Male Mediterranean fruit flies prefer warmer temperatures that improve sexual performance

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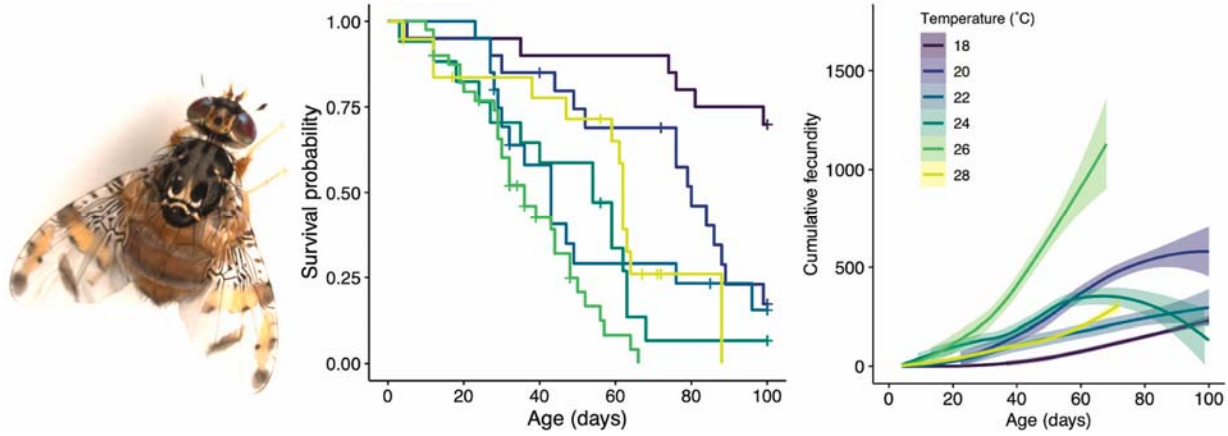
Highlights

- Temperatures preferred by males were higher than by females.
- Males optimised mating performance at or higher than the temperature they preferred.
- Sperm storage was reduced at the highest test temperature of 28 °C.
- Only lifetime fecundity was optimised at the temperature preferred by females.
- Avoidance of extremes may drive a mismatch in thermal preference and optima.

Abstract

Females and males have divergent strategies of energy investment, so the thermal preference of each sex in insects may differ because energetic conversion of metabolic reserves is dependent on temperature. We determined the thermal preference of virgin, sexually mature Mediterranean fruit flies, *Ceratitis capitata*, and found that males preferred a significantly higher temperature (23.8 ± 0.3˚C) than that of females (22.1 ± 0.3˚C). We then tested predictions for the difference in thermal preference related to the energetic demands of reproduction over a range of temperatures. The frequency and duration of calling bouts by male *C. capitata* were optimal at 26˚C. Mating propensity and latency, and copula duration, were optimal over the range of 22-28˚C. When mating occurred, temperature had little effect on the incidence of sperm storage by females, but there was a notable decline in the number stored at 28˚C. Female lifespan was highest at 18˚C, but lifetime egg production was optimal at 24˚C. These results illustrate temperature-related differences in the reproductive fitness of the sexes in *C. capitata*, although the optima for male traits align best with their thermal preference. They also support the theoretical prediction that insect thermal preference should be lower than the optimum for fitness.

Graphical Abstract



Keywords: Thermal preference, Sexual performance, Sperm storage, Tephritidae

1. Introduction

Optimal expression of behavioural, physiological and ecological traits by ectotherms depends on their body temperature being maintained within certain limits (Huey and Stevenson, 1979). Ectotherms can thermoregulate behaviourally to maintain their body temperatures at those ensuring optimal fitness (Fey et al., 2019). However, certain traits may have thermal optima that differ from

others (Andrew et al., 2013; Davidowitz and Nijhout, 2004), so trade-offs can exist where the temperature favouring one trait (e.g., activity, growth, reproduction) may lead to suboptimal expression of another (e.g., nutrient assimilation, body size, longevity) (Buchan and Sohal, 1981; Coggan et al., 2011; Jerbi-Elayed et al., 2015; McCue et al., 2016; Plasman et al., 2019). Over the lifespan of an individual, decisions to move to warmer or cooler locations may be integrated in a way to maximise lifetime fitness despite competing thermal demands (Coggan et al., 2011; Killen, 2014; Shinner et al., 2020). This situation is complicated by the currently developing climate crisis. Even if ectotherms are able to thermoregulate to avoid excessively high temperatures, this may have consequences for their ability to obtain sufficient nutrients, interact with conspecifics to find mates, or maintain rates of reproduction high enough to maintain local populations under certain situations (Basson et al., 2016; Huey and Kingsolver, 2019). It is also apparent that in some ectotherm species, one sex can be more or less susceptible to temperature change than the other (Darnell et al., 2013; Huey and Pianka, 2007; Lailvaux, 2007).

The reproductive interactions of females and males have evolved as a consequence of the confluence of interests, but asymmetric costs borne by each sex (Venkateswaran et al., 2021). The two sexes produce different-sized gametes, invest differently over the stages of offspring development, and have correspondingly divergent life histories (e.g., Andersson, 1994; Flatt and Heyland, 2011; Lailvaux and Husak, 2014). This divergence is evident in the energetic investment of the sexes. Females usually divert limited resources into pre-zygotic development of gametes to enhance survival of offspring, whereas males tend to invest in ejaculate, sexual advertisement and copulation to maximise paternity (Alexander et al., 1997). There can also be sex-specific differences in metabolic rate that relate to maternal inheritance of mitochondrial DNA haplotypes (Nagarajan-Radha et al., 2019).

Energetic conversion of metabolic reserves in insects is dependent on temperature (Clarke and Fraser, 2004; Rho and Lee, 2017). By extension, the differing modes of reproductive investment of each sex may be reflected in their thermal preference, which then optimises certain temperature-dependent metabolic processes (Forsman, 2018; Rogowitz and Chappell, 2000; Shillington, 2005). While we are unaware of studies to test this prediction, females could seek to maintain body temperatures that facilitate efficient use of nutrients for reproductive output. In *Glossina brevipalpis*, flies exhibit postprandial thermophily where warmer temperatures are preferred after a blood meal to improve digestion (both sexes blood-feed) (McCue et al., 2016). Females of *Callosobruchus maculatus* prefer warmer temperatures than males but the reason for this is unknown (Malek and Czarnoleski, 2021). In male *Locusta migratoria* and both sexes of *Tenebrio*

molitor, individuals thermoregulate behaviourally to optimise assimilation of dietary intake (Coggan et al., 2011; Rho and Lee, 2017). In contrast, we would expect males to exhibit behaviours optimal for securing mates at temperatures that enhance their activity. For example, in two long-horned Eucalyptus-boring beetle species, mass-specific metabolic rate during running is higher in males than females, which matches the need for males to search actively for mates (Rogowitz and Chappell, 2000). Thorax temperature and metabolic rate also need to be elevated in male crickets and katydids to permit acoustic signalling that attracts females (Erregger et al., 2017). However, temperatures higher and lower than optimal can lead to depletion of nutrient reserves, which then affects fitness-related traits like survival and reproduction. In *Drosophila melanogaster*, lipid and glycogen reserves decline at temperatures higher than 27˚C, which also aligns with higher metabolic and food consumption rates (Klepsatel et al., 2019). These results were thought to result from reduced assimilation efficiency, with energy gain per unit of food consumed being negatively affected as temperatures increase above an optimum (Klepsatel et al., 2019). If temperatures change and the capacity for thermoregulation differ between the sexes, with consequences for nutrient intake and assimilation, this could then affect survival and population growth (Huey and Berrigan, 2001; Huey and Kingsolver, 2019).

The role of energy in male reproductive success is evident in male Mediterranean fruit flies, *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae). In this widespread pest, which damages fruit cultivation in tropical and temperate regions of the world (Malacrida et al., 2007), mating strategy (i.e. calling and participation in mating aggregations) depends on stored lipid and sugar levels (Warburg and Yuval, 1997). Sexually mature male *C. capitata* exhibit elevated expression of genes associated with protein and carbohydrate metabolism, energy production, muscle activity and flight (Gomulski et al., 2012). In females, transcription of genes associated with fatty acid metabolism is increased, which reflects their need for nutrient storage and egg development for optimal reproductive success (Gomulski et al., 2012). However, it is not clear whether the mating behaviour, and consequently, the fitness of female and male *C. capitata* are affected differently by temperature and if they actively seek out temperatures that will optimise these traits. This not only has implications for the dynamics of *C. capitata* populations, but also the effectiveness of control tactics like the sterile insect technique where successful mating by sterile males is key to population suppression (Knipling, 1959; Lance and McInnis, 2021).

Here, we therefore tested the idea that sex-related variation in thermal preference results from different reproductive benefits for each sex. Specifically, we determined whether sexually mature, virgin male *C. capitata* prefer higher temperatures to facilitate increased calling behaviour and

sexual performance, and whether females prefer lower temperatures to improve reproductive output through more efficient conversion of food to eggs. By doing so, we sought to establish whether the females and males of an important pest species could respond differently to variation in temperature.

2. Materials and methods

2.1. Insect rearing

Flies developed to the adult stage in a laboratory at 25±1˚C and 65% relative humidity using standard rearing procedures for *Ceratitis* fruit flies (Weldon et al., 2016). The laboratory had a 14:10 LD photo cycle, with the first and last hour of light increasing and decreasing gradually to simulate dawn and dusk. On the day of adult emergence, the flies were sorted by sex and maintained in separate cages to ensure that they remained virgin unless otherwise noted. New genetic material was introduced to the culture on an annual basis (approximately every 12 generations) by mating wild-collected males with laboratory females to avoid inbreeding.

2.2. Thermal preference

At 2 days after adult emergence, flies were distributed to six plastic cages of mixed sex (45 flies per cage). Three cages were furnished with granulated table sugar and hydrolysed yeast (Yeast Extract Powder; Biolab; Merck, Germany) as food in separate Petri dishes, whereas the other three had only sugar. Both cages also contained a source of water. One cage of each diet was placed in constant temperature cabinets and acclimated for 5 days at 20, 25 or 30˚C and 65% relative humidity. At 7 days after adult emergence, acclimated females and males were placed at random on a thermal gradient (Appendix A.1) ranging from 17.8±2.4 to 33.5±0.1˚C to test their thermal preference. The temperatures on the thermal gradient were within the critical thermal limits for *Ceratitis capitata*, which has a CT_{min} of 5-7˚C and CT_{max} of 42-43˚C, depending on experimental context (Nyamukondiwa and Terblanche, 2009). By using this temperature range, we minimised the risk of flies being trapped by cold temperatures at the lower end of the gradient that has been raised as a concern in ectotherm thermal preference studies by Anderson et al. (2007). Tested individuals were sexually mature because it takes only 2-3 days for *C. capitata* to reach sexual maturity under standard laboratory conditions (Papadopoulos et al., 1998) so may have already mated at the time of testing. Tests were conducted in darkness. A programmable digital camera with a flash was set to photograph the location of each fly after 30 minutes. This time period was selected based on the expected time needed for *D. melanogaster*, a much smaller fly, to achieve reach equilibrium on a thermal gradient (Dillon et al., 2012). A control for the temperature gradient was also run where the gradient apparatus had a uniform temperature of 23.2±0.1˚C. This was to understand the

distribution of flies in the absence of thermal variation. Nine cohorts of flies, handled as described above, resulting in selected temperature estimates for between 32-56 age-matched individuals for each combination of diet, acclimation temperature and sex on the temperature gradient. For the same combination of treatments, we obtained location records for 17-25 individuals. Sample sizes were unequal due to random selection of flies from cages and some escaping from the gradient apparatus during testing.

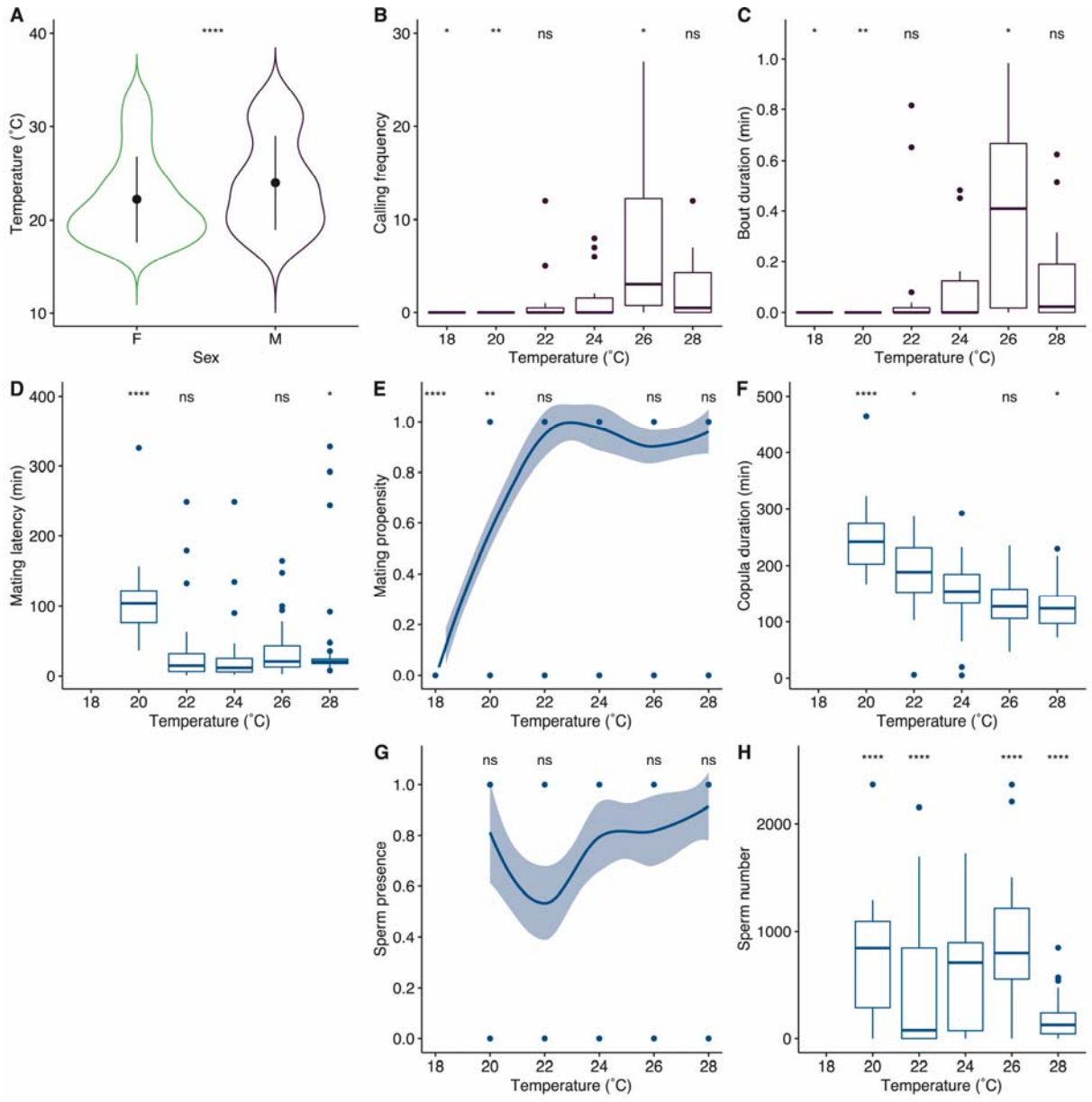


Fig. 1. Thermal preference and effects of temperature on male calling and reproductive traits in *C. capitata*. (A) Violin plots showing the distribution, mean and standard error of temperatures selected by sexually mature female and male flies on a thermal gradient. Sexes compared with Wilcoxon's signed-rank test. (B) Frequency of male calling and (C) duration of male calling bouts in relation to temperature. Post-hoc comparisons (Wilcoxon's signed rank tests) are shown relative to 24 °C. (D) Mating latency, (E) mating propensity, (F) copula duration, (G) sperm presence in

spermathecae, and (H) number of sperm in spermathecae in relation to temperature. Box plots show the median, first and third quartiles (lower and upper box edges), 1.5 × interquartile range (whiskers), and outliers (individual points) at each temperature. Post-hoc comparisons (Tukey's tests) are shown relative to 24 °C. For post-hoc comparisons: ns (P) > 0.05; * < 0.05; ** < 0.01; *** < 0.001; **** <0.0001.

The distance of flies along the temperature gradient was measured using Image J (Schneider et al., 2012). Best-fit regression models (Appendix A.1) were used to estimate the temperature experienced by flies based on their distance along the gradient. Male temperature preference was bimodal (Figure 1A), so within each sex the temperature selected by flies was compared using Kruskal-Wallis tests among diets (female: $\chi^2 = 0.073016$, df = 1, P = 0.787 ; male: $\chi^2 = 1.0957$, df = 1, P = 0.2952) and acclimation temperatures (female: $\chi^2 = 0.431$, df = 2, P = 0.0806; male: $\chi^2 = 4.106$, df = 2, P = 0.128). Thereafter, with diet and acclimation treatments having no effect on selected temperatures, the data were pooled (female n = 232; male n = 275) and unpaired two-sample Wilcoxon tests were used to evaluate the effect of sex on selected temperatures and the distance along the gradient apparatus when the temperature was uniform. Owing to the non-parametric approaches that were used and unequal representation of treatments within the tested cohorts, we could not account for batch effects within this analysis.

2.3. Male calling behaviour

Six day-old, virgin male *C. capitata* that had been sorted from females on adult emergence were collected in pre-weighed plastic vials, individually weighed on an analytical balance (to 0.1 mg; AR0640, Ohaus Corp., Pine Brook, NJ, USA), and then placed on ice for exactly 2 minutes. The immobilised flies were then placed on paper towel and a wooden toothpick was used to apply a small dot of enamel paint to the thorax. As only four temperatures could be tested per day (testing performed between 0800-1300 South African Standard Time), each batch comprised only 50 males, half of which were marked with black paint (Gloss Black, Spraymate, Daleside, South Africa), and the other half were marked with white paint (Gloss White, Spraymate, Daleside, South Africa). The marked flies were transferred to cages according to the colour of their mark where they recovered (at 25±1˚C) before being used for calling behaviour tests the following day.

At 7 days after adult emergence, calling bouts of virgin male *C. capitata* were determined at temperatures of 18, 20, 22, 24, 26 and 28˚C for 30 min. Calling behaviour was recorded in four circular observation arenas (35 mm diameter, 15 mm depth) set into an aluminium block (155 × 155 × 40 mm). Channels were bored into the block, through which a 1:1 ratio of water:propylene glycol mix of known temperature was circulated from a temperature-controlled water bath (K6-cc-NR, Huber Kältemaschinenbau GmbH, Offenburg, Germany). Two arenas were each fitted with an

exposed junction thermocouple capable of operating within a temperature range of -75 to 250˚C (copper/constantan, type T, 36 SWG) attached to a temperature data logger (TC-08, Pico Technology Ltd., Cambridgeshire, UK) to monitor the actual temperature in the arenas. Prior to commencing an experiment, the arenas were brought to one of the test temperatures (18, 20, 22, 24, 26 and 28˚C). Temperatures were tested in random order and only between 0800-1300 South Africa Standard Time (SAST) to coincide with peak mating time (Blay and Yuval, 1997). Two male *C. capitata*, one marked black and the other white, were introduced to each arena, which was then sealed with a cover of double-glazed glass. Pairs were used in the arena because the presence of conspecific facilitates calling behaviour (McDonald, 1987). Flies were given 5 minutes to acclimatise to the test arena prior to recording. Spontaneous activity of individuals was recorded for 30 minutes using a high definition webcam (C270, Logitech, 7700 Gateway Blvd Newark, CA 94560 USA) positioned above the temperature-controlled arenas. A total of four recordings were made at each temperature (temperature n=16, total n = 96), resulting in 12 hours of video footage. Test flies used for the locomotor trial were exposed to only one test temperature and discarded after each observation. Video recordings were observed using JWatcher (version 1.0) (Blumstein and Daniel, 2007) to score the frequency and duration of calling bouts of one focal fly in each arena (randomly selected by colour mark). The effect of temperature on the frequency and duration of calling bouts was analysed using Kruskal-Wallis tests and post-hoc Wilcoxon signed-rank tests relative to the temperature closest to that preferred by males (24˚C; Figure 1A).

2.4. Sexual performance

The mating propensity, time until mating (mating latency), and copula duration of *C. capitata* were determined at temperatures of 18, 20, 22, 24, 26 and 28˚C. At 6 days after adult eclosion, virgin female and male *C. capitata* were weighed before being paired in individual clear plastic sample containers at night to prevent mating until the following day. Sample containers were placed in incubators set at one of the test temperatures (n = 40 per temperature) with 14:10 LD photocycle. Observations of mating were made through the glass internal door of the incubators between 0800-1300 hours SAST. All mated females were immobilised with aerosol freeze spray and dissected within two hours of copulation ending to remove their spermathecae (excluding those that escaped during the procedure). The two spermathecae were macerated in separate drops of water on a clean slide, covered with coverslips, allowed to dry and then inspected under a phase contrast microscope following established methods (Pérez-Staples et al., 2010). The effects of temperature, and female and male body weight, on mating propensity and sperm presence were determined using generalized linear models (GLM) with binomial errors. The effects of the same predictor variables

Table 1. Model summaries for effects of temperature and other predictor variables on reproductive traits in *C. capitata*. GLM: generalised linear model; MANOVA: multivariate analysis of variance

Dependent variable	Test or model (error distribution, link function)	Predictor variables	df	Test statistic		*P*	R^2
Number of male calling bouts (Figure 1b)	Kruskal-Wallis	Temperature	5	χ^2	27.2	<0.001	
Duration of male calling bouts (Figure 1c)	Kruskal-Wallis	Temperature	5	χ^2	28.2	<0.001	
Mating latency (Figure 1d)	GLM (Gaussian, identity)	Temperature	4	χ^2	54.6	<0.001	0.25
		Residual	165				
Mating propensity (Figure 1e)	GLM (binomial, logit)	Temperature	5	χ^2	164.2	<0.001	0.57
		Residual	234				
Copula duration (Figure 1f)	GLM (Gaussian, identity)	Temperature	4	χ^2	101.3	<0.001	0.38
		Residual	165				
Sperm presence (Figure 1g)	GLM (binomial, logit)	Temperature	4	χ^2	10.0	0.040	0.11
		Male body weight	1	χ^2	2.9	0.087	
		Residual	142				
Sperm number (Figure 1h, Figure 2)	GLM (negative binomial, log)	Temperature	4	χ^2	17589.2	<0.001	0.20
		Female body weight	1	χ^2	506.8	<0.001	
		Male body weight	1	χ^2	837.9	<0.001	
		Residual	140				
Female survival (Figure 3a)	Log-rank	Temperature	5	χ^2	53.7	<0.001	
Lifetime fecundity (Figure 3c)	GLM (Poisson, log)	Temperature	5	χ^2	53.7	<0.001	0.32
		Female survival	1	χ^2	60.4	<0.001	
		Residual	129				

Lifetime diet consumption (Figure 3d)	MANOVA	Temperature	10	F	23.0	<0.001	
				Pillai's trace	1.0		
		Female survival	2	F	9.8	<0.001	
				Pillai's trace	0.1		
		Residual	127				
Lifetime sucrose consumption	Linear model	Temperature	5	F	17.1	0.003	0.45
		Female survival	1	F	4.2	<0.001	
		Residual	127				
Lifetime yeast consumption	Linear model	Temperature	5	F	20.0	<0.001	0.56
		Female survival	1	F	5.7	<0.001	
		Residual	129				
Diet conversion efficiency (Figure 3e)	MANOVA	Temperature	10	F	7.1	<0.001	
				Pillai's trace	0.5		
		Female survival	2	F	13.5	<0.001	
				Pillai's trace	0.2		
		Residual	121				
Sucrose conversion efficiency	Linear model	Temperature	5	F	6.4	<0.001	0.31
		Female survival	1	F	5.0	<0.001	
		Residual	125				
Yeast conversion efficiency	Linear model	Temperature	5	F	8.3	<0.001	0.25
		Female survival	1	F	3.3	<0.001	
		Residual	125				
Abdominal nutrients (Figure 4)	MANOVA	Age	2	F	6.5	<0.001	
				Pillai's trace	0.3		
		Temperature	5	F	1.6	0.114	
				Pillai's trace	10.6		
		Abdomen weight	1	F	9.9	<0.001	

				Pillai's trace	0.2	
		Eggs laid	1	F	5.6	0.004
				Pillai's trace	0.1	
		Residual	80			
Lipid content	Linear model	Age	2	F	1.4	0.260
		Temperature	5	F	1.8	0.127
		Abdomen weight	1	F	1.5	0.227
		Eggs laid	1	F	2.7	0.107
		Residual	81			
Protein content (log10)	Linear model	Age	2	F	6.6	0.002
		Temperature	5	F	1.6	0.168
		Abdomen weight	1	F	28.9	<0.001
		Eggs laid	1	F	11.5	0.002
		Residual	81			

on mating latency (log10-transformed) and copula duration were assessed using GLMs with Gaussian errors, and on the number of sperm transferred using a GLM with negative binomial errors due to overdispersion of data. The minimal adequate models (MAM), determined by stepwise removal of least significant terms and significant reduction of Akaike's information criterion, are reported (table 1). Likelihood ratio tests were used to summarise the effects of predictor variables. Post-hoc comparisons relative to 24˚C were performed using Tukey's tests.

2.5. Female diet consumption and fecundity

Newly emerged, unfed females were transferred to individual cages (n = 210) that comprised two stacked clear plastic cups, with the bottom cut out of the inner cup. Mesh was secured over the top of the inner cup and an oviposition substrate placed on the floor of the intact outer cup (Fanson et al., 2009). Oviposition substrates comprised a Parafilm-covered Petri dish (55 mm) containing 7 mL of 0.1% lemon essence solution. Solutions of sucrose and hydrolysed yeast ('yeast') (45 g L^{-1} for each) and water were provided in three separate pipette tips with ends loosely capped with moulding clay.

Cages were placed in incubators held at 18, 20, 22, 24, 26 or 28˚C with 14:10 photocycle (n = 30 per temperature, plus an extra 30 at 26˚C). Daily survival, and lifetime food consumption (Appendix A.2) and fecundity (number of eggs laid) of each female was recorded for up to 100 days. To evaluate temperature effects on ovarian development, females surviving from a pre-assigned subset of five individuals were harvested at 4 and 12 days after adult emergence and frozen at -80˚C. An additional 10 newly emerged females were immediately frozen at -80˚C as a reference. The abdomens of frozen females were assayed for soluble lipid and protein content (per mg wet weight) using established methods (Weldon et al., 2016).

Kaplan-Meier survival curves were built, and log-rank tests were used to compare the effects of temperature on survival. The effects of temperature and lifespan on lifetime fecundity (total number of eggs) were determined using a GLM with Poisson errors. After accounting for evaporation (Appendix A.3), lifetime sucrose and yeast intake (log x+1-transformed) and efficiency of conversion to eggs (lifetime fecundity divided by lifetime sugar or yeast consumption; log x+1-transformed) were compared using multivariate analysis of variance (MANOVA) with temperature and lifespan as predictors. Lipid and protein content (μg) of female abdomens at each temperature and age were also compared using MANOVA, with number of eggs already laid as a covariate, and abdomen weight as a covariate interacting with temperature and age. The interaction of age and temperature was not significant, so was removed from the model. Each MANOVA was followed by

univariate linear models with the same effects to identify which response variables contributed to multivariate effects. Post-hoc comparisons relative to 20˚C (the test temperature closest to that preferred by females; Figure 1A) were performed using Tukey's tests. All statistical analyses were implemented in R (version 3.6.2).

3. Results

The temperature selected by female *C. capitata* (mean ± SE = 22.1 ± 0.3˚C, median = 20.9˚C, IQR = 5.6˚C) was cooler than that of males (23.8 ± 0.3˚C, median = 23.4˚C, IQR = 8.2˚C). Despite the wider distribution of temperatures selected by males (Figure 1A), this difference was still highly significant ($W = 25008$, $P < 0.001$). The distribution of females and males when temperature was uniform along the apparatus did not differ ($W = 6835$, $P = 0.647$; Appendix A.1).

Temperature significantly affected (Table 1) the number (Figure 1B) and duration of male calling bouts (Figure 1C), and was optimised at 26˚C. Males did not call and no mating occurred at 18˚C. Mating latency, mating propensity, and copula duration varied considerably with temperature. The male selected temperature was within the range of optimal temperatures for mating latency (22-26˚C; Figure 1D) and mating propensity (20-28˚C; Figure 1E). Copula duration declined as temperature increased, with the lowest mean copula duration (127.3 ± 6.8 min) recorded at 28˚C (Figure 1F). The presence of sperm in the spermathecae of females was marginally affected by temperature, but sperm presence at 24˚C (79.4% of mated females) did not differ from that at other temperatures (Figure 1G). The MAM for sperm presence included male body weight but it was not significant. The number of sperm decreased significantly as female body weight increased (estimate = -0.101 ± 0.005), but increased significantly as male body weight increased (estimate = 0.150 ± 0.005) (Figure 2). When taking the covariates into account, mean sperm number at 24˚C was lower than at 20 or 26˚C, but significantly greater than at 22˚C (Figure 1H). The lowest mean sperm count was at 28˚C (188.6 ± 33.7).

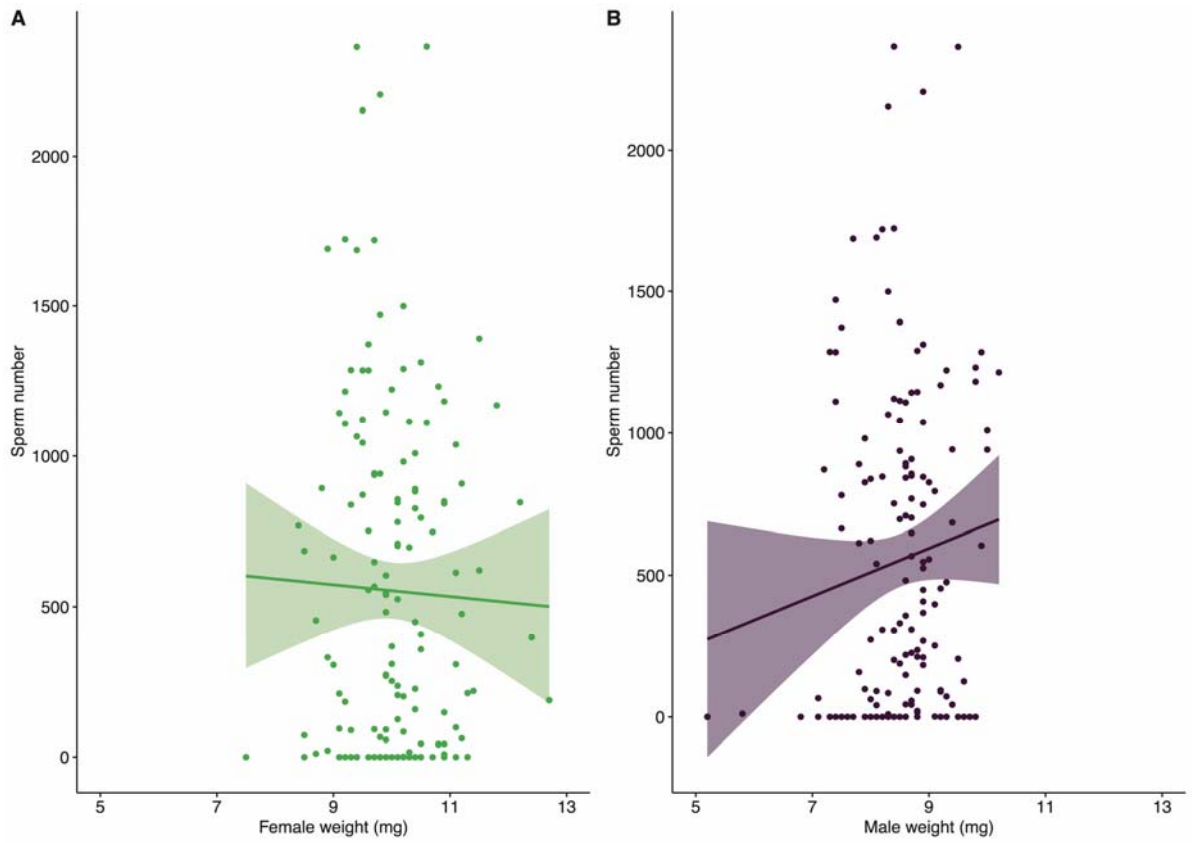


Figure 2. Relationship between female (A) and male (B) weight and the number of sperm stored in the spermathecae of female *C. capitata*.

Female survival was greater and lower than expected at 18˚C and 26˚C, respectively (table 1, Figure 3A). In contrast, cumulative egg production as females aged was at its lowest at 18˚C and highest at 26˚C (Figure 3B). The range of temperatures with the highest mean lifetime fecundity encompassed the temperature selected by females in the thermal preference assay (Figure 3C). Lifetime fecundity also significantly increased with female survival (coefficient = 0.044 ± 0.004). There was a significant effect of temperature and lifespan on multivariate lifetime diet consumption. Consumption of both sucrose (estimate = 0.008 ± 0.002) and yeast (estimate = 0.009 ± 0.002) increased with survival. When taking survival into account, lifetime consumption of sucrose at 22˚C was lower than at 20˚C, and that of yeast over the range of 22 - 28˚C was lower than at 20˚C (Figure 3D). There was a significant effect of temperature and female survival on multivariate efficiency of diet conversion to eggs. Conversion of sucrose (estimate = 0.009 ± 0.002) and yeast (estimate = 0.007 ± 0.001) to eggs increased with female survival. Sucrose conversion to eggs followed the same pattern as lifetime consumption, but yeast conversion at 18-22˚C did not differ, and was less than at temperatures in the range 24-26˚C (Figure 3E). Multivariate abdominal nutrients were significantly affected by female age, abdomen weight and the number of eggs that had been laid but not temperature. Weight-corrected abdominal lipid content was not affected by

any of the predictor variables (Figure 4). Female abdominal protein content increased with abdomen weight (estimate = 0.106 ± 0.020). Weight-corrected abdominal protein content was highest in newly-emerged (2.30 ± 0.03 μg mg^{-1}) and 4 day-old females (2.25 ± 0.02 μg mg^{-1}), which was higher than at 12 days (2.16 ± 0.03 μg mg^{-1}).

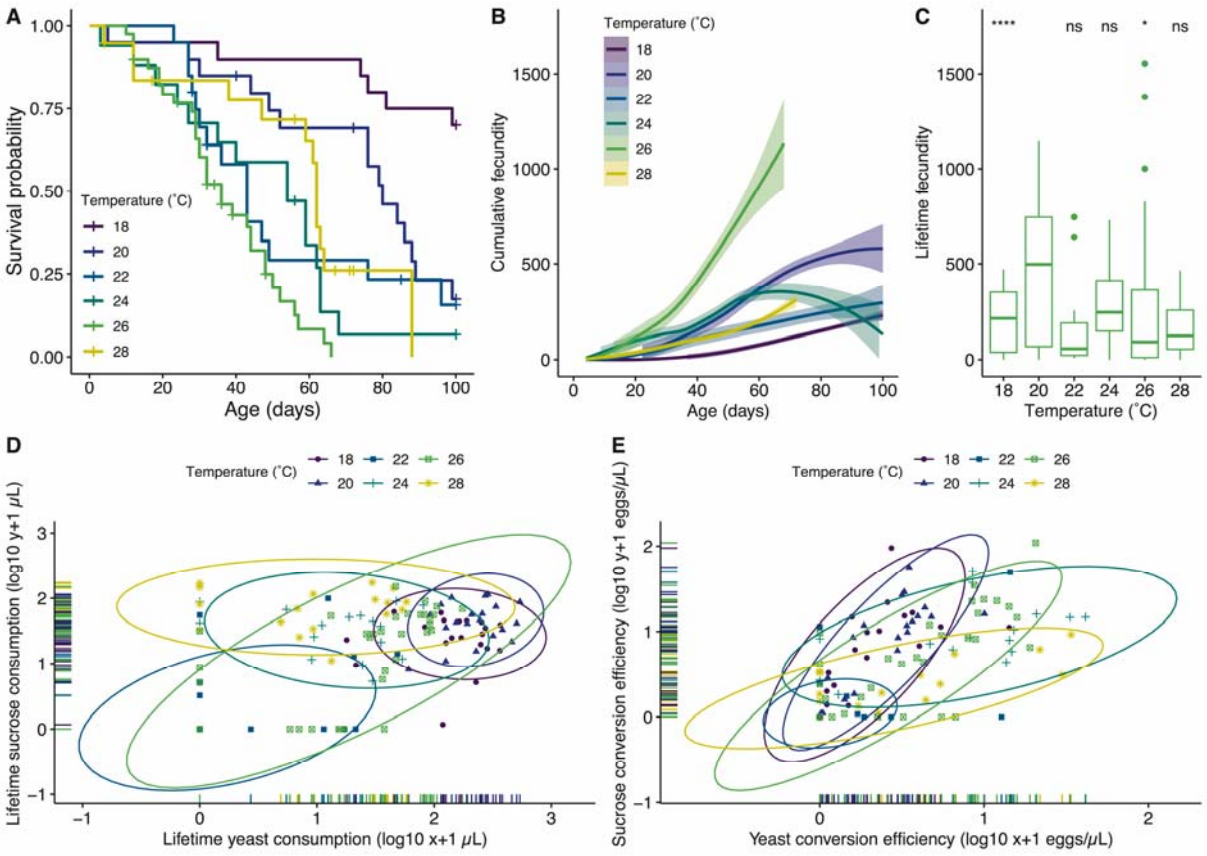


Figure 3. Effects of temperature on survival and reproductive traits in virgin *C. capitata*. (A) Kaplan-Meier survival curves for females held at different temperatures for up to 100 days. (B) Cumulative fecundity over the lifespan of females held at different temperatures. Smooth functions and standard errors were plotted using local regression (loess). (C) Effect of temperature on lifetime fecundity. The median, first and third quartiles (lower and upper box edges), 1.5 × interquartile range (whiskers), and outliers (individual points) at each temperature. Post-hoc comparisons (Tukey's tests) are shown relative to 20˚C. For post-hoc comparisons: ns (*P*) > 0.05; * < 0.05; ** < 0.01; *** < 0.001; **** <0.0001. (D) Lifetime diet consumption and (E) lifetime efficiency of diet conversion to eggs at different temperatures. Ellipses were drawn assuming a multivariate *t* distribution.

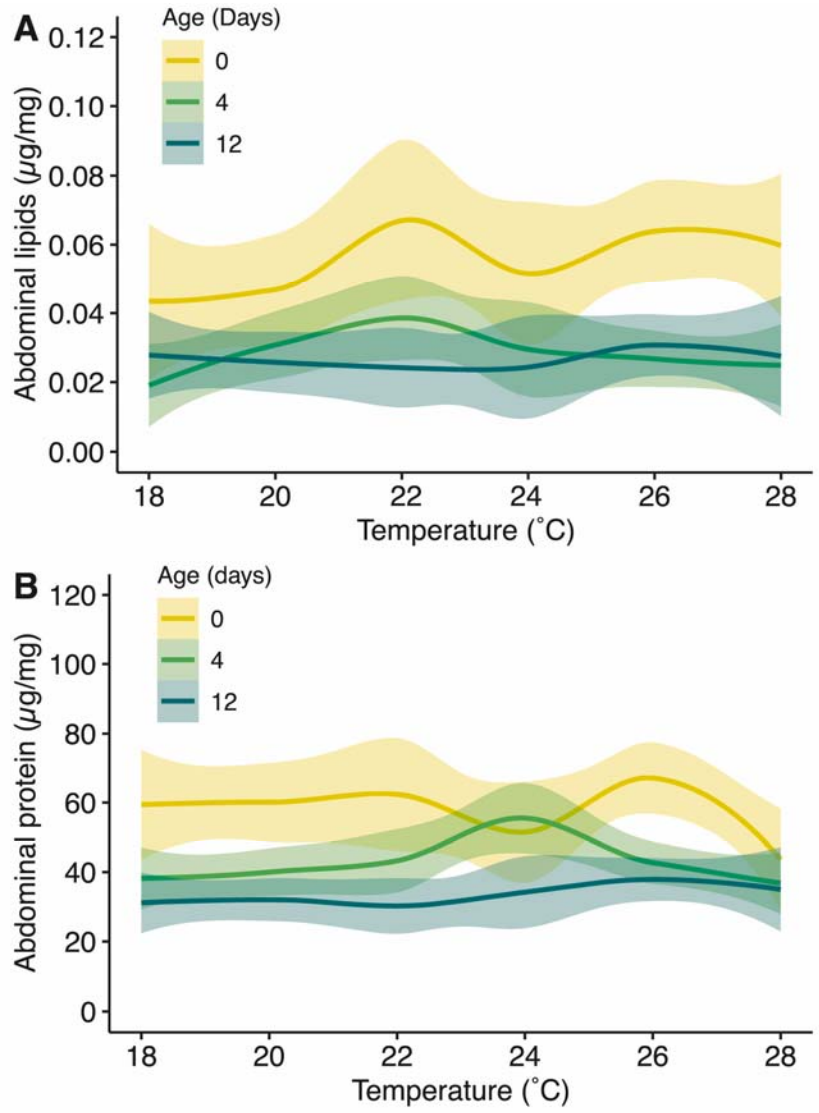


Figure 4. Abdominal (A) lipid and (B) protein content in relation to temperature and age of female *C. capitata*.

4. Discussion

The thermal optimum for *C. capitata* egg-to-egg development is 28˚C (Grout and Stoltz, 2007). However, our results demonstrate that the species has different thermal optima for its sexual performance and life history traits. Male *C. capitata* optimised their reproductive performance at or slightly higher than their preferred temperature. This suggests that males might exhibit higher fitness at higher temperatures. However, the number of sperm stored declined markedly when mating occurred at 28˚C. This is probably not due to shorter copulations at 28˚C because copula duration is not associated with sperm storage in *C. capitata* (Taylor and Yuval, 1999). Another possibility is that sperm may be damaged by even 10-15 hours of exposure to elevated temperatures, as heatwaves can reduce insect sperm transfer, viability and storage (Sales et al.,

2018). How higher temperatures ultimately affect fitness of wild males, and those released in sterile insect technique programmes, is not certain and requires investigation in a warming world.

Only lifetime fecundity was optimised at the temperature preferred by females. In addition, there was evidence for a temperature-mediated trade-off between female survival and cumulative egg production. Females held at 26˚C had the lowest survival and laid eggs rapidly, but due to their shortened lifespans, these females did not reach the highest lifetime fecundity. The highest survival was at 18˚C but at this temperature fecundity was also low. Similar results have been observed before in *C. capitata* (Vargas et al., 1997), but the trade-off was not discussed. Our data support the hypothesis that lifespan in the Natal fly, *Ceratitis rosa*, increases with altitude as a consequence of temperature-related declines (Duyck et al., 2010). Declines in adult lifespan as temperature increases have also been found in other insects (Xiao et al., 2016), and are associated with temperature-mediated lifespan-reproduction trade-offs (Lee and Roh, 2010; Norry et al., 2006). More broadly, there is a clear relationship with increasing temperature and reduced insect lifespan across latitude that scales with metabolic rate (Munch and Salinas, 2009). This reduction in lifespan that is predicted by the metabolic theory of ecology suggests that an increase in average temperature of 1.1˚C or 2.9˚C could reduce insect lifespan by 3-19% or 8-42%, respectively (Munch and Salinas, 2009).

Temperature preference deviation from the optimal temperature is not unexpected. The best body temperature for an insect to maintain is predicted to be a suboptimal one (Martin and Huey, 2008). Ectotherms are not perfect thermoregulators (e.g., Bonebrake et al., 2010; Wilson et al., 2020) and thermal performance curves tend to be non-linear and highly asymmetric (Huey and Stevenson, 1979), so an insect attempting to perfectly match body temperature to the optimum for a trait risks suffering fitness costs if an inappropriately high body temperature is selected (Martin and Huey, 2008). In *Drosophila* it has even been shown that the internal thermoreceptors function to avoid elevated temperatures (Hamada et al., 2008). Our data provide evidence for imperfect thermoregulatory capacity in *C. capitata*, with sexually mature males selecting a wider range of temperatures during thermal preference tests.

Ceratitis capitata has a wide thermal tolerance range (Nyamukondiwa and Terblanche, 2009) that is presumed to underlie its wide geographic range (Weldon et al., 2018), but in this study we found that their optimal reproductive performance is constrained to temperatures between 22-28˚C. Given these observations, survival during extreme temperature events are likely to be possible, but mating and perhaps fertility could be suppressed until more favourable conditions are encountered. Indeed,

when held at temperatures fluctuating between 35 and 24˚C, *C. capitata* exhibited a high intrinsic rate of increase (Vargas et al., 2000). Nevertheless, longer-term effects of stressful temperatures are possible with consequences for female and male fitness even when optimal conditions return (Klepsatel et al., 2016; Krebs and Loeschcke, 1994). *Ceratitis capitata* could overcome the negative effects of higher temperatures in a changing climate by seeking sheltered, cooler microclimates (Ma and Ma, 2022). Behavioural rescue from extreme conditions is particularly likely in heterogenous environments where opportunities to find suitable microclimates exist (Fey et al., 2019). For fruit flies, even within orchards planted with single cultivars, there are opportunities to locate cooler, more sheltered microhabitats due to the complex three-dimensional structure and evaporative cooling afforded by tree canopies (Pincebourde et al., 2012). Thermoregulatory behaviour has been noted in orchard tree canopies by *C. capitata*, and also in the Queensland fruit fly, *Bactrocera tryoni*, where flies moved lower in the canopy to avoid the heat of the day (Inskeep et al., 2021; Kaspi and Yuval, 1999).

In conclusion, male *C. capitata* prefer slightly warmer temperatures than females. The warmer temperatures favoured by males align with those that support improved sexual performance. Females on the other hand appear to have multiple reproductive and life history traits each with diverse thermal optima. In those cases, suboptimal may be optimal, and rather than risking injurious or potentially fitness compromising overheating, they target lower body temperatures.

Data accessibility statement

Data and R script for this study are openly available in figshare at http://doi.org/10.25403/UPresearchdata.16725130, reference number 16725130.

Declaration of competing interests

The authors declare no competing interests.

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Funding sources

C.W.W. was supported by a DST-NRF Centre for Invasion Biology Postdoctoral Research Fellowship bursary and a University of Pretoria (UP) Research Development Programme grant.

Acknowledgments

Thanks to Lanha Burger, Ruben Cruywagen, Matshidiso Hlalele, Erika Nortje, Petrus Roets and Jean Saayman for technical assistance. We are also grateful for comments from the editors and two anonymous reviewers that helped to improve the manuscript.

References

Alexander, R.D., Marshall, D.C., Cooley, J.R., 1997. Evolutionary perspectives on insect mating, in: Choe, J.C., Crespi, B.J. (Eds.), The Evolution of Mating Systems in Insects and Arachnids. University Press, Cambridge, pp. 4-31.

Anderson, J.L., Albergotti, L., Proulx, S., Peden, C., Huey, R.B., Phillips, P.C., 2007. Thermal preference of *Caenorhabditis elegans*: a null model and empirical tests. J. Exp. Biol. 210, 3107-3116.

Andersson, M., 1994. Sexual Selection. Princeton University Press, Princeton, New Jersey.

Andrew, N.R., Hart, R.A., Jung, M.P., Hemmings, Z., Terblanche, J.S., 2013. Can temperate insects take the heat? A case study of the physiological and behavioural responses in a common ant, *Iridomyrmex purpureus* (Formicidae), with potential climate change. J Insect Physiol 59, 870-880.

Basson, C.H., Levy, O., Angilletta, M.J., Clusella-Trullas, S., Herrell, A., 2016. Lizards paid a greater opportunity cost to thermoregulate in a less heterogeneous environment. Funct. Ecol. 31, 856-865.

Blay, S., Yuval, B., 1997. Nutritional correlates of reproductive success of male Mediterranean fruit flies (Diptera: Tephritidae). Animal Behaviour 54, 59-66.

Blumstein, D.T., Daniel, J.C., 2007. Quantifying Behavior the JWatcher Way. Sinauer Associates, Inc., Sunderland.

Bonebrake, T.C., Boggs, C.L., McNally, J., M, Ranganathan, J., Ehrlich, P.R., 2010. Oviposition behavior and offspring performance in herbivorous insect: consequences of climatic and habitat heterogeneity. Oikos 119, 927-934.

Buchan, P.B., Sohal, R.S., 1981. Effect of temperature and different sex ratios on physical activity and lifespan in the adult housefly, *Musca domestica*. Experimental Gerontology 16, 223-228.

Clarke, A., Fraser, K.P.P., 2004. Why does metabolism scale with temperature? Funct. Ecol. 18, 243-251.

Coggan, N., Clissold, F.J., Simpson, S.J., 2011. Locusts use dynamic thermoregulatory behaviour to optimize nutritional outcomes. Proceedings of the Royal Society B 278, 2745-2752.

Darnell, M.Z., Fowler, K.K., Munguia, P., 2013. Sex-specific thermal constraints on fiddler crab behavior. Behav. Ecol. 24, 997-1003.

Davidowitz, G., Nijhout, H.F., 2004. The physiological basis of reaction norms: The interaction among growth rate, the duration of growth and body size. Integr. Comp. Biol. 44, 443-449.

Dillon, M.E., Liu, R., Wang, G., Huey, R.B., 2012. Disentangling thermal preference and the thermal dependence of movement in ectotherms. J. Therm. Biol. 37, 631-639.

Duyck, P.-F., Kouloussis, N.A., Papadopoulos, N.T., Quilici, S., Wang, J.-L., Jiang, C.-R., Müller, H.-G., Carey, J.R., 2010. Lifespan of a *Ceratitis* fruit fly increases with higher altitude. Biol. J. Linn. Soc. 101, 345-350.

Erregger, B., Kovac, H., Stabentheiner, A., Hartbauer, M., Römer, H., Schmidt, A.K.D., 2017. Cranking up the heat: relationships between energetically costly song features and the increase in thorax temperature in male crickets and katydids. J. Exp. Biol. 220, 2635-2644.

Fanson, B.G., Weldon, C.W., Pérez-Staples, D., Simpson, S.J., Taylor, P.W., 2009. Nutrients, not caloric restriction, extend lifespan in Queensland fruit flies (*Bactrocera tryoni*). Aging Cell 8, 514-523.

Fey, S.B., Vasseur, D.A., Alujevic, K., Kroeker, K.J., Logan, M.L., O'Connor, M.I., Rudolf, V.H.W., DeLong, J.P., Peacor, S., Selden, R.L., Sih, A., Clusella-Trullas, S., 2019. Opportunities for behavioral rescue under rapid environmental change. Global Change Biol. 25, 3110-3120.

Flatt, T., Heyland, A., 2011. Mechanisms of Life History Evolution: The Genetics and Physiology of Life History Traits and Trade-Offs. Oxford University Press, Oxford.

Forsman, A., 2018. On the role of sex differences for evolution in heterogeneous and changing fitness landscapes: insights from pygmy grasshoppers. Philosophical Transactions of the Royal Society B 373, 20170429.

Gomulski, L.M., Dimopoulos, G., Xi, Z., Scolari, F., Gabrieli, P., Siciliano, P., Clarke, A.R., Malacrida, A.R., Gasperi, G., 2012. Transcriptome profiling of sexual maturation and mating in the Mediterranean fruit fly, *Ceratitis capitata*. PLoS One 7, e30857.

Grout, T.G., Stoltz, K.C., 2007. Developmental rates at constant temperatures of three economically important *Ceratitis* spp. (Diptera: Tephritidae) from southern Africa. Environ. Entomol. 36, 1310-1317.

Hamada, F.N., Rosenzweig, M., Kang, K., Pulver, S.R., Ghezzi, A., Jegla, T.J., Garrity, P.A., 2008. An internal thermal sensor controlling temperature preference in *Drosophila*. Nature 454, 217-220.

Huey, R.B., Berrigan, D., 2001. Temperature, demography, and ectotherm fitness. Am. Nat. 158, 204-210.

Huey, R.B., Kingsolver, J.G., 2019. Climate warming, resource availability, and the metabolic meltdown of ectotherms. Amercan Naturalist 194, E140-E150.

Huey, R.B., Pianka, E.R., 2007. Lizard thermal biology: do genders differ? Am. Nat. 170, 473-478.

Huey, R.B., Stevenson, R.D., 1979. Integrating thermal physiology and ecology of ectotherms: A discussion of approaches. Am. Zool. 19, 357-366.

Inskeep, J.R., Allen, A.P., Taylor, P.W., Rempoulakis, P., Weldon, C.W., 2021. Canopy distribution and microclimate preferences of sterile and wild Queensland fruit flies. Scientific Reports 11, 13010.

Jerbi-Elayed, M., Lebdi-Grissa, K., Le Goff, G., Hance, T., 2015. Influence of temperature on flight, walking and oviposition capacities of two aphid parasitoid species (Hymenoptera: Aphidiinae). J. Insect Behav. 28, 157-166.

Kaspi, R., Yuval, B., 1999. Mediterranean fruit fly leks: factors affecting male location. Functional Ecology 13, 539-545.

Killen, S.S., 2014. Growth trajectory influences temperature preference in fish through an effect on metabolic rate. J. Anim. Ecol. 83, 1513-1522.

Klepsatel, P., Galikova, M., Xu, Y., Kuhnlein, R.P., 2016. Thermal stress depletes energy reserves in *Drosophila*. Scientific Reports 6, 33667.

Klepsatel, P., Wildridge, D., Galikova, M., 2019. Temperature induces changes in Drosophila energy stores. Sci Rep 9, 5239.

Knipling, E.F., 1959. Sterile-male method of population control. Science 130, 902-904.

Krebs, R.A., Loeschcke, V., 1994. Costs and benefits of activation of the heat-shock response in *Drosophila melanogaster*. Funct. Ecol. 8, 730-737.

Lailvaux, S.P., 2007. Interactive effects of sex and temperature on locomotion in reptiles. Integr. Comp. Biol. 47, 189-199.

Lailvaux, S.P., Husak, J.F., 2014. The life history of whole-organism performance. Q. Rev. Biol. 89, 285-318.

Lance, D.R., McInnis, D.O., 2021. Biological basis of the sterile insect technique, in: Dyck, V.A., Hendrichs, J., Robinson, A.S. (Eds.), Sterile Insect Technique. Principles and Practice in Area-Wide Integrated Pest Management, 2nd edition ed. CRC Press, Boca Raton, Florida, USA, pp. 113-142.

Lee, K.P., Roh, C., 2010. Temperature-by-nutrient interactions affecting growth rate in an insect ectotherm. Entomol. Exp. Appl. 136, 151-163.

Ma, G., Ma, C.S., 2022. Potential distribution of invasive crop pests under climate change: incorporating mitigation responses of insects into prediction models. Current Opinion in Insect Science 49, 15-21.

Malacrida, A.R., Gomulski, L.M., Bonizzoni, M., Bertin, S., Gasperi, G., Guglielmino, C.R., 2007. Globalization and fruitfly invasion and expansion: the medfly paradigm. Genetica 131, 1-9.

Malek, D.K., Czarnoleski, M., 2021. Thermal preferences of cowpea seed beetles (*Callosobruchus maculatus*): Effects of sex and nuptial gift transfers. Insects 12, 310.

Martin, T.L., Huey, R.B., 2008. Why "suboptimal" is optimal: Jensen's inequality and ectotherm thermal preferences. Am. Nat. 171, E102-E118.

McCue, M.D., Boardman, L., Clusella-Trullas, S., Kleynhans, E., Terblanche, J.S., 2016. The speed and metabolic cost of digesting a blood meal depends on temperature in a major disease vector. J. Exp. Biol. 219, 1893-1902.

McDonald, P.T., 1987. Intragroup stimulation of pheromone release by male Mediterranean fruit flies (Diptera: Tephritidae). Annals of the Entomological Society of America 80, 17-20.

Munch, S.B., Salinas, S., 2009. Latitudinal variation in lifespan within species is explained by the metabolic theory of ecology. Proceedings of the National Academy of Sciences 106, 13860-13864.

Nagarajan-Radha, V., Aitkenhead, I., Clancy, D.J., Chown, S.L., Dowling, D.K., 2019. Sex-specific effects of mitochondrial haplotype on metabolic rate in *Drosophila melanogaster* support predictions of the Mother's Curse hypothesis. Philosophical Transactions of the Royal Society B 375, 20190178.

Norry, F.M., Sambucetti, P., Scannapieco, A.C., Loeschcke, V., 2006. Altitudinal patterns for longevity, fecundity and senescence in *Drosophila buzzatii*. Genetica 128, 81-93.

Nyamukondiwa, C., Terblanche, J.S., 2009. Thermal tolerance in adult Mediterranean and Natal fruit flies (*Ceratitis capitata* and *Ceratitis rosa*): Effects of age, gender and feeding status. J. Therm. Biol. 34, 406-414.

Papadopoulos, N.T., Katsoyannos, B.I., Kouloussis, N.A., Economopoulos, A.P., Carrey, J.R., 1998. Effect of adult age, food, and time of day on sexual calling incidence of wild and mass-reared *Ceratitis capitata* males. Entomologia Experimentalis et Applicata 89, 175-182.

Pérez-Staples, D., Weldon, C.W., Radhakrishnan, P., Prenter, J., Taylor, P.W., 2010. Control of copula duration and sperm storage by female Queensland fruit flies. Journal of Insect Physiology 56, 1755-1762.

Pincebourde, S., Woods, H.A., Fox, C., 2012. Climate uncertainty on leaf surfaces: the biophysics of leaf microclimates and their consequences for leaf-dwelling organisms. Functional Ecology 26, 844-853.

Plasman, M., McCue, M.D., Reynoso, V.H., Terblanche, J.S., Clusella-Trullas, S., 2019. Environmental temperature alters the overall digestive energetics and differentially affects dietary protein and lipid use in a lizard. J. Exp. Biol. 222.

Rho, M.S., Lee, K.P., 2017. Temperature-driven plasticity in nutrient use and preference in an ectotherm. Oecologia 185, 401-413.

Rogowitz, G.L., Chappell, M.A., 2000. Energy metabolism of Eucalyptus-boring beetles at rest and during locomotion: Gender makes a difference. J. Exp. Biol. 203, 1131-1139.

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. Nature Communications 9.

Schneider, C.A., Rasband, W.S., Eliceiri, K.W., 2012. NIH Image to ImageJ: 25 years of image analysis. Nature Methods 9, 671-675.

Shillington, C., 2005. Inter-sexual differences in resting metabolic rates in the Texas tarantula, *Aphonopelma anax*. Comparative Biochemistry and Physiology A 142, 439-445.

Shinner, R., Terblanche, J.S., Clusella-Trullas, S., 2020. Across-stage consequences of thermal stress have trait-specific effects and limited fitness costs in the harlequin ladybird, *Harmonia axyridis*. Evol. Ecol. 34, 555-572.

Taylor, P.W., Yuval, B., 1999. Postcopulatory sexual selection in Mediterranean fruit flies: advantages for large and protein-fed males. Animal Behaviour 58, 247-254.

Vargas, R.I., Walsh, W.A., Kanehisa, D., Jang, E.B., Armstrong, J.W., 1997. Demography of four Hawaiian fruit flies (Diptera: Tephritidae) reared at five constant temperatures. Annals of the Entomological Society of America 90, 162-168.

Vargas, R.I., Walsh, W.A., Kanehisa, D., Stark, J.D., Nishida, T., 2000. Comparative demography of three Hawaiian fruit flies (Diptera: Tephritidae) at alternating temperatures. Annals of the Entomological Society of America 93, 75-81.

Venkateswaran, V.R., Roth, O., Gokhale, C.S., 2021. Consequences of combining sex-specific traits. Evolution 75, 1274-1287.

Warburg, M.S., Yuval, B., 1997. Effects of energetic reserves on behavioral patterns of Mediterranean fruit flies (Diptera: Tephritidae). Oecologia 112, 314-319.

Weldon, C.W., Boardman, L., Marlin, D., Terblanche, J.S., 2016. Physiological mechanisms of dehydration tolerance contribute to the invasion potential of *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae) relative to its less widely distributed congeners. Frontiers in Zoology 13, 15.

Weldon, C.W., Nyamukondiwa, C., Karsten, M., Chown, S.L., Terblanche, J.S., 2018. Geographic variation and plasticity in climate stress resistance among southern African populations of Ceratitis capitata (Wiedemann) (Diptera: Tephritidae). Scientific Reports 8, 9849.

Wilson, E.S., Murphy, C.E., Rinehart, J.P., Yocum, G., 2020. Microclimate temperatures impact nesting preference in *Megachile rotundata* (Hymenoptera: Megachilidae). Environ. Entomol. 49, 296-303.

Xiao, L., He, H.-M., Huang, L.-L., Geng, T., Fu, S., Xue, F.-S., 2016. Variation of life-history traits of the Asian corn borer, *Ostrinia furnacalis*, in relation to temperature and geographical latitude. Ecology and Evolution 6, 5129-5143.