Appendix A

Male Mediterranean fruit flies prefer warmer temperatures that improve sexual performance

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Appendix A.1. Thermal gradient

A bench comprising a single sheet of aluminium was used to establish a thermal gradient (Figure S1.1). A linear thermal gradient along the top of the bench (760 mm in length) was produced by placing one leg of the bench in ice water while the other was fitted with a Peltier heating module. A plexiglass cover with ten channels confined flies to the gradient with one fly in each channel. Temperature along the gradient was measured at eight points along the bench (separated by 100 mm) with copper-constantan (type T) thermocouples attached to a data logger (TC-08, Pico Technology Ltd., Cambridgeshire, UK) to ensure that it was linear, stable and reproducible (Figure S1.2). Relative humidity within the channels was kept at close to 100% by lining the top of the aluminium bench with damp sheets of paper. Flies were introduced at random distances along the gradient by using an aspirator.

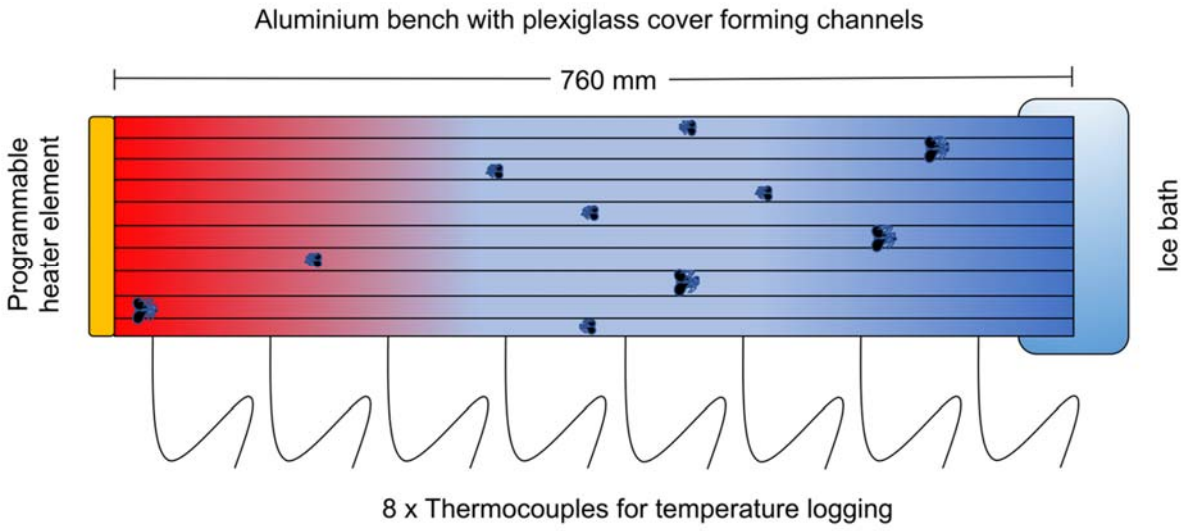

Figure A.1.1. Diagram of the aluminium bench used to test the thermal preference of female and male *C. capitata*.

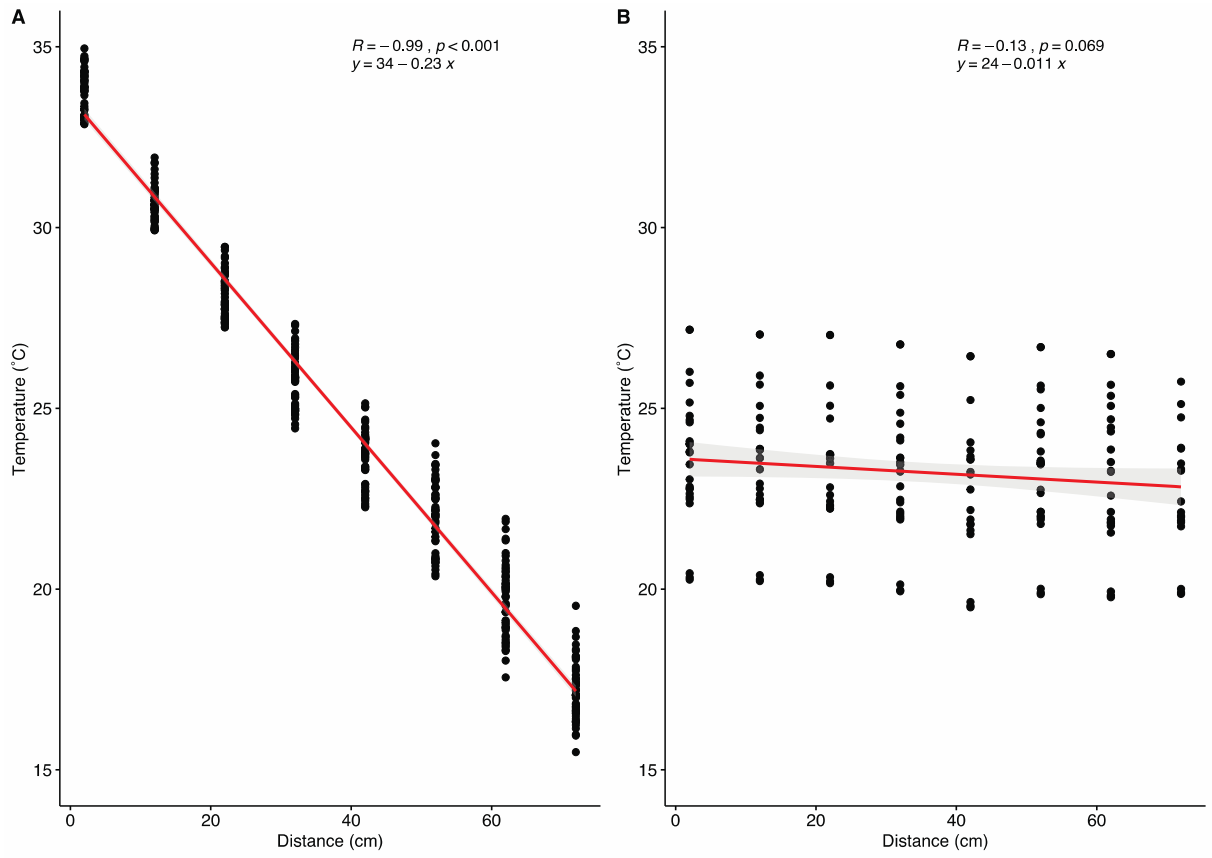

Figure A.1.2. Relationship between distance and temperature along an apparatus used to establish a linear thermal gradient. (A) Temperatures recorded when one end of the apparatus was heated with a Peltier heating module and the other was placed in ice water; (B) Temperatures when no heating or cooling was applied. The regression equations shown provide the highest R^2 value for the data. Grey shading shows the 95% confidence interval for the regression equation.

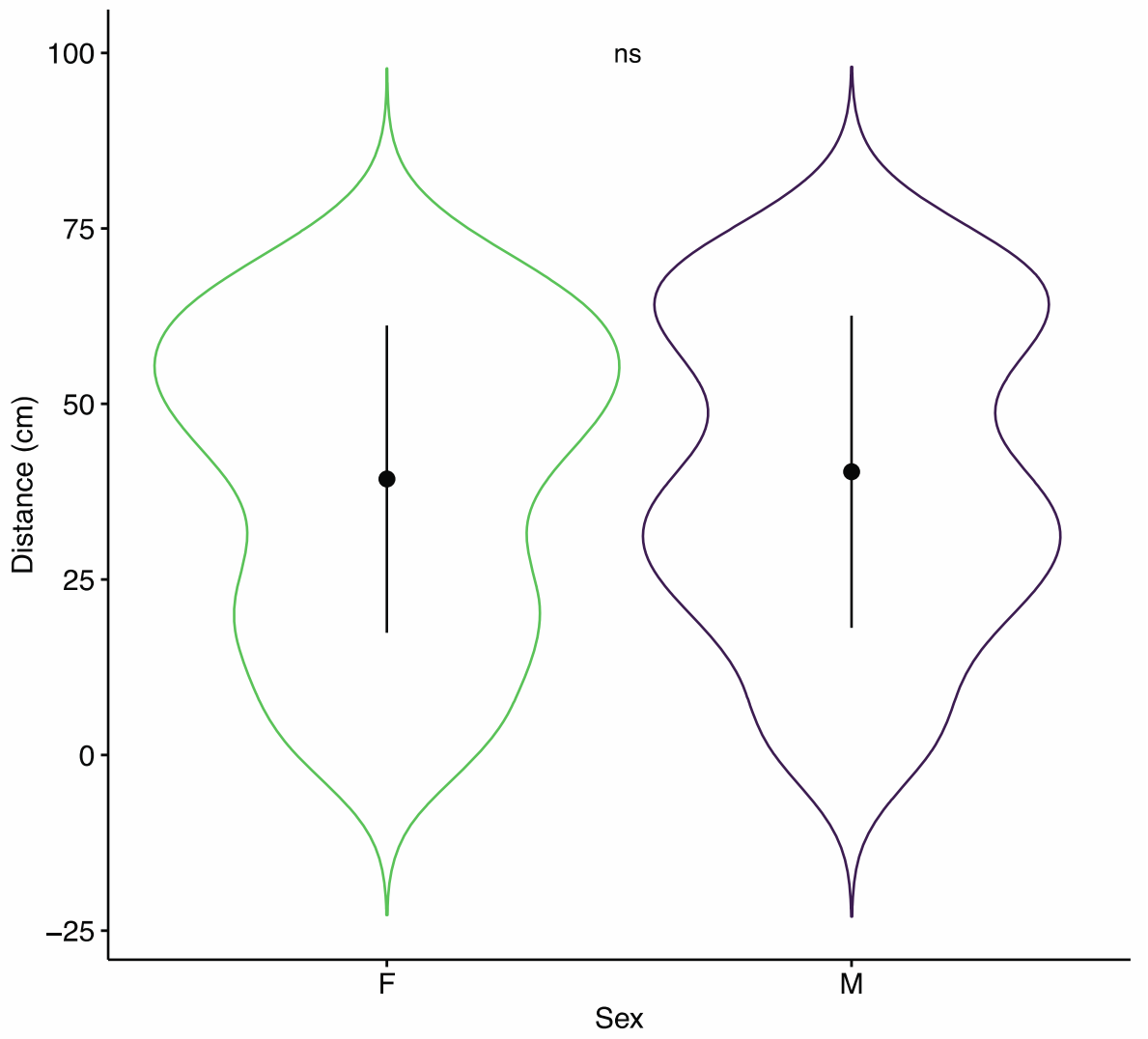

Figure A.1.3. Violin plots showing the distribution, mean and standard error of locations of sexually mature female and male flies on a thermal gradient apparatus when no heating or cooling was applied (as in Figure A.1.2). Sexes compared with Wilcoxon's signed-rank test.

Appendix A.2. Female food 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 (45 g L^{-1} for each) and water were provided in three separate pipette tips (200 µL in each) with ends loosely capped with molding clay (Prestik, Bostik, Montague Gardens, South Africa). 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).

Food and water were checked every 1-2 days and replenished if depleted. Food remaining in the pipette tips was measured with a high accuracy digital caliper to 0.001 mm (1101-150, Insize Co., Ltd., P.R. China) every 4 days (until females died or up to 100 days) before providing fresh tips of food and water. The linear distance of food remaining was converted to a volume using Equation S3.1, where d is the linear distance in µL:

$$Volume = 0.0566 \times d^{2.1941}$$

Equation A.2.1

This relationship was established by relating known tip volume to linear distance measured using the same digital caliper and using the regression model with the highest R^2 value. Oviposition substrates were removed and replaced the same day. Eggs were counted under a dissecting stereomicroscope.

At 4 and 12 days after adult emergence, females surviving from a pre-assigned subset of five were frozen at -80˚C in a freezer. An additional 10 newly emerged females were immediately frozen at -80˚C. Later, females were weighed on a microbalance (to 0.1 mg) before the abdomen was removed from each female and weighed. The abdomens of frozen females were dried to constant weight with silica gel (at 25˚C) before being assayed for dissolvable carbohydrate, lipid and protein content using colorimetric methods optimised for *Ceratitis* fruit flies by Weldon et al. (Weldon et al., 2016).

Appendix A.3. Evaporation of sugar and hydrolysed yeast solutions

Evaporation from sucrose and hydrolysed yeast solutions was accounted for by using the predictable relationship between temperature, vapor pressure deficit and volume loss in the incubators used in the study. Yellow (200 µL) pipette tips were filled with 200 µL of either a sucrose or hydrolysed yeast (HG000BX6.500, Merck, Wadesville, South Africa) solution (45 g L^{-1} for each), but with a small "boundary layer" at the tip end. After filling, the tips were loosely plugged at their wide end with molding clay. One sucrose- and one yeast-filled-tip were each inserted into the mesh top of five insect cages comprising two stacked clear plastic cups. Mesh was secured over the top of the inner cup, from which the base had been removed, and an oviposition substrate was placed on the floor of the intact outer cup. The cages were placed in an incubator at one test temperature for 24 hours. Test temperatures were 18, 20, 22, 24, 26 or 28˚C. The temperature in the incubator was verified with a copper-constantan thermocouple attached to a temperature recorder (TC-08, Pico technology Ltd., Cambridgeshire, UK) before beginning the tests, and then temperature and relative humidity was recorded during 24-hour exposure (iButton DS1923, Maxim, Sunnyvale, CA, USA). The volume lost was measured using the same method described in Appendix S3. This procedure was repeated at each temperature three times.

Relative humidity was converted to vapour pressure (kPa) using Equation S4.1, where $e^{\circ}(T)$ is the saturation vapour pressure at air temperature T (kPa):

$$VP = \left(\frac{relative\ humdity}{100}\right) \times\ e^{\circ}(T)$$

Equation A.3.1

Linear regression was used to predict the relationship between temperature and vapor pressure, as well as temperature and volume loss due to evaporation. These relationships in the incubators were quite stable and predictable (Figure S4.1). The linear regression equations for daily volume loss of sucrose and yeast solutions due to evaporation in relation to temperature were used to estimate volume lost over four days (to the last full four-day period that the fly was alive) during the female feeding experiments. Consumption by females (µL) was calculated using Equation S4.2, where V_i is initial volume, V_r is volume remaining, and E is estimated evaporation. If $E > (V_i - V_r)$, consumption was set at 0 µL:

$$Consumption = (V_i - V_r) - E$$

Equation A.3.2

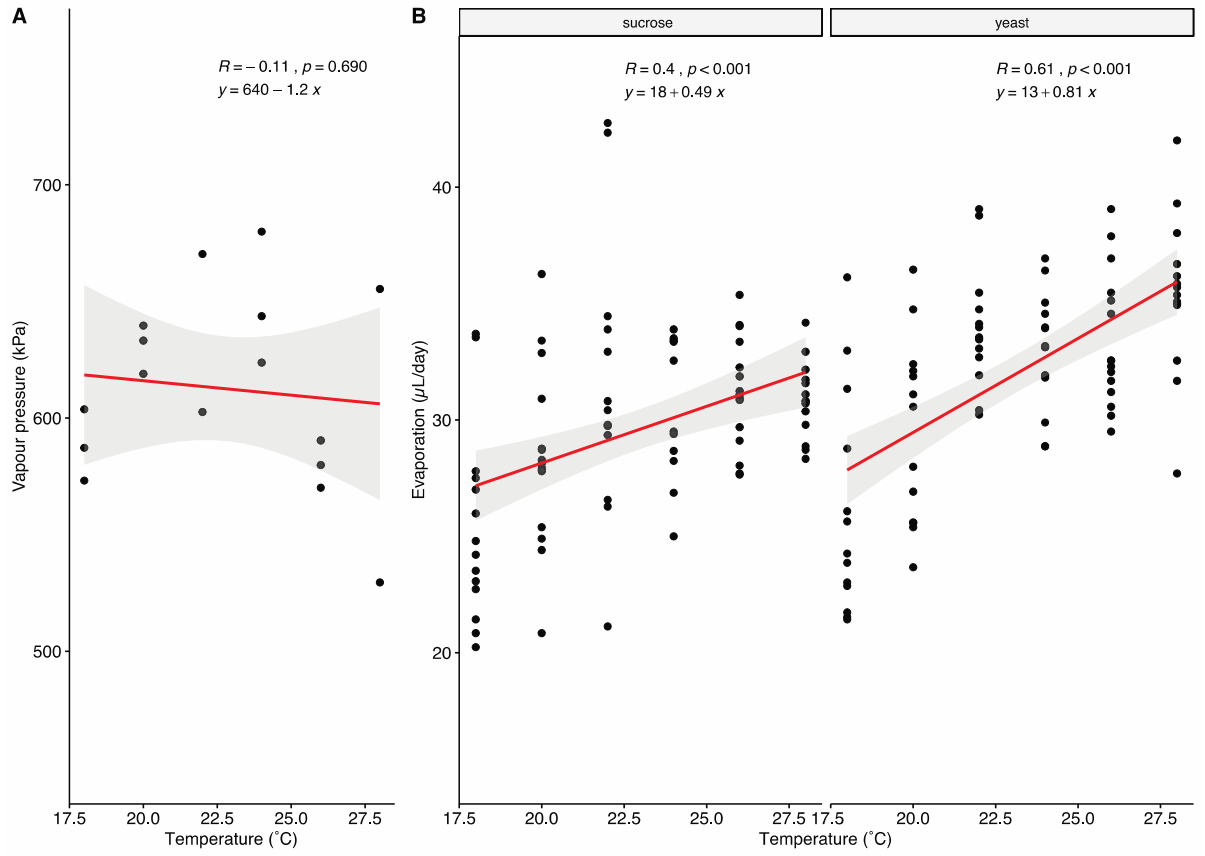

Figure A.3.1. Relationship between incubator temperature and (A) vapour pressure, and (B) volume loss from a pipette tip containing 200 µL of sucrose or yeast hydrolysate solution (45 g L^{-1} for both). The R^2 value for the linear regression is presented. Grey shading shows the 95% confidence interval for the regression equation.

References

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.

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