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The effect of short-term exposure to high temperatures on male courtship behaviour and mating success in the fruit fly *Drosophila virilis*

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ABSTRACT

Human-induced climate change is leading to higher average global temperatures and increasingly extreme weather events. High temperatures can have obvious effects on animal survival, particularly in ectotherms. However, the temperature at which organisms become sterile may be significantly lower than the temperature at which other biological functions are impaired. In the fruit fly *Drosophila virilis*, males are sterilized at temperatures above 34 °C, but are still active and able to mate normally. We investigated the male behavioural changes associated with high-temperature fertility loss. We exposed males to a warming treatment of 34.4 °C or 36.6 °C for 4 h, and then recorded their mating behaviour after being allowed to recover for 24 h. Previous work in this species suggests that males exposed to 34.4 °C lose the ability to produce new sperm, but can utilize mature sperm produced before the heat shock. We therefore predicted that these males would increases their courtship rate, and reduce their choosiness, in order to try to ensure a mating before their remaining mature sperm die. In contrast, over two-thirds of males exposed to 36.6 °C are completely sterile. In standard mating trials, earlier exposure to 34.4 °C or 36.6 °C did not affect male courtship behaviour when compared to control males kept at 23 °C. Exposure to high temperatures also did not alter the extent to which males directed courtship toward females of the same species. However, males exposed to 36.6 °C were significantly slower to mate, and had a reduced likelihood of mating, when compared to control males. Overall, exposure to high temperatures did not alter male courtship behaviour, but did lower their likelihood of mating. This suggests that females can distinguish between normal and heat-sterilized males before mating, and that female mate choice may at least partly mitigate the population-level consequences of high-temperature induced male sterility in this species.

1. Introduction

Climate change around the globe has intensified in the last century, with the global average temperature increasing by 1.1 °C in the last 100 years (Lindasey and Dahlman, 2021), and predicted to potentially exceed 4 °C by 2100 (Collins et al., 2013). Besides the rise in global average temperature, heatwaves have also increased in frequency, intensity, and duration due to climate change (Meehl and Tebaldi, 2004; Sales et al., 2018; USEPA, 2021). One of the concerns raised by the changing climate is its effects on wildlife. Crucially, all organisms have a temperature above which they cannot complete basic biological functions, known as the critical thermal limit (Kingsolver et al., 2013; Parratt et al., 2021). Importantly, high temperatures can also reduce the fertility of both endotherms and ectotherms (Skinner and Louw, 1966; Yaeram et al., 2006; Takahashi, 2011; Parratt et al., 2021; Sales et al., 2021). Notably, male fertility appears to be more susceptible to impairment at high temperatures than female fertility, due to the sensitivity of male gametes (Takahashi, 2011; Sage et al., 2015; Iossa, 2019). For example, heat stress frequently leads to a decline in sperm count and sperm viability, and alteration of ejaculate composition (Jørgensen et al., 2006; Prasad et al., 2011; Boni et al., 2016; Green et al., 2019; Sales et al., 2021). At very high temperatures, many organisms reach a thermal fertility limit (also known as the TFL), which is the temperature above which an individual loses its ability to produce offspring (Walsh et al., 2019). This is significant, because in terms of fitness complete sterility is as deleterious to an organism as death. The ecological significance of high-temperature induced sterility is not yet fully clear (Walsh et al., 2019). However, recent work in *Drosophila* fruit flies has shown that fertility limits may be several degrees below critical limits (Parratt et al., 2021), and that information on fertility limits substantially improves predictions on the highest temperatures species occur at worldwide (Parratt et al., 2021). Thus, the loss of fertility due to high temperatures

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may be a major threat to the viability of wild populations and the maintenance of biodiversity.

In many species, heat stress may also impair reproductive behaviour in ways that reduce reproductive output (Leith et al., 2021; Walsh et al., 2021). For example, heat stress has been shown to reduce male display behaviour (Gerhardt and Mudry, 1980; Fasolo and Krebs, 2004), mating frequency (Zizzari and Ellers, 2011; Fraser and Chan, 2019), and mating duration (Katsuki and Miyatake, 2009; Chirault et al., 2015). However, courtship or mating behaviour is not considered in standard measures of thermal fertility loss (Leith et al., 2021; Walsh et al., 2019), and temperature effects on mating behaviour and fertility are typically considered in isolation (but see Sutter et al., 2019; Vasudeva et al., 2021; Macchiano et al., 2023). Further, in many species females assess male mating behaviour during mate choice (Andersson 1994; Rosenthal 2017). Therefore, any sexual behavioural changes between fertile and sterile males would enable females to discriminate between the two, and avoid mating with sterile males. If at least some males in the population remained fertile, female mate choice in favor of these males could mitigate the harmful effects of thermal fertility loss on population fitness.

Another mating behaviour that may change in response to temperature is the strength of mate choice (García-Roa et al., 2020; Pilakouta and Baillet, 2022). While there are clear fitness benefits to choosing high-quality or compatible mating partners (Andersson 1994; Rosenthal 2017), the extent to which both males and females express their mating preference is inherently dependent on the environment and their current state (Jennions and Petrie, 1997; Cotton et al., 2006; Dougherty, 2021). High temperatures could alter the strength of male mate choice for several reasons. First, high temperatures could potentially reduce the energetic resources available to invest into reproduction, for example because of reduced food intake (Hansen, 2009). Theory suggests that animals in poorer condition should reduce how choosy they are during mating in order to increase their chances of producing some offspring (Cotton et al., 2006; but see Dougherty, 2023). Second, high temperatures could disrupt the production of male or female sexual signals, making it more difficult for either sex to choose their preferred partner (Candolin, 2019; Leith et al., 2021). Third, high temperature stress may impose a time cost on males, in terms of reduced survival or reproductive function. For example, in the fruit fly *Drosophila virilis* mature sperm can survive temperatures that disrupt spermatogenesis, leaving males with enough sperm for only a few matings, and no ability to produce more (Walsh et al., 2021). Here, high temperatures impose a strict time cost on males; his window of fertility is reduced to around one week. Therefore, one potential adaptive strategy for these males is to mate indiscriminately in order to ensure some matings (Sullivan, 1994; Jennions and Petrie, 1997).

In this study we investigated the effect of a short (4-h) heatwave on male courtship behaviour, mate choice, and fertility in the fruit fly *D. virilis*. *Drosophila virilis* is relatively cold-adapted, being found in temperate montane forests in eastern Asia, including Japan and eastern China (Makino and Kawata, 2012). Notably, urban populations in eastern and southern China likely experience temperatures over 35 °C in summer heatwaves, and the maximum temperature can reach 42.7 °C in Chongqing (Yang et al., 2019). Previous work has shown that 80% of males exposed to 34 °C for 4 h lose fertility after a seven-day delay, suggesting that this temperature permanently disrupts their ability to produce new sperm (Parratt et al., 2021). In contrast, 4 h at 35 °C or above both kills mature sperm and permanently disrupts males' ability to produce new sperm, rendering 80% of males sterile (Parratt et al., 2021). Hence, the temperatures recorded in urban areas of eastern and southern China likely cause the loss of fertility in the majority of *D. virilis* males during summer heatwaves.

We exposed males to elevated temperatures of either 34.4 °C or 36.6 °C for 4 h, and then recorded their courtship behaviour, mating success and mate choice one day later. 34.4 °C was chosen in order to produce delayed male sterility (mature sperm survive, but new sperm are not made) and 36.6 °C to produce immediate male sterility (Parratt et al., 2021). We recorded male behaviour in a post-heatwave setting, which allows us to measure behavioural changes caused by impairment of male fertility, rather than those caused by the more general effect of heat stress. We use this study to ask two questions. First, do males experiencing high-temperature fertility loss also show concurrent changes in their pre-copulatory behaviour, which would allow females to avoid them during courtship and before mating? Second, do males without the ability to make new sperm increase their mating effort and reduce their choosiness in order to secure a mating? Based on a previous study (Parratt et al., 2021), the two heat treatments were predicted to influence male fertility in different ways. We predicted that males exposed to 36.6 °C will be immediately sterile, and that this will lead to a concurrent reduction in courtship activity and mating success, and a longer mating latency, when compared to control males. However, we predicted that heat treatment at 36.6 °C will not influence the strength of male mate choice. We predicted that males exposed to 34.4 °C will be fertile for around seven days, but new sperm production will be impaired. This results in males having a short time-window in which to mate, and a hard limit on the maximum number of matings they can achieve. As a result, we predicted these males will show an increase in courtship behaviour, and so have higher mating success and a shorter mating latency when compared to control males. Additionally, we predicted these males will show weaker mate choice when compared to control males, in order to ensure mating.

2. Methods

2.1. Male courtship choice

In order to test for changes in the strength of male mate choice, we, simultaneously presented males with a choice of either a conspecific female or a heterospecific *D. novamexicana* female, a related but not geographically concurrent species. We focused on male mate choice for conspecific over heterospecific females for two reasons. First, because *D. virilis* males have a strong mating preference for conspecific females over *D. novamexicana* females (Watanabe and Kawanishi, 1979). Second, because hybrid matings between *D. virilis* males and *D. novamexicana* females result in the production of fertile F1 offspring (Orr and Coyne, 1989). This means that a change in male mating preference in favor of heterospecific matings could potentially be adaptive in this species during heat stress. We thus predicted that 34.4 °C males will be more likely to court heterospecific females than control males. We expect no change in the strength of male mate choice between control and 36.6 °C males.

2.2. Fly population and maintenance

D. virilis (S-4; Cambridge Fly Facility) were kept in vials with propionic food (1 L water, 10 g agar, 20 g yeast, 70 g maize, 10 g soya flour, 80 g malt extract, 22 g molasses, 14 ml 10% Nipagin, 6.2 ml propionic acid). The breeding stock was stored in a temperature-controlled room at 18 °C, with a 12:12 h day/night cycle. *D. novamexicana* (15,010–1031.04; *Drosophila* Species Stock Center) were kept on banana food (1 L water, 15 g agar, 30 g yeast, 150 g banana, 50 g molasses, 30 g malt extract, 25 ml 10% Nipagin). All adults were virgins collected and sexed within 72 h of emergence. Adult males and females were kept separately at a density of 10 males per vial and 30 females per vial to prevent stress from overcrowding. All experimental adult flies were kept at 23 °C, 12:12 h day/night cycle. All males and females used in the mating trials were at least 14 days old by the time of temperature treatments to ensure that they were sexually mature (Walsh et al., 2021).

2.3. Temperature treatments

Adult males were randomly allocated to one of three temperature treatments: 23.0 °C (control), 34.4 °C, and 36.6 °C. These temperatures were chosen to mimic those that resulted in 80% delayed fertility, or 80% immediate fertility, after 4 h exposure (Parratt et al., 2021). From this prior data, we expected the treatments to result in.

1. 23.0 °C (control): normal fertility
2. 34.4 °C: delayed sterility in 80% of males. Mature sperm survive, but males cannot make new sperm
3. 36.6 °C: immediate sterility in 80% of males

We also checked the fertility of all males after performing mating trials, in order to confirm that the temperature treatments worked as expected (see below).

Males were moved to ASG double yeast food (1 L water, 10 g agar, 85 g sugar, 60 g maize, 40 g yeast, 25 ml 10% Nipagin) immediately before experimental temperature exposure because proprionic food melts at high temperatures. Vials containing males were placed into 3D-printed floating racks held in preheated water baths with precise temperature control (Walsh et al., 2021). All temperature treatments were carried out for 4 h. All flies were moved back to vials containing rearing food immediately after the temperature treatments.

2.4. RING test

Rapid Iteration Negation Geotaxis (RING) tests were performed to examine the physical mobility of males that have undergone different temperature treatments (Gargano et al., 2005). This was to ensure that any differences shown in later experiments would not be due to the differences in physical abilities between different types of males. After exposure to water baths, 10 males from each treatment were immediately placed into a vial containing propionic food. Males were tapped to the bottom of the vials and were allowed to climb from the bottom. Photos were taken with a 3s timer with a height indicator on the side of the vial, repeated five times for each vial. After the photos were taken, the maximum heights that the males climbed were measured and recorded. A total of eight tests (80 males total) were done for each male treatment. RING tests were not blind to male treatment.

2.5. Mating trials

Immediately after temperature treatments, males were put into individual vials to reduce stress, and untreated females were put into the experimental vials used during the mating trials to acclimatize to the environment. One day later, male mating behaviour was observed in standard mating trials. All trials were carried out in a temperature-controlled room set at 23 °C (±1 °C) and started at 10:00 a.m. when the light went on in the room. During the mating trials, each male was presented with two females, a conspecific female and a heterospecific (*D. novamexicana*) female. All individuals were able to fully interact in the vials. Males were added to vials with females in a fully randomized order, and all observations were blind to the male temperature treatment.

Vials were observed for 60 min, and the following behavioural data was recorded.

1. Male courtship latency: the time from introducing a male into a vial to the first male courtship attempt
2. Male courtship choice: the identity of the female first courted by the male. This is used as a proxy for male mate choice. We assumed that a greater courtship bias towards conspecific females reflects stronger mate choice
3. The number of courtship attempts directed towards each female. New courtship attempts were only considered when intervals of two attempts exceed 30s, or when the males courted another female
4. Mating latency: the time from first courtship to mating
5. The identity of the female for a successful mating
6. Mating duration

Finally, after the observation period of the trials, males were kept in the vials with the two females overnight (this species is still able to mate in the dark). This allowed males to mate with females outside of the observation period, in order to increase the chances of male mating and improve our ability to measure the fertility of males. This allowed us to confirm that the chosen experimental temperatures affected male fertility in the same way as in Parratt et al. (2021). Male fertility was defined as: “the ability to produce offspring with a female after housing together for 24 h”. Males were removed from the vials and discarded the next morning. Females were then transferred to new individual vials with propionic food for *D. virilis* females and banana food for *D. novamexicana* females, allowing the laying of eggs. The presence of offspring was checked after two weeks post-trial. We only observed one *D. novamexicana* female producing offspring after two weeks, after being housed with a control *D. virilis* male. We therefore did not consider heterospecific matings any further.

2.6. Data analysis

All statistical tests were carried out using R Version 4.0.4 (R Development Core Team, 2021). ANOVA was used to compare the height differences reached by males from the three temperature treatments in the RING test. To compare the courtship latency and mating latency between male temperature treatments, we performed survival analyses (Cox regression model) using the Survminer package v0.4.9. Pairwise comparisons between treatments were performed using log-rank tests. We compared the mating duration between male temperature treatments using analysis of variance (type III). Finally, we used the lme4 v1.1–27.1 (Bates et al., 2015) and Car v3.1–0 (Fox and Weisberg, 2019) packages to run generalized linear mixed models comparing the proportion of males that courted conspecific over heterospecific females first (male mate choice), and the proportion of males that mated with conspecific over heterospecific females, between the three temperature treatments. Each model containing a single fixed factor, with date as a random effect, and female species (*D. virilis* or *D. novamexicana*) as a binomial response variable.

3. Results

The RING test confirmed that the temperature treatments do not influence general male activity: there was no significant difference in the average height climbed between males from the three temperature treatments ($F_{2,\,116} = 2.44$, $P = 0.09$) (Figure S1).

The total sample size for the mating trials was 222 (control males: N = 77; 34.4 °C males: N = 77; 36.6 °C males: N = 68). In order to confirm that the temperature treatments affected male fertility as expected, we first compared the percentage of vials containing conspecific females housed with males for 24 h that produced offspring, regardless of whether mating was observed during the trial. There was no significant difference in the proportion of conspecific females producing offspring after being housed with 34.4 °C males or control males (Table 1; 34.4 °C males had a 9.4% reduction in fertility compared to control males). In contrast, conspecific females housed with 36.6 °C males were significantly less likely to produce offspring compared to control males (Table 1), resulting in a 70.3% reduction in fertility.

The experimental temperature treatment did not significantly influence male courtship latency (cox regression: $X^2_{\;2} = 1.41$, $P = 0.49$, $N = 222$) (Fig. 1). There was no difference in the likelihood of courtship during the 1-h mating trial between control males and either 34.4 °C males or 36.6 °C males (Table 1). Temperature treatment was not a significant predictor of the total number of male courtship attempts (GLMM: $X^2_{\;2} = 0.85$, $P = 0.76$, $N = 222$). The experimental temperature treatment did not influence male courtship preference for conspecific females: males in all three treatments preferred to court conspecific

Table 1
Differences in the likelihood of *D. virilis* females producing offspring, the likelihood of courtship and the likelihood of mating for males exposed to three experimental temperatures: 23.0 °C (control males), 34.4 °C and 36.6 °C. Chi-squared test results are shown for comparisons between the control treatment and each heated treatment. Significant results are highlighted in bold. Likelihood of mating was calculated only for those males that were observed courting females (hence smaller sample sizes).

	23.0 °C males		34.4 °C males				36.6 °C males			
	N	%	N	%	X^2	P	N	%	X^2	P
Produced offspring	77	54.5	77	49.9	0.42	0.52	68	16.2	**22.9**	$<$ **0.001**
Likelihood of courtship	77	61.0	77	54.5	0.96	0.33	68	50.0	2.24	0.13
Likelihood of mating	48	70.8	42	50.0	4.09	0.43	34	23.5	**17.83**	$<$ **0.001**

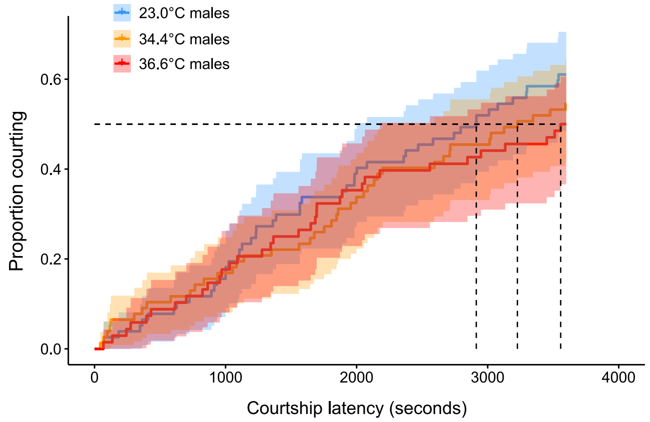

Fig. 1. Cumulative proportion of *D. virilis* males that were observing courting either of two females following exposure to one of three temperature treatments: 23.0 °C (control; blue line), 34.4 °C (orange line), and 36.6 °C (red line). Trials were run for 1 h (3600 s), and contained one male, one conspecific female and one heterospecific *D. novamexicana* female. Dotted lines show the time at which 50% of males had been observed courting. Shaded regions represent the 95% confidence region. Mating trials were stopped after 60 min. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

females, and although the proportion of males that courted heterospecific females first increased from control males to 36.6 °C males (Fig. 2a), the differences were not significant (GLMM: $X^2{}_2 = 3.75$, $P = 0.15$, $N = 124$).

When considering only those males that courted during the mating trial (control males: N = 48; 34.4 °C males: N = 42; 36.6 °C males: N = 34), the likelihood of a 36.6 °C male mating was significantly lower than the likelihood for a control male (Table 1). In contrast, exposure to 34.4 °C did not significantly influence male mating success compared to control males (Table 1). Moreover, males were more likely to mate with conspecific females across all three treatments (Fig. 2b), and the proportion of males mating with conspecific females did not differ significantly across the three temperature treatments (GLMM: $X^2{}_2 = 4.89$, $P = 0.087$, $N = 63$). Experimental temperature treatment significantly influenced mating latency (Cox regression: $X^2{}_2 = 21.1$, $P < 0.001$, $N = 222$) (Fig. 3). Pairs containing control males had the shortest mating latency and pairs containing 36.6 °C males had the longest mating latency. For those matings observed during the 1-h mating trial, there was no significant difference in mating duration between the three male treatments ($F_{2,60} = 3.04$, $P = 0.055$, $N = 63$; Fig. 4).

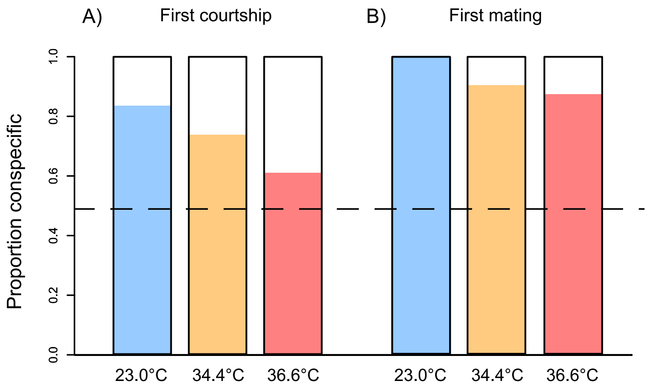

Fig. 2. Proportion of *D. virilis* males that A) first courted a conspecific *D. virilis* female, or B) first mated to a conspecific *D. virilis* female, in relation to the three experimental heat treatments: 23.0 °C (control, blue bar), 34.4 °C (orange bar), and 36.6 °C (red bar). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

4. Discussion

This study investigated the effects of heat-induced fertility loss on male mating behaviour, mating success and mate choice in the fruit fly *Drosophila virilis*. We exposed males to temperatures that reduced their chances of producing offspring by 10% (34.4 °C) or 70% (36.6 °C). We thus confirm the results from a previous study, suggesting that exposure to 36.6 °C for 4 h results in immediate sterility in 80% of males (Parratt et al., 2021). However, exposure to high temperatures had no effect on male courtship behaviour when compared to control males. Earlier exposure to high temperatures also did not alter the extent to which males directed courtship toward or mated with females of the same species, therefore showing no change in the strength of male mate

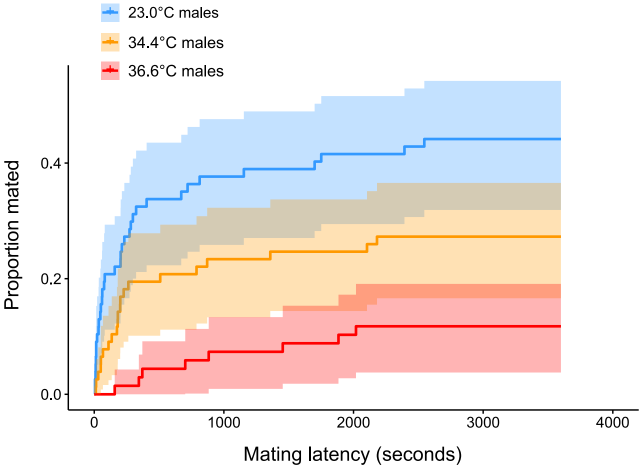

Fig. 3. Cumulative proportion of *D. virilis* males that were observed mating over time, following exposure to one of three experimental heat treatments: 23.0 °C (control; blue line), 34.4 °C (orange line), and 36.6 °C (red line). Trials were run for 1 h (3600 s), and contained one male, one conspecific female and one heterospecific *D. novamexicana* female. Shaded regions represent the 95% confidence region. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

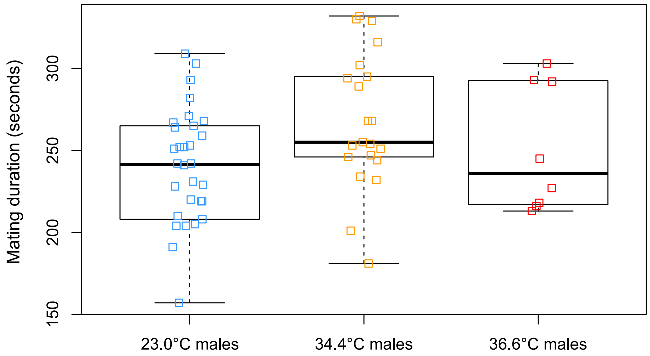

Fig. 4. Mating duration (seconds) of *D. virilis* males following exposure to one of three experimental heat treatments: 23.0 °C (control males, blue squares), 34.4 °C (orange squares), and 36.6 °C (red squares). Boxes show the median and inter-quartile range, and whiskers show the furthest data point within 1.5 times the inter-quartile range. Letters above each column represent the result of a post-hoc Tukey test. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

choice. In contrast, males exposed to 36.6 °C were significantly slower to mate, and had a reduced likelihood of mating within the 1-h observation period when compared to control males. This suggests that females can distinguish between normal and heat-sterilized males before mating, and that female mate choice may mitigate some of the population-level consequences of high-temperature induced male sterility in this species.

We did not detect any differences in male courtship behaviour following heatwave exposure, in contrast to other studies examining temperature effects on mating behaviour in *Drosophila* (e.g. Patton and Krebs, 2001; Fasolo and Krebs, 2004), or other ectotherms (Leith et al., 2021). This discrepancy may be because here mating trials were performed the day after heatwave exposure, potentially giving males time to recover from heat stress. This is in contrast to previous studies in which mating trials were done during or shortly after heat shock treatments (Patton and Krebs, 2001; Fasolo and Krebs, 2004). Most importantly, this means that even males that are completely sterilized by high temperatures do not stop courting females. This has two important consequences. First, it means that females should not use male courtship rate as an indicator of his fertility. Second, the similar level of sexual activity between fertile and sterile males might be potentially deleterious to females if male harassment is costly to females, as is seen in other insect species (Arnqvist and Rowe, 2005).

We also investigated whether experimental temperature treatment influenced male mate choice for conspecific over heterospecific females. We predicted that males exposed to 34.4 °C would reduce their choosiness compared to control males, because there is evidence from a previous study that this temperature impairs spermatogenesis, leaving males with less than a week's worth of mature sperm (Parratt et al., 2021). When faced with such a reduction in long-term mating potential, theory suggests that males should reduce choosiness in order to ensure as many matings as possible in the short term (Sullivan, 1994; Jennions and Petrie, 1997). However, we found no significant change in the proportion of males that first courted a conspecific female. One reason for this could be simply that in *D. virilis* the male mating preference for conspecifics is invariably strong. Indeed, we know of no studies testing the plasticity of male mate choice in relation to any other environmental conditions in this species, and we cannot rule out the possibility that male preferences for other female phenotypes (e.g. female body size) might be more environment-dependent. However, this lack of an effect is also supported by recent meta-analyses which found that the strength of mate choice is not significantly affected by temperature (Pilakouta and Baillet, 2022) or the time cost of mating (Dougherty, 2021).

Despite male mating behaviour appearing unchanged by the temperature treatments, 36.6 °C males mated significantly slower, and were less likely to mate overall, compared to control males. How females distinguish between control and heat-treated males is not clear. Females may be able to detect subtle behavioural changes we did not measure, perhaps due to neurological damage caused by heat stress (Robertson, 2004). Alternatively, females may assess non-behavioural sexual signals. For example, both wing song and cuticular hydrocarbons (CHC) profiles are affected by high temperatures in *Drosophila* (Patton and Krebs, 2001; Etges et al., 2017), and CHC expression is expected to be especially sensitive to temperature changes given its role in preventing desiccation resistance in insects (Chung and Carroll, 2015). Regardless of how females discriminate males, the discrimination itself is important, because it means that female choice could potentially mitigate some of the population-level costs of heat-induced sterility. However, this mitigation was not absolute: 8 of 68 females mated with 36.6 °C males within the 1-h mating trial, and a further 8 were not observed mating but did produce offspring after being housed with a 36.6 °C male for 24 h. Further, given that we observed no reduction in courtship behaviour in 36.6 °C males and that most 36.6 °C males are sterile, we suggest the actual number of females that mated to a 36.6 °C male is likely much higher. While in some cases these matings did lead to the production of some offspring, in most cases such matings are a waste for females. Females may also have additional mitigation strategies not tested here; for example, female *D. pseudoobscura* mated to sterile males remate more quickly (Sutter et al., 2019), and even monandrous *D. subobscura* females will remate after a sterile mating (Fisher et al., 2013).

One key outstanding question is the extent to which the thermal fertility loss observed in this study could have consequences for female fitness and population health in the natural environment. Clearly, female reproductive output will be lower if they are not able to consistently avoid mating with sterile males. Females may also suffer indirect costs, such as the cost of harassment from sterile or low-fertility males (Arnqvist and Rowe, 2005), or the time and energy costs of having to find fertile males. All these costs have the potential to reduce female fecundity, and therefore reduce overall population growth rate. Importantly, our results show that female mate choice may mitigate the costs of male fertility loss to some extent, but not absolutely. However, we note five reasons why the results seen here may not match patterns in natural populations. First, the temperature treatments used in this experiment were based on experimental data, and may not be ecologically realistic. Second, in the wild females also experience temperature changes. While male fertility appears to be more sensitive to heat stress than female fertility in many organisms (Takahashi, 2011; Sage et al., 2015; Iossa, 2019), temperature may affect male and female mating behaviour in similar ways (García-Roa et al., 2020; Leith et al., 2021). Therefore, to fully understand the impact of climate change on natural populations we need to consider the potentially additive effects of changing behaviour or reduced fertility in both sexes. Third, males could potentially recover their fertility following a heat wave as long as testes function is not damaged (e.g. Sales et al., 2021; Canal and Fricke, 2022). Fourth, the effects of high temperatures on male fertility may depend on when in their life males are exposed. For example, larval and pupal exposure to high temperatures has been shown to reduce fertility to a greater extent compared to adult exposure in insects (Sales et al., 2021; Walsh et al., 2021). In contrast, lower-magnitude or ramping temperature increases during development could facilitate heat hardening of exposed individuals, leading to a greater ability to function in higher temperatures as adults (e.g. Stazione et al., 2019). However, previous work in *D. virilis* found that early exposure to moderate high temperatures does indeed lead to increased lethal temperature limits, but it had no impact on the temperature at which fertility was lost (Walsh et al., 2021). Fifth, not all males were sterilized by our test treatments, suggesting there is individual heterogeneity in temperature tolerance. Overall then, the fitness costs of high-temperature fertility loss for females, and the population as a whole, will likely depend on the proportion of males in the population that are affected, and the ability of females to quickly locate and identify fertile males. In order to fully assess the population-level consequences of thermal fertility loss, future studies should therefore investigate this phenomenon in a range of ecologically-relevant scenarios.

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CRediT authorship contribution statement

Kwan Wai Mak: Investigation, Formal analysis, Writing – original draft, Writing – review & editing, Visualization. **Tom A.R. Price:** Conceptualization, Methodology, Writing – review & editing, Supervision. **Liam R. Dougherty:** Conceptualization, Methodology, Writing – original draft, Writing – review & editing, Supervision.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.SMARTQC

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jtherbio.2023.103701.

References

Andersson, M.B., 1994. Sexual Selection. Princeton University Press, Princeton.

Arnqvist, G., Rowe, L., 2005. Sexual Conflict. Princeton University Press, Princeton.

Bates, D., Mächler, M., Bolker, B., Walker, S., 2015. Fitting linear mixed-effects models using lme4. J. Stat. Software 67 (1), 1–48.

Boni, R., Gallo, A., Montanino, M., Macina, A., Tosti, E., 2016. Dynamic changes in the sperm quality of *Mytilus galloprovincialis* under continuous thermal stress. Mol. Reprod. Dev. 83, 162–173.

Canal, B., Fricke, C., 2022. Recovery from heat-induced infertility – a study of reproductive tissue responses and fitness consequences in male *Drosophila melanogaster*. Ecol. Evol. 12 (12), e9563.

Candolin, U., 2019. Mate choice in a changing world. Biol. Rev. 94 (4), 1246–1260.

Chirault, M., Lucas, C., Goubault, M., Chevrier, C., Bressac, C., Lécureuil, C., 2015. A combined approach to heat stress effect on male fertility in *Nasonia vitripennis*: from the physiological consequences on spermatogenesis to the reproductive adjustment of females mated with stressed males. PLoS One 10, e0120656.

Chung, H., Carroll, S.B., 2015. Wax, sex and the origin of species: dual roles of insect cuticular hydrocarbons in adaptation and mating. Bioessays 37 (7), 822–830.

Collins, M., Knutti, R., Arblaster, J., et al., 2013. Long-term climate change: projections, commitments and irreversibility. In: Stocker, T.F., Qin, D., Plattner, G.-K., et al. (Eds.), Climate Change 2013: the Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA.

Cotton, S., Small, J., Pomiankowski, A., 2006. Sexual selection and condition-dependent mate preferences. Curr. Biol. 16, 755–765.

Dougherty, L.R., 2021. Meta-analysis reveals that animal sexual signalling behaviour is honest and resource based. Nature Ecology & Evolution 5, 688–699.

Dougherty, L.R., 2023. The Effect of Individual State on the Strength of Mate Choice in Females and Males. Behavioral Ecology, arac100.

Etges, W.J., De Oliveira, C.C., Rajpurohit, S., Gibbs, A.G., 2017. Effects of temperature on transcriptome and cuticular hydrocarbon expression in ecologically differentiated populations of desert *Drosophila*. Ecol. Evol. 7, 619–637.

Fasolo, A.G., Krebs, R.A., 2004. A comparison of behavioural change in *Drosophila* during exposure to thermal stress. Biol. J. Linn. Soc. 83, 197–205.

Fisher, D.N., Doff, R.J., Price, T.A., 2013. True polyandry and pseudopolyandry: why does a monandrous fly remate? BMC Evol. Biol. 13 (1), 1–11.

Fraser, C.M.L., Chan, B.K.K., 2019. Too hot for sex: mating behaviour and fitness in the intertidal barnacle *Fistulobalanus albicostatus* under extreme heat stress. Mar. Ecol. Prog. Ser. 610, 99–108.

Fox, J., Weisberg, S., 2019. An R Companion to Applied Regression, third ed. Sage, Thousand Oaks CA.

García-Roa, R., Garcia-Gonzalez, F., Noble, D.W., Carazo, P., 2020. Temperature as a modulator of sexual selection. Biol. Rev. 95 (6), 1607–1629.

Gargano, J.W., Martin, I., Bhandari, P., Grotewiel, M.S., 2005. Rapid iterative negative geotaxis (RING): a new method for assessing age-related locomotor decline in Drosophila. Exp. Gerontol. 40 (5), 386–395.

Gerhardt, H.C., Mudry, K.M., 1980. Temperature effects on frequency preferences and mating call frequencies in the green treefrog, *Hyla cinerea* (Anura: hylidae). J. Comp. Physiol. 137, 1–6.

Green, C., Moore, P.J., Sial, A.A., 2019. Impact of heat stress on development and fertility of *Drosophila suzukii* Matsumura (Diptera: drosophilidae). J. Insect Physiol. 114, 45–52.

Hansen, P.J., 2009. Effects of heat stress on mammalian reproduction. Philosophical Transactions of the Royal Society B 364, 3341–3350.

Iossa, G., 2019. Sex-specific differences in thermal fertility limits. Trends in Ecology &amp. Evolution 34 (6), 490–492.

Jennions, M.D., Petrie, M., 1997. Variation in mate choice and mating preferences: a review of causes and consequences. Biol. Rev. 72, 283–327.

Jørgensen, K.T., Sørensen, J.G., Bundgaard, J., 2006. Heat tolerance and the effect of mild heat stress on reproductive characters in *Drosophila buzzatii* males. J. Therm. Biol. 31, 280–286.

Katsuki, M., Miyatake, T., 2009. Effects of temperature on mating duration, sperm transfer and remating frequency in *Callosobruchus chinensis*. J. Insect Physiol. 55, 113–116.

Kingsolver, J.G., Diamond, S.E., Buckley, L.B., 2013. Heat stress and the fitness consequences of climate change for terrestrial ectotherms. Funct. Ecol. 27 (6), 1415–1423.

Leith, N.T., Macchiano, A., Moore, M.P., Fowler-Finn, K.D., 2021. Temperature impacts all behavioral interactions during insect and arachnid reproduction. Current Opinion in Insect Science 45, 106–114.

Lindasey, R., Dahlman, L., 2021. *Climate Change: Global Temperature* [Online]. NOAA Climate.Gov. https://www.climate.gov/news-features/understanding-climate/climate-change-global-temperature. Accessed 29 Oct 2021.

Macchiano, A., Miller, E., Agali, U., Ola-Ajose, A., Fowler-Finn, K.D., 2023. Developmental temperature alters the thermal sensitivity of courtship activity and signal–preference relationships, but not mating rates. Oecologia 202 (1), 97–111.

Makino, T., Kawata, M., 2012. Habitat variability correlates with duplicate content of *Drosophila* genomes. Mol. Biol. Evol. 29, 3169–3179.

Meehl, G.A., Tebaldi, C., 2004. More intense, more frequent, and longer lasting heat waves in the 21st century. Science 305, 994–997.

Orr, H.A., Coyne, J.A., 1989. The genetics of postzygotic isolation in the Drosophila virilis group. Genetics 121 (3), 527–537.

Parratt, S.R., Walsh, B.S., Metelmann, S., White, N., Manser, A., Bretman, A.J., Hoffmann, A.A., Snook, R.R., Price, T.A.R., 2021. Temperatures that sterilise males better match global species distributions than lethal temperatures. Nat. Clim. Change 11, 481–484.

Patton, J.Z., Krebs, A.R., 2001. The effect of thermal stress on the mating behavior of three *Drosophila* species. Physiol. Biochem. Zool. 74, 783–788.

Pilakouta, N., Baillet, A., 2022. Effects of temperature on mating behaviour and mating success: a meta-analysis. J. Anim. Ecol. 91 (8), 1642–1650.

Prasad, A., Croydon-Sugarman, M.J.F., Murray, R.L., Cutter, A.D., 2011. Temperature-dependent fecundity associates with latitude in *Caenorhabditis briggsae*. Evolution 65, 52–63.

R Development Core Team, 2021. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria.

Robertson, R.M., 2004. Thermal stress and neural function: adaptive mechanisms in insect model systems. J. Therm. Biol. 29, 351–358.

Rosenthal, G.G., 2017. Mate Choice. Princeton University Press, Princeton.

Sage, T.L., Bagha, S., Lundsgaard-Nielsen, V., Branch, H.A., Sultmanis, S., Sage, R.F., 2015. The effect of high temperature stress on male and female reproduction in plants. Field Crops Res. 182, 30–42.

Sales, K., Vasudeva, R., Dickinson, M.E., Godwin, J.L., Lumley, A.J., Michalczyk, Ł., Hebberecht, L., Thomas, P., Franco, A., Gage, M.J.G., 2018. Experimental heatwaves compromise sperm function and cause transgenerational damage in a model insect. Nat. Commun. 9, 4771.

Sales, K., Vasudeva, R., Gage, M.J.G., 2021. Fertility and mortality impacts of thermal stress from experimental heatwaves on different life stages and their recovery in a model insect. Royal Society Open Science 8, 201717.

Sullivan, M.S., 1994. Mate choice as an information gathering process under time constraint: implications for behaviour and signal design. Anim. Behav. 47, 141–151.

Sutter, A., Travers, L.M., Oku, K., Delaney, L., Store, K.J., , S., Price, T.A., Wedell, N., 2019. Flexible polyandry in female flies is an adaptive response to infertile males. Behav. Ecol. 30 (6), 1715–1724.

Skinner, J.D., Louw, G.N., 1966. Heat stress and spermatogenesis in *Bos indicus* and *Bos taurus* cattle. J. Appl. Physiol. 21, 1784–1790.

Stazione, L., Norry, F.M., Sambucetti, P., 2019. Heat-hardening effects on mating success at high temperature in Drosophila melanogaster. J. Therm. Biol. 80, 172–177.

Takahashi, M., 2011. Heat stress on reproductive function and fertility in mammals. Reprod. Med. Biol. 11, 37–47.

USEPA, 2021. *Climate Change Indicators: Heat Waves* [Online]. United States Environmental Protection Agency. https://www.epa.gov/climate-indicators/climate-change-indicators-heat-waves. Accessed 29 Oct 2021.

Vasudeva, R., Dickinson, M., Sutter, A., Powell, S., Sales, K., Gage, M.J.G., 2021. Facultative polyandry protects females from compromised male fertility caused by heatwave conditions. Anim. Behav. 178, 37–48.

Walsh, B.S., Parratt, S.R., Hoffmann, A.A., Atkinson, D., Snook, R.R., Bretman, A., Price, T.A.R., 2019. The impact of climate change on fertility. Trends Ecol. Evol. 34, 249–259.

Walsh, B.S., Mannion, N.L., Price, T.A., Parratt, S.R., 2021. Sex-specific sterility caused by extreme temperatures is likely to create cryptic changes to the operational sex ratio in *Drosophila virilis*. Current Zoology 67, 341–343.

Watanabe, T.K., Kawanishi, M., 1979. Mating preference and the direction of evolution in Drosophila. Science 205 (4409), 906–907.

Yaeram, J., Setchell, B.P., Maddocks, S., 2006. Effect of heat stress on the fertility of male mice in vivo and in vitro. Reprod. Fertil. Dev. 18, 647–653.

Yang, J., Yin, P., Sun, J., Wang, B., Zhou, M., Li, M., Tong, S., Meng, B., Guo, Y., Liu, Q., 2019. Heatwave and mortality in 31 major Chinese cities: definition, vulnerability and implications. Sci. Total Environ. 649, 695–702.

Zizzari, Z.V., Ellers, J., 2011. Effects of exposure to short-term heat stress on male reproductive fitness in a soil arthropod. J. Insect Physiol. 57, 421–426.