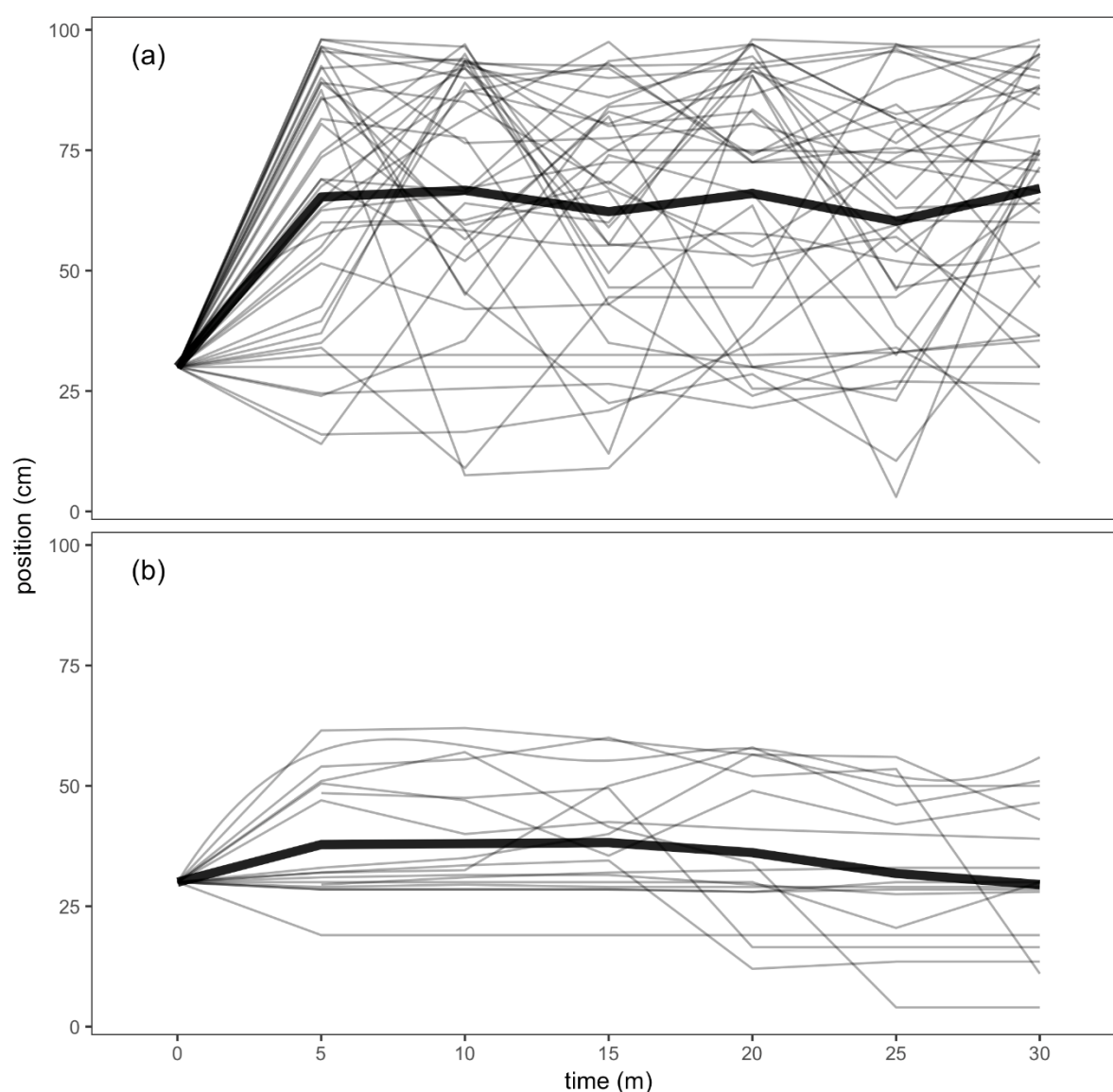


Figure 3: The positions of *B. tryoni* within the choice apparatus (Fig. 1). Panel (a) shows the location of flies over time when no heat gradient is applied and the apparatus is kept at a constant temperature, while (b) indicates fly positions with a heat gradient applied. Grey lines denote individuals while black lines indicate overall means.

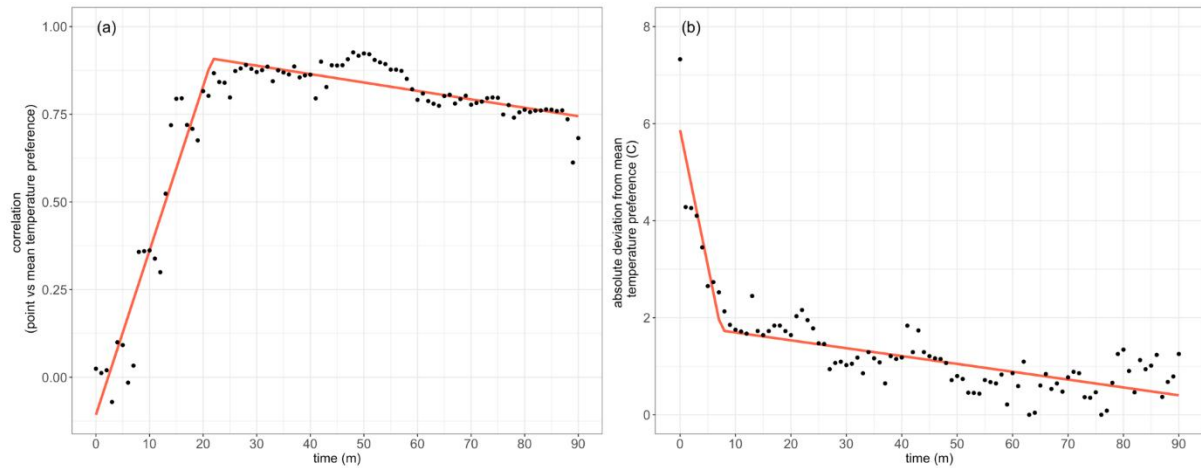


Figure 4: (a) The Pearson's correlation between individual *B. tryoni*'s mean thermal preference, and their preference at every minute across the 90-minute pilot trial. (b) The absolute deviation from individuals' mean preference, at every minute across the 90-minute pilot trial. Red lines indicate maximum-likelihood fits from segmented GLM's.

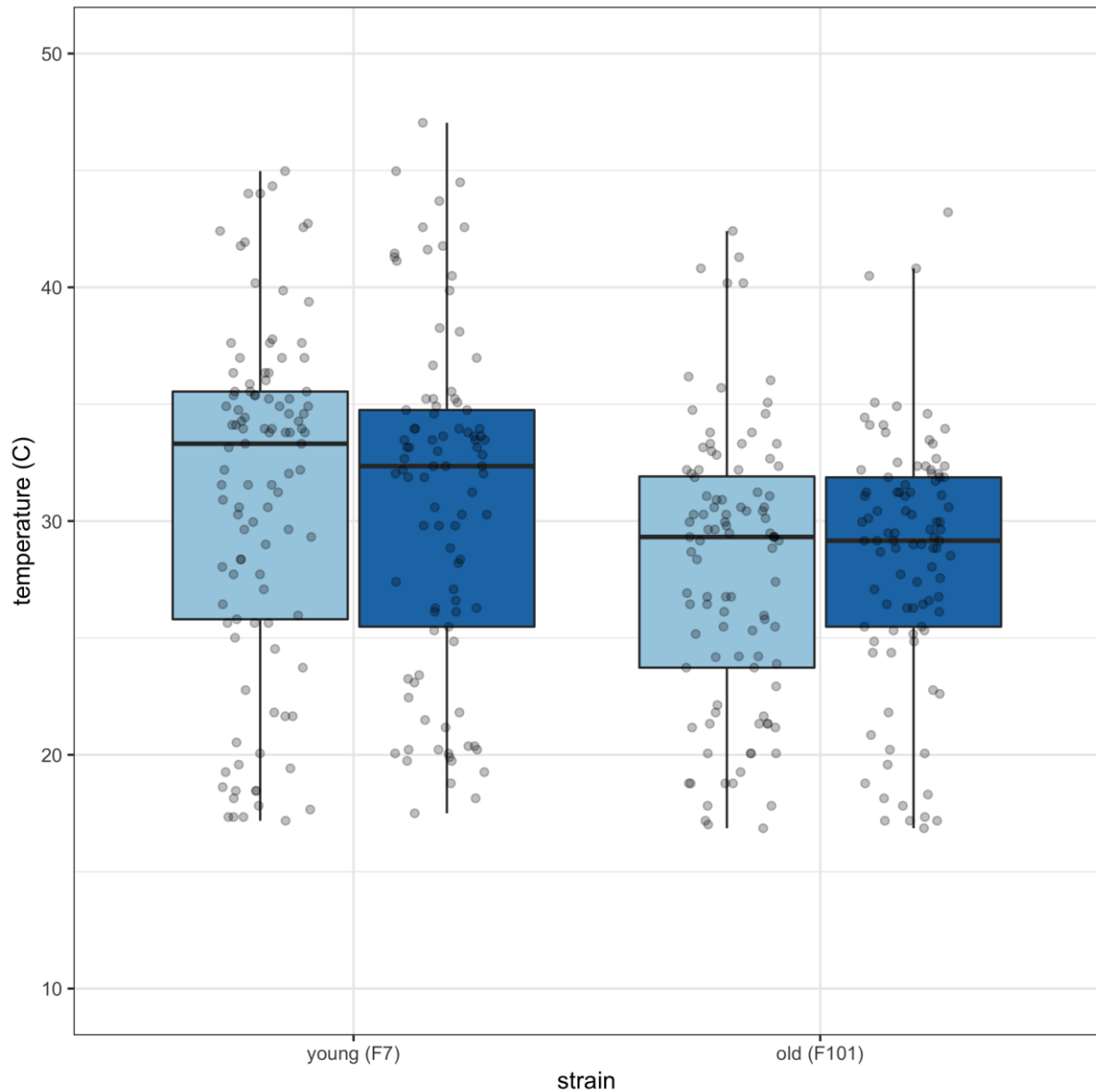


Figure 5: The thermal preferences of female (light blue) and male (dark blue) young and old *B. tryoni* from a population in Sydney, Australia, as recorded after 25 minutes. Boxes enclose the 25th and 75th percentiles, internal lines indicate the median, and whiskers extend to values spanning 1.5x the inter-quartile range. Points denote the raw preferences of individual flies.

Table 1: GLMM fit and parameter estimates for the effects of sex and colony on the thermal preferences of *B. tryoni*.

Model	df	L. L.	LL-ratio	P
<i>null</i>	3	-1301.748		
<i>full</i>	7	-1286.428	30.640	< 0.001
Parameter	Est.	SE	t	p
<i>Intercept</i>	30.956	0.763	40.554	< 0.001
<i>Sex</i>	-0.212	1.038	-0.204	0.838
<i>Colony</i>	-3.069	0.906	-3.385	< 0.001
<i>Sex x Colony</i>	0.647	1.314	0.492	0.623

Highlights

- *Bactrocera tryoni* behaviourally thermoregulate
- Flies from older colonies preferred lower temperatures than flies from newer colonies
- Flies from older colonies displayed less variation in thermal preference compared to flies from newer colonies
- Thermal preference is not related to sex